

Contribution of eye retraction to swallowing performance in the northern leopard frog, *Rana pipiens*

Robert P. Levine^{1,*}, Jenna A. Monroy² and Elizabeth L. Brainerd¹

¹*Biology Department and Organismic and Evolutionary Biology Program, University of Massachusetts Amherst, 611 North Pleasant St, Amherst, MA 01003, USA* and ²*Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA*

*Author for correspondence (e-mail: robblevine@mac.com)

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Summary

Most anurans retract and close their eyes repeatedly during swallowing. Eye retraction may aid swallowing by helping to push food back toward the esophagus, but this hypothesis has never been tested. We used behavioral observations, cineradiography, electromyography and nerve transection experiments to evaluate the contribution of eye retraction to swallowing in the northern leopard frog, *Rana pipiens*. Behavioral observations of frogs feeding on 1.5 cm long crickets reveal a high degree of variability in eye retraction and swallowing. Eye retraction can occur bilaterally or unilaterally, and both swallowing movements and eye retraction can occur separately as well as together. During swallowing, cineradiography shows that the eyes and associated musculature retract well into the oropharynx and appear to make contact with the prey item. This contact appears to help push the prey toward the esophagus, and it may

also serve to anchor the prey for tongue-based transport. Electromyographic recordings confirm strong activity in the retractor bulbi muscles during eye retraction. After bilateral denervation of the retractor bulbi, frogs maintain the ability to swallow but show a 74% increase in the number of swallows required per cricket (from a mean of 2.3 swallows to a mean of 4.0 swallows per cricket). Our results indicate that, in *Rana pipiens* feeding on medium-sized crickets, eye retraction is an accessory swallowing mechanism that assists the primary tongue-based swallowing mechanism.

Movies available on-line

Key words: eye retraction, swallowing, feeding, frog, *Rana pipiens*, Anura, denervation, retractor bulbi, biomechanics.

Introduction

It is often suggested that eye retraction contributes to swallowing in frogs and toads (e.g. Dickerson, 1906; Regal and Gans, 1976; Nishikawa, 2000; Schwenk, 2000a) but, to our knowledge, this hypothesis has never been tested. The mechanism by which eye retraction might aid swallowing is stated charmingly in the following passage from Mary Dickerson's *The Frog Book* (Dickerson, 1906; pp. 82-83):

“[A May beetle] furnishes a mouthful that makes the toad shut his eyes hard several times to get the big thing swallowed. For, strange as it may seem, the large eyes of the toad can be pressed down into the mouth as far below its roof as they rise above the head, and the movement aids effectually in swallowing.”

There are two hypotheses embedded in this passage. The first is that the eyes actually do retract far enough down into the oropharynx to contact the prey item, and the second is that eye retraction contributes to swallowing performance. The goal of the present study is to test these two hypotheses.

An alternative hypothesis is that eye retraction does not

contribute to swallowing and instead may be a non-functional consequence of other feeding movements. Eye retraction also occurs during prey capture (Nishikawa, 2000). In this context, eye retraction may serve to protect the eyes, and eye retraction during swallowing could be a byproduct of mechanical linkages developed for prey capture. It is also possible that subambient pressure in the oropharynx during swallowing could result in passive eye retraction.

The feeding behaviors of frogs have been studied extensively over the past decade and provide an excellent model system for applying integrative approaches to the study of functional morphology and neural control (reviewed in Nishikawa, 2000). While most frogs seem to be similar upon superficial inspection, closer examination reveals an array of morphological and functional specializations, especially in their feeding apparatus. Frogs feed using coordinated movements of the cranium, mandibles, hyoid and tongue. The biomechanics and kinematics of these elements and their associated muscles have been studied extensively during prey capture. Recent studies by Nishikawa and colleagues have

furthered our understanding of frog feeding behavior by combining nerve transection with kinematic analysis to examine the neural networks underlying feeding behavior (Nishikawa and Roth, 1991; Nishikawa et al., 1992; Anderson and Nishikawa, 1993; Nishikawa and Gans, 1996; Anderson et al., 1998; Nishikawa, 1999). By contrast, only a few studies have touched upon the mechanics of prey manipulation and swallowing (Regal and Gans, 1976; Ritter and Nishikawa, 1995; Gray et al., 1997; Valdez and Nishikawa, 1997).

Regal and Gans (1976) concluded that frogs use the tongue as the primary organ for manipulating and swallowing prey but suggested that movements of the head, jaws and eyes may also contribute to prey transport. Denervation of the hyoglossus muscle, responsible for retracting the tongue, demonstrated that the tongue plays an important role in oral transport and swallowing (Ritter and Nishikawa, 1995; Tso et al., 1995). Gray et al. (1997) expanded our understanding of prey manipulation by showing that forelimb movements can also play an important role in both prey capture and transport. Gray and colleagues proposed that while lingual movements may be the primary mechanism for transporting small prey, forelimb movements may facilitate transport of larger prey.

In this study we used cineradiography, electromyography, behavioral observations and muscle denervation experiments to investigate the contribution of eye retraction to swallowing performance in the northern leopard frog, *Rana pipiens*. We used cineradiography to determine whether the eyes retract far enough down into the oropharynx to contact the prey, and electromyography of retractor bulbi muscles to determine whether eye retraction is active or passive. Muscle denervation was chosen because it provides a direct test of whether a particular muscle, in this case the retractor bulbi muscle, is necessary for performing a given behavior (Nishikawa and Roth, 1991). If the elimination of eye retraction by denervation of the retractor bulbi muscles inhibits the frog's ability to swallow, then we shall conclude that eye retraction is essential for swallowing. If swallowing is still possible, but performance decreases, then we shall conclude that eye retraction contributes to swallowing. If denervation has no effect on swallowing performance, then we shall conclude that eye retraction does not contribute to swallowing.

Materials and methods

Study animals

Male, adult northern leopard frogs (*Rana pipiens* Schreber 1782), with a snout–vent length ranging from 5.5 cm to 7.0 cm ($N=24$), were purchased from a commercial dealer (J. M. Hazen Frog Co., Alburg, VT, USA). Frogs were housed at room temperature (21–23°C) in individual plastic containers lined with moist paper towels. Animals were fed a diet of crickets (*Gryllus*) twice a week and were deprived of food for 5 days before all experimental observations to standardize motivation level and to ensure that all frogs performed multiple feeding trials. All procedures were approved by the

Institutional Animal Care and Use Committee of the University of Massachusetts Amherst (approval number 20-10-06).

Two specimens were dissected to determine the anatomical position and fiber orientation of the retractor bulbi muscle. The Sudan Black B method for nerve staining followed by whole specimen clearing was performed on one specimen to trace the path of the abducens nerve and its rami innervating the retractor bulbi muscle (Filipski and Wilson, 1984).

Cineradiography

Cineradiographic images were used to determine whether the eyes are retracted into the oral cavity during feeding. For three individuals, the positions of the eyes were marked using a single lead ball (≤ 0.5 mm in diameter) implanted into each retractor bulbi muscle at the point of insertion onto the medial surface of the eyeball. The retractor bulbi muscles were accessed by making a 3 mm mid-sagittal incision into the palatal skin overlying the parasphenoid bone. A 1.0 mm incision was made in the epimysium of each retractor bulbi muscle, and a pair of Dumont fine-tip forceps were used to implant the lead balls into the belly of each muscle, near its point of insertion. The incision in the palatal skin was closed using 6.0 silk surgical suture.

Five feeding sequences for each individual were filmed in lateral view. To visualize the prey's position, the dorsal side of each cricket was marked with barium powder using cyanoacrylate glue. X-ray videos were recorded using a Siemens cineradiographic unit with a Sirecon image intensifier. A Sony miniDV camcorder with a shutter speed of 1/250 s was used to record the images from the cineradiographic unit at a time resolution of 30 frames s^{-1} .

Electromyography

Electromyographic (EMG) recordings of the retractor bulbi muscles of one individual during feeding were used to determine whether the retractor bulbi muscles are active during eye retraction. Two bipolar, fine-wire hook electrodes were constructed from 0.05 mm nichrome insulated wire (California Fine Wire Co., Grover Beach, CA, USA). The frog was anesthetized in a solution of 0.1 g tricaine methanesulfonate (MS-222) per liter of water, buffered to a pH of 7 using sodium bicarbonate. During implantation of each electrode, the frog was placed on its back with its mouth propped open, and all but the upper jaw was covered with moist toweling to avoid desiccation. With a 23-gauge hypodermic needle, the electrodes were inserted through the skin of the palette and into each retractor bulbi muscle. The electrode leads were passed through the nares and sutured to the skin just posterior to the narial openings using 6.0 silk surgical suture. All four electrode leads were bound together and secured to the back of the frog using a central suture. Following feeding trials, the animal was euthanized and electrode placement was verified by dissection.

Signals were amplified 1000–10 000 times and filtered with a 60 Hz notch filter, a 10 Hz high-pass filter and a 5000 Hz low-pass filter (model number 1700; A-M Systems AC Amplifier, Everett, WA, USA). Each signal was digitized at

1000 samples s^{-1} using an InstruNet analog-to-digital converter with Superscope II software (GW Instruments, Somerville, MA, USA).

Electromyograms from five feeding sequences were recorded and synchronized with standard video to confirm the presence and extent of eye retraction. EMGs and feeding sequences were temporally correlated to determine the stage in the feeding sequence in which the retractor bulbi muscles were active.

Behavioral observations

Eight individuals were observed during trials of normal feeding. For each individual, five feeding sequences were filmed in lateral view at 30 frames s^{-1} using a Sony miniDV camcorder. During filming, frogs were placed in a glass aquarium (25 cm \times 25 cm \times 30 cm) with a black background that was lit from above by a fiber optic illuminator. Frogs were fed one cricket at a time with at least 10 min between prey capture and the onset of the next feeding trial to allow time for complete swallowing of each cricket. The size of the crickets (1.5 \pm 0.2 cm) was also controlled, as it has been shown that *Rana pipiens* will modulate the mechanism it uses to capture, and potentially swallow, prey based on prey size and type (Anderson, 1993; Anderson and Nishikawa, 1993).

Each feeding sequence was viewed frame-by-frame to determine the number of swallows and eye retractions used to ingest each cricket. For the purpose of this study, we defined swallowing as any elevation of the floor of the buccal cavity that was greater than what is normally seen in breathing or buccal oscillation. Along with analyzing the number of swallows and eye retractions for each feeding trial, the movements of the head, mouth and body were qualitatively described. Cineradiographic sequences were also used to supplement the standard video for behavioral analysis.

The duration of eye retractions ($N=40$ retractions in eight individuals) and the position of the eye during retraction ($N=10$ retractions in five individuals) were analyzed. Video sequences were imported into Image J (version 1.29; rsb.info.nih.gov/ij/) and the distance from the top of the eye to the bottom of the eye socket was measured for each frame.

Denervation experiments

In order to determine whether eye retraction contributes to swallowing performance, 24 frogs were subjected to three experimental procedures: control filming, denervation of the retractor bulbi and sham denervation of the retractor bulbi. Frogs were separated randomly into three treatment groups comprising eight frogs each. Each frog was subjected to only one treatment. For all treatment groups, the number of swallows per cricket, as defined in the behavioral analysis, was quantified as a measure of performance.

Filming of the control group was used to establish a baseline measure of swallowing performance in frogs that were able to retract their eyes normally. Each frog was placed in a glass aquarium (25 cm \times 25 cm \times 30 cm) and filmed in lateral view with a Sony miniDV camcorder. For each trial, frogs were fed one cricket at a time, and the number of swallows was counted

for 10 min following the onset of prey capture. An equal number of feeding sequences (five) was recorded for each frog to control for satiety.

The second treatment group was the experimental group, consisting of eight frogs that underwent bilateral denervation of the retractor bulbi muscle. The retractor bulbi muscle is innervated by a large branch of the abducens nerve (Duellman and Trueb, 1986). Each frog was anesthetized and the retractor bulbi muscle was accessed as described for implantation of radiopaque markers. The branch of the abducens nerve innervating the retractor bulbi muscle was exposed near the point where it enters each muscle. Each nerve was transected, and a 1.0–2.0 mm section was removed to ensure effectiveness of denervation (Nishikawa and Roth, 1991). The incision was then closed using 6.0 silk suture and surgical adhesive. Frogs were allowed to recover from the anesthesia for at least 2 h and then filmed during feeding by the same method as described for the control group. After feeding sequences were filmed, frogs were euthanized and nerve transection was verified by dissection.

The last treatment group was a sham group, used to test the effect of surgical trauma on swallowing performance. This group also consisted of eight frogs that underwent a surgery to mimic the actual denervation surgery in every way, excluding cutting of the nerve. After the sham-operated frogs recovered from the anesthesia, feeding behaviors were filmed as described above.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to test for significant differences between the control, sham and denervation treatment groups. The mean number of swallows per cricket for each individual was used as the measure of performance. A Tukey *post-hoc* test was used for pairwise comparisons between treatment groups (Zar, 1999). All statistical analyses were performed in JMP (SAS Institute Inc., Cary, NC, USA) using a significance level of $P<0.05$.

Results

Behavioral observations

Observations from 11 individuals (eight in standard video and three in x-ray video) revealed that swallowing behaviors involve movements of the head, hyobranchial apparatus, jaws, tongue and eyes (Fig. 1). Swallowing movements generally began within 2 s of prey capture and continued for a highly variable amount of time, ranging from 22 s to 7.15 min. A typical swallowing sequence began with slight lordosis of the vertebral column and extension of the head. Eye retraction then began as the head reached full extension (Fig. 1B) and continued as the head and spine returned to their rest positions (Fig. 1C). The eyes remained retracted for a variable length of time and then returned to their rest position (Fig. 1D).

The first swallowing movement after prey capture was stereotypical for every individual and was accompanied by strong bilateral eye retraction. This stereotypical first swallow

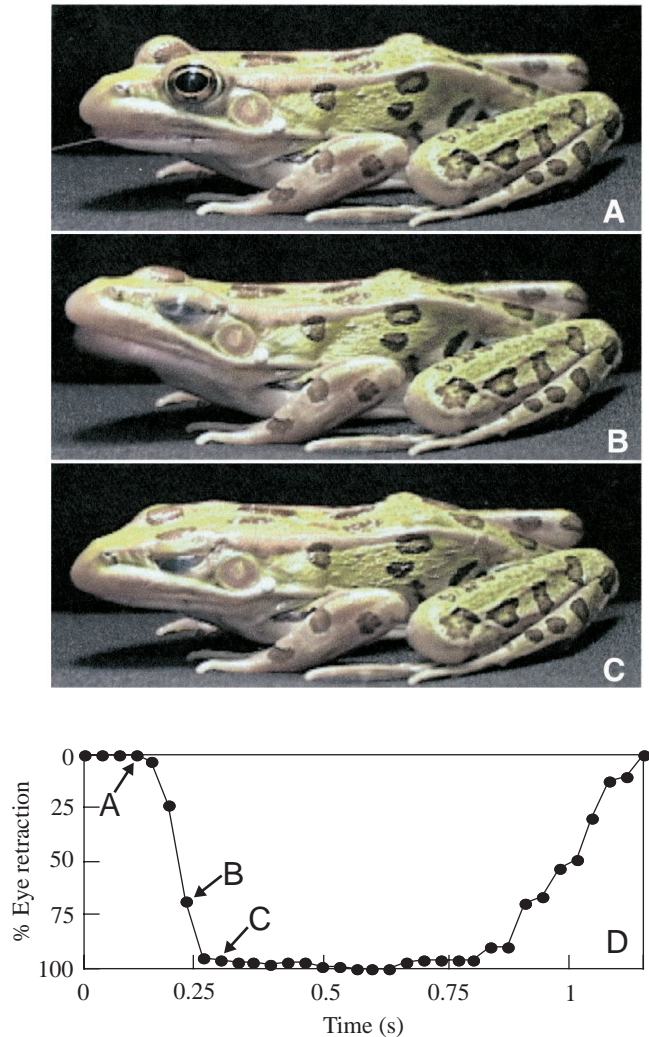


Fig. 1. Video frames from a swallowing sequence illustrating (A) the normal position of the eyes; (B) head extension and lordosis of the vertebral column during initial eye retraction; (C) full eye retraction and (D) a kinematic trace of the percent eye retraction *versus* time for the same swallowing sequence. The arrows in D, labeled A, B and C, correspond with the frames from the swallowing sequence. Note that eye retraction is rapid, the eyes remain retracted for a relatively long time, more than 0.5 s in this example, and then eye protraction is slower than eye retraction. A representative video of *Rana pipiens* swallowing a cricket can be viewed online (<http://jeb.biologists.org>).

was followed by variable bouts of eye retraction and swallowing. Eye retraction and swallowing occurred both simultaneously and independently and were coupled with movements of the body. Bilateral eye retraction comprised 92% of all retractions, while unilateral retraction seemed to occur more frequently when the prey was positioned on one side of the mouth. However, the latter observation could not be quantified.

Our video observations show that eye retraction and swallowing are not obligately coupled. In 42% of the observed

swallows, swallowing occurred without eye retraction ($N=90$ swallows, 40 trials from eight individuals). In addition, frogs at rest (with no food in the oropharynx) retract their eyes at regular intervals. Eye retraction at rest may function to moisten and clean the eyes, much like the function of blinking in mammals. These non-feeding retractions are briefer, and eye depression is less pronounced, than in the eye retractions associated with swallowing.

The duration of eye retraction during swallowing was variable, with a range of 0.19–3.05 s and a mean of 1.25 ± 0.11 s (mean \pm S.E.M.; $N=40$ retractions in eight individuals). Eye retraction occurred in three distinct phases (Fig. 1). The initial phase, consisting of eye depression, was rapid and generally lasted less than 0.1 s. This phase was followed by a period of maximal retraction that was the basis for much of the variability seen in total duration. In the final phase, the eyes returned to their resting position. The speed at which the eyes returned to the resting position was slower and more variable than the initial phase of retraction (Fig. 1D).

In the swallowing sequences, two distinct body postures were observed. In the first and most common one, the frog sat upright with its forelimbs only slightly bent. In the second, the frog was in a crouched position with its abdomen and chest resting on its forelimbs, which were slightly tucked under the body. Eye retraction and swallowing occurred in both postures but was less dramatic in the latter.

During prey capture, eye retraction sometimes occurred as the frog lunged forward and protracted the tongue. We found that eye retraction occurred during the strike in 46% of normal prey capture sequences ($N=40$ strikes in eight individuals).

Cineradiography

X-ray videos of *R. pipiens* feeding on barium-marked crickets revealed that the eyes and associated musculature are retracted deep into the oropharynx during swallowing ($N=15$ feeding sequences from three individuals). In all trials, frogs had no difficulty capturing prey or swallowing, suggesting that the implantation procedure and the radiopaque markers did not affect normal feeding behaviors.

During eye retraction associated with swallowing, the eyes moved ventrad and caudad, came in contact with the prey and appeared to force it toward the pharynx (Fig. 2). Once crickets passed into the esophagus, eye retraction and swallowing movements ceased, and rhythmic prey movements, presumably associated with peristalsis, completed swallowing of the cricket.

Electromyography

Dissection of two individuals confirmed that the retractor bulbi muscle originates from the orbitosphenoid, with its dorsal margin abutting the prootic (Duellman and Trueb, 1986). Parallel muscle fibers run anteriomedially and insert on the ventral and medial surfaces of the eyeball.

Electromyograms were recorded from hook electrodes implanted into the right and left retractor bulbi muscles of one individual ($N=5$ feeding trials). EMG activity was observed

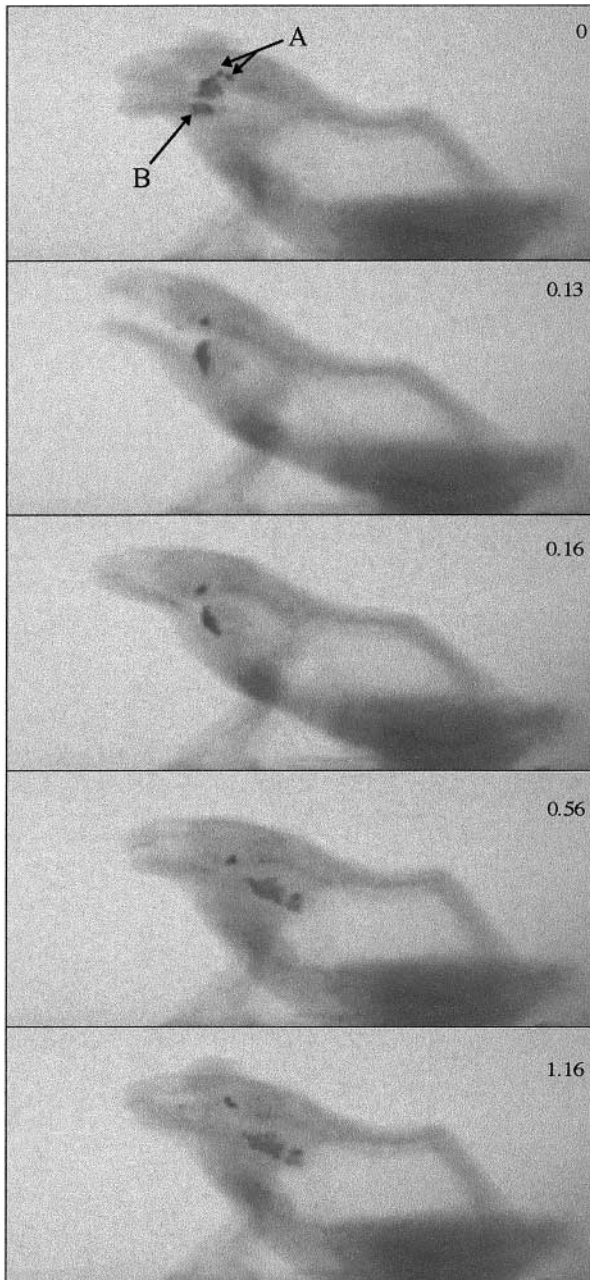


Fig. 2. Selected x-ray video frames of *Rana pipiens* swallowing a cricket, illustrating the movement of the eyes during feeding. Lead balls (arrow A) mark the insertion of the retractor bulbi on the ventral surface of the eyes. A cricket has been marked with barium powder (arrow B) to facilitate viewing in x-ray. Note that as the eyes retract into the oropharynx, the cricket moves back towards the esophagus. Time (seconds) is indicated on each frame. A representative x-ray video of *Rana pipiens* swallowing a cricket can be viewed online (<http://jeb.biologists.org/>).

during every eye retraction ($N=16$ retractions) but not during normal breathing or swallowing without eye retraction. The onset of muscle activity occurred shortly before the beginning of eye retraction (Fig. 3). A strong burst of muscle activity was observed at the onset, with diminishing intensity throughout

Table 1. Results from ANOVA and pairwise, post-hoc tests on mean number of swallows

Source	d.f.	F	P
Model	2	54.42	<0.001
Error	21		
Total	23		

Pairwise comparisons	Q	P
Control vs sham	0.172	0.99
Control vs denervation	12.862	<0.001
Sham vs denervation	12.691	<0.001

the retraction event. Our results confirm that the retractor bulbi muscles are active during the eye retraction associated with swallowing in *R. pipiens*.

Denervation experiments

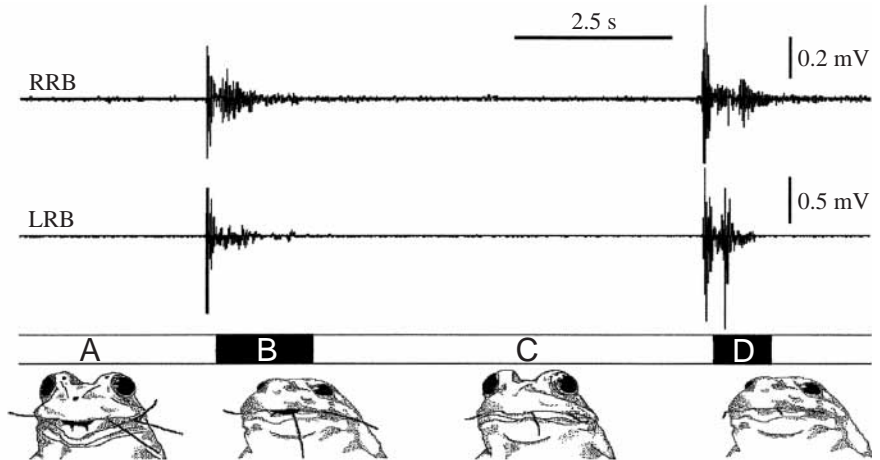
Nerve staining combined with dissection revealed that the abducens nerve (VI) originates from the ventral surface of the hindbrain and travels rostrad through the optic foramen before bifurcating and innervating the rectus lateralis and the retractor bulbi muscles. The ramus innervating the retractor bulbi runs along the dorsomedial surface of the muscle before terminating at the muscle's most anterior end. Thus, the branch of the abducens nerve innervating the retractor bulbi muscle can be accessed surgically through the roof of the oral cavity with little damage to the surrounding tissues.

Following transection of the branch of the abducens innervating the retractor bulbi muscle, frogs were not able to perform eye retraction. Other movements associated with swallowing did not appear to be affected by nerve transection. Results from the analysis of variance (ANOVA) show a statistically significant effect of the treatment group (control, sham and denervation) on mean number of swallows per cricket ($F_{2,21}=54.42$, $P<0.0001$; Fig. 4). Tukey *post-hoc* tests did not show a statistically significant difference between the control and the sham treatments ($P=0.99$) but did reveal a significant difference between the control and denervation treatments ($P<0.0001$), as well as a significant difference between the sham and denervation treatments ($P<0.0001$) (Table 1; one-way ANOVA with Tukey pairwise *post-hoc* tests).

Discussion

For eye retraction to contribute to swallowing performance, we hypothesized that the eyes and associated muscles must move down into the oropharynx during swallowing. From external views, it is clear that the eyes retract into the head (Fig. 1), but it is possible that they do not move all the way down into the oropharynx. Frogs have large palatal vacuities that potentially allow the eyes to retract below the palate, but x-ray imaging is required to see whether the eyes actually do. Our x-ray videos show that the eyes are retracted well into the

Fig. 3. Electromyographic (EMG) activity from the right (RRB) and left (LRB) retractor bulbi muscles of *Rana pipiens* during swallowing. Black bars represent the duration of each eye retraction and were calculated from synchronized light video. Line drawings represent the position of the eyes during periods of relaxation (A,C) and during active retraction of the eyes (B,D). Note that bilateral EMG activity starts shortly before eye retraction begins and ends as the eyes return to their rest positions.



oropharynx and come in contact with the prey bolus during swallowing (Fig. 2).

During eye retraction, the eyes move both ventrad and caudad, indicating that they may act to push the prey toward the esophagus (Fig. 2). An alternative or additional function of eye retraction may be to assist the tongue-based transport mechanism by anchoring the prey at appropriate times during cycles of tongue protraction and retraction. In this case, the function of eye retraction would be analogous to the function of palatopterygoid teeth in lizards that use lingual prey transport (Bramble and Wake, 1985; Schwenk, 2000b). The x-ray video sequences that we collected appear to be consistent with both hypotheses. The prey moves ventrad and caudad as the eyes retract, and then the eyes remain retracted while the tongue is presumably protracting in preparation for the next cycle of swallowing. However, we did not implant radiopaque markers in the tongue so we cannot confirm the relative timing of tongue and eye movements.

We then tested whether the eye retraction observed in x-ray video results from active contraction of the retractor bulbi muscles or whether it may result from subambient pressures in the oropharynx pulling the eyes in during swallowing. Gans and Pyles (1983) showed that frogs have the ability to seal the oral cavity and, as a result, to generate positive pressures during buccal pumping and breathing (Martin and Gans, 1972). Their ability to seal this cavity makes it theoretically possible for frogs to generate sufficient subambient pressure in the oropharynx during swallowing to produce passive eye retraction. To explore this possibility, we recorded the electrical activity of the retractor bulbi muscles during swallowing. EMG recordings show that the retractor bulbi muscles are active during eye retraction (Fig. 3), indicating that this behavior is not a passive consequence of low pressure in the oropharynx.

After confirming that the eyes are actively retracted into the oropharynx, we then used bilateral denervation of the retractor bulbi muscles to test whether swallowing can occur without eye retraction, and, if so, to quantify the contribution of eye retraction to swallowing performance. We found that swallowing is still possible in the absence of eye retraction but

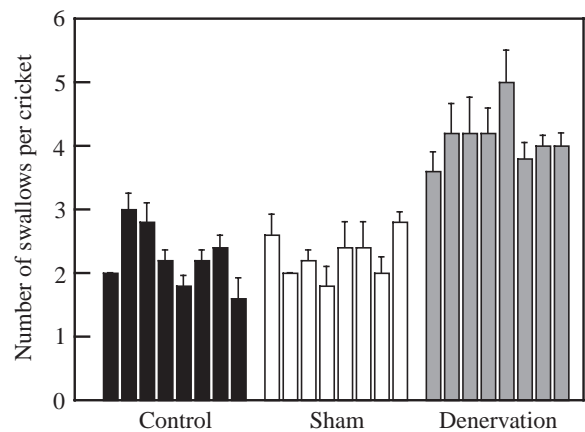


Fig. 4. Bar chart of the mean number of swallows for each individual frog for the three treatment groups: control, sham and denervation. Each bar represents the mean (\pm S.E.M.) of five feeding trials for one individual ($N=8$ individuals per treatment, five feedings per individual). One-way ANOVA with Tukey *post-hoc* tests revealed a significant difference between the control and the denervation groups and the sham and the denervation groups but no difference between the control and sham group (see Table 1).

that denervation increased the mean number of swallows per cricket from 2.3 to 4.0 (a mean increase of 74% above the control condition; Fig. 4).

We considered using other measures of swallowing performance in addition to number of swallows per cricket but found none that seemed appropriate. The total amount of time that it takes to swallow a cricket might be a good measure, but we found that this time was highly variable, ranging from 22 s to 7.15 min (in control treatment frogs). This wide range of total swallowing times results from variability in the lengths of pauses between swallowing movements. It is unclear why these pauses are so variable. We also considered using time per swallow and distance that the prey is transported per swallow, but this would have required x-ray studies of control, sham-operated and denervated frogs, and this was precluded by practical considerations.

Because we found that frogs can still swallow without eye retraction, it seems likely that other movements, such as those of the tongue, serve as the primary swallowing mechanism, as suggested by Regal and Gans (1976), with eye retraction serving as an accessory mechanism. However, we propose one caveat to this conclusion: the prey crickets used in this study were fairly small and easily swallowed by the frogs. The contribution of eye retraction might be greater when frogs feed on larger prey.

Variability in swallowing behavior

Behavioral observations reveal a high degree of variability in frog swallowing. For example, frogs not only have the ability to retract both eyes at the same time (bilateral retraction) but can also retract one eye independently from the other (unilateral retraction). We also found that eye retraction and swallowing are not obligately coupled; 42% of the time, swallowing movements occur without eye retraction, and eye retraction occurs regularly when no food is present in the oropharynx. The ability of frogs to perform a variety of swallowing behaviors suggests that these swallowing behaviors may be modulated by the size or position of the prey in the oropharynx.

Information on prey capture in frogs supports this suggestion. For example, Nishikawa (2000) has shown that prey capture is a dynamic behavior that is modulated by sensory feedback such as visualization of prey type and tactile assessment of prey. Other studies have shown that differences in prey size, orientation or distance from the frog may produce differences in the kinematics and motor patterns of prey capture (Anderson, 1993; Anderson and Nishikawa 1993, 1997; Gray and Nishikawa, 1995; Valdez and Nishikawa, 1997). Therefore, it is not surprising that swallowing behaviors also show variability that may result from behavioral modulation. Our results suggest that frogs have the ability to modulate prey transport and swallowing depending on prey characteristics in much the same way that they are able to modulate prey capture.

Behavioral observations show that the eyes also sometimes retract during prey capture. One possible function for eye retraction during the initial strike would be to protect the eyes. However, our results show that the eyes only retract about 50% of the time during prey capture, and when they do, they do not retract as deeply into the head as they do during swallowing.

Eye retraction and swallowing in other vertebrates

Along with addressing the proximate role of eye retraction, this study also provides an opportunity to examine behavioral and functional conservation during evolution. Eye retraction can be observed in most families of frogs (Nishikawa, 2000), in some families of salamanders (R. P. Levine, personal observation) and, in a modified form, in caecilians (O'Reilly et al., 1996). The large palatal vacuities of frogs and salamanders make it possible for the eyes to retract into the oropharynx and therefore to contribute to prey manipulation and swallowing. Eye reduction has occurred in some families of frogs and salamanders (e.g. Pipidae and Ambystomatidae). We might not expect the eyes to contribute substantially to

swallowing in these lineages, even though the eyes sometimes still retract during swallowing (e.g. in *Ambystoma tigrinum*; R. P. Levine, personal observation).

In extant caecilians, the eyes are highly reduced and the skulls are well ossified to facilitate fossoriality (O'Reilly, 2000). Most of the extrinsic eye muscles have been either reduced in size or co-opted to function with a specialized sensory organ, the tentacle. In many species of caecilians, instead of retracting the eyes during feeding, the modified extrinsic eye muscles serve to protract and retract the tentacle (Billo and Wake, 1987), and in some cases both the tentacle and the eye (O'Reilly et al., 1996). The evolutionary consequence of eye reduction as an adaptation to burrowing has constrained the use of the eyes in feeding mechanics. Therefore, in caecilians, it seems unlikely that eye retraction would contribute to swallowing performance.

In a study of the cranial nerves of the coelacanth *Latimeria chalumnae*, Bemis and Northcutt (1991) found that the basicranial muscle of *Latimeria* is innervated by cranial nerve VI (abducens) and, as a result, may be homologous with the retractor bulbi muscle in tetrapods (Bemis and Northcutt, 1991; Northcutt and Bemis, 1993). The basicranial muscle spans the intracranial joint of *Latimeria* and is hypothesized to play a role in cranial kinesis during feeding (Bemis and Northcutt, 1991). During the evolution of land vertebrates, the intracranial joint was lost, but the basicranial muscle, and its potential role in feeding, may have been retained within Lissamphibia as the retractor bulbi. Our results confirming the contribution of the retractor bulbi muscle to swallowing performance provide support for the hypothesis that the original role of the basicranial muscle in sarcopterygians was feeding.

Concluding remarks

Behavioral observations, EMGs, x-ray video and denervation experiments all support the hypothesis that eye retraction contributes to swallowing performance in the northern leopard frog, *Rana pipiens*. Frogs are able to swallow medium-sized crickets after eye retraction is prevented by bilateral denervation of the retractor bulbi muscle, but 74% more swallows per cricket are required. The use of eye retraction as an accessory swallowing mechanism varies with different prey characteristics and may contribute more to swallowing larger prey than smaller prey.

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