How a Frog, Pipa pipa, Succeeds or Fails in Catching Fish

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Edward Fernandez[1,2], Frances Irish[3], and David Cundall[1]

We quantified factors contributing to failed or successful prey captures by *Pipa pipa*, a permanently aquatic, tongueless frog widely distributed in Amazonian South America. *Pipa* catches fish by entraining and ingesting large volumes of water and by limiting fish escape with its fingers. Based on analysis of high-speed video (250 and 500 fps), feeding attempts appeared superficially stereotyped, but many features were modulated and slower than in other suction feeders. For both successful and unsuccessful capture attempts, the entire frog might or might not move and fingers might or might not contact the prey. Mouth opening generated initial suction but continued movement of entrained water depended on actively enlarging the volume of the anterior trunk by depressing the ventral pectoral girdle. Although captured fish varied in size and position relative to the frog, both fish size and the distance of the fish from the frog's mouth at the initiation of mouth opening were significantly greater for unsuccessful attempts. Our data suggest that capture success depends partly on sensory evaluation of prey size and distance that initiate capture movements and partly on the independent probabilities of rapidly moving parts in two different organisms favoring the predator or its prey.

HOW the aglossal pipid frogs in the genus *Pipa* catch elusive prey has long puzzled biologists. *Pipa pipa* is the largest of the South American pipids and one of the most bizarre-looking frogs in the world (Duellman and Trueb, 1986; Trueb et al., 2000). Captive *Pipa* have been reported to have a highly variable diet and to eat virtually anything, including frogs (Deckert, 1917), strips of beef muscle and liver, and live guppies (*Poecilia reticulata*; Rabb and Snedigar, 1960). However, field-caught *P. pipa* have been found with two erythrinid fishes about 30 mm long, and a relatively small *P. pipa* (78 mm SVL) from Santa Cecilia, Ecuador contained a smaller pimelodid catfish (Duellman, 1978), but others from various localities in Brazil contained only arthropods and amphibian skin (Alves-Pinto et al., 2014). The latter study supported the contention, based largely on studies of the African dwarf clawed frog *Hymenochirus* (Sokol, 1969; O'Reilly et al., 2002; Dean, 2003), that pipids have limited ability to exploit elusive prey. There remain few reports of stomach contents from *P. pipa* or field observations of its natural history (Trueb, 2003; Alves-Pinto et al., 2014), so the range of prey selected in the field by *Pipa* remains largely unknown.

Although much of our current understanding of pipid feeding behavior is based on *Hymenochirus*, Carreño and Nishikawa (2010) reported that *P. pipa* not only generates rapid negative pressure in its buccal cavity but also modulates both suction and forelimb movements during prey capture in response to variations in prey position, prey type, and prey size. They provided kinematic and pressure measurements only for captures of earthworms but noted that *P. pipa* were able to catch fish, a departure from a previous study suggesting that *P. pipa* used only forelimb scooping to catch prey (O'Reilly et al., 2002).

Features that correlate with *Pipa pipa*'s permanent occupation of aquatic environments include robust hind legs, fully webbed hind feet, flattening of the trunk and skull (Trueb et al., 2000), and a sliding iliosacral joint (Videler and Jorna, 1985). *Pipa pipa* also retains a lateral line system and neuromast organs (Russell, 1976), which presumably help it locate prey and predators (Roth et al., 1992; Nishikawa, 2000). Pipids use a bidirectional suction mechanism (Sokol, 1969; Lauder, 1985) in which water volume entrained during suction is limited by the ability of the frog to actively increase its body volume and by the potential volume that the frog can hold. Lacking gills and having a relatively short, wide head, *P. pipa* would appear superficially to be anatomically constrained to move limited volumes during suction. This in turn should constrain the size and nature of elusive prey they can capture.

Studies of the performance of aquatic ambush predators attempting to catch elusive prey have concentrated on the feeding and locomotor apparatus of the predator (Higham, 2007; Holzman et al., 2007; Day et al., 2015). Despite some early efforts to relate kinematics to the success of a capture attempt (e.g., Hoff et al., 1985; Drost, 1987), laboratory studies of aquatic suction-feeders rarely compare successful and unsuccessful prey capture attempts (Higham, 2007; Van Wassenbergh and De Rechter, 2011, but see Nauwelaerts et al., 2008). Given that *P. pipa* has been reported to generate rapid suction (Carreño and Nishikawa, 2010), our questions were 1) what is the timing and nature of movements generating suction during capture of elusive prey, and 2) what differentiates successful and unsuccessful capture attempts. We tested the hypotheses that 1) *Pipa* uses a highly stereotyped suction mechanism and that 2) failure of a capture attempt has numerous correlates, i.e., that failure can result from more than one factor.

MATERIALS AND METHODS

Behavioral recording.—Eight live *Pipa pipa* (69–135 mm snout–vent length; see Table 1) were obtained from commercial suppliers and housed individually in 40 l aquaria at 23–30°C under a natural light cycle. They were maintained primarily on live goldfish (*Carassius auratus*), but one was recorded eating two red worms (*Eisenia fetida*). Initial feeding trials for frogs 1 and 2 were recorded in 2012 at 250 frames per second with a Redlake MotionMeter (Redlake MASD, Inc., San Diego, CA). All eight specimens were recorded feeding in 2013 and/or 2014 with a Fastec TS3 100-L high-speed video camera (Fastec Imaging Corporation, San Diego, CA) at 500 fps and illuminated with two lights, a focused Lowell Pro

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Table 1. Frogs, their size, number of recorded fish capture attempts, and views analyzed for each recording condition.

Frog #	SVL (mm)	Events	Views
Recording condition 1			
1	102	8	7 lateral, 1 anterolateral
2	83	6	3 ventrolateral, 2 lateral, 1 anterolateral
3	85	11	5 lateral, 2 ventral + lateral, 2 anterior + ventral, 2 anterior + lateral
4	84	5	2 lateral, 1 dorsal + lateral, 1 anterior + lateral, 1 ventral
5	77	3	1 lateral, 2 posterior + ventral
6	69	2	1 lateral, 1 anterodorsal
Recording condition 2			
1		15	1 anterior, 6 lateral, 8 anterior + lateral
7	135	2	2 anterior + lateral
8	126	6	6 anterior + lateral
Recording condition 3			
1		11	9 anterior + dorsal, 2 dorsal + lateral
8		4	3 dorsal + lateral, 1 dorsal + posterolateral

(Tiffen Company, Hauppauge, NY) 250W halogen lamp and a more diffuse 250W ECT incandescent flood lamp.

Captures of goldfish (*Carassius auratus*, widely used as elusive prey: e.g., Van Wassenbergh and De Rechter, 2011) were recorded with frogs in a variety of orientations relative to the camera because movements of all parts of the body are not visible or measurable from a single viewpoint. Furthermore, questions arising during analysis of early video records, and our experience with frog behavior, encouraged us to try other arrangements of arenas and mirrors (summarized in Table 1). For the first arrangement, used during initial recording sessions of six frogs in late 2012 and early 2013, frogs were moved to an aquarium containing a small acrylic chamber (35x12.5x9 cm) with a white opaque rear wall, a mirror below it at 35° to the horizontal base and a mirror at one end at 55° to the vertical focal plane. Thirty-five capture attempts were recorded with this arrangement showing six frogs in eight different orientations. Because the frogs moved infrequently in their aquaria and could usually be placed at almost any location, for the second recording arrangement, we left the frogs in their own aquaria and inserted a vertical mirror at the left end of the aquarium angled at 50° to the front of the aquarium. Frogs were gently moved to a location near the mirror before releasing fish. Twenty-three capture attempts showing three different frog orientations were recorded from three frogs using this arrangement. In an effort to measure pectoral adduction and abduction during prey capture, we used a third recording arrangement in which a mirror was placed above the frog at 50° below the vertical axis to give dorsal as well as anterior or lateral views. Fifteen strikes were recorded from two frogs showing three different orientations of the frogs, all including dorsal views.

Our analysis of movements during capture attempts is based on external evidence of bone positions and pressure changes. These are possible because the skin covering most of the trunk is thin, attached to underlying tissues only along the lateral edge of the trunk, and the body wall muscles are all relatively thin (pers. obs.). During periods when fluid pressures in the body cavity are negative relative to external ambient pressure, the skin is pushed onto underlying tissues, making some skeletal outlines (notably the parts of the pectoral and pelvic girdles) visible despite the existence of large lymphatic spaces between the skin and body wall muscles. When body cavity pressure rises above ambient, the skin and body wall muscles bow outward, or inflate. These

features allowed estimates of the timing of bone movements and associated pressure changes.

To record prey capture, three to six fish of varying sizes were introduced into the acrylic chamber or the aquarium and allowed to swim freely until one was captured. *Pipa* is an ambush predator, remaining stationary until a fish swims close to its mouth. Multiple fish were presented to increase the probability that a fish would swim close enough to elicit a capture attempt and to reduce experimenter movements near the aquarium. The use of a mirror allowed two views of frog movements for 47 of the 73 records.

When resting normally, the frog's mouth is concealed by the forelimbs in most lateral views (e.g., Carreño and Nishikawa, 2010). The complexity and variety of movements used by the frogs predicated our attempts to obtain capture records from as many different views as possible. Because the frogs typically attempted capture only when fish were close, we waited until one of the fish swam near the frog's mouth. We recorded both successful (48) and unsuccessful (25) capture attempts. Fewer unsuccessful attempts were recorded because both cameras require significant delays in downloading and/or restoring recording capability after a recording event. Because our initial goal was to elucidate the suction mechanism of *Pipa*, to maximize the probability of recording successful captures, unsuccessful attempts were recorded only if all the fish immediately left the area of the frog.

Kinematic analysis.—Prey capture began with movement of a finger, a toe, or the lower jaw. Prey captures were analyzed by determining the time in milliseconds from the first detectable movement, which was the beginning of mouth opening, the beginning of finger movement, or the beginning of hind foot or toe movement. These three events (numbered 1–3) were always the earliest events but could occur in any order. The timing of additional features or events exploited anatomical features described by Sokol (1969) and Trueb et al. (2000). These included: 4) beginning of clavicle/hyoid depression, as visible in the floor of the oral or buccopharyngeal cavity (we use the term hyoid despite the fact that adult *Pipa* lack most derivatives of the hyoid arch except the stapes and possibly the medial structures lying anterior to the hyoglossal foramen [Ridewood, 1897]; the clavicles, anterior epicoracoids, and hyoid apparatus are shown in Fig. 1), 5) onset of body lengthening, as seen from beginning of head movement away from hind leg or anterior
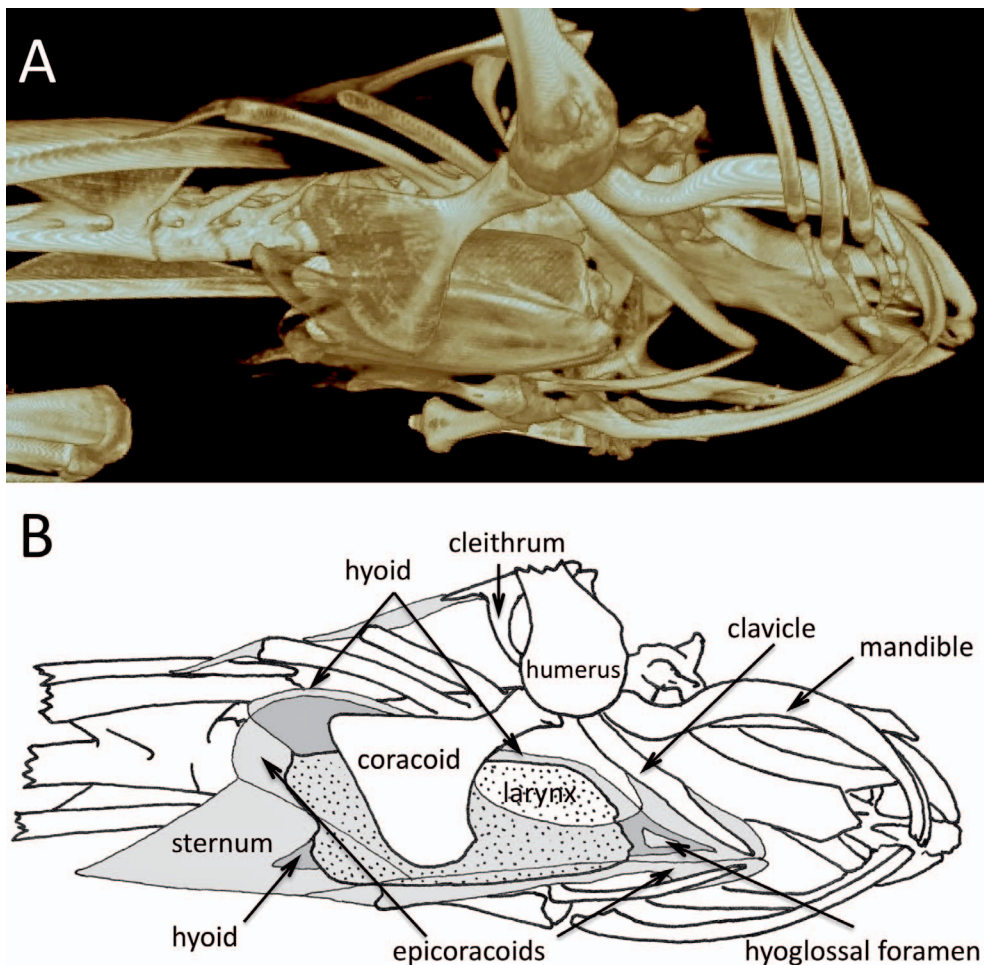
Fig. 1. (A) Oblique ventrolateral view of a CT scan of *Pipa pipa,* MCZ A7646, showing positions of the larynx, clavicles, and coracoids of the ventral pectoral girdle. (B) A drawing of the same view of the skeleton showing the positions of the cartilaginous epicoracoids (from Trueb et al., 2000 and AMNH 58075) and the hyoid and hyoglossal foramen lying dorsal to the epicoracoids along the dorsolateral edge of the larynx (stippled), as determined from dissections and Ridewood (1897).

movement of the end of the urostyle under the skin, 6) end of clavicle or hyoid depression, again as visible in the floor of the oral or buccopharyngeal cavity, 7) beginning of negative pressure in the trunk, as seen from deformations of the skin around the lateral pectoral elements, 8) beginning of postclavicular pectoral depression, visible as movements of the median ventral surface of the frog, 9) time at which fingers reach the mouth, 10) time of peak gape, 11) initial dorsal bending movement of the frog's vertebral column, 12) maximum extension of hind limb, 13) peak pectoral depression, 14) end of trunk negative pressure, seen as beginning of outward skin movement causing reduction and ultimate loss of shadows around pectoral elements, 15) onset of body shortening, and 16) total duration of capture event, measured from first movement to time at which the frog's forelimbs returned to their approximate resting position. Complete deflation usually took much longer.

Successful and unsuccessful capture attempts were compared using one-way ANOVA in SPSS 22. Because some frogs attempted to catch a number of fish during a recording session, we compared means and standard deviations for the timing of suction events to determine if repeated attempts influenced capture success. We also analyzed the temporal relationship between frog movements and movement of the focal fish the frog attempted to capture, noting 17) time of initial movement of the fish from the beginning of frog movement, typically the beginning of a C-start, the first bend formed during an escape response by the fish; and 18) time of initial movement of the fish towards the mouth of the frog,

again from the beginning of frog movement. In those strikes with appropriate views, we also measured, directly on the monitor image using an SPI dial caliper to the nearest 0.01 mm: 1) distance from the closest part of the prey to the edge of the frog's mouth, and 2) prey length. Both measurements were converted to relative values by dividing by the length of the frog's head measured in the same fashion.

Capture sequence effects.—Some *Pipa* ate numerous fish during a single recording session and these were initially analyzed as independent events. To test if sequence number (1st fish capture, 2nd capture, 3rd, etc.) was related to success, we compared sequence means for the beginning of depression of the hyoid and shoulder girdle, the beginning of trunk negative pressure and the end of shoulder girdle depression for all capture events, and for successful and unsuccessful attempts separately.

Buccopharyngeal volume measurements.—To measure the change in volume of frogs during a suction event, we used those records in which the maximum height, snout–vent length, and width behind the forelimbs of the frogs could all be measured and assumed the frog was an approximate rectangle. We then measured a resting view prior to prey capture and a view in a frame at maximum pectoral girdle expansion. These measurements were possible for five of the eight frogs in 45 prey capture attempts, 28 successful events and 17 unsuccessful ones. We analyzed the relative increase in volume (maximum/resting volume) for each event.
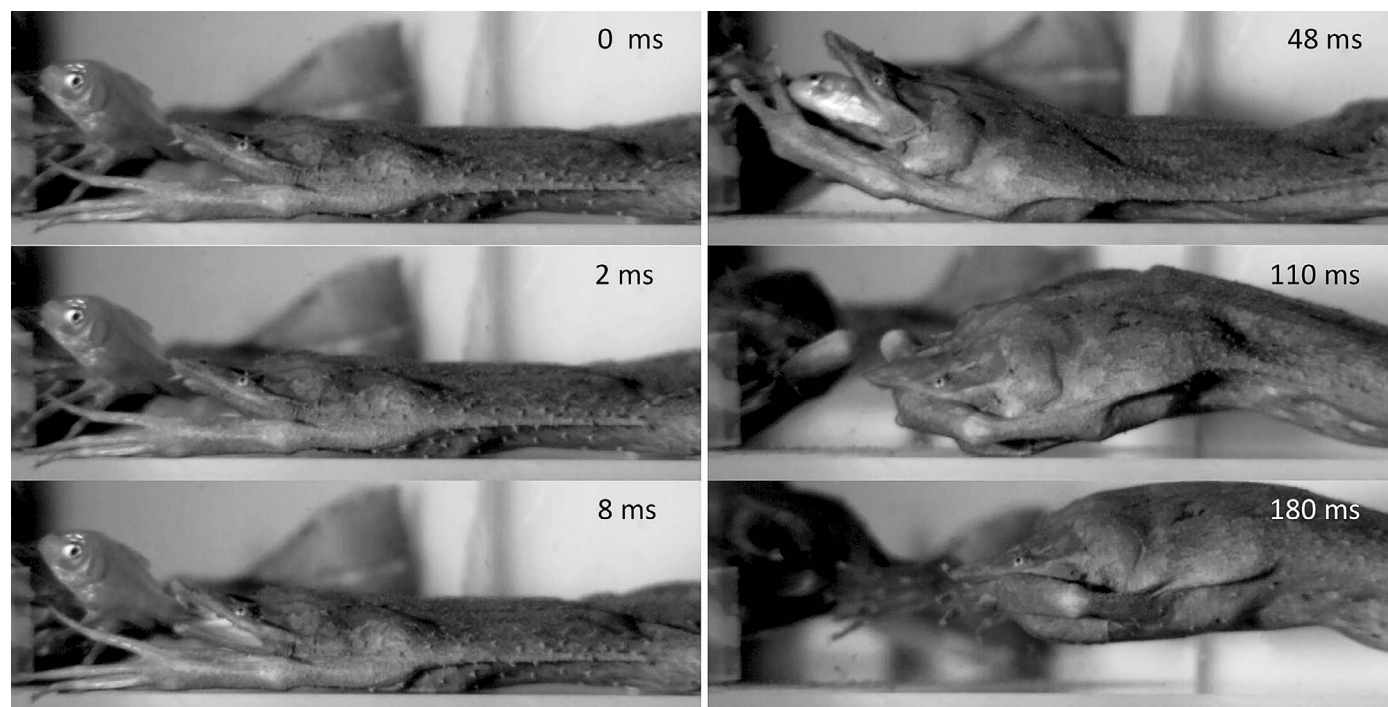
Fig. 2. Six frames from high-speed video of an anterolateral view of a lunging capture of a *Carassius auratus* by *Pipa pipa*, showing, at 0 ms, beginning of toe movement; 2 ms, mandible depression; 8 ms, middle of mandible depression, beginning of intraoral suction and forward lunge; 48 ms, beginning of pectoral girdle depression and trunk negative pressure, movement of fish into frog's mouth; 110 ms, dorsal bending of the trunk, continued trunk negative pressure, beginning of reversal of body movement and peak shoulder girdle depression, entry of front feet into mouth, although the fingers did not contact the prey; and 180 ms, inflation of trunk shortly following the end of trunk negative pressure and removal of front feet from mouth.

To determine the volume of water that can be ingested at one time by *Pipa pipa*, specimens 1 and 8 were anesthetized in an un-buffered solution of 0.5% tricaine methanesulfonate (MS-222) made from the same water used for housing the frogs. After the frogs showed no righting response, each was weighed on an Ohaus TS400 top-loading balance (Ohaus Corporation, Parsippany, NJ) to the nearest 0.1 g and its resting volume was obtained by volumetric displacement. Each was then held vertically by grasping it behind its forelimbs and its mouth was carefully opened. Water was poured into the mouth until it flowed from the corners of the mouth, after which the frog was upended over a large beaker that was then emptied into a graduated cylinder. This procedure was repeated three times for each frog. Weights of the frogs before and after filling with water were essentially the same (within a gram, variations likely due to different amounts of water on the frog's skin). Frogs were then placed in water without anesthetic, monitored until they regained swimming function and returned to their aquaria. Both frogs resumed feeding two days after the procedure.

RESULTS

Feeding kinematics.—The frogs exhibited no movement prior to the initiation of a capture event, which was usually marked by a flaring of one or more finger or toe tips accompanied or followed by observable contraction of the mandibular depressor muscle. The next movement was depression of the mandibles (Figs. 2, 3; see also videos in Supplementary Materials for these and all remaining figures). As the mandibles dropped, anterior views showed the medial region of the hyoid apparatus pressed against the roof of the mouth (Fig. 3). As the mouth opened, the frog's toes flared and the hind feet often started moving caudally as the head and trunk began moving forward toward the prey, except in 18 cases (25%) in which the frog's position changed little and the prey moved into the frog.

The timing of major kinematic events is summarized in Table 2 for successful and unsuccessful capture attempts. After finger movement and mouth opening, the next major events are hyoid depression and retraction and the beginning of body elongation (Table 2, Fig. 4). The latter occurs as a result of the ilia sliding caudally on the sacrum. The fingers usually continue to flare and typically extend beyond the prey, acting as a barrier. They are used frequently to scoop (terminology following Gray et al., 1997) the prey into the mouth. Hence, after mouth opening, hyoid and laryngeal retraction combined with elongation of the trunk increase the volume of the buccopharyngeal cavity and, potentially, the entire trunk.

The mouth begins closing while the ventral pectoral elements begin moving ventrally and the vertebral column begins bending dorsally (Fig. 2). During all of these events, the interior of the trunk is experiencing negative pressure based on deformation of the skin. Pectoral depression accompanies expansion of the buccopharyngeal cavity, which increases the volume of water drawn into the frog. The posterior body ceases extending caudally as the prey and surrounding water move into the frog to fill the expanding buccopharyngeal cavity. The average volume of the frog's body at maximum pectoral depression, calculated from displacements of the dorsal and ventral surfaces of the trunk (Table 3), was approximately 1.5 times the resting volume of the trunk.

The speed at which water is evacuated from the gut varies as a function of capture success. If successful, the frogs
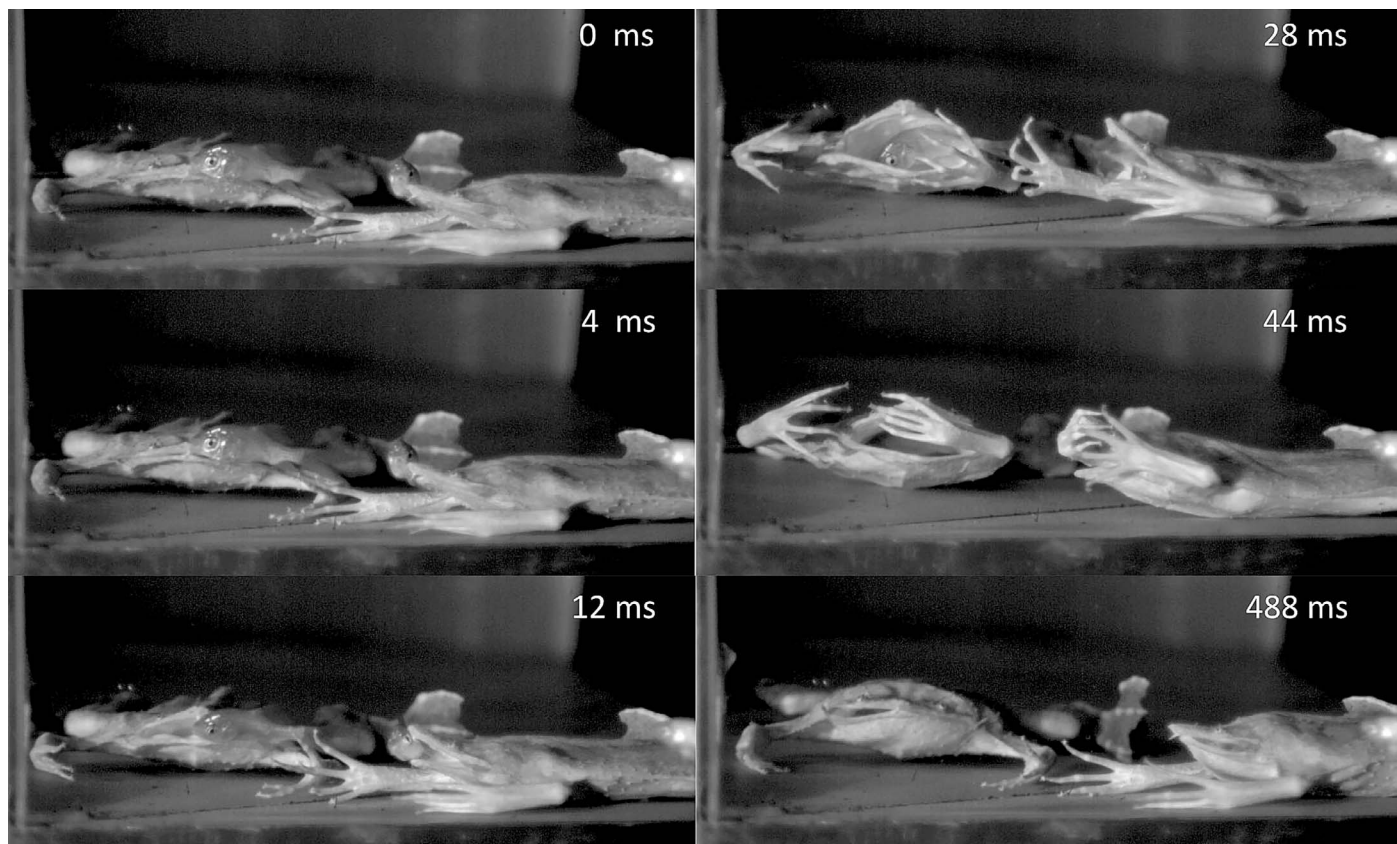
Fig. 3. Six frames of anterior and anterolateral views of a stationary capture event by *Pipa pipa* with finger movement but with no contact of fingers with the prey (*Carassius auratus*). The final frame shows asymmetric jaw position with prey in the oral cavity during expulsion of water. 0 ms, beginning of finger movement; 4 ms, 1 frame (2 ms) after beginning of mouth opening; 12 ms, beginning of clavicle and hyoid retraction and depression and beginning of fish (*Carassius auratus*) movement into oral cavity; 28 ms, fish (except tail) within oral cavity, middle of pectoral depression; 44 ms, fish in caudal region of buccopharyngeal cavity, peak pectoral depression; 488 ms, middle of water expulsion from buccopharyngeal cavity that combined with slight rearward movement of the whole frog.

opened only the lateral edges of the mouth, often asymmetrically (Fig. 3), restraining the prey in the mouth, and water flowed out slowly based on the rate at which the body deflated. In some captures the prey would remain in the caudal buccopharyngeal cavity (Fig. 5); in others it would reappear in the oral cavity (Fig. 3).

Volume measurements.—Volume measurements from video records of prey capture attempts (Table 3) show extraordinary variation in the relative volume change for the two frogs (1, 8) with more than five measures. The results suggest little correlation between volume change and success, or between volume change and the relative size of the fish. Pearson pairwise correlation between relative fish size and frog volume changes among 40 events in which both could be measured gave a non-significant but slightly inverse relationship (-0.23, $P = 0.16$).

Wet weights of the two anesthetized *P. pipa* were 58 g and 94 g, their mean initial total volumes 57 and 96 cm^3, respectively, and the maximum volumes of water held were 54 and 93 cm^3, 95% and 97% of their respective total volumes.

Unsuccessful vs. successful capture attempts.—Apart from a slight delay in mouth opening and a longer delay in pectoral girdle depression during unsuccessful capture attempts (Figs. 6, 7), the timing of all other movement variables did not differ significantly from successful events (Table 2). The only

other variables that differed significantly between successful and unsuccessful capture attempts were size and distance of the prey, mean prey size and distance being significantly greater in unsuccessful attempts (Table 4, Figs. 6, 7). Because Frog 1 accounted for nearly half of both successful and unsuccessful capture events, its values for fish size and distance are given to show that variance for individual frogs approximates that for all frogs recorded.

Sequence effects.—There were no significant differences among means for the timing of selected kinematic events in sequential capture attempts when all capture attempts were compared or between means for successful or unsuccessful events alone, with one exception. Shoulder girdle depression differed significantly among successful capture attempt sequences (Table 5: values for seq. 6 > seq. 1 and 3) but, because sample sizes decreased as sequence number increased, these differences have questionable biological relevance. Despite a general trend for shoulder girdle depression to be delayed in later sequences, this did not necessarily prevent success.

Behavioral (kinematic) variation.—*Pipa* caught fish using two different strategies, one using forward movement of the whole body and one with no measurable body movement. The former incorporated elements of ram and suction feeding, the latter used no ram effects. Of 73 analyzed capture attempts, 18 showed no movement toward the fish,

Table 2. Timing of kinematic events for successful and unsuccessful capture attempts from first detectable movement, with *P* values from ANOVA. All times rounded to nearest millisecond.

Movement variable	Successful captures			Unsuccessful attempts			
	Mean±SD	Range	*n*	Mean±SD	Range	*n*	*P*
Mouth open	2±2	0–8	48	3±2	0–6	23	0.2
Finger movement	7±3	2–16	47	6±2	4–10	25	0.3
Hind foot	12±13	4–58	32	9±5	4–24	23	0.3
Hyoid depression	12±4	4–24	26	11±3	6–14	9	0.5
Begin trunk neg. press.	18±6	4–28	45	17±5	10–32	19	0.7
Body lengthening	18±10	8–54	37	24±28	6–122	21	0.2
End hyoid depression	20±6	8–36	26	22±11	8–42	9	0.3
Pectoral depression	31±12	12–62	48	39±20	12–94	24	0.06
Fingers reach mouth	39±13	12–76	47	38±13	20–82	25	0.7
Peak gape	47±15	24–120	48	55±23	24–102	26	0.1
Vertebral bending	69±17	36–106	43	65±25	28–124	19	0.5
Max extens. hindlimb	66±32	20–144	27	72±43	36–192	20	0.6
Peak pectoral depress.	111±47	58–352	46	118±69	44–376	23	0.6
End trunk neg. press.	115±32	56–180	46	121±51	48–250	17	0.5
Onset body shorten.	169±81	10–338	27	183±71	18–280	14	0.6
Capture event duration	222±156	70–914	48	261±260	50–1272	24	0.4

and of these, 15 were successful. Correlated with absence of forward body movement was absence of hind limb extension in all but two events, but the toes of the hind limb moved slightly in five of the 18 events. Hence, in a quarter of the capture attempts, the hind limbs were essentially decoupled from movements of the rest of the body. Interestingly, entrainment and engulfment of a volume of water approximately equal to the mass of the frog did not result in appreciable displacement of the frog.

One other kinematic variation involved use of the forelimbs and fingers in scooping or corralling fish. Of 65 attempts for which finger use could be seen clearly, fingers contacted the focal fish in 46 of them. In 19 attempts there was no contact between the fingers and the fish. In one attempt the forelimbs did not sweep medially at all and in several the movement was abbreviated.

DISCUSSION

Pipa pipa captures elusive prey using a suction method that appears superficially to be stereotyped but actually varies in both timing and the nature of specific movements.

Ram vs. ram-free events.—Although we have no data on fluid displacement patterns during suction events, the assumption that the water entrained by suction lies near and in front of the frog's mouth is undoubtedly correct in that the prey lying in that volume disappears into the frog during successful capture events. To determine why the frog is not sucked forward during ram-free events is another matter. To resolve it would require visualization techniques we did not use (e.g., Lauder and Clark, 1984; Day et al., 2005). The frog generates suction by opening its mouth, initiating suction, but the oral cavity is small and prey rarely show any movement into the frog until the frog begins enlarging its anterior trunk. Because the frog moves very little, we assume that water displacement around the frog must compensate for water entering the frog. Trunk expansion from cranial to caudal combined with maintenance of hind foot position, despite caudal and then cranial displacement of the pelvic girdle, apparently contribute to equalizing fluid displacements such that the frog ends

up moving very little in those capture attempts showing no rapid forward movement.

The volumes of water that *Pipa pipa* can potentially house during feeding events are probably similar to those obtained by our manipulations of anesthetized animals. Those measurements reveal two features of *P. pipa* important to understanding its suction feeding. The buccopharyngeal cavity is amazingly distensible and expands to limits presumably determined by the body wall. Additionally, the central viscera (hyoid and larynx, heart, lungs, liver, esophagus, and stomach) are apparently arranged to allow all of it to move caudally approximately a third of the length of the trunk, providing the space for expansion of the buccopharyngeal cavity. Fish that are ingested appear to lie in the middle of the trunk (Fig. 5), a position we initially mistook for the stomach. The fact that the frog's trunk can expand to house a volume of water equal to its entire tissue volume is part of the structural modification allowing inertial suction feeding. However, the rate of expansion is relatively slow, potentially limiting its effectiveness in sucking in elusive prey. It is here that forelimb movements may be critical in providing a corralling function that offsets some of the variations in timing of the suction mechanism.

Success vs. failure.—As mentioned in the methods, we did not record every failed capture attempt because our initial goal was to determine how *Pipa* caught fish. Because the high-speed video cameras we used were triggered after captures, if the frog failed to catch a fish during an attempt, and some fish remained near the frog, we did not record the failed attempt because downloading the file or rearming the camera took too long. We estimate that we failed to record approximately half of all unsuccessful attempts. Given our recorded sample sizes for each category (48 successful, 25 unsuccessful), the frogs were successful in approximately half of their attempts. However, given that all the frogs occasionally ingested numerous fish (maximum of seven in 32 minutes) in relatively short time spans, the limits to their exploitation of elusive prey probably relates to their success in crypsis and the density of suitable prey. In the artificial setting that we created to record their feeding behavior, the only element of crypsis the frogs could use was immobility,
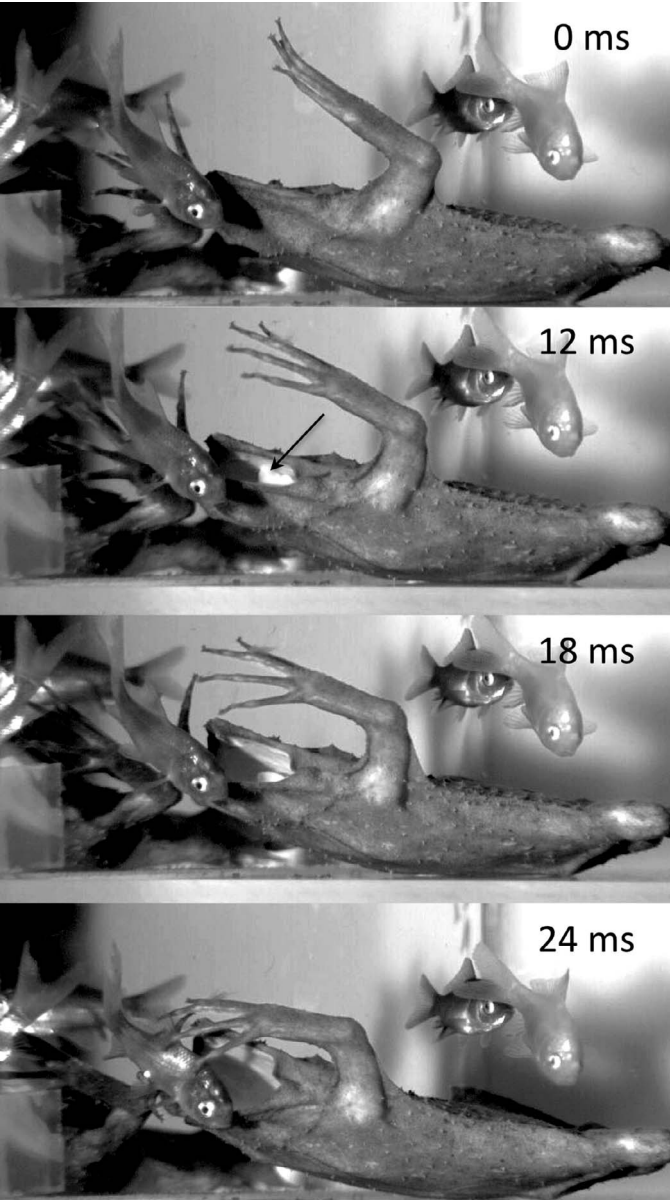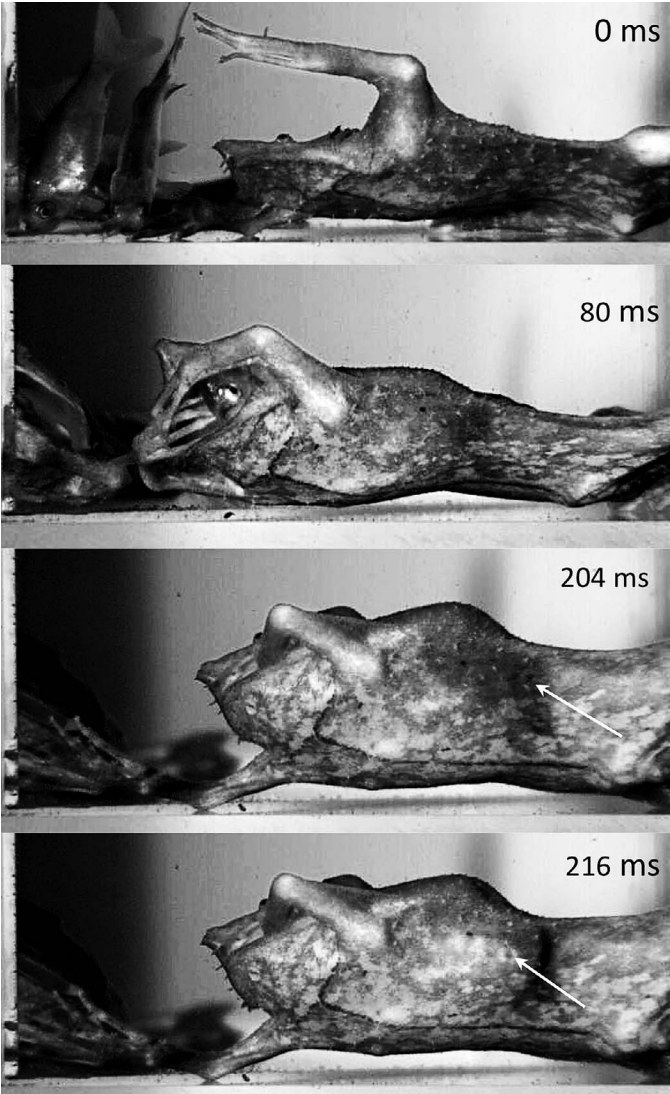
Fig. 4. Clavicle and hyoid movement: 0 ms, beginning of finger movement by *Pipa pipa*; 12 ms, mouth partially open, clavicle and hyoid pressed against roof of oral cavity, but beginning of clavicle and hyoid retraction and depression; 18 ms, clavicle and hyoid in process of depression and retraction; 24 ms, clavicle and hyoid no longer visible, one finger almost in contact with fish (*Carassius auratus*). The fish was caught.


Fig. 5. Fish (*Carassius auratus*) in body of *Pipa pipa*: 0 ms, beginning of capture; 80 ms, peak gape and pectoral girdle depression; 204 ms, inflation of buccopharyngeal cavity with fish near its caudal border; 216 ms, fish eye visible through body wall of frog (*Pipa pipa*). This sequence also shows the approximate caudal limit of water inflow, which matches the border of the inflated buccopharyngeal cavity. Fish movement inside the frog is clearly evident in the video although fish position is difficult to see in single frames.

which apparently sufficed for juvenile *Carassius auratus*. Other fish species might differ.

Our comparison of successful and unsuccessful capture attempts also made clear that the timing of suction events was not stereotyped and displayed extraordinary ranges of values, even for critical early events, such as hyoid and pectoral depression (see also Nauwelaerts et al., 2008). Our initial hypothesis was that delays in initiating any movements associated with suction would increase the probability that the prey could escape before being entrained in the volume sucked into the frog. Given the range of values for initiating depression of the hyoid and pectoral girdle (Table 2), and the absence of significant differences between successful and unsuccessful capture attempts in values for any of the timing variables measured, there is little support

for our hypothesis. We conclude that for *Pipa,* success in catching fish is not tightly correlated with the timing of suction events but, as found by Nauwelaerts et al. (2008), depended on correctly judging the distance of the prey.

Table 3. Relative volume changes (maximum/resting) during prey capture events.

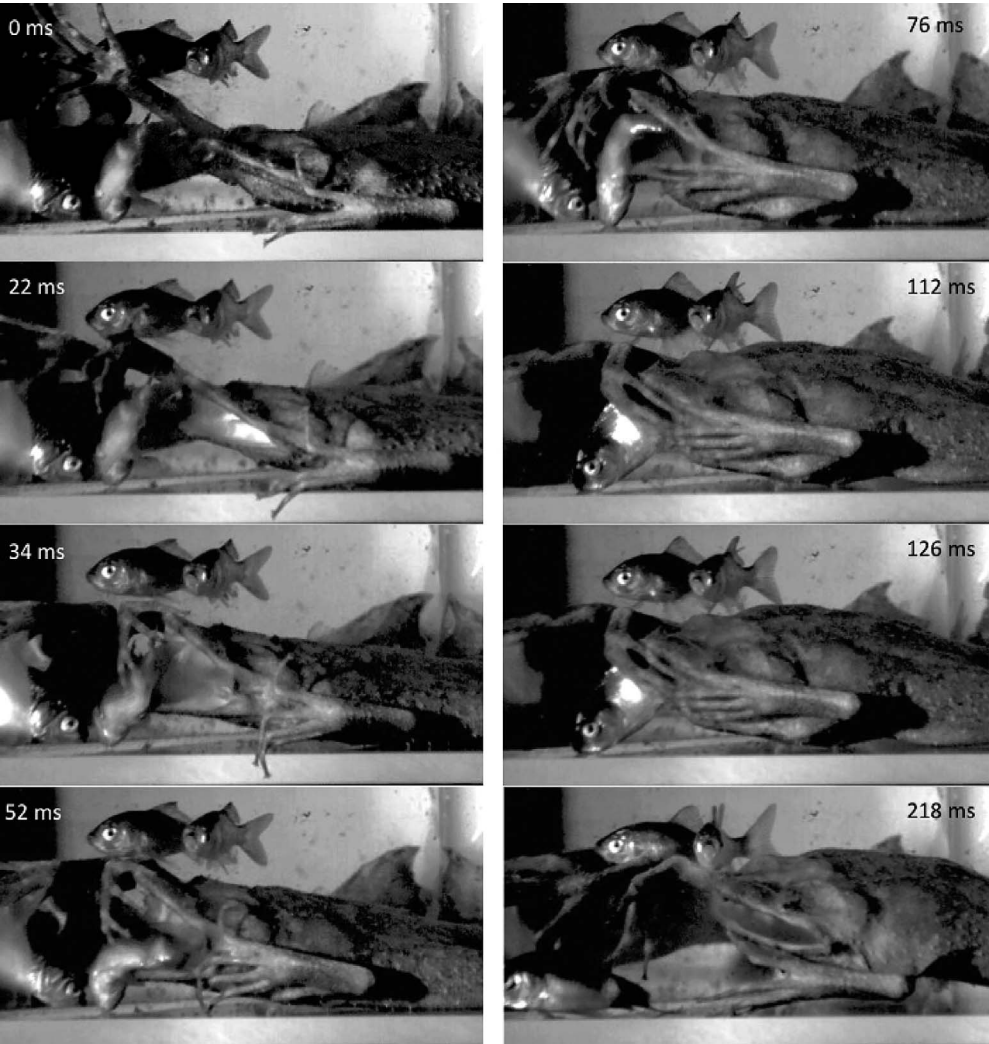|	Successful			Unsuccessful		
Frog	n	Mean±SD	range	n	Mean±SD	range
1	17	1.53±0.18	1.30–1.83	12	1.47±0.15	1.17–1.65
3	0			2	1.45±0.21	1.30–1.60
4	1	1.30		2	1.29±0.02	1.27–1.30
5	1	1.84		1	1.60	
8	9	1.50±0.17	1.29–1.82	0		
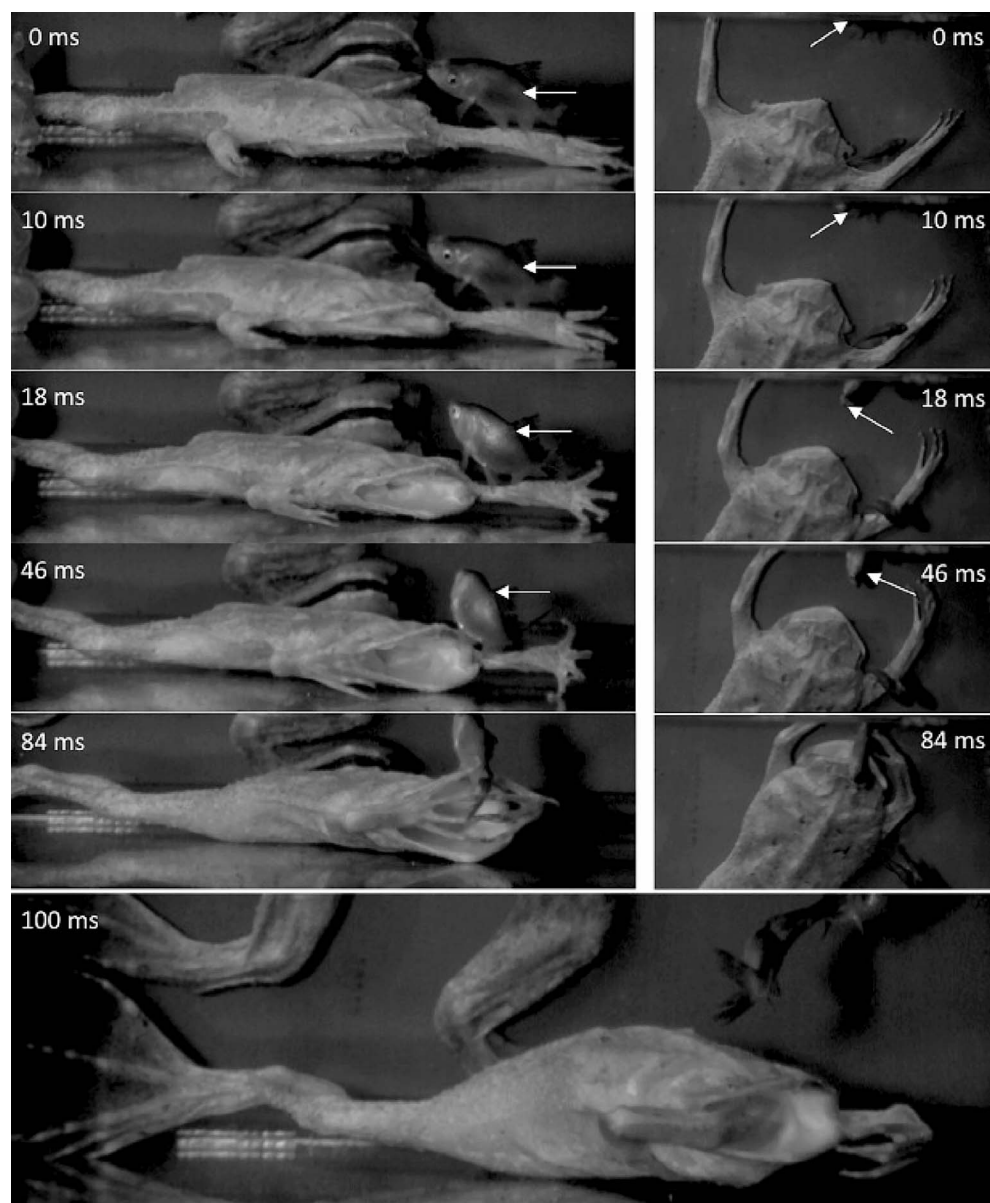
Fig. 6. Eleven views (five anterolateral, five dorsal from the same video frames, and one of the frog [*Pipa pipa*] at peak inflation) of an unsuccessful capture attempt on a fish (*Carassius auratus*: arrows) too far from the frog at the initiation of the event and during which the frog delayed pectoral girdle depression by more than 50 ms.

Feeding behavior.—Our video records confirm that *P. pipa* is an opportunistic ambush predator that modulates critical elements of prey-capture kinematics, as seen by Carreño and Nishikawa (2010) in their records of *Pipa* feeding on worms. However, whereas suction generation during ingestion of worms would appear to be extremely rapid and of very short duration, our kinematic data for capture of fish suggest that suction is prolonged. Carreño and Nishikawa's (2010) graphs of buccopharyngeal pressure profiles in four species of pipids show *P. pipa* generating maximum suction in approximately 6 ms and mouth opening and closing to be completed in about 12 ms. In their graph, intrabuccal pressure returns to ambient very rapidly. In our analysis, peak gape was reached 24–120 ms after capture initiation (Table 2). Judging from skin profiles, negative pressure in the trunk persisted until shortly after pectoral depression ceased, invariably over 50 ms after the beginning of the capture attempt. These values are similar to Carreño and Nishikawa's times on high-speed video images (their fig. 4) of worm capture but differ from their figure 6 of a pressure profile of *P. pipa* during feeding on a worm.

From our kinematic data, we propose that *Pipa* differs from most other suction feeders in three respects. First, in its earliest movements, *Pipa* frequently uses asymmetric or asynchronous jaw opening. During 25% (18 of 73) of capture attempts, one side of the lower jaw depressed 2–4 ms before the other (Fig. 8), and all of these were to fish located off the midline of the frog's head on the side opening first. Although four of these 18 were failed attempts, the success rate for asynchronous jaw opening (78%), compared to the overall rate (66%), suggests that asynchrony enhances the probability of capture. Rapid asynchronous movements of the right and left mandibles have been demonstrated previously only in aquatic salamanders (*Cryptobranchus*) among lissamphibians (Cundall et al., 1989; Elwood and Cundall, 1994), although Carreño and Nishikawa (2010) noted its use in *P. pipa* during both capture and manipulation of prey.

Secondly, *Pipa*'s use of forelimbs to corral elusive prey appears unique among suction-feeding vertebrates. Among the other pipids whose feeding has been studied, *Hymenochirus* does not use its forelimbs during initial capture (Sokol, 1969; Dean, 2003), but *Xenopus* uses its fingers to scoop prey into the mouth (Avila and Frye, 1977a, 1977b; Gray et al.,

Fig. 7. Eight views of an unsuccessful attempt to capture a large fish (*Carassius auratus*) during which the right manus of *Pipa pipa* is repositioned (52–76 ms) after the fish initiates a C-start (34–76 ms) that drives its head below the major volume of water entrained by suction.

1997; Carreño and Nishikawa, 2010). O'Reilly et al. (2002) claimed that *Pipa* always used its forelimbs and hands to scoop prey into its mouth, but we noted considerable variation in the use of the forelimbs by most of the *Pipa* recorded. The frogs usually, but not always, combined scooping movements of one or both forelimbs with suction in capture attempts. In only one of 73 attempts did a frog show no movement of its forelimbs. In all others, the fingers flared slightly and usually moved toward the prey. However, in nearly a third of the attempts, the fish were sucked into the mouth before the fingers reached them. It appears that simple movement of the fingers toward the prey while the

mouth is opening might generate competing sensory input that either delays or redirects the prey's motor responses. The fact that the fingers actually did contact the fish in approximately two-thirds of capture attempts, and that it was usually the distal ends of the fingers that made contact, support our proposition that *Pipa* belongs in a suction category different from those suction-feeding vertebrates previously described (e.g., Deban and Wake, 2000; O'Reilly et al., 2002; Day et al., 2015).

Thirdly, the timing of suction events directed at elusive prey in *Pipa* appears similar to but more variable than that used by suction-feeding salamanders directed at non-elusive

Table 4. Effects of prey size, distance (both measured in relative frog head lengths), and behavior (timing of fish C-start and movement toward the frog relative to beginning of frog movement in ms) on capture success for all frogs and for frog #1 alone, with *P* values from one-way ANOVA.

Variable	Successful			Unsuccessful			
	Mean±SD	range	*n*	Mean±SD	range	*n*	*P*
Fish size, all	1.24±0.33	0.61–2.24	44	1.54±0.42	0.75–2.49	20	<0.01
Fish size, frog 1	1.28±0.34	0.77–2.24	22	1.77±0.39	1.16–2.49	8	<0.01
Fish distance, all	0.67±0.43	0.10–2.05	48	0.93±0.50	0.32–2.13	22	0.03
Fish distance, frog 1	0.79±0.50	0.14–2.05	23	1.21±0.52	0.63–2.13	9	0.04
Begin C-start, all	13.3±7.8	4–46	41	15.5±10.3	6–42	23	0.34
Begin C-start, frog 1	13.7±9.9	4–46	20	9.3±3.3	6–18	12	0.15
Begin move, all	19.7±11.6	6–58	47	23.9±21.3	8–94	18	0.31
Begin move, frog 1	15.1±8.4	6–36	22	14.8±8.9	8–30	10	0.94

Table 5. Sequence effects on the initiation of hyoid and shoulder girdle depression, trunk negative pressure, and on the time of maximum shoulder girdle depression.

| | Begin hyoid depression | | | | | | Begin shoulder girdle depression | | | | | |
| | All | | Success | | Failure | | All | | Success | | Failure | |
Seq. #	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
1	14±6	10	13±7	7	11±4	2	31±12	23	30±10	14	35±14	8
2	12±2	5	11±1	2	12±3	2	31±20	18	29±13	12	40±33	5
3	10±3	6	10±3	5	10	1	33±15	13	28±9	9	45±19	4
4	10±2	5	11±1	2	9±3	3	29±11	6	22±14	2	33±9	4
5	10±3	5	10±3	4	12	1	43±19	7	39±8	4	47±31	3
6	13±4	4	13±4	4			42±2	4	42±2	4		
7	15±7	2	15±7	2			54±11	2	54±11	2		

| | Begin trunk negative pressure | | | | | | Maximum shoulder girdle depression | | | | | |
| | All | | Success | | Failure | | All | | Success | | Failure | |
	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
1	17±7	20	16±7	14	19±8	6	115±68	22	123±77	14	101±49	8
2	17±5	17	17±4	12	17±6	5	103±27	16	103±20	11	102±40	5
3	18±4	11	18±5	8	18±4	3	108±33	3	101±27	9	124±45	4
4	15±4	6	17±7	2	14±3	4	108±24	6	101±1	2	112±31	4
5	17±3	6	19±3	4	14±0	2	146±105	7	120±33	4	182±169	3
6	17±7	4	17±7	4			103±18	4	103±18	4		
7	25±4	2	25±4	2			125±50	2	125±50	2		
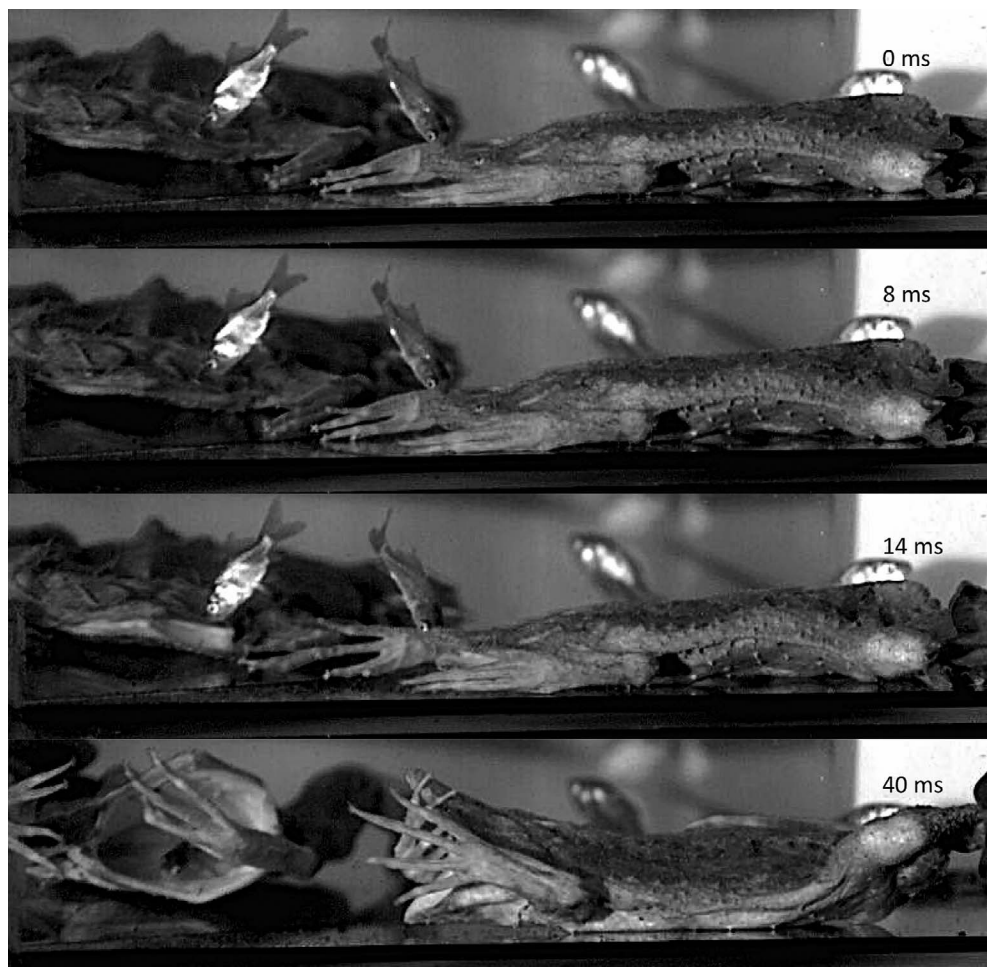
Fig. 8. Four video frames showing asymmetric mouth opening by *Pipa pipa* and the behavior of the mandibular symphysis during capture of *Carassius auratus*. 0 ms is the frame before the first frame showing movement of the frog's right mandible, which is depressing at 8 ms, prior to depression of the left mandible, which began at 12 ms and shows clearly at 14 ms. At 40 ms the mouth has reached peak gape, the relative cross-sectional area of which is increased by flexion around the symphysis. The frog's right manus is adducted during suction but its fingers never contact the fish.

prey (Reilly and Lauder, 1992). Many prior studies have not quantified the timing of movements of the prey, and hence we cannot be certain that *Pipa* differs significantly from other suction-feeding amphibians in its timing of prey entrainment. However, during most attempts, movement of the fish occurs between clavicle and hyoid depression/retraction and depression of more posterior shoulder girdle elements, but well after mouth opening begins. Heiss et al. (2013) showed "prey" (pieces of dead fish hanging from a thread) movement beginning as the mouth opened and well before noticeable hyoid depression in juvenile *Andrias*. The inflation of the whole body of *Pipa* suggests, however, that suction feeding mechanisms in tetrapods are more diverse than currently recognized (Wainwright et al., 2015).

DATA ACCESSIBILITY

Supplemental information is available at http://www.copeiajournal.org/ch-16-510.

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