universität
wien

DISSERTATION

Funktional – morphologische Untersuchung des Fressapparates bei vier cryptodiren Schildkröten: ein Vergleich zwischen *Cuora amboinensis*, *Cuora flavomarginata*, *Cuora galbinifrons* und *Sternotherus odoratus*.

angestrebter akademischer Grad
Doktor der Naturwissenschaften (Dr. rer.nat.)

Verfasserin / Verfasser:	Nikolay Natchev
Matrikel-Nummer:	9802229
Dissertationsgebiet (lt. Studienblatt):	Zoologie
Betreuerin / Betreuer:	A.O. Prof. Dr. J. Weisgram

Wien, im

Inhaltverzeichnis:

I. Einleitung

I. 1. Die Problematik der Schildkrötenphylogenie und -paläoökologie

Die wohl markanteste Synapomorphie aller Schildkröten ist ihr Panzer. Bei der ältesten bekannten Schildkröte, *Odontochelys semitestacea,* war der Panzer vermutlich noch unvollständig entwickelt (Li et al., 2008); die zweitälteste bekannte Art, *Proganochelys,* ein Fossil aus der oberen Trias, besaß hingegen schon einen typischen Panzer bestehend aus Carapax und Plastron (Gaffney, 1990). Da noch keine Übergangsformen zwischen verschiedenen triassischen Amniotengruppen und den ersten echten Schildkröten bekannt sind, ist eine morphologische Beschreibung der ancestralen Form und deren biologische Besonderheiten mehr oder weniger rein spekulativ. Molekularbiologische Untersuchungen deuten auf eine hohe Affinität der Chelonia entweder zu Archosauria (Zardoya & Mayer, 1998; Hedges & Poling, 1999; Rest et al., 2003), oder zu Lepidosauriformes (Rieppel & De Braga, 1996; De Braga & Rieppel, 1997) hin. Eine Alternative zu diesem „chelonia as diapsid crown group“ - Konzept ist die Positionierung der Schildkröten innerhalb der Anapsida - „Parareptilia“ (siehe Benton, 2006).

Über die Lebensweise ancestraler Chelonier existieren verschiedene Hypothesen. Während *Odontochelys semitestacea* höchstwahrscheinlich aquatisch lebte (Li et al., 2008), vermutet Gaffney (1990) für *Proganochelys quenstedty* einen terrestrischen, oder semi-terrestrischen Lebensraum. Laut Rieppel (2000), stellen die Schildkröten möglicherweise eine Schwestergruppe der Sauropterygia dar; diese triassische diapside Reptiliengruppe war rein marin, die ersten Schildkröten wären also Meeresbewohner gewesen. Vergleichende morphometrische Untersuchungen an den Vorderextremitäten der Fossilienformen von *Proganochelys quenstedty*, *Palaeochersis talampayensis* und rezenter Schildkrötenarten demonstrieren, dass diese ancestralen Chelonier bereits rein terrestrische Tiere waren (Joyce & Gauthier, 2004). Da keine Extremitäten der ältesten bekannten pleurodiren Schildkröte, *Proterochersis robusta* (siehe Gaffney & Meylan, 1988; Gaffney et al., 2006) gefunden worden sind, kann diese Methode keinerlei Auskunft über ihre Lebensweise liefern. Terrestrische Lebensweise wurde durch histologische Analysen der Panzerknochen (Scheyer & Sanders, 2007) von *Proterochersis robusta* und *Proganochelys quenstedty* nachgewiesen. Obwohl also die Habitatpräferenzen der ganz basalen Cheloniergruppen noch nicht völlig geklärt sind, ist es unbestritten, dass der gemeinsame Vorfahr aller rezenten Schildkröten im Süßwasser lebte (Joyce & Gauthier, 2004). Alle rezenten Pleurodira sind rein aquatisch. Auch unter den heute lebenden Cryptodira sind nur wenige, hoch evoluierte Testudinoidea rein terrestrisch. Die Superfamilie Testudinoidea beinhaltet drei Familien: Testudinidae (Tortoises), Emydidae und Geoemydidae. Innerhalb dieser drei Taxone sind nur die Testudinidae obligatorische Landbewohner. Innerhalb der Emydidae und Geoemydidae sind rein aquatische, aber auch einige semiaquatische Formen zu finden (siehe Pritchard, 1979; Ernst & Barbour, 1989; Rogner, 1995; Claude et al., 2003). Da alle drei Testudinoidea Familien ancestral aquatisch sind, formulierten Summers et al. (1998) die Hypothese, dass terrestrische Nahrungsaufnahme innerhalb der rezenten Schildkröten mehrmals unabhängig voneinander entstanden ist.

I.2. Grundbegriffe und Terminologie zur Nahrungsaufnahme der gnathostomen Vertebrata

I.2.1. Aquatische Fressvorgänge

Laut Schwenk (2000a), wird der Fressvorgang bei niederen Tetrapoden in vier Hauptphasen unterteilt: 1. Transport des Futters von der Aussenwelt in die Mundhöhle, Nahrungsaufnahme („prey capture“, oder „ingestion“); 2. Nahrungstransport (mit oder ohne Manipulation der Nahrung) durch den Oropharyngealraum („prey transport“); 3. Verdichtung der Nahrung zum „Bolus“ im Pharynxbereich („pharyngeal packing“) und 4. Beförderung der Nahrung vom Pharynx in und durch den Ösophagus („swallowing“ ; „deglutation“).

Zwei Hauptmechanismen der aquatischen Nahrungsaufname bei Gnathostomata sind beschrieben:

1. Einsaugen der Nahrung (Beute) durch Erzeugung von Unterdruck im Buccopharyngealraum. Bei diesem Mechanismus wird die Nahrung durch einen, vom Tier erzeugten Wasserstrom in Richtung Mund befördert, während der Kopf relativ unbeweglich zur Beute bleibt. Das bedeutet eigentlich, dass die Nahrung aktiv, durch Wirkung hydraulischer Kräfte zum Tier bewegt wird. Diese Art von Beutefang ist bekannt als „suction feeding“ (Gans, 1969; Norton & Brainerd, 1993).

2. Beutefang durch Vorstoßen des ganzen Körpers oder nur Kopf und Hals über die Nahrung. Diese Methode wird „inertial feeding“ (Gans, 1969), oder auch „ram feeding“ genannt (siehe z.B. Liem, 1980; Lauder & Prendergast, 1992; Norton & Brainerd, 1993).

Die ersten Untersuchungen von Nahrungsaufnahme unter Wasser sind an Fischen durchgeführt worden (Alexander, 1970; Liem, 1970), bei denen ein „unidirektionaler“

Wasserstrom gegeben ist (siehe Lauder & Shaffer, 1993). Das eingesaugte Wasser strömt durch die Mundöffnung in den Oropharyngealraum ein und verlässt diesen wieder durch die Kiemenspalten. Die Nahrung wird durch verschiedene Mechanismen zurückgehalten. Der Wassersog während der „suction" ist nicht konstant stark und seine Geschwindigkeit und Richtungsbeschleunigung variieren vor und innerhalb des Mundraums (Muller et al., 1982; Muller & Osse, 1984; Muller & Van Leeuwen, 1985; Van Wassenbergh et al., 2006; Van Wassenbergh & Aerts, 2008). Mit zunehmender Distanz des Futters zur Mundöffnung verliert der Saugeffekt drastisch an Kraft (Muller & Osse, 1984). Die meisten Actinopterygier protrahieren Ober- und Unterkiefer während der „ingestion" und formen dabei einen Art Rohr, das, bei gleichzeitiger seitlicher Spreizung des Suspensoriums, Richtung Nahrung gestoßen wird. Da die Branchiostegalmembran noch angelegt ist, entsteht innerhalb der Mundhöhle ein Unterdruck. Durch Ausstrecken des Mundrohres wird der Weg zur Beute verkürzt, die Beute wird eingesaugt und anschließend wird die mitströmende Wassermenge durch die Kiemenspalten ausgepresst. Die „Bugwelle", die durch die Kieferprotraktion verursacht wird, ist sehr klein und kann ignoriert werden.

Die Muränen *Muraena retifera* und *Echidna nebulosa* besitzen z.B. eine Reihe von Reduktionen in den morphologischen Strukturen die für Erzeugung von Unterdruck im Mund- und Pharyngealraum dienen (Metha & Wainwright, 2007a). Diese Fische haben alternativ zum „suction feeding" einen Beutefangmodus entwickelt - das sogenannte „lunge and biting". Dabei sind die „biting" - Kieferzyklen sehr variabel, was ihre Kinematik betrifft. Der Schädel wird beim Zubeißen zurückgezogen, eine Hyoidabsenkung fehlt völlig (Metha & Wainwright, 2007a). Die Stärke der „Bugwelle" die die Beute vom Räuber wegschwemmen würde, ist durch den relativ langsamen „prey capture" - Vorstoß der Muränen (z.B. 10 mal langsamer als beim „suction-feeder" *Anguilla rostrata*) klein gehalten. Die Muränen sind die einzigen bekannten

Actinopterygii, die keine hydrodynamischen Mechanismen für „food transport“ verwenden (Metha & Wainwright, 2007b). In *Muraena retifera* z.B., sind die Kiemenbögen zu einer Art Pharyngealkiefer umgebaut, die weit nach rostral protrahierbar sind. Diese Pharyngealkiefer dienen gleichzeitig zum imobilisieren und zum Transport der Beute nach posterior.

Salamanderlarven sowie alle neotenen Salamanderarten verwenden obligatorisch „suction feeding“ durch Expansion des Hyobranchialraumes (siehe Deban & Wake, 2000; Deban et al., 2001). Adult benutzen nur Arten mit gut entwickelter Zunge andere Nahrungsaufnahme-Strategien unter Wasser. Plethodontiden und einige andere Salamanderarten stoßen („lunging“) mit dem ganzen Körper zur Beute vor (siehe Wake, 1982; Deban, 1997), und fangen und immobilisieren diese mit den Kiefern („jaw prehension“ oder „biting“). Reilly & Lauder (1992) berichten, dass bei Salamandern wie z.B. *Siren sp*, *Necturus sp.*, *Ambystoma sp.* und *Dicamptodon sp.* (die noch zwei oder mehr Kiemenöffnungen besitzen), das Wasser während der Kompressionsphasen beim „suction feeding“ unidirectional fliesst, ein Mechanismus der auch bei Teleostei und Urodelenlarven exsistiert. Bei Arten wie *Amphiuma sp.* und *Cryptobranchus sp.*, die einen caudal geschlossenen Oropharyngealraum besitzen, muss die während der „ingestion“ aufgenommene Wassermenge auch wieder durch die Mundöffnung ausgestoßen werden („bidirectional feeding“). Andere „bidirectional feeding“ Formen, wie manche Plethodontiden benutzen „lingual prehension“ beim Unterwasserbeuterwerb (siehe Deban, 1997). Bei „lingual prehension“ erfolgt der erste Kontakt zur Beute mit der Zunge. Bei *Ambystoma tigrinum* ist der Nahrungstransport durch den Mundraum hydraulisch und involviert keine Kopf- und Körperbewegungen nach vorne. Der „prey transport“ erfolgt durch reines „suction feeding“ (Reilly & Lauder, 1990).

Die meisten Anurenlarven sind filtrierende Formen. Die Branchialbögen sind zu einer Art Filterkorb modifiziert, durch welchen der Wasserstrom fließt. Die sogenannte „Buccalpumpe" (siehe Wassersug & Hoff, 1979) funktioniert durch rhythmische Kontraktionen der orbito-hyoid Muskulatur (Filterkorb-Erweiterung) sowie der M. intermandibularis und M. interhyoideus (Elevation des Zungenbeines). Manche Larven der Familie Pipidae sind räuberisch (Sokol, 1962) und benutzen einen ähnlichen „suction feeding" Mechanismus wie die Teleostei (Deban & Olson, 2002). Metamorphisierten Froschlurche besitzen gut ausgebildete Zungen und Arten welche auch adult unter Wasser fressen, weisen „jaw prehension" auf. Die einzige Ausnahme sind wiederum die Pipidae, deren Zungen stark reduziert sind, und die ihre Beute durch „suction feeding" fangen (Deban et al., 2001).

„Prey ingestion" durch „suction feeding" wird auch für die pelagischen Gymnophionenlarven berichtet (O''Reilly, 2000). Der Kopf bewegt sich während des Fressvorgangs nicht in Richtung Beute und der Nahrungsaufnahme-Zyklus ist extrem schnell. Bei *Epicrionops* Larven dauert „prey capture" unter 20 Millisekunden (siehe O''Reilly, 2000; Deban et al., 2001), der weitere „prey transport" erfolgt hydraulisch. Formen die adult rein aquatisch leben, findet man unter den Gattungen *Typhlonectes*, *Potomotyphlus* und *Atretochoana* (Wilkinson & Nussbaum, 1999); trotz der starken Hyoidabsenkung während des Beutefanges, wird die Nahrung nicht eingesaugt (O'Reilly, 2000), sondern der ganze Körper wird gewissermaßen nach vorne geschleudert. Der eigentliche Beuteerwerb erfolgt durch „jaw prehension" (Wilkinson, 1991; O'Reilly, 2000). Ein derartiger „prey capture" Modus wird von O'Reilly (2000) als „compensatory suction" (sensu Van Damme & Aerts, 1997) bezeichnet. Gemeint ist wohl, dass bei schnellem ram-feeding zur Vermeidung einer, die Beute wegspülenden Bugwelle, "compensatory suction" (kompensiert eben diese Bugwelle) angewandt wird.

„Compensatory suction“ ist charakteristisch für Tetrapoden bei denen beim Fressen ein „bidirectionaler Wasserstrom" auftritt (siehe Reilly & Lauder, 1992a). Dieser Begriff (compensatory suction) beschreibt den Prozess, als flüssigkeitsdynamisches Phänomen, sehr korrekt (siehe Van Damme & Aerts, 1997; Aerts et al., 2001; siehe auch weiter unten). Beim Beutetransport und beim Schluckakt der Gymnophionen treten koordinierte Saug-, Zungen- und Trägheitsbewegungen auf (Bemis et al., 1983; O’Reilly, 2000). Größere Beute wird durch Rollbewegungen des ganzen Körpers zerkleinert (Deban et al., 2001).

Das Unterwasser-Fressverhalten der Lepidosauria ist nur wenig untersucht. Obwohl manche scleroglosside Echsen sehr wassergebunden leben (wie z.B. der asiatische Wasserwaran *Varanus salvator*), existieren keine Beweise, dass diese Tiere auch unter Wasser fressen.

Aquatische Nahrungsaufnahme ist mehrmals unabhängig bei verschiedenen Schlangenfamilien entstanden (siehe Cundall & Greene, 2000). Als Folge der Spezialisierung des Hyolingualapparats der Schlangen zur Chemorezeption, ist der Hyoidkörper in seinem Volumen stark reduziert (Schwenk, 1994). Auch sind Schlangen nicht in der Lage durch Hyoidabsenkung einen Unterdruck in der Buccalhöhle zu produzieren; damit sind kein „suction feeding“ oder „compensatory suction“ möglich (Vincent et al., 2004, 2007). Die Wasserschlangen stoßen den Kopf in lateraler Haltung zur Beute (Smith et al., 2002; Alfaro, 2002, 2003), die Kiefer werden geöffnet noch bevor die Beschleunigung des Kopfes nach vorne beginnt. Hydrodynamische Untersuchungen haben bewiesen, dass auf diese Weise die Bugwelle kleiner gehalten wird als bei einen „Strike“ mit geschlossenem Maul

(Cundall & Greene, 2000). Die einzige Vipernart, die regelmäßig Fische jagt (*Agkistrodon piscivorus*) benutzt einen frontalen „Strike“, wie beim Fressen an Land (siehe Kardong, 1982). Jedoch sind manche Generalisten wie z.B. *Nerodia fasciata* in der Lage, in Bezug auf Beutetypus und Fressmedium sechs alternierende Fangstrategien zu benutzen: „lateral striking“, „open-mouth searching“, „forward striking“ und „aerial atack“ unter Wasser; „slow approach“ und „fast approach“ an Land (Daghfous et al., 2008).

Der Nahrungsaufnahmeapparat der meisten Schildkröten ist zum Unterwasserfressen konstruiert (Lauder & Prendergast, 1992; Claude et al., 2004). Die spezialisierten, aquatisch lebenden Arten besitzen ein oft zur Gänze verknöchertes Hypoglossum und einen großen und flachen Hyoidkörper, ausgestattet mit zwei kleinen rostrolateral liegenden Cornu hyale und zwei Paar langen, dorsalgebogenen Branchialhörnern: Cornu branchiale I (CnB I) und Cornu branchiale II (CnB II) (siehe Schumacher, 1973; Van Damme & Aerts, 1997; Lemell & Weisgram, 1997; Lemell et al., 2002). Der Hyoidkörper ist stark verknöchert und besitzt einen rostralen Processus lingualis. Die distalen Enden der CnB I und CnB II sind knorpelig und werden als Epibranchiale I (EB I) und Epibranchiale II (EB II) bezeichnet; diese entsprechen dem Epibranchiale II und III der Teleostei. Die proximalen Elemente der Hyoidhörner sind normalerweise verknöchert und werden als Ceratobranchiale I (CB I) und Ceratobranchiale II (CB II) bezeichnet. Die Zunge ist klein und die dorsale Zungenmucosa ist mit relativ wenigen Drüsen und Papillen ausgestattet (Winokur, 1988; Iwasaki, 1992, Iwasaki et al., 1992, Iwasaki et al., 1996; Van Damme & Aerts, 1997; Beisser et al., 1995; Beisser et al., 1998; Lemell et al., 2002). Die hyobranchiale Muskulatur ist sehr kräftig entwickelt, dadurch wird eine plötzliche und schnelle Absenkung des Hyoidaparates ermöglicht. Der Schädel ist relativ flach und breit, ohne palatale Wölbung. Laut Bramble (1973) mindert ein flaches Munddach die Wirbelbildungen im Wasserstrom die bei heftigem Einsaugen von Wasser („suction feeding“) entstehen.

Natürlich ist die Untersuchung der Wassersogkomponenten und der Druckgradienten bei Tieren ohne Kiemenöffnungen viel komplizierter als z.B. bei Teleostei. Die theoretischen Kalkulationen werden durch die Tatsache, dass auch die Zunge umströmt wird, zusätzlich extrem erschwert (die „Kegelmodelle“ von Muller & Osse, 1984 und Osse et al., 1985 sind nur begrenzt anwendbar). Durch allgemeine Kenntnisse der Strömungsdynamik, sind für aquatische Nahrungsaufnahme bei Schildkröten zwei grundsätzliche Modelle entwickelt worden (Lauder & Prendergast, 1992; Van Damme & Aerts, 1997). Lauder & Pendergast (1992) untersuchten den Beuteerwerb bei *Chelydra serpentina* – eine carnivore cryptodire Schildkröte - mittels Hochgeschwindigkeitsvideos mit 200 fr/s. *C. serpentina* wird als „ram-feeder“ beschrieben - die Hyoidretraktion hat keine Bewegung der Beute zum Räuber zur Folge. Die Würmer und die Fische die als Nahrung verwendet wurden, werden durch die Kiefer erfasst („jaw prehension“) und bleiben in Relation zum Hintergrund unbeweglich. Das kinematische Muster dieses Fressvorganges zeigt gravierende Ähnlichkeit zu den Beutefangbewegungen bei manchen Fischen. Lauder & Prendergast (1992) formulierten die Hypothese, dass diese Ähnlichkeit durch eine hydrodynamische Beschränkung (wegen der hohen Dichte und Viskosität des Wassers), die allgemein auf aquatische Nahrungsaufnahme-Systeme wirkt, erklärbar ist. Die kinematischen Unterschiede sind demzufolge ein Produkt der morphologischen Differenzen zwischen „uni-„ und „bidirectional feeders“. Drei kinematische Charakteristiken, die für Teleostei, Urodela und Chelonia allgemein gültig sind, wurden postuliert: 1. Hyoidretraktion startet zusammen mit der Kieferöffnung („fast opening“); 2. auf die größte Kieferöffnung („peak gape“) folgt die größte Hyoidabsenkung („peak hyoid“); 3. die Kieferöffnung wird durch

Unterkieferabsenkung und Kopfelevation gewährleistet. Als „suction feeding“ wird nur ein Fressakt bezeichnet, bei dem in einem bestimmten Zeitabschnitt die Beute in Richtung Räuber bewegt wird.

Im Widerspruch zu Lauder & Prendergast (1992) sind Van Damme & Aerts (1997) der Meinung, dass bei der aquatischen Nahrungsaufnahme der Schildkröten, allein durch An- oder Abwesenheit von sichtbaren Beutebewegungen keine endgültigen Rückschlusse über Stärke und Richtung des Wasserstromes gemacht werden können. Der Kopf produziert bei seiner Bewegung in Richtung Beute eine bedeutende Bugwelle („pushing“ sensu Van Leuwen, 1984). Diese Bugwelle wird durch den saugenden Gegenstrom, der wiederum durch die Hyoidabsenkung produziert wird, gewissermaßen neutralisiert (Van Damme & Aerts, 1997). Diese Fressaktkomponente wird als „compensatory suction“ bezeichnet. Wenn der Wassersog, erzeugt durch Hyoidretraction, die Beute Richtung Räuber mitbeschleunigt sprechen die Autoren über „inertial suction“. Das Begriff „ram feeding“ wird nur in Fällen benutzt, in denen sich das Tier aktiv bewegt wobei das Wasser ungehindert durch den Oropharyngealraum fliesst und das System durch die Kiemenspalten verlässt (z.B. manche filtrierende Fische). Schildkröten besitzen keine Kiemenöffnungen, die Kopfbewegung in Richtung Beute muss also von „compensatory suction“ begleitet werden (Van Damme & Aerts, 1997). "Compensatory suction" ist also ein essentieller Teil des "ram-feeding" - Verhaltens bei bidirectionaler Nahrungsaufnahme. Laut Summers et al. (1998) trifft ebendies auch für die aquatischer Nahrungsaufnahme von *Terrapene carolina*, *Kinosternon leucostomum, Platysternon megacephalum*, *Heosemys grandis* zu. Eine ähnliche Nahrungsaufnahmestrategie wurde auch für *Malaclemys terrapin* beschrieben (Bels et al., 1998), jedoch der Begriff „compensatory suction“ wurde aus mir unbekannten Gründen nicht verwendet. Bels et al. (1998) sind der Meinung, dass die

Bugwellenminimierung auch dazu dient, etwaige drucksensible Sensoren der Beute zu überlisten. Eine interessante Stelle in diesem Diskurs nimmt die Fransenschildkröte (*Chelus fimbriatus*) ein. Diese südamerikanische pleurodire Schildkröte besitzt eine extrem hydrodynamische Schädelkonstruktion und eine Bugwelle bei Vorbeschleunigung des Kopfes ist wahrscheinlich nicht vorhanden (siehe Lemell et al., 2002) oder jedenfalls sehr gering. Die Hyoidretraction startet hier erst spät während dem „fast opening", da keine (oder fast keine) „compensatory suction" notwendig ist. Die Arten, die im Rahmen dieser Dissertation untersucht wurden, sind omnivore Generalisten und bezüglich ihrer Kopfmorphologie nicht so spezialisiert wie *C. fimbriatus*. Da keine Klarheit über die Hydrodynamik des Schildkröten-Fressvorganges herrscht, werden die Begriffe „ram feeding" (sensu strictu Lauder & Prendergast, 1992), „compensatory suction" und „inertial suction" (sensu Van Damme & Aerts, 1997) für diese Arbeit akzeptiert und werden für die Beschreibung des Beutefanges („prey capture") benutzt. Die Begriffe „compensatory suction" und „inertial suction" (sensu Aerts et al., 2001) werden für die Analyse von „prey transport" (Transport der Nahrung durch die Mundhöhle) verwendet.

Für Schildkrötenarten mit einem sehr schnellen „strike" (wie *Chelodina longicolis*) wird von Aerts et al. (2001) eine Kopfbeschleunigung am Ende der „fast opening" Phase vermutet, die nicht durch Halsmuskelaktivität bedingt ist. Die Beschleunigungskraft wäre entgegengesetzt dem Wasserstrom, der bei der raschen und voluminösen Hyoidabsenkung erzeugt wird. Das Momentum sei groß genug um den craniocervicalen Komplex in Richtung Beute zu schleudern. Eine solche, gewissermaßen einem "inverse rocket drive" (Weisgram, pers.com.) entsprechende Kraft konnte allerdings nicht nachgwiesen werden und tritt jedenfalls bei den hier untersuchten Arten nicht auf. In den vorliegenden Arbeiten wird demzufolge die Rolle einer hydrodynamischen „pulling force" (siehe Aerts et al., 2001) nicht anerkannt.

I.2.2. Terrestrische Fressvorgänge

Terrestrische Nahrungsaufnahme ist während der Entwicklungsgeschichte der Gnathostomen mehrmals unabhängig entstanden. Bekannt ist, dass viele Teleostei zur terrestrischen Nahrungsaufnahme fähig sind (zur Übersicht siehe Sponder & Lauder, 1980). Der in flachen, schlammigen Gewässern lebende afrikanische Wels *Channallabes apus* z.B. ist in der Lage, Insekten am Uferrand außerhalb des Wassers zu erbeuten (Van Wassenberg et al., 2006a). Der Wels kann seinen langen Körper stark nach ventral krümmen („dorso-ventral flexion“) und damit die Beute von oben gegen das Substrat pressen. Diese „dorso-ventrale flexion“ des vorderen Abschnittes des Fischkörpers ist wichtig für terrestrische „prey capture“. Die Schlammspringer (Periophthalminae) aus den Mangrovenwäldern Afrikas, Asiens und Australiens biegen ihren ganzen Körper bogenartig, um die Beute gegen den Grund zu fixieren (Sponder & Lauder, 1980). Im Gegensatz zu *C. apus*, kann *Periophthalmus koelreuteri* seine Beute am Land durch koordinierte Bewegungen seiner Schlundzähne (siehe Sponder & Lauder, 1980) transportieren und schlucken. Es ist bekannt, dass die Axialskelettkonstruktion des devonischen Tetrapoden *Ichthyostega*, dorso-ventrale und nicht laterale Bewegungen erlaubte (Ahlberg et al., 2005). Dies wurde aber als Adaptation für terrestrische Fortbewegung und nicht für terrestrische Nahrungsaufnahme interpretiert.

Terrestrische Nahrungsaufnahme ist, bedingt durch die unterschiedliche Dichte und Viskosität von Luft und Wasser, grundsätzich von aquatischer Nahrungsaufnahme zu unterscheiden (Lauder, 1985). Saugen ist an Land nur begrenzt und im Zusammenhang mit Flüssigkeiten möglich (z.B. beim Trinken) und energetisch ungünstig. Die unterschiedlichen Mechanismen der Nahrungsaufnahme werden durch gravierende Unterschiede im morphologischen Design des Nahrungsaufnahmeapparates reflektiert. Da auf die Nahrung wirkende Gravitationskräfte an Land nicht durch die Tragkraft der Luft verringert werden können (wie das im Wasser der Fall ist), haben die terrestrischen Tetrapoden ein zusätzliches Stützorgan entwickelt – die Zunge. Ob die ersten devonischen Vierbeiner, wie z.B., *Acanthostega* und *Ichthyostega,* oder aber auch oberdevonische Sarcopterygii wie z.B. *Tiktaalik* (siehe Daeschler et al., 2006) Zungen oder zungenähnliche Organe besassen ist nicht bekannt. Die ersten Formen, die den Übergang von Wasser zum Land geschafft haben, waren carnivor (siehe De Vree & Gans, 1989), es ist jedoch nicht bekannt, ob diese ersten semi-terrestrischen oder rein terrestrischen Tetrapoden ihre Beute mit den Kiefern oder mit der Zunge gefangen haben. Laut Bramble & Wake (1985) soll der gemeinsame Ancestor aller rezenten Tetrapoden „lingual prehension“ (also Nahrungsaufnahme mit Hilfe der Zunge) benutzt haben.

Bei der terrestrischen Nahrungsaufnahme der primitivsten rezenten Tetrapoden, den Urodelen, erfolgt der erste Kontakt zur Beute durch die Zunge („lingual prehension“). Die Zunge wird protrahiert oder sogar aus der Mundhöhle geschleudert (Wake & Deban, 2000; Deban et al., 2001). Die meisten terrestrischen Salamander besitzen nur schwach ausgebildete Kiefer und Zähne. Bei diesen Arten wird die Beute durch die Zungenretraktion in den Mundraum befördert. Die Zunge kann aber auch nur zum „immobilisieren“ der Beute benutzt werden und nicht zur „ingestion“. In diesem Fall erfolgt der erste Kontakt zur Nahrung durch die Zunge, der eigentliche Fang involviert dann die Kiefer („jaw prehension“). Reine „jaw prehension“ benutzen Arten mit kräftiger entwickelten Kiefern (Wake & Deban, 2000). Koordinierte Kiefer- und

Zungenbewegungen ersetzen die „hydraulischen Effekte“ beim terrestrischen Beutetransport.

Die meisten adulten Frösche benutzen zum Beutefang einen Körpersprung nach vorne („lunge“) bei einer gleichzeitigen Zungenprotraktion durch „mechanical pulling“ (Deban et al., 2001). Dies entspricht wahrscheinlich dem ancestralen Beutefangmodus der Anuren. Weitere zwei Mechanismen der Zungenprotraktion bei Fröschen können unterschieden werden: „inertial elongation“ und „hydrostatic elongation“. Da in diesen Fällen die Zunge weit aus der Mundhöhle gestreckt werden kann, wird der „lunge“ überflüssig (Nishikawa, 2000; Deban et al., 2001). Anderson (1993) berichtet, dass bei Froschlurchen der erste Kontakt zur Beute obligatorisch lingual erfolgt, doch können die Frösche ihr Fressverhalten je nach Nahrungsgröße ändern. Kleinere Beute wird nur durch Zungenretraktion in die Mundhöhle befördert, dabei sind die Kiefer nicht involviert („lingual prehension“). Größere Beute wird mit der Zunge kontaktiert, dann wird der Kopf nach unten gesenkt und die Kiefer umschließen die Nahrung („lingual fixation“ und „jaw prehension“). Zum „prey positioning“ werden oft die Vorderextremitäten benutzt. Für manche aboricole Arten ist sogar „prey prehension“ mittels der Vorderbeine berichtet (siehe Deban et al., 2001).

Gymnophionen sind die einzigen Amphibien die obligatorisch „jaw prehension“ an Land benutzen. Dieser Mechanismus unterscheidet sich jedoch grundsätzlich von der „jaw prehension“ der Urodelen und Anuren. Beim Beutefang von Caecilien (erdwühlen) wird die voluminöse Zunge nicht protrahiert - der erste Beutekontakt erfolgt mit den Kiefern (Bemis et al., 1983; O’Reilly, 2000). Interessant bei Gymnophionen ist ihr „prey transport“. Kleine Nahrungspartikel werden nur mit der Zunge transportiert, für größere Beute wird auch „inertial transport“ benutzt (Bemis et al., 1983; O’Reilly, 2000). Dabei wird die Beute kurzfristig von den Kiefern befreit und der Kopf wird gewissermaßen "darüber geschleudert". Dieser Form des Nahrungstransportes ist ansonsten bei Echsen und Archosauria weit verbreitet (siehe unten).

Bei der Nahrungsaufnahme der Lepidosauria, ist das Organ, mit dem der erste Kontakt zur Beute hergestellt wird, gleichzeitig das Organ der „prey prehension" („ingestion"). Die Lepidosauria sind bezüglich ihres „prehension modes" scharf in zwei, auch phylogenetisch exakt abgrenzbare Gruppen, nämlich Iguania versus Scleroglossa, (Schwenk, 2000b) getrennt.

Viele Untersuchungen beschreiben die Verwendung der Zunge für „ingestion" innerhalb der Iguania: Schwenk & Throckmorton, (1989), Bels & Baltus (1987), Bels (1990), Bels & Delheusy, (1992), Meyers & Nishikawa (2000) Meyers & Herrel (2005) - für Iguanidae; Schwenk & Throckmorton (1989), Kraklau (1991), Herrel et al. (1995), Meyers & Nishikawa (2000), Schaerlaeken et al. (2007) – für Agamidae; Bramble & Wake (1985), Schwenk & Throckmorton (1989), Bell (1990), Wainwright et al. (1991), Herrel et al. 2001b, Herrel et al. 2001c – für Chamaeleonidae. Nur in der vergleichenden Studie von Bels & Goose (1990) wird über "jaw prehension" bei Iguania berichtet. Laut Schwenk & Throckmorton (1989) ist Zungenprotraktion umso kürzer, je grösser die Beute ist. Dadurch kann es passieren, dass sehr große Beute gleichzeitig mit der Zunge und mit den Kiefern berührt wird.

Scleroglosse Echsen benutzen obligatorisch „jaw prehension" (Condon, 1987; Goose & Bels, 1992; Schwenk & Throckmorton, 1989; Schwenk 2000b). Nur für wenige Arten ist eine Teilnahme der Zunge bei der „ingestion" beschrieben (Urbani & Bels, 1995; Smith et al., 1999). Da „lingual prehension" innerhalb der Scleroglossa nur isoliert vorkommt, ist die Involvierung der Zunge vielleicht sekundär.

Laut Schwenk (2000b) benutzten die ancestralen Lepidosauria „lingual prehension“, doch früh in der Evolution haben sich die großen Gruppen Iguania und Scleroglossa formiert. Die Iguania haben den „lingual prehension“ - Mechanismus ihre lange Evolution hindurch beibehalten. Wagner & Schwenk (2000) vermuten, dass die Funktionalität des Fressapparates der Iguania unter „internal selection“ steht. Das bedeutet, dass jedes neue Merkmal, welches die Funktion des gesamten „ingestion“ Systems negativ beeinträchtigen könnte, ausselektiert wird. Nur Merkmale, die die Funktionalität verbessern (oder beibehalten) stabilisieren sich innerhalb der Population. Solche Systeme sind „evolutionary stable configuration (ESC)“ und Wagner & Schwenk (2000) stufen den Iguania „lingual prehension“ Mechanismus als solchen ein. Da „internal selection“ relativ unabhängig von Umwelteinflüssen wirkt, sind die ESC phylogenetisch stabil und werden in der Evolution für lange Perioden beibehalten. Eine Auflösung solcher ESC passiert selten. Als Hauptgrund für die Entwicklung von „jaw prehension“ an der Basis der Scleroglossa-Linie wird ein Habitatwechsel vermutet (Schwenk, 2000b).

Obwohl die Zunge ihre Bedeutung als „prehensive organ“ verliert, bleibt der Nahrungstransport auch bei Scleroglossa hauptsächlich lingual wie bei den Iguania (siehe Herrel et al., 1999; Goose & Bels, 1992; Smith, 1984; Urbani & Bels, 1995; Elias et al, 2000, Schaerlaeken et al., 2008). Die einzigen Echsen die hauptsächlich „inertial transport“ benutzen sind die Varanidae (Smith, 1986; Elias et al., 2000). Bei ihrer „prey transport“ Untersuchung von Scleroglossa-Arten, die hoch spezialisierte chemorezeptorische Zungen besitzen, fanden Elias et al. (2000), dass nur die ersten Zyklen einen reinen „inertial transport“ aufweisen. Bei mehr als der Hälfte der folgende Zyklen war die Zunge involviert und die letzten Zyklen modulierten zum reinen „lingual transport“.

Dem Transport folgt eine Phase, während der die Nahrung im Bereich des Pharynx zum „Bolus“ verformt wird. Diese wird als „pharyngeal packing“ bezeichnet (Bramble & Wake, 1985; Smith, 1984; Herrel et al., 1999). Die „pharyngeal packing“ - Zyklen unterscheiden sich kinematisch oft von den „transport“ - Zyklen. Posthyoidale Muskulatur wird während der Kieferöffnung aktiviert und der hyolinguale Komplex wird angehoben aber nicht protrahiert. Die hinteren Zungenabschnitte („hindtongue“) pressen die Nahrung an den Gaumen und befördern sie tiefer in den Pharynx (Bramble & Wake, 1985). Bei Arten die keine voluminösen Zungen besitzen übernimmt das ganze Hyobranchium die Rolle der „hindtongue“ (Smith, 1986). Laut Smith (1986) erfolgt der Übergang des Bolus in den Ösophagus bei Varaniden durch eine gut abgrenzbare „pharyngeal compression“ - Phase. Bei „pharyngeal compression“ wird der Kopf ventral gesenkt, dabei verlässt die Nahrung den Pharyngealbereich („pharyngeal emptying“ sensu Smith, 1992). Der Schluckakt erfolgt durch peristaltische Kontraktionen der Constrictormuskulatur. Für „nonmammalian“ Tetrapoden wird der Begriff „swallowing“ vorgeschlagen (siehe Schwenk, 2000a). Bei den Säugern wird der Bolus durch eine reflexartige Kontraktion der internen Pharyngealmuskulatur geschluckt – „deglutation“ sensu Smith (1992).

Die terrestrischen Nahrungsaufnahmestrategien der Schildkröten sind nur lückenhaft erforscht. Weisgram (1985) berichtet, dass die kinosternide *Claudius angustatus* „jaw prehension“ an Land benutzt. Diese Schildkröte ist jedoch nicht in der Lage ihre Beute außerhalb des Wassers zu transportieren und zu schlucken. Die einzige emydide Art die bis dato kinematisch untersucht ist – *Terrapene carolina* - ergreift die Beute mit den Kiefern, dabei wurde auch eine deutliche Protraktion des Hyolingualkomplexes festgestellt (Bels et al., 1997; Summers et al., 1998). Dass diese Tiere die Zunge während „prey capture" protrahieren lässt vermuten, dass sie unter anderen Umständen auch „lingual prehension" benutzen. Als Auslöser für „prehension" - Modulationen wird die Art der Nahrung vermutet (Summers et al., 1998). Andererseits wurde „lingual prehension" bei *Terrapene carolina* nie nachgewiesen. Alle drei bis dato untersuchten Vertreter der Unterfamilie Testudinidae (*Testudo hermannii*; *Geochelone radiata* und *Kinixys belliana*) benutzen hingegen obligatorisch ihre Zungen zum Nahrungserwerb (Wocheländer et al., 1999; Bels et al, 2008). Bis jetzt wurde eine detaillierte kinematische Analyse nur für *T. hermanii* (Wocheländer et al., 1999; 2000) publiziert. Diese Studien zeigen, dass sich Nahrungsaufnahme und Nahrungstransportzyklen nicht bedeutend unterscheiden. Die Motorik der Kiefer, des Hyolingualkomplexes und des gesamten Craniocervicalkomplexes (Hals- und Kopfbewegungen) ist hoch integriert, stereotyp und rhythmisch. Die kinematischen Muster entsprechen ziemlich genau den hypothetischen Mustern des „Generalized Feeding Cycle Model" (GFCM), Bramble & Wake (1985). Die qualitative Beschreibung der Nahrungsaufnahme bei *Geochelone radiata* und *Kinixys belliana* zeigt, dass „slow opening" (SO) und „fast opening" (FO) Phasen auch bei diesen Arten präsent sind (Bels et al., 2008). Eine gravierende kinematische Änlichkeit zwischen „prey capture" und „prey transport" Zyklen wurde auch für die aquatischen Arten *Dermochelys coriacea* und *Malaclemys terrapin* berichtet (Bels et al., 1998). In *T. carolina* hingegen, öffnen sich die Kiefer bei „prey capture" gleichmäßig, eine Unterteilung in verschiedene Phasen ist ziemlich undeutlich. Doch bei allen „prey transport" Sequenzen wurde eine zweiteilige „SO" Phase vor „FO" nachgewiesen (Bels et al., 1997). Bis jetzt existieren genaue kinematische Analysen von terrestrischem „prey transport" bei Schildkröten nur für *T. hermannii* (Wocheländer et al, 2000) und *T. carolina* (Bels et al., 1997). Keine Beschreibungen von „pharyngeal packing" und „swallowing" sind bis dato veröffentlicht. Die Arbeit von Summers et al. (1998) zeigt sich als einziger Vergleich der aquatischen und terrestrischen „prey capture“ Strategien bei ein und derselben Schildkrötenart, ein Vergleich der Transportkinematik fehlt.

I.3. Ziele und Arbeitshypothesen

In Gegensatz zur aquatischen Nahrungsaufnahme, deren Kinematik relativ gut untersucht ist (Bramble, 1978; Weisgram, 1985; Bels & Renous, 1992; Lauder & Prendergast, 1992; Bels et al., 1997; Lemell & Weisgram, 1997; Van Damme & Aerts, 1997; Bels et al., 1998; Summers et al., 1998; Aerts et al.,2001; Lemell et al., 2000; Lemell et al., 2002), ist der Mechanismus des terrestrischen Fressens der Schildkröten nahezu unbekannt (siehe Bels et al., 1997, 2008; Summers et al., 1998; Wocheslãnder et al, 1999, 2000). Die einzige vergleichende Arbeit über terrestrische und aquatische Nahrungsaufnahme bei Schildkröten ist jene von Summers et al. (1998). Leider fehlen bis heute Informationen über die Mechanismen welche die heute lebenden Chelonier für Nahrungtransport und Schlucken in den verschiedenen Medien verwenden. Im Rahmen dieser Dissertation wird eine detaillierte, vergleichend- und funktionsmorphologische Analyse des Fressvorgangs unter Wasser und an Land bei drei Arten von Geoemydidae und einer Spezies der Kinosternidae durchgeführt.

Die Anatomie des Craniums und des Hyolingualkomplexes von *Cuora galbinifrons*, *Cuors amboinensis*, *Cuora flavomarginata* und *Sternotherus odoratus* werden beschrieben. Bezüglich der Kopfmorphologie der drei Geoemydidae Arten der Gattung *Cuora.*, konnten einige Ungenauigkeiten der bisherigen Beschreibungen korrigiert werden. Die gewonnene Information über die Konstruktion des

Neurocraniums, Kiefer- und Hyolingualapparates wird funktionell - morphologisch ausgewertet um Korrelationen zwischen „Bauplan“ und Habitatpräferenzen besser verstehen zu können. Die gravierend unterschiedlichen physikalischen Eigenschaften der zwei Medien (Luft und Wasser), fordern entsprechende Anpassungen im Kopfdesign. Da alle drei untersuchten *Cuora* Arten in der Lage sind, an Land wie auch unter Wasser zu fressen, sind keine „morphologischen Extreme“ zu erwarten. Eine vergleichend-morphologische Untersuchung beantwortet die Frage, wie die Strukturen des Fressapparates bei eng verwandten Arten mit alternierenden Habitatpräferenzen ausgebildet sind und welche Funktion sie besitzen. Weil “within Testudinoidea, phylogeny seemed to constrain only localized features of the skull and remained of minor influence” (Claude et al., 2004), wird innerhalb der Gattung *Cuora* eine hohe interspezifische Variation im Kopfdesign erwartet.

Die vorliegenden Studien beinhalten die erste kinematische Analyse des Fressvorganges bei Vertretern der größten rezenten Schildkrötengruppe Geoemydidae. Die morphologischen und ökologischen Unterschiede zwischen den untersuchten Arten lassen auch Unterschiede in der Fresskinematik erwarten. Nachdem die Gattung *Cuora* ursprünglich aquatisch ist, wird in beiden Fressmedien „jaw prehension“ vermutet. Aufgrund des niedrigen Spezialisierungsgrades ist keine Stereotypie der Fressbewegungen zu erwarten. Der Fressvorgang befindet sich wahrscheinlich permanent unter sensomotorischer Kontrolle.

Die vorliegende Arbeit beinhaltet auch die erste, auf high-speed Videos (500 fr/s) basierende, Untersuchung der Nahrungsaufnahme von einem Vertreter der Kinosternidae (*Sternotherus odoratus*). Experimentell wird untersucht, ob diese hoch aquatischen Tiere in der Lage sind auch an Land zu fressen. Juvenile *S. odoratus* sind sehr aktiv und können sich an Land sehr gut bewegen, sie sind fähig zu klettern und sind oft außerhalb des Wassers anzutreffen. Man kann vermuten, dass die Moschusschildkröten ihre Beute terrestrisch wohl fangen können, „prey transport“ ist jedoch nur im Wasser möglich.

Beim Fressvorgang der meisten Tetrapoden sind Lokomotion und Nahrungserwerb stark korreliert (siehe Montuel et al., 2008). Innerhalb der Schildkröten ist der Beuteerwerb durch "lunge" jedoch nicht bekannt (siehe Lemell et al., 1997; Bels et al., 2008). Obwohl alle Schildkröten sich mehr oder weniger gut an Land bewegen und sogar fast alle Testudinidae schwimmen, manche sogar tauchen können ist ein Zusammenhang zwischen Fortbewegung und Nahrungsaufnahme nicht ersichtlich. Als Haupteinschränkung für amphibischen Nahrungserwerb in Chelonia wird der Bauplan des Kopfes und besonders des Hyoides und der Zunge vermutet. Der Fressvorgang an Land stellt für jene Schildkröten, deren Fressapparat vorwiegend an die aquatische Nahrungsaufnahme angepasst ist, ein Problem dar, weil die Möglichkeit fehlt die Beute während der Kieferöffnung ("FO") beim ersten Transportzyklus mit Hilfe der Zunge am Gaumen zu fixieren. Die rein terrestrischen Arten hingegen besitzen eine hypertrophierte Zunge und einen verkleinerten Hyobranchialkomplex, sodass sie beim Fressen unter Wasser bei der "prey capture" scheitern würden, da sie "compensatory suction" nur ansatzweise und "inertial suction" keinesfalls anwenden können.

II. Eingereichte Arbeiten:

II.1. Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. Zoology (2009).

Nikolay Natchev, Egon Heiss, Patrick Lemell, Daniel Stratev, Josef Weisgram

Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns

Nikolay Natchev[a], Egon Heiss[a], Patrick Lemell[a], Daniel Stratev[b], Josef Weisgram[a] *

[a] *Department of Theoretical Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*

[b] *University of Chemical Technology and Metallurgy* , *8 St. Kliment Ohridski blvd., 1756 Sofia, Bulgaria*

Received 14 January 2008; received in revised version 3 April 2008; accepted 22 May 2008

* Corresponding author.

E-mail address: josef.weisgram@univie.ac.at (J. Weisgram).

number of text pages: 26

number of tables: 4

number of figures: 10

Abstract

This study examines the kinematics and morphology of the feeding apparatus of two geoemydid chelonians, the Malayan (Amboina) box turtle (*Cuora amboinensis*) and the yellow-margined box turtle (*Cuora flavomarginata*). Both species are able to feed on land as well as in water. Feeding patterns were analysed by high-speed cinematography. The main focus of the present study is on the terrestrial feeding strategies in both Asian box turtles, because feeding on land has probably evolved *de novo* within the ancestrally aquatic genus *Cuora.* During terrestrial feeding (analysed for both species), the initial food prehension is always done by the jaws, whereas intraoral food transport and pharyngeal packing actions are tongue-based. The food uptake modes in *Cuora*s differ considerably from those described for purely terrestrial turtles. Lingual food prehension is typical of all tortoises (Testudinidae), but is absent in *C. amboinensis* and *C. flavomarginata*. A previous study on *Terrapene carolina* shows that this emydid turtle protrudes the tongue during ingestion on land, but that the first contact with the food item occurs by the jaws. Both Asian box turtles investigated here have highly movable, fleshy tongues; nonetheless, the hyolingual complex remains permanently retracted during initial prey capture. In aquatic feeding (analysed for *C. amboinensis* only), the prey is captured by a fast forward strike of the head (ram feeding). As opposed to ingestion on land, in the underwater grasp the hyoid protracts prior to jaw opening. The head morphology of the investigated species differs. In contrast to the Malayan box turtle, *C. flavomarginata* exhibits a more complexly structured dorsal lingual epithelium, a considerable palatal vault, weaker jaw adductor muscles and a simplified trochlear complex. The differences in the hyolingual morphology reflect the kinematic patterns of the terrestrial feeding transport.

Keywords: Box turtles; Feeding mode; Food prehension; Prey capture; Head morphology

Introduction

Terrestrial feeding has evolved several times among vertebrates in connection with the shift from water to land: e.g. Actinopterygii *vs.* Tetrapoda (Sponder and Lauder, 1980; Van Wassenbergh et al., 2006); terrestrial feeding also probably evolved independently within turtles (Summers et al., 1998). The stem turtles were terrestrial organisms, but the common ancestor of all living forms lived in fresh water (Joyce and Gauthier, 2004; Scheyer and Sander, 2007).

Aquatic feeding in chelonians is well studied (Bramble, 1978; Weisgram, 1985; Bels and Renous, 1991; Lauder and Prendergast, 1992; Bels et al., 1997; Lemell and Weisgram, 1997; Van Damme and Aerts, 1997; Bels et al., 1998; Summers et al., 1998; Lemell et al., 2000; Aerts et al., 2001; Lemell et al., 2002). Two main mechanisms for underwater food uptake and transport have been recognised so far – compensatory suction and inertial suction (*sensu* Van Damme and Aerts, 1997; Aerts et al., 2001).

In contrast to aquatic feeding, only few studies treat the issues of terrestrial feeding kinematics in turtles (Bels et al., 1997; Summers et al., 1998; Wocheslãnder et al., 1999, 2000, Bels et al., 2008). There are two methods of food uptake on land: lingual and jaw prehension (see Bels et al., 2008). The terrestrial intraoral food transport in tortoises is lingual-based. Highly stereotypical, coordinated hyolingual and jaw movements convey the food to the pharynx (Wocheslãnder et al., 2000). A slow jaw opening phase prior to reaching peak gape is present in all terrestrial turtles (Bels et al., 2008).

Our study focuses on species that are able to complete the whole feeding process, including ingestion (initial prey capture), processing, transport and swallowing (see Schwenk, 2000), on land and in water. Our general question was how both investigated Asian box turtles handle the different physical requirements of the feeding media (high density and viscosity of water compared to air; Gans, 1969). In water the food items are buoyant, but when feeding on land, the turtles have to handle the weight of the prey. If the tongue is not large or movable enough to ensure the lingual prey fixation in the buccal cavity during jaw opening, the food cannot be transported (terrestrial "inertial" food transport is not found in chelonians). Laboratory experiments on the Mexican mud turtle, C*laudius angustatus,* confirm that prey caught on land can be processed only by using hydrodynamic mechanisms, when the head is put below the water level (Weisgram, 1985). Bramble and Wake (1985) also reported that some aquatic chelonians can take food on land but are unable to handle the items and to transport them through the oral cavity.

Among living chelonians, species with terrestrial habitat preferences are found only within the three testudinoid families: Emydidae, Testudinidae and Geoemydidae. The Malayan box turtle and the yellow-margined box turtle belong to a subtaxon within the geoemydid genus *Cuora* (see Stuart and Parham, 2004). *Cuora amboinensis* is closely related to water, but *Cuora flavomarginata* is a "terrestrial omnivorous generalist" (see Winokur, 1988). Within the superfamily Testudinoidea the form of the skull is closely correlated with ecological factors, but "phylogeny seemed to constrain only localized features of the skull and remained of minor influence …" (Claude et al., 2004). Considering the diversity of habitat and food types exploited by the different *Cuora* species, we propose that the construction of the hyolingual complex and the jaw adductors can also vary considerably within the genus. A comparative morphological investigation of the head should answer how the environmental shift is reflected by the "Bauplan" of the feeding apparatus in the investigated species.

The common ancestor of the testudinoids was an aquatic form (see Joyce and Gauthier, 2004, for overview and cladogram). Currently, within Testudinoidea, species exist that feed terrestrially (all tortoises), aquatically (most emydids and geoemydids), or both (few emydids and geoemydids). We have analysed the terrestrial feeding kinematics in two geoemydid chelonians to compare their food prehension modes to those described for tortoises and for the semi-terrestrial turtle *Terrapene carolina*. All testudinids studied to date obligatorily use lingual food prehension (Wocheslânder et al., 1999; Bels et al., 2008). Jaw prehension is typical of turtles that feed in water. *Terrapene carolina* belongs to a primitively aquatic clade within Emydidae (see Summers et al., 1998). Terrestrial food uptake in this species involves the jaws (Bels et al., 1997). The genus *Cuora* is ancestrally aquatic (see Claude et al., 2003 for overview). We propose that the Asian box turtles, analogous to *T. carolina*, use jaw prehension when feeding on land. Nonetheless, we expect kinematic differences in the prey capture modes of emydids and geoemydids because terrestrial feeding presumably evolved secondarily within these groups.

The present study is also designed to determine the differences in the strategies used for aquatic and terrestrial feeding in *Cuora amboinensis*. Some semi-aquatic turtles are able to modulate their feeding kinematics in response to prey capture in different media (Summers et al., 1998). We predict the same for the food transport.

Material and methods

Both of the Southeast-Asian box turtles examined in this study, *Cuora amboinensis* (Daudin, 1802) and *Cuora flavomarginata* (Grey, 1863), live in semi-aquatic habitats and are omnivorous. *Cuora amboinensis* prefers shallow, stagnant or slow-flowing waters, although adult animals also wander far from any water source. In the literature these turtles are regarded as primarily herbivorous in the wild (Ernst and Barbour, 1989). In captivity, *C. amboinensis* is highly carnivorous and feeds even on fast-swimming prey such as frogs and fish. The home range of *Cuora flavomarginata* includes southeast China, Taiwan and the Ryukyu Islands of Japan. This species is less closely associated with water than *Cuora amboinensis*. It has been found in rice paddies and in forested hills (Ernst and Barbour, 1989).

The animals used for the present study were kept in glass aquariums at 25 °C constant temperature with 12h/12h light/dark photoperiod and were fed vegetables (bananas, cucumbers, apples, tomatoes, etc.), small invertebrates (mealworms, snails and land worms), but also fish and pieces of liver.

Anatomical observations were made on three *C. amboinensis* specimens – two females and one male (carapace lengths 16.4, 15.9, and 16.9 cm) – and three female *C. flavomarginata* specimens (carapace lengths 16, 16.5, and 17.2 cm.). The animals were killed by intraperitoneal injection of a lethal dose of sodium pentabarbitol. Immediately after death the skin was removed and the morphology of the skeletal elements and the cranial, hyoidal and cervical muscles were investigated using a Wild M5A dissecting microscope. The anatomical research focused on the structures involved in feeding. For both species we investigated the "Bauplan" of the cranium, the hyolingual complex and the head musculature. The description of the musculature and the tendon systems is based on the terminology of Schumacher (1973).

The tongue, part of the oesophagus and the trachea were fixed using Bouin's solution for histological investigations. The material was embedded in paraffin and the tongue was sectioned longitudinally. For light microscopic observation, the sections (8 µm) were stained with Azan after Heidenhain and were observed and photographed using a Nikon Eclipse E800 light microscope.

High-speed videos

Four subadult *Cuora amboinensis* and three subadult *Cuora flavomarginata* were used to investigate terrestrial feeding. The animals were filmed in lateral view in an empty aquarium (size 40 cm x 16 cm x 25 cm) with a reference grid (1 cm x 1 cm) in the background using a NAC-1000 high-speed video system at 250 frames per second. We offered the food items (one by one, or arranged in rows) on the bottom of the aquarium in front of the animals. From each specimen, a minimum of 15 feeding events were filmed. The food items were pellets, mealworms, earthworms and bananas. A total of 36 video sequences of *C. amboinensis* and 27 of *C. flavomarginata* feeding on pellets or small mealworms were selected for qualitative and quantitative analyses. We detected two different transport modes here termed as “prey positioning” and “lingual transport". We quantified their occurrence by recording the number of times each individual utilised a pure form of prey positioning or lingual transport and calculated the mean percentage occurrence for each species. For our transport kinematics analysis we selected 36 sequences per transport type in *C. amboinensis* and 27 for *C. flavomarginata* (the same number as for prey capture for both species). The transport cycles in both investigated turtles could well be differentiated from “pharyngeal packing” cycles, because in pharyngeal packing the gape is quite modest and the head is slightly elevated.

For filming aquatic feeding, the aquarium was filled with 15 cm of water and the food items were suspended in the water on a thin thread in front of the animals. The turtles were fed with pieces of fish. Three specimens of *C. amboinensis* were filmed in lateral view (10 films per specimen), and 21 videos were selected for analysis. Our *C. flavomarginata* specimens refused to feed under water under the strong light needed for filming.

The videos were digitised using Adobe Premiere, and horizontal and vertical coordinates of specific anatomical landmarks (Fig. 1) were matched using an AVI-digitiser (© Peter Snelderwaard, University of Leiden, Leiden, The Netherlands). Based on the landmarks’ displacement in the bi-directional level, we were able to calculate: a) the gape amplitude – distance between the tips of the upper and lower jaw; b) ventral hyoid movement – dorso-ventral height of the head at the level of the origin of the ceratobranchiale II; c) the anterior-posterior movements of the tongue – the distance between the tip of the tongue and the line connecting the tips of the upper and lower jaw; d) the extension and retraction of the neck – the distance between the point on the parietal surface of the cranium and the anterior tip of the carapace; e) the distance predator to prey – the distance between the centre of mass of the prey and the tip of the upper jaw; f) the maximal gape angle – the angle between the jaws at maximum gape. These data enabled us to calculate the duration of the different feeding stages, the time at which the peak gape was reached, the duration and the velocity of hyoid retraction, the delay between peak hyoid depression and peak gape, the time of pro- and retraction of the head, and the total duration of the cycles.

Results

Anatomy

In both species the skull is anapsid and akinetic. The temporal roof is ventrally and dorsally open due to emarginations (see Fig. 2). The temporal arch is complete and consists of three elements: os postorbitale, os jugale and os quadratojugale. An os quadratojugale was also found in *C. flavomarginata* (in contrast to Wermuth and Mertens, 1961 and Ernst and Barbour, 1989). No direct connection between the jugal and the quadratojugal bones was detected. The supraoccipital bone bears a crista supraoccipitalis. The dorsal ridge is straight and extends behind the foramen magnum occipitale in *C. amboinensis* (Fig. 2C). In *C. flavomarginata*, the crista supraoccipitalis is shorter, convex and has fenestrations (Fig. 2A). The jaw articulation, as typical of the Cryptodira, allows no lateral mandible movements. The trochlear process is formed only by the quadrate bones. The palate is flat in *C. amboinensis* but concave in *C. flavomarginata* (see also Heiss et al., 2008). The palatines are bright bony plates, they are robust in the Malayan box turtle but thin and transparent in *C. flavomarginata.*

The hyoid is relatively large compared to cranium length and only partially ossified (see Fig. 3C, D). The hyoid corpus is square and lacks fenestrations. The lingual process is elongated, cartilaginous and elastic. The branchial horns I (CB I) are caudo-dorsally extended and ossified. The epibranchialia I are cartilaginous. On the origin of the cornu branchiale II (CB II) on the hyoid corpus there are two islands of ossification. The branchial horns II are shorter than CB I, and their caudal ends are strongly laterally divided in *C. amboinensis*. The hypoglossum (Fig. 3C, D) has one caudal and one rostral process. It is a relatively big (compared to *Testudo hermanii;* see Wocheslander et al., 1999) and flat cartilaginous plate without any ossifications. *C. flavomarginata* exhibits two typical fenestrations in the rostral part.

A schematic illustration of the most important jaw and hyoid muscles is shown in figure 3A and B. The m. adductor mandibulae externus fills the whole upper temporal fossa. The mm. add. mand. ext. pars superficialis and pars profunda are strongly developed in *C. amboinensis* (Fig. 2D) and relatively small in *C. flavomarginata* (Fig. 2B). The external tendon system is well developed and has a myovector-changing function (see Bramble, 1974; Jordanskii, 1990). *C. amboinensis* possesses a large transiliens bulge (3.4 ± 0.3 mm in diameter) within the external adductor tendon. The m. adductor mandibulae posterior has no insertion on the temporal bone, and we were unable to detect a margin splitting that muscle into caudal and rostral portions (see Schumacher, 1956). No clear morphological separation of the m. add. mand. internus into a pars pseudotemporalis and pars pterygoideus was possible.

The tongue in both species is developed differently. The lingual glands in *C. flavomarginata* are concentrated anteriorly on the origins of the high and slender lingual papillae. The lingual papillae are well vascularised, and the blood vessels form lacunae (Fig. 4). The tongue of *C. amboinensis* is smaller and the ridge-like lingual papillae are broader and shorter (Beisser and Weisgram, 2001). Lingual papillae on the ventral side of the tongue were found in *C. flavomarginata*.

Kinematics

Terrestrial feeding

In 83.3 % of our film sequences of *Cuora amboinensis*, the plastron contacted the bottom of the aquarium after the forwards locomotion of the body stopped. The limbs were stretched to the side (see Fig. 5B). Throughout the feeding process the animals "lay" on the ground. *Cuora flavomarginata* exhibited different behaviour. Its plastron never contacted the ground during ingestion (Fig. 6B).

The static phase of the "final head fixation" was taken as "time zero" for our kinematic analyses. All kinematic patterns during ingestion and oropharyngeal transport were analysed frame by frame (4 ms steps). Jaw opening is characterised by lower jaw depression, which coincides with neck protraction and head depression. No protraction of the hyoid complex was recorded. The gape opened gradually (Figs. 5A, 6A). During jaw opening, before reaching maximum gape, the lower jaw can contact the ground. This occurred in 100 % of the prey capture sequences of *C. amboinensis* (Fig. 5B) but only in 14.8 % of *C. flavomarginata*.

The peak gape was reached in 0.267 ± 0.041 s in *C. amboinensis* and in 0.360 ± 0.026 s in *C. flavomarginata*. Even at maximal gape amplitude (the maximal gape angle measured for *C. amboinensis* was 58°), the large tongue was not visible in lateral view (see Figs. 5B, 6B). The diameter in the larynx region of the neck increased. After reaching maximum gape the whole head rotated ventrally. During head rotation, the jaw amplitude remained constant. A static gape phase of maintaining the maximal jaw amplitude (here termed MG phase = maximal gape phase) was detected in all cycles of prey capture and oropharyngeal transport. The duration of the MG phase during ingestion was 0.068 ± 0.008 s in *C. amboinensis*, but shorter by almost half in *C. flavomarginata*: 0.042 ± 0.008 s. The gape closing was followed by a neck retraction and the hyoid was then lifted to its zero position.

In *Cuora flavomarginata* (number of transport cycles per food item: 5.22 ± 1.23) and in *C. amboinensis* (number of transport cycles per food item: 6.94 ± 2.47), we detected two different transport pattern modes. In the first mode the lower jaw depressed gradually. Peak gape was reached in 0.148 ± 0.030 s in *C. flavomarginata* and in 0.169 ± 0.021 s in *C. amboinensis*. The neck remained motionless (Fig. 7) and was permanently retracted, which hindered precise observation of the hyoid movements. Discrete gape phases lacked prior peak gape. We have termed such transport cycles “lingual transport”.

Symptomatic of the second detected mode was that the neurocranium and the neck did not remain static. After the food item was pressed to the upper jaw by the tongue, the lower jaw depressed slowly to approximately half the maximal gape. A static gape phase in which the jaw amplitude remained constant followed. The duration of that static phase was variable (see Tables 1 and 2), not only in the two species and the different specimens, but in some cases also between two successive transport cycles. In the next phase, the gape increased rapidly. The end of the fast jaw opening phase coincided with the start of a rapid retraction of the hyolingual complex (velocity in *C. amboinensis* V = 9.6 ± 1.4 cm/s). Almost simultaneously a neck extension occurred (Fig. 8A). The food item was moved forward and did not lose contact with the tongue. The MG phase was relatively short (0.018 ± 0.002 s) in *C. flavomarginata* and 0.024 ± 0.003 s in *C. amboinensis*). The MG phase was followed by rapid mouth closing (Tables 1 and 2). We recorded such transport cycles (up to 12 for some feeding events in *C. amboinensis*) as “prey positioning”.

In *C. flavomarginata*, from a total of 141 transport cycles in 27 film sequences, 61.8 % were recognised as “lingual transport”. In *C. amboinensis*, from a total of 250 transport cycles in 36 film sequences, 66.8 % were “prey positioning”. *C. flavomarginata* used lingual transport immediately after prey capture in 7 of our sequences (n = 27), or after 1 to 4 (average = 2.00 ± 1.39) prey positioning cycles. In *C. amboinensis* (n = 36) we detected 4.64 ± 2.41 prey positioning cycles prior to the first lingual transport cycle. In only two sequences was lingual transport used directly after prey capture.

Aquatic feeding

Aquatic feeding modes were investigated only in *C. amboinensis*. Six kinematic variables are represented in table 3. The turtles approached the food item slowly. When the tip of the upper jaw was at 1.32 ± 0.34 cm from the fish, the forward locomotion stopped. The body and the head remained static. The prey capture started with a hyoid protraction and lifting, followed by neck extension (Fig. 9A, B). Jaw opening started during the fast neck extension. Upon reaching peak gape, the jaws were moved over the prey. No division into different gape phases was detected during mouth opening. The extension of the neck continued after the peak gape was reached. The gape remained for a maximum of 0.012 ± 0.002 s and, during this phase, the hyoid retraction started. Hyoid retraction lasted 0.042 ± 0.006 s. The peak ventral hyoid depression was reached during the jaw closing phase. Jaw closing phase was very short: 0.033 ± 0.004 s. During ingestion, the prey remained static. No inertial suction effect was detected (Fig. 9B2, B3). The first contact with the prey always occurred at the jaws. Due to the impulse of the fast forward strike of the head, the grasped food item moved in the same direction. Ingestion ended with head retraction. The gape could not be completely closed because the prey was not entirely in the oral cavity. The water volume taken up during prey capture was expelled by protracting the hyoid complex to its resting position.

The following cycle starts the transport phase (Fig. 10). The neck is retracted at the beginning of the cycle. The oropharyngeal transport was hydrodynamic and occurred without reduction; no crushing actions were recognised. The prey was freed shortly from the jaws (Fig. 10B2) during the mouth opening (duration: 0.028 ± 0.004 s). The time to peak gape was nearly twice as short as the same phase during prey capture. The end of jaw opening coincided with head protraction and hyoid retraction (duration: 0.048 ± 0.008 s). The rush neck extension and the abrupt hyoid depression help reposition the food deeper within the oral cavity. Hyoid depression reached its peak at the end of gape closing. In transport cycles where the prey was almost completely taken into the oral cavity, neck extension was smaller, but the hyoid depression was still considerable.

Discussion

Previous research has shown that feeding modes and head morphology are well correlated in turtles (Bramble, 1973; Claude et al., 2004). Although *C. amboinensis* feeds aquatically as well as terrestrially, our morphological study points to a predominantly aquatic lifestyle within this ecomorphological framework. The tongue, with short and broad lingual papillae, and the flat roof of the mouth cavity indicate inefficient food transport on land. The strongly developed jaw adductors, the large transiliens tendon bulge and the hard palatinal bones correlate with the feeding ecology and the diet of the species – the Amboina box turtles need to bite hard to hold elusive prey. The elongated and stilted hyoid horns support the enlargement of the anterior oesophagus in aquatic feeding with a bidirectional flow system (Lauder and Shaffer, 1986; Van Damme and Aerts, 1997; Aerts et al., 2001). Both investigated species have a relatively large hyoid complex to produce sufficient suction when feeding under water. Nonetheless, the elasticity of the anterior part of the hyoid body enables the tongue to be highly mobile. The tongue movements in *C. flavomarginata* are additionally facilitated by the considerable dorsal vault of the mouth roof.

The tongue morphology in turtles can vary considerably (Winokur, 1988; Weisgram et al., 1989; Iwasaki, 1992; Beisser et al., 1995, 1998, 2001, Beisser and Weisgram, 2001; Beisser et al., 2004). The tongue of specialised suction feeders such as *Chelus fimbriatus* is tiny and lacks lingual papillae or glands (Lemell et al., 2002). Terrestrial species possess a large, fleshy tongue with numerous large lingual papillae (Winokur, 1988). The secretion of the well-developed lingual glands in *C. flavomarginata* promotes oropharyngeal food transport on land. Most lingual glands are concentrated at the base of the anterior papillae, as opposed to *Testudo hermannii* (Wocheslӓnder et al., 1999), whose glands are concentrated at the posterior surface of the tongue; this is also different from *Rhinoclemmys pulcherrima incisa*, where the lingual glands are distributed over the whole dorsal lingual surface (Beisser et al., 2004).

The geoemydids originated in the Eocene (Claude et al., 2003) as an aquatic turtle lineage and are considered to be a sister group of the testudinids (Claude et al., 2004) or the emydids (Joyce, 2007). In the two geoemydid species examined here, the tongue is not used for food uptake in terrestrial feeding, as opposed to tortoises (Wocheslӓnder et al., 1999; Bels et al., 2008). Although *C. flavomarginata* and

C..amboinensis possess highly mobile tongues that can be extended outside the oral cavity, initial prey capture is never tongue-based. We found no exceptions concerning the different food types. During mouth opening and the MG phase in ingestion, a strong depression rather than protraction of the hyoid complex occurs. We propose that this is correlated to the mechanism of gape opening. The simultaneous contraction of the m. coracohyoideus, m. depressor mandibulae and m. branchiomandibularis, the low activity of the m. geniohyoideus, m. genioglossus and m. Intermandibularis, and the deep depression of the anterior tip of the head help explain the extraordinary hyoid position during the MG phase. Posterior fixation of the hyoid during jaw opening has not been reported for chelonians that are able to feed on land. Presumably the increased impact of the m. coracohyoideus in jaw opening promotes a larger gape. Bels et al. (1997) report a maximal gape angle of 40° in *T. carolina.* The maximum angle measured in *C. amboinensis* was almost 60°.

Descriptions of prey capture kinematics in emydids are restricted to a single species, *T. carolina* (Bels et al., 1997; Summers et al., 1998). As the Malayan box turtle and the yellow-margined box turtle, *T. carolina* uses jaw prehension when feeding on land. The kinematic patterns of the jaws and the hyolingual complex in both Asian box turtles diverge notably from those in *T. carolina*. As discussed above, hyoid protraction is absent in *Cuora*s. In *T. carolina* the hyolingual complex protracts prior to peak gape, and slow and fast jaw opening phases are determinable (Bels et al., 1997). Based on our results, we conclude that at least three different modes of terrestrial food uptake can now be recognised within the superfamily Testudinoidea: 1. Lingual prehension, which is obligatory in testudinids (Wocheslӓnder et al., 1999, 2000; Bels et al., 2008); 2. Jaw-based prehension involving hyolingual protraction (found in the emydid *T. carolina;* Bels et al., 1997); 3. Prey capture via jaw prehension without hyolingual protraction (in the geoemydids studied here). It remains open whether any species exhibits facultative tongue prehension as stated by Bels et al. (1997) and Summers et al. (1998). Because of the limited information on the prey capture modes in emydids and geoemydids, we are unable to determine if there is a phylogenetic signal concerning the terrestrial food uptake kinematics in testudinoids.

As opposed to prey capture on land, in the underwater grasp the hyoid is protracted prior to jaw opening. The hyoid depresses considerably during prey capture. The anterior part of the oesophagus in *C. amboinensis* is distensible and serves as a reservoir for the swallowed water; still we were not able to detect inertial suction effects like in ingestion modes of *Chelodina longicollis* (Van Damme and Aerts, 1997) and *Chelus fimbriatus* (Lemell et al., 2002). *C. amboinensis* seems to be predominantly a ram-feeder using a similar aquatic prey capture strategy as *Chelydra serpentina* (see Lauder and Prendergast, 1992). As presented in table 4, the strike of the Malayan box turtle is slower than that of the specialised aquatic predators. It is faster than the strikes in the less specialised, purely aquatic, carnivorous forms *Pelusios castaneus* (Lemell and Weisgram, 1997), *Malaclemys terrapin* and *Dermochelys coriacea* (Bels et al., 1998).

For terrestrial food transport, the kinematic patterns in tetrapods have been proposed to adhere to a generalised cyclic model (Bramble and Wake, 1985) of five phases: "slow open I" (SO-I), "slow open II" (SO-II), "fast open" (FO), "fast close" (FC) and "slow close-power stroke" (SC-PS). The terrestrial transport modes described in turtles to date correspond with the generalised cyclic model concerning gape, neck and hyoid kinematics (see Bels et al., 1997, Wocheslӓnder et al., 2000; Bels et al., 2008). Discrete SO-I, SO-II and FO gape phases are well determinate. Fast jaw closing starts immediately after the peak gape is reached. Ventral head rotation starts at the end of SOII and the hyoid retraction begins early in the FO phase.

In *C. amboinensis and C. flavomarginata* we have detected certain deviations from the proposed conservative kinematic feeding patterns (Alfaro and Herrel, 2001): during lingual transport, the neurocranium remains almost completely motionless. In contrast to tortoises (Bels et al., 2008), there is no clear kinematic distinction between SO and FO phases (Fig. 7A). We propose that food transport is effected predominantly by intrinsic lingual movements without major hyoid displacement. Such a transport mechanism is facilitated in *C. flavomarginata* because of the large, movable tongue and the well-developed dorsal lingual papillae. The gape kinematic profiles in prey positioning are almost similar to those predicted by the generalised cyclic model of Bramble and Wake (1985). The main difference is the occurrence of a static MG phase after fast opening. The hyoid and the neck kinematic patterns in prey positioning are quite different from those proposed by the cyclic model. The retraction of the hyoid complex starts at the beginning or during the static MG phase rather than at the beginning of FO, and is abrupt and rapid. The end of fast jaw opening is correlated with extension of the neck. The head and the food are moved forwards. Despite the rapid neck protraction, we cannot categorise the process as cranial inertial feeding (Bramble and Wake, 1985) because the food is accelerated together with the head (no inertial moment). The lack of posterior head movement immediately before neck extension does not allow the transport mode to be defined as “It”-type (*sensu* Elias et al., 2000) either. Prey positioning cycles seem to be used by the turtles to position the prey on the dorsal tongue surface. The further transport of the food item toward the oesophagus involves mainly lingual transport.

When feeding on fish, the aquatic food transport in *C. amboinensis* involves compensatory suction. Hyoid retraction starts shortly prior to reaching peak gape, or during the MG phase. The food displacement is promoted by forward movement of the head. There are certain kinematic similarities between the aquatic transport and terrestrial prey positioning in *C. amboinensis*. The start of the neck extension, as well as the start of the hyoid retraction, correlate with the same gape phases. Nevertheless, the duration of all phases measured in aquatic transport is shorter than in terrestrial transport. The difference in the duration of the gape cycles is more than four-fold. In underwater transport the slow opening gape phases are absent.

Most of the measured parameters varied considerably. The food transport in the investigated *Cuora* species seems to be permanently modulated, fine-tuned and optimised by sensory feedback. The sensorimotor feedback impacts on prey capture are sufficiently studied for lower tetrapods (for references see Deban et al., 2001; Schaerlaeken et al., 2007). The existence of modulations in reptilian feeding transport is evidenced in lizards (Bels and Baltus, 1988; Schwenk and Throckmorton, 1989; Herrel and De Vree, 1999, Herrel et al., 1999; Schwenk, 2000; Herrel et al., 2001; Lappin and German, 2005; Ross et al., 2007) and snakes (Kardong and Berkhoudt, 1998) as well as in aquatic feeding in turtles (Lauder and Prendergast, 1992; Lemell and Weisgram, 1997; Bels et al., 1998).

The formation of high and slender dorsal tongue papillae, cranial flexure and a palatinal vault which are morphological features typical of tortoises are present in *C. flavomarginata* but almost absent in *C. amboinensis*. Our results therefore support the statement of Claude et al. (2004) that differences in the habitats correspond to important and rapid morphological changes within Testudinoidea.

Jaw prehension is obligate in both investigated species when feeding on land and in water. Typical of terrestrial prey capture in *C. amboinensis* and *C. flavomarginata* is the occurrence of a prolonged maximal gape phase (MG phases) and the absence of hyoid protraction. Such kinematic patterns have not been detected in other turtles to date. A topic for further investigation will be the range in which this prey capture mode is distributed within those geoemydids able to feed on land. The jaw, neck and tongue movements during terrestrial food transport are not stereotypical in the investigated *Cuoras.* In both Asian box turtles examined in this study, sensory feedback plays an important role in feeding, and the process itself is very flexible.

Acknowledgements

We would like to thank Prof. Ken Kardong and Prof. Vincent Bels for devoting so much time during the 8th Int. Congress of Vertebrate Morphology to the discussion of our work. Richard Gemel and Dr. Walter Joyce provided useful information on turtle ecology and phylogeny. Dr. Christan Beisser and two anonymous reviewers are acknowledged for helpful comments on the manuscript. We thank Dr. Michael Stachowitsch for language corrections. Supported by FWF Austrian Science Fund Project P20094-B17.

References

Aerts, P., Van Damme, J., Herrel, A., 2001. Intrinsic mechanics and control of fast cranio-cervical movements in aquatic feeding turtles. Am. Zool. 41, 1299–1310.

Alfaro, M.E., Herrel, A., 2001. Introduction: major issues of feeding motor control in vertebrates. Am. Zool. 41, 1243-1247.

Beisser, C.J., Weisgram, J., 2001. Dorsal tongue morphology and lingual glands in Chelonians. J. Morph. 248, 205.

Beisser, C.J., Weisgram, J., Splechtna, H., 1995. The dorsal lingual epithelium of *Platemys pallidipectoris* (Pleurodira, Chelidae). J. Morph. 226, 267–276.

Beisser, C.J., Weisgram, J., Hilgers, H., Splechtna, H., 1998. Fine structure of the dorsal lingual epithelium of *Trachemys scripta elegans* (Chelonia: Emydidae). Anat. Rec. 250, 127–135.

Beisser, C.J., Lemell, P., Weisgram, J., 2001. Light and electron microscopy of the tongue of *Pelusios castaneus* (Chelonia: Pleurodira). Tissue and Cell 33, 63–71.

Beisser, C.J., Lemell, P., Weisgram, J., 2004. The dorsal lingual epithelium of *Rhinoclemmys pulcherrima incisia* (Chelonia, Cryptodira). Anat. Rec. 277A, 227–235.

Bels, V., Baltus, I., 1988. The influence of food items on the feeding cycle in *Anolis equestris* (Reptilia: Iguanidae). Copeia 2, 479–481.

Bels, V.L., Renous, S., 1991. Kinematics of feeding in two marine turtles (*Chelonia mydas* and *Dermochelys coriacea*). Proc. 6th Ord. Gen. Meeting of the Soc. Eur. Herpetol., Hung. Nat. Hist. Mus., Budapest, pp. 73–78.

Bels, V.L., Davenport, J., Delheusy, V., 1997. Kinematic analysis of the feeding behaviour in the box turtle *Terrapene carolina* (L.), (Reptilia: Emydidae). J. Exp. Zool. 277, 198–212.

Bels, V.L., Davenport, J., Renous, S., 1998. Food ingestion in the estuarine turtle *Malaclemys terrapin*: comparison with the marine leatherback turtle *Dermochelys coriacea*. J. Mar. Biol. Ass. U.K., 78, 1–20.

Bels, V.L., Baussart, S., Davenport, J., Shorten, M., O'Riordan, R.M., Renous, S., Davenport, J., 2008. Functional evolution of feeding behaviour in turtles. In: Wyneken, J., Godfrey, M.H., Bels, V. (Eds.), Biology of Turtles. CRC Press, Boca Raton, pp. 189–212.

Bramble, D.M., 1973. Media dependent feeding in turtles. Am. Zool. 13, 1342.

Bramble, D.M., 1974. Occurrence and significance of the os transiliens in gopher tortoises. Copeia 74, 102–108.

Bramble, D.M., 1978. Functional analysis of underwater feeding in the snapping turtle. Am. Zool. 18, 623.

Bramble, D.M., Wake, D.B., 1985. Mastication, food transport, and swallowing. In: Hildebrand, M., Bramble, D.M., Liem, K.F., Wake, D.B. (Eds.), Functional Vertebrate Morphology. Belknap Press of Harvard University, Cambridge, Massachusetts, pp. 230–261.

Claude, J., Paradis, E., Tong, H., Auffray, J.C., 2003. A geometric morphometric assessment of the effects of the environment and cladogenesis on the evolution of the turtle shell. Biol. J. Linn. Soc. 79, 585–501.

Claude, J., Pritchard, P. C. H., Tong, H., Paradis, E., Auffray, J.C., 2004. Ecological correlates and evolutionary divergence in the skull of turtles: a geometric morphometric assessment. Syst. Biol. 53, 933–948.

Deban, S.M., O'Reilly, J.C., Nishikawa, K.C., 2001. The evolution of the motor control of feeding in amphibians. Am. Zool. 41, 1280–1298.

Elias, J.A., McBrayer, L.D., Reilly, M.S., 2000. Prey transport kinematics in *Tupinambis teguixin* and *Varanus exanthematicus*: conservation of feeding behaviour in “chemosensory-tongued” lizards. J. Exp. Biol. 203, 791–801.

Ernst, C.H., Barbour, R.W., 1989. Turtles of the World. Smithsonian Institution Press, Washington, D.C.

Gans, C., 1969. Comments on inertial feeding. Copeia 1969, 855–857.

Heiss, E., Plenk, H., Jr., Weisgram, J., 2008. Microanatomy of the palatal mucosa of the semiaquatic Malayan box turtle, *Cuora amboinensis*, and functional implications. Anat. Rec. 291, 876–885.

Herrel, A., De Vree, F., 1999. Kinematics of intraoral transport and swallowing in the herbivorous lizard *Uromastix acanthinurus*. J. Exp. Biol. 202, 1127–1137.

Herrel, A., Verstappen, M., De Vree, F., 1999. Modulatory complexity of the feeding repertoire in scincid lizards. J. Comp. Physiol. A. 184, 501–518.

Herrel, A., Meyers, J.J., Nishikawa, K.C., De Vree, F., 2001. The evolution of feeding motor patterns in lizards: modulatory complexity and constraints. Am. Zool. 4, 1311–1320.

Iwasaki, S., 1992. Fine structure of the dorsal epithelium of the tongue of the freshwater turtle, *Geoclemys reevesii* (Chelonia, Emydinae). J. Morph. 211, 125–135.

Jordanskii, N., 1990. Evolucija kompleksnaich adaptacii: chelustnoi aparat amfibii i reptilii. Izdatelstvo Nauka, Moskva.

Joyce, W., 2007. A phylogeny of Mesozoic turtles. Bull. Peabody Mus. Nat. Hist. 48, 3–102.

Joyce, W., Gauthier, J., 2004. Paleoecology of Triassic stem turtles sheds new light on turtle origins. Proc. of the Royal Society of London B 271, 1–5.

Kardong, K.V., Berkhoudt, H., 1998. Intraoral transport of prey in the reticulated python: tests of a general tetrapod feeding model. Zoology 101, 7–23.

Lappin, A.K., German, M., 2005. Feeding behavior modulation in the leopard lizard (*Gambelia wislizenii*): effects of noxious versus innocuous prey. Zoology 4, 287–295.

Lauder, G.V., Prendergast, T., 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. J. Exp. Biol. 164, 55–78.

Lauder, G.V., Schaffer, H.B., 1986. Functional design of the feeding mechanism in lower vertebrates: unidirectional and bidirectional flow systems in the tiger salamander. Zool. J. Linn. Soc. 88, 277–290.

Lemell, P., Weisgram, J., 1997. Feeding patterns of *Pelusios castaneus* (Chelonia: Pleurodira). Neth. J. Zool. 47, 429–441.

Lemell, P., Beisser, C.J., Weisgram, J., 2000. Morphology and function of the feeding apparatus of *Pelusios castaneus* (Chelonia: Pleurodira). J. Morph. 244, 127–135.

Lemell, P., Lemell, C., Snelderward, P., Gumpenberger, M., Wocheländer, R., Weisgram, J., 2002. Feeding patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). J. Exp. Biol. 205, 1495–1506.

Ross, C.F., Eckhardt, A., Herrel, A., Hylander, W.L., Metzger, K.A., Schaerlaeken, V., Washington, R.L., Williams, S.H., 2007. Modulation of intra-oral processing in mammals and lepidosaurs. Integr. Comp. Biol. 47, 118–136.

Schaerlaeken, V., Meyers, J.J., Herrel, A., 2007. Modulation of prey capture kinematics and the role of lingual sensory feedback in the lizard *Pogona vitticeps*. Zoology 110, 127–138.

Scheyer, T.M., Sander, P.M., 2007. Terrestrial palaeoecology for basal turtles indicated by shell bone histology. Proc. Roy. Soc. Lond. B 274, 1885–1893.

Schumacher, G.H., 1956. Morphologische Studie zum Gleitmechanismus des M. adductor mandibulae externus bei Schildkröten. Anat. Anz. 103, 1–12.

Schumacher, G.H., 1973. The head muscles and hyolaryngeal skeleton of turtles and crocodilians. In: Gans, C., Parsons, T.S. (Eds.), Biology of the Reptilia, Vol. 4. Academic Press, New York, pp. 101–199.

Schwenk, K., 2000. Feeding. Academic Press, San Diego.

Schwenk, K., Throckmorton, G.S., 1989. Functional and evolutionary morphology of lingual feeding in squamate reptiles: phylogenetics and kinematics. J. Zool. Lond. 219, 153–175.

Sponder, D., Lauder, G.V., 1980. Terrestrial feeding in the mudskipper *Periophtalmus* (Pisces: Teleostei): a cineradiographic analysis. J. Zool. Lond. 193, 517–530.

Stuart, B.L., Parham, J.F., 2004. Molecular phylogeny of critically endangered Indochinese box turtle (*Cuora galbinifrons*). Mol. Phylogenet. Evol. 31, 164–177.

Summers, A.P., Darouian, K.F., Richmond, A.M., Brainerd, E.L., 1998. Kinematics of aquatic and terrestrial prey capture in *Terrapene carolina*, with implications for the evolution of feeding in cryptodire turtles. J. Exp. Zool. 281, 280–287.

Van Damme, J., Aerts, P., 1997. Kinematics and functional morphology of aquatic feeding in Australian snake-necked turtles (Pleurodira; Chelodina). J. Morph. 233, 113–125.

Van Wassenbergh, S., Herrel, A., Adriaens, D., Huysentruyt, F., Devaere, S., Aerts, P., 2006. Evolution: a catfish that can strike its prey on land. Nature 440, 881.

Weisgram, J., 1985. Feeding mechanics of *Claudius angustatus* Cope 1865. In: Duncker, H.R., Fleischer, G. (Eds.), Fortschritte der Zoologie, Vol. 30. Gustav Fischer Verlag, Stuttgart, pp. 257–260.

Weisgram, J., Dittrich, H., Splechtna, H., 1989. Comparative functional study of the oral cavity in two turtle species. Plzen. Lek. Sborn., Suppl. 59, 117–122.

Wermuth, H., Mertens, R., 1961. Schildkröten, Krokodile, Brückenechsen. VEB Gustav Fischer Verlag, Jena.

Winokur, B.M., 1988. The buccopharyngeal mucosa of the turtles (Testudines). J. Morph. 196, 33–52.

Wochesländer, R., Hilgers, H., Weisgram, J., 1999. Feeding mechanism of *Testudo hermanni boettgeri* (Chelonia, Cryptodira). Neth. J. Zool. 49, 1–13.

Wochesländer, R., Gumpenberger, M., Weisgram, J., 2000. Intraoral food transport in *Testudo hermanni* (Chelonia, Cryptodira) – a radiographic video analysis. Neth. J. Zool. 50, 445–454.

Table 1. Mean ± standard deviation for nine kinematic variables (measured in seconds) from terrestrial feeding events of *Cuora flavomarginata*. SO-I = slow open I; SO-II = slow open II; FO = fast open; MG = maximal gape; FC = fast close.

Variable	Capture (N=27)	Lingual Transport (N=27)	Prey Positioning (N=27)
Duration of gape cycle	0.444 ± 0.046	0.242 ± 0.042	0.454 ± 0.086
Time to peak gape	0.360 ± 0.026	0.148 ± 0.030	0.414 ± 0.064
Duration of SO-I phase			0.096 ± 0.008
Duration of SO-II phase			0.264 ± 0.062
Duration of FO phase			0.036 ± 0.005
Duration of MG phase	0.042 ± 0.008	0.016 ± 0.002	0.018 ± 0.002
Duration of FC phase	0.042 ± 0.004	0.038 ± 0.004	0.040 ± 0.004
Duration of hyoid retraction			0.050 ± 0.004
Peak hyoid delay to end of MG phase			0.036 ± 0.008

Table 2. Mean ± standard deviation for nine kinematic variables (measured in seconds) from terrestrial feeding events of *Cuora amboinensis*. SO-I = slow open I; SO-II = slow open II; FO = fast open; MG = maximal gape; FC = fast close.

Variable	Capture (N=36)	Lingual Transport (N=36)	Prey Positioning (N=36)
Duration of gape cycle	0.429 ± 0.078	0.225 ± 0.022	0.510 ± 0.096
Time to peak gape	0.267 ± 0.041	0.169 ± 0.021	0.424 ± 0.088
Duration of SO-I phase			0.086 ± 0.009
Duration of SO-II phase			0.308 ± 0.066
Duration of FO phase			0.040 ± 0.004
Duration of MG phase	0.068 ± 0.008	0.020 ± 0.002	0.024 ± 0.003
Duration of FC phase	0.077± 0.012	0.045 ± 0.006	0.045 ± 0.005
Duration of hyoid retraction			0.048 ± 0.008
Peak hyoid delay to end of MG phase			0.038 ± 0.006

Table 3. Mean ± standard deviation for six kinematic variables (measured in seconds) from aquatic feeding events of *Cuora amboinensis*. MG = maximal gape; FC = fast close.

Variable	Capture (N=21)	Transport (N=21)
Duration of gape cycle	0.120 ± 0.015	0.073 ± 0.009
Time to peak gape	0.067 ± 0.014	0.028 ± 0.004
Duration of MG phase	0.012 ± 0.002	0.014 ± 0.002
Duration of FC phase	0.032 ± 0.004	0.031 ± 0.002
Duration of hyoid retraction	0.042 ± 0.006	0.048 ± 0.008
Peak hyoid delay to end of MG phase	0.024 ± 0.008	0.028 ± 0.012

Table 4. Comparison of gape cycle duration in prey capture of four aquatic feeding specialists: *Chelus fimbriatus, Chelodina longicollis, Dermochelys coreacea* and *Chelydra serpentina*; two aquatic generalists*: Pelusios castaneus* and *Malaclemys terrapin*; and the semi-aquatic generalist *Cuora amboinensis.* cr, crab; f, fish; m, pieces of meat; mf, mussel flesh.

((Footnote below the table:))
Data from Lauder and Prendergast (1992), Van Damme and Aerts (1997), Bels et al. (1998), Lemell and Weisgram (1997); Lemell et al. (2002).
* No SD given by the author (Lemell and Weisgram, 1997).

Species	Prey capture cycle duration (s) Mean ± SD N=Number of feeding events	Prey type
Cuora amboinensis	0.120 ± 0.015 (N=21)	f
Chelydra serpentina	0.078 ± 0.002 (N=15)	f
Chelus fimbriatus	0.083 ± 0.020 (N=20)	f
Chelodina longicollis	0.110 ± 0.015 (N=8)	m
Malaclemys terrapin	0.300 ± 0.154 (N=10)	cr
Dermochelys coriacea	0.615 ± 0.196 (N=10)	mf
Pelusios castaneus	0.280 ± * (N=4)	f

Figure legends

Fig. 1. Points used for kinematic analyses of feeding cycles of *Cuora amboinensis* and *Cuora flavomarginat*a. A, ventral margin of tympanum; C, anterior tip of carapace; F, centre of mass of the feeding items; H, hyoid; L, anterior tip of lower jaw; P, parietal bone; T, anterior tip of tongue; U, anterior tip of the upper jaw; e, imaginary line connecting U and L.

Fig. 2. Head morphology of *C. flavomarginata* and *C. amboinensi*s. (A) *Cuora flavomarginata,* cranium lateral view; (B) *Cuora flavomarginat*a, m. adductor mandibulae externus dorsal view; (C) *Cuora amboinensi*s, cranium lateral view; (D) *Cuora amboinensis*, M. adductor mandibulae externus dorsal view. Scale bar = 10 mm. aep, m. add. mand. externus pars profunda; aes, m. add. mand. externus pars superficialis; bo, os basioccipitale; cso, crista supraoccipitalis; exo, os exoccipitale; f, os frontale; j, os jugale ; mx, os maxillare; q, os quadratum; qj, os quadratojugale; p, os parietale; pdp, processus descendens ossi parietale (dorsal part); pf, os praefrontale; pl, os palatinum ; pmx, os praemaxillare ; po, os postorbitale; ppe, processus pterigoideus externus (vertical plate); s, os squamosum; v, os vomer.

Fig. 3. *Cuora sp*., schematic illustration of head morphology. (A) Osteology and mycology of the head; (B) direction of forces during contraction of the jaw and hyoid muscles; (C) hyoid and hypoglossum, lateral view; (D) hyoid and hypoglossum, dorsal view. ame, m. add. mand. externus; amem, m. add. mand. externus pars media; bmd, m. branchiomandibularis; cbI, cornu branchiale I; cbII, cornu branchiale II; ch, corpus hyoidei; chd, M. coracohyoideus; chy, cornu hyale; dm, m. depressor mandibulae; epI, epibranchiale I; gg, m. genioglossus; gh, m. geniohyoideus; hg, hypoglossum; hh, m. hyohyoideus; im, m. intermandibularis; j, os jugale; md, mandibula; mx, os maxillare; oss, ossifications on the hyoid corpus; p, os parietale; pf, os praefrontale; pl, os palatinum; po, os postorbitale; prl, processus lingualis hyoidei; pt, m. add. mand. internus pars pterygoideus; q, os qudratum; qj, os quadratojugale; rm, ramphotheca; s, os squamosum; ty, tympanum.

Fig. 4. *Cuora flavomarginata,* light micrograph; tongue and hyoid, median-longitudinal cut, 8 mm; Azan staining. ar, cartilago arytaenoidea; chy, corpus hyoidei; cr, cartilago cricoidea; gg, m. hypoglossoglossus; gh, m. geniohyoideus; gl, glandulae

linguales; hg, hypoglossum; hgl, m. hyoglossus; im, m. intermandibularis; lp, lingual papillae (dorsal); lpv, lingual papillae (ventral).

Fig. 5. (A) Kinematic profiles from a terrestrial food ingestion event in *Cuora amboinensi*s, based on cinematography (250 fr/s). Top to bottom: first line = kinematics of head extension; second line = gape; third line = dorsoventral hyoid movements. (B) Video frames from a terrestrial food ingestion event in *Cuora amboinensis* based on cinematography (250 fr/s); B1, final head approach; B2, beginning of MG phase; B3, end of MG phase; B4, end of fast closing phase.

Fig. 6. (A) Kinematic profiles from a terrestrial food ingestion event in *Cuora flavomarginata* based on cinematography (250 fr/s). Top to bottom: first line = kinematics of head extension; second line = gape; third line = dorsoventral hyoid movements. (B) (B) Video frames from a terrestrial food ingestion event in *Cuora flavomarginata* based on cinematography (250 fr/s); B1, final head approach; B2, beginning of MG phase; B3, end of MG phase; B4, end of fast closing phase.

Fig. 7. (A) Kinematic profiles from a terrestrial pure lingual food transport ("lingual transport") event in *Cuora amboinensis* based on cinematography (250 fr/s). Top to bottom: first line = kinematics of head extension; second line = gape; third line = anterior/posterior movement of tongue related to imaginary line connecting U and L; e, imaginary line connecting U and L. (B) Video frames from a terrestrial pure lingual food transport (lingual transport) event in *Cuora amboinensis* based on cinematography (250 fr/s); B1, prior to jaw opening; B2, jaw opening; B3, beginning of MG phase; B4, end of MG phase; B5, jaw closing; B6, end of transport cycle.

Fig. 8. (A) Kinematic profiles from a terrestrial food "prey positioning" event in *Cuora amboinensis* based on cinematography (250 fr/s). Top to bottom: first line = kinematics of head extension;second line = gape; third line = dorso/ventral hyoid movement; fourth line = anterior/posterior movement of tongue related to imaginary line connecting U and L; e, imaginary line connecting U and L. (B) Video frames from a terrestrial food "prey positioning" event in *Cuora amboinensis* based on cinematography (250 fr/s); B1, beginning of jaw opening; B2, end of slow opening

phase; B3, beginning of MG phase; B4, end of MG phase; B5, end of fast closing phase.

Fig. 9. (A) Kinematic profiles from an aquatic food ingestion event in *Cuora amboinensis* based on cinematography (250 fr/s). Top to bottom: first line = kinematics of head extension; second line = gape; third line = dorsoventral hyoid movements. (B) Video frames from an aquatic food ingestion event in *Cuora amboinensis* based on cinematography (250 fr/s); B1, final head approach (corresponds to the zero line on the x-axis); B2, beginning of MG phase; B3, end of MG phase; B4, end of fast closing phase.

Fig. 10. (A) Kinematic profiles from an aquatic food transport event in *Cuora amboinensis* based on cinematography (250 fr/s). Top to bottom; first line = kinematics of head extension; second line = gape; third line = dorsoventral hyoid movements. (B) Video frames from an aquatic food transport event in *Cuora amboinensis* based on cinematography (250 fr/s); B1, beginning of jaw opening; B2, beginning of MG phase; B3, end of MG phase; B4, end of fast closing phase.

Fig. 1

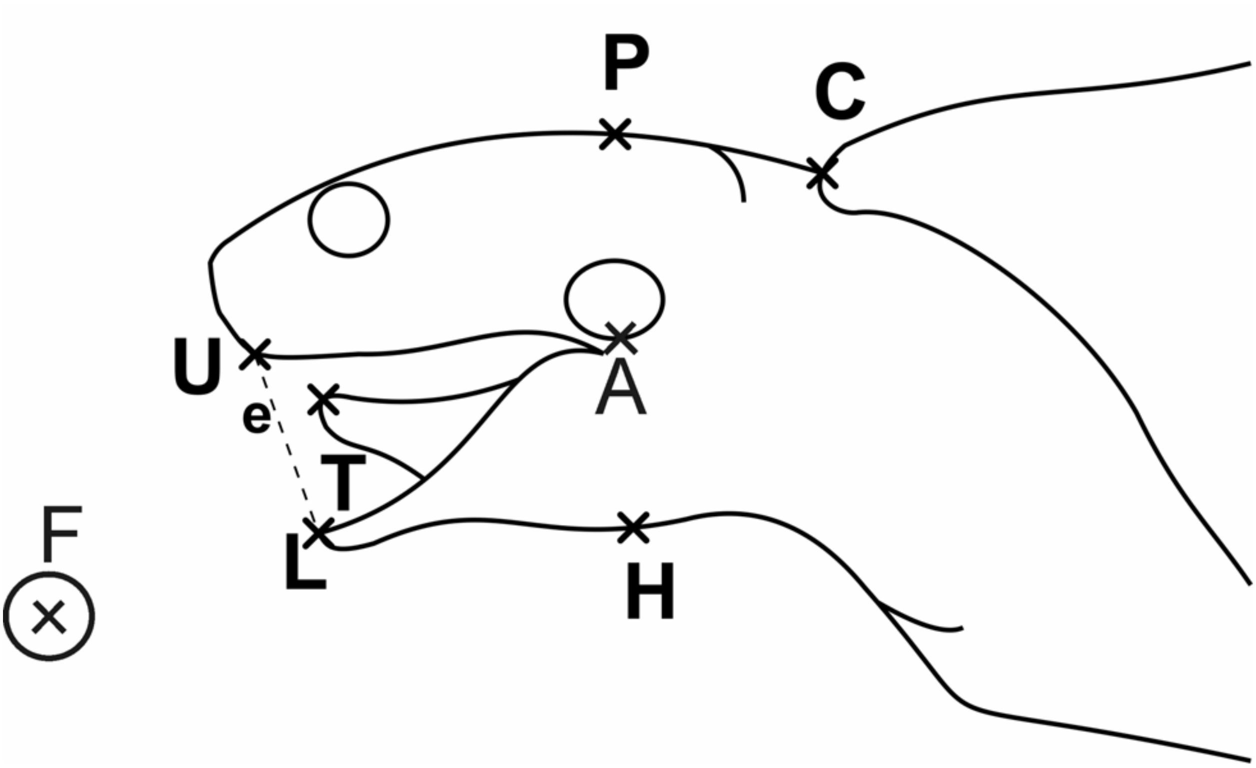

P
C
U
e
A
T
F
L
H

Fig. 2

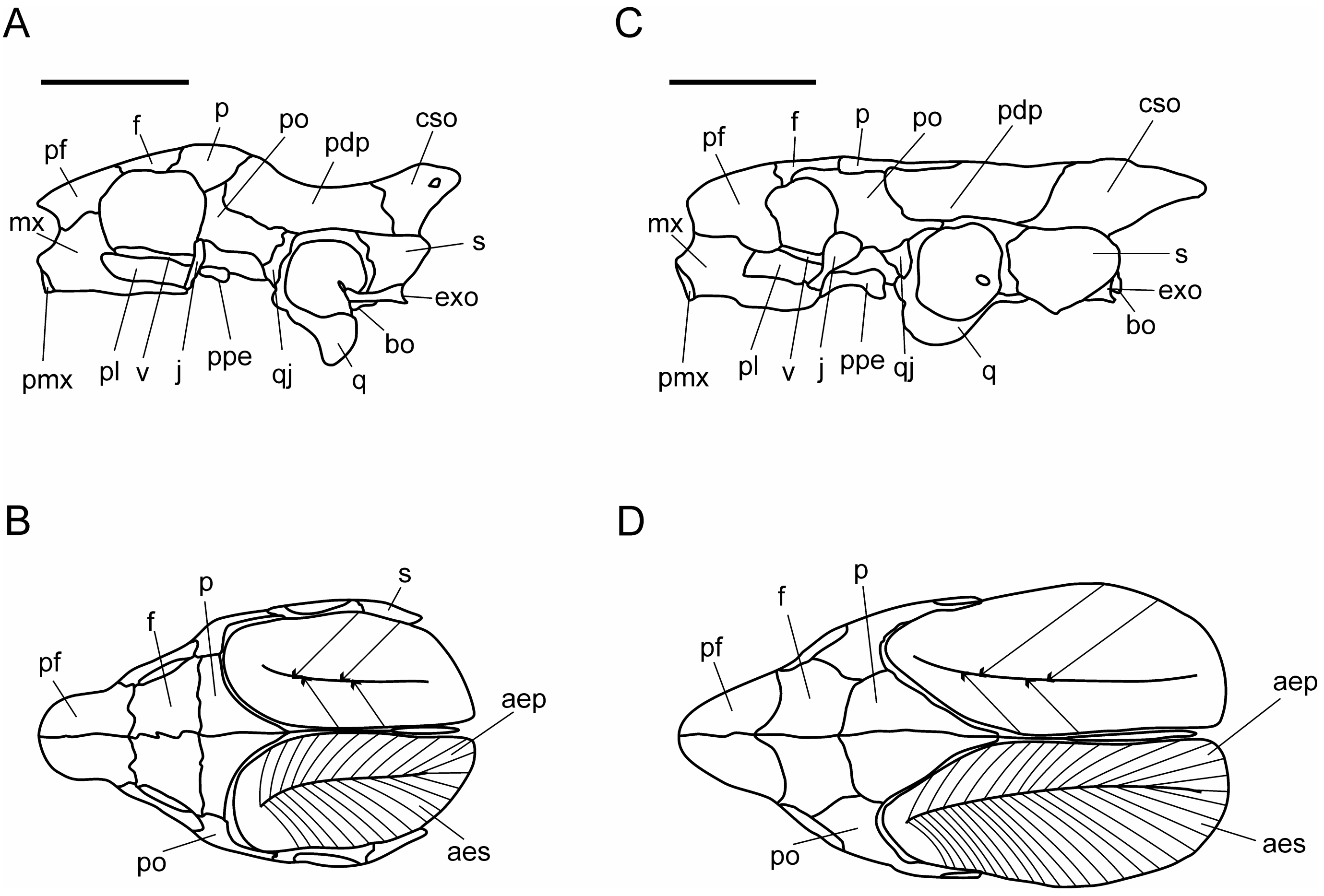

A
C
pf
f
p
po
pdp
cso
mx
s
exo
bo
pmx
pl
v
j
ppe
qj
q
pf
f
p
po
pdp
cso
mx
s
exo
bo
pmx
pl
v
j
ppe
qj
q
B
D
p
s
f
pf
aep
po
aes
f
p
pf
aep
po
aes

Fig. 3

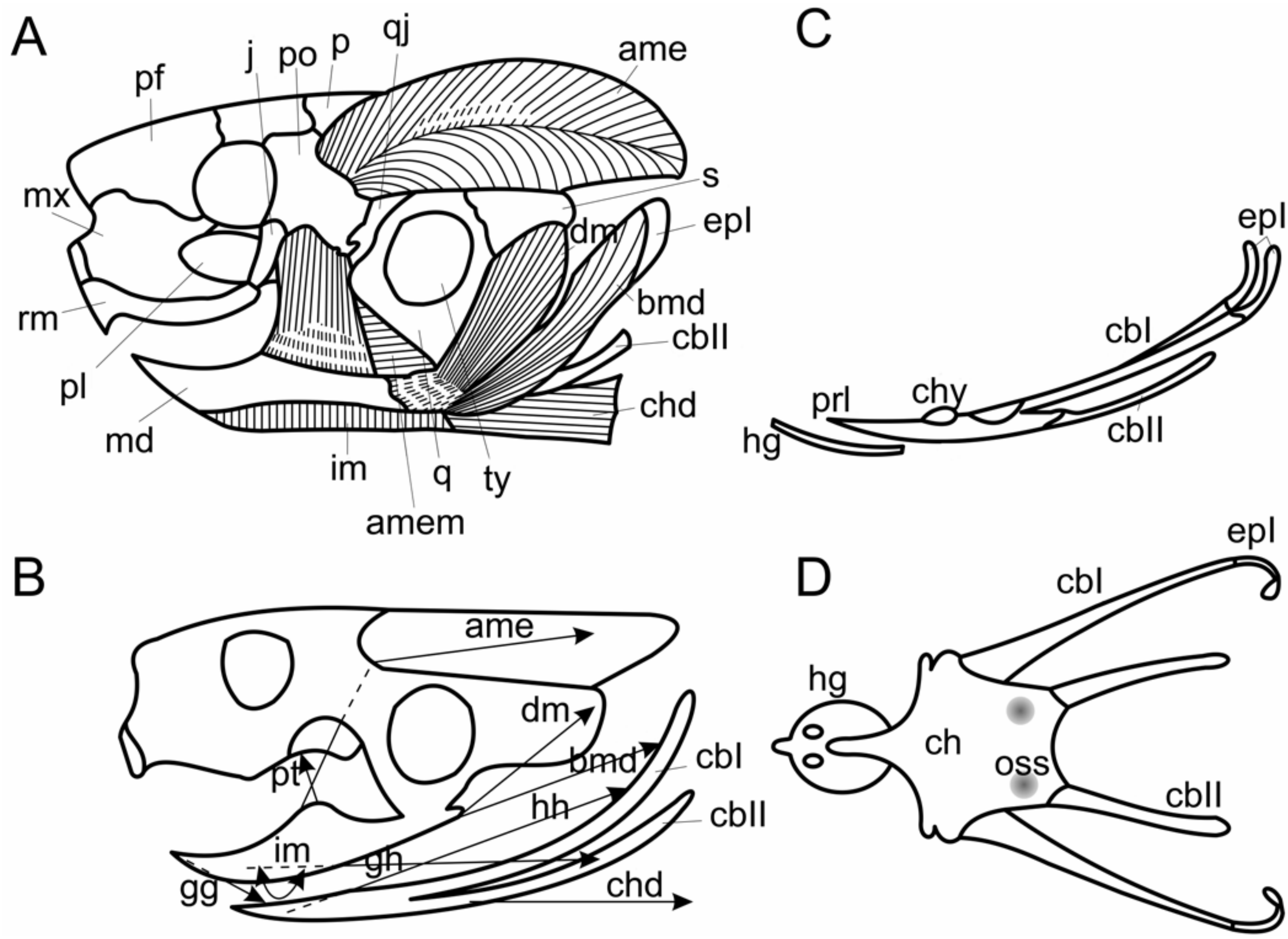

A
pf
j
po
p
qj
ame
s
mx
dm
epI
bmd
cbII
rm
pl
chd
md
im
q
ty
amem
C
epI
cbI
prl
chy
hg
cbII
B
ame
dm
pt
bmd
cbI
hh
cbII
im
gh
gg
chd
D
epI
cbI
hg
ch
oss
cbII

Fig. 4

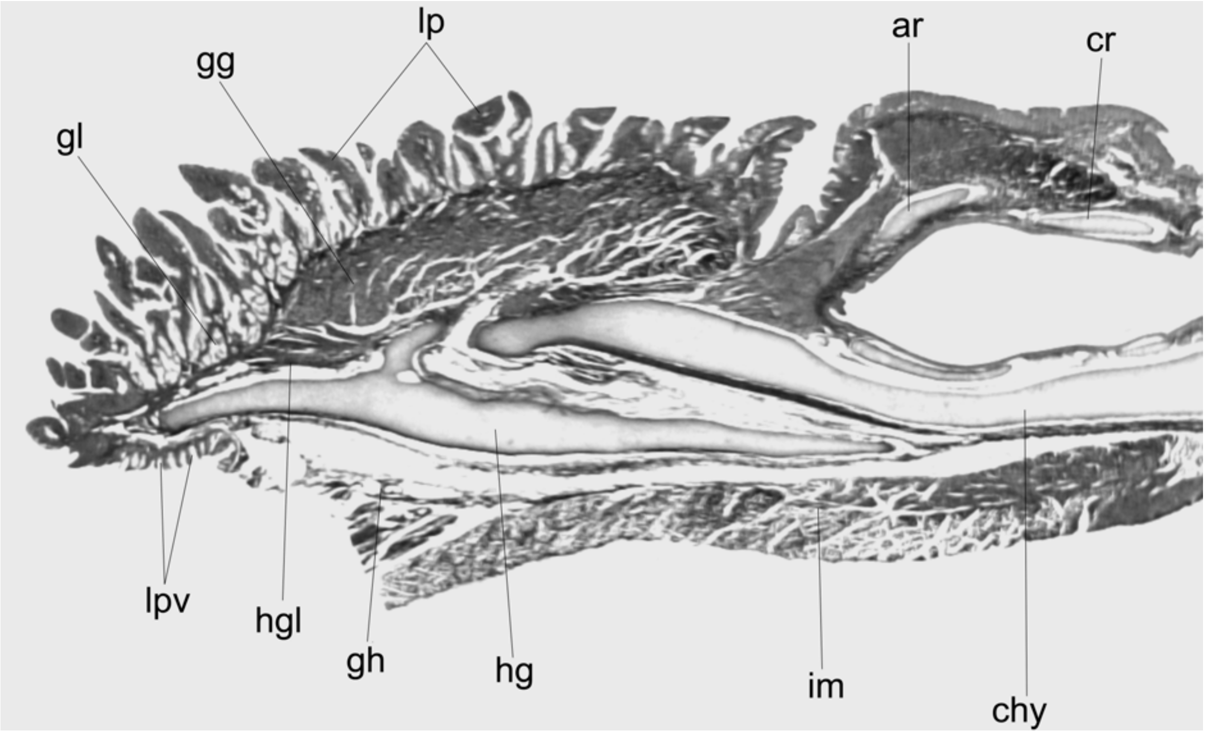

lp
gg
ar
cr
gl
lpv
hgl
gh
hg
im
chy

Fig. 5

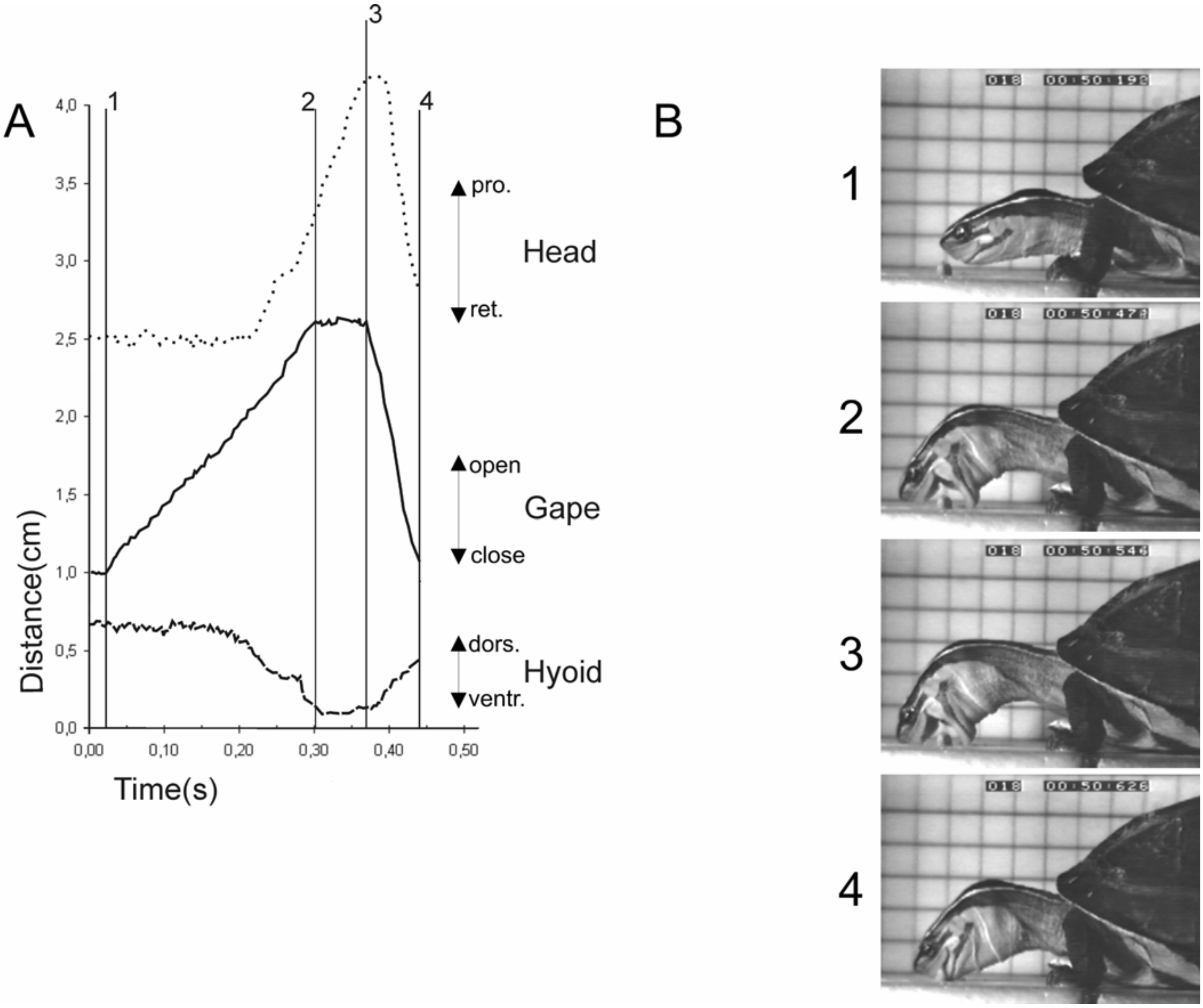

A
B
1
2
3
4
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Distance(cm)
0,00
0,10
0,20
0,30
0,40
0,50
Time(s)
pro.
Head
ret.
open
Gape
close
dors.
Hyoid
ventr.
018
00:50:193
00:50:478
00:50:546
00:50:626

Fig. 6

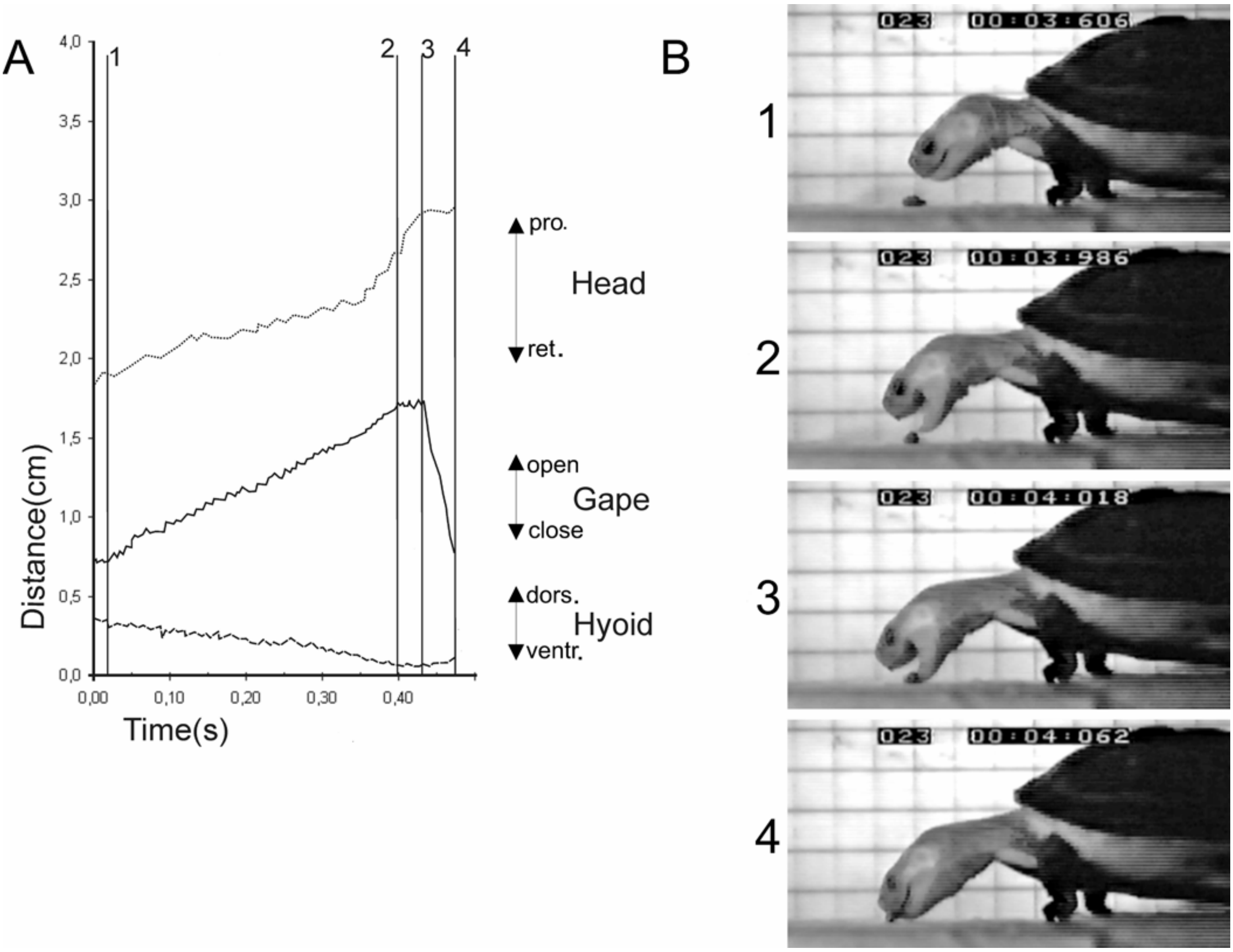

A
B
1
2
3
4
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
Distance(cm)
0,00
0,10
0,20
0,30
0,40
Time(s)
▲pro.
Head
▼ret.
▲open
Gape
▼close
▲dors.
Hyoid
▼ventr.
023 00:03:606
023 00:03:986
023 00:04:018
023 00:04:062

Fig. 7

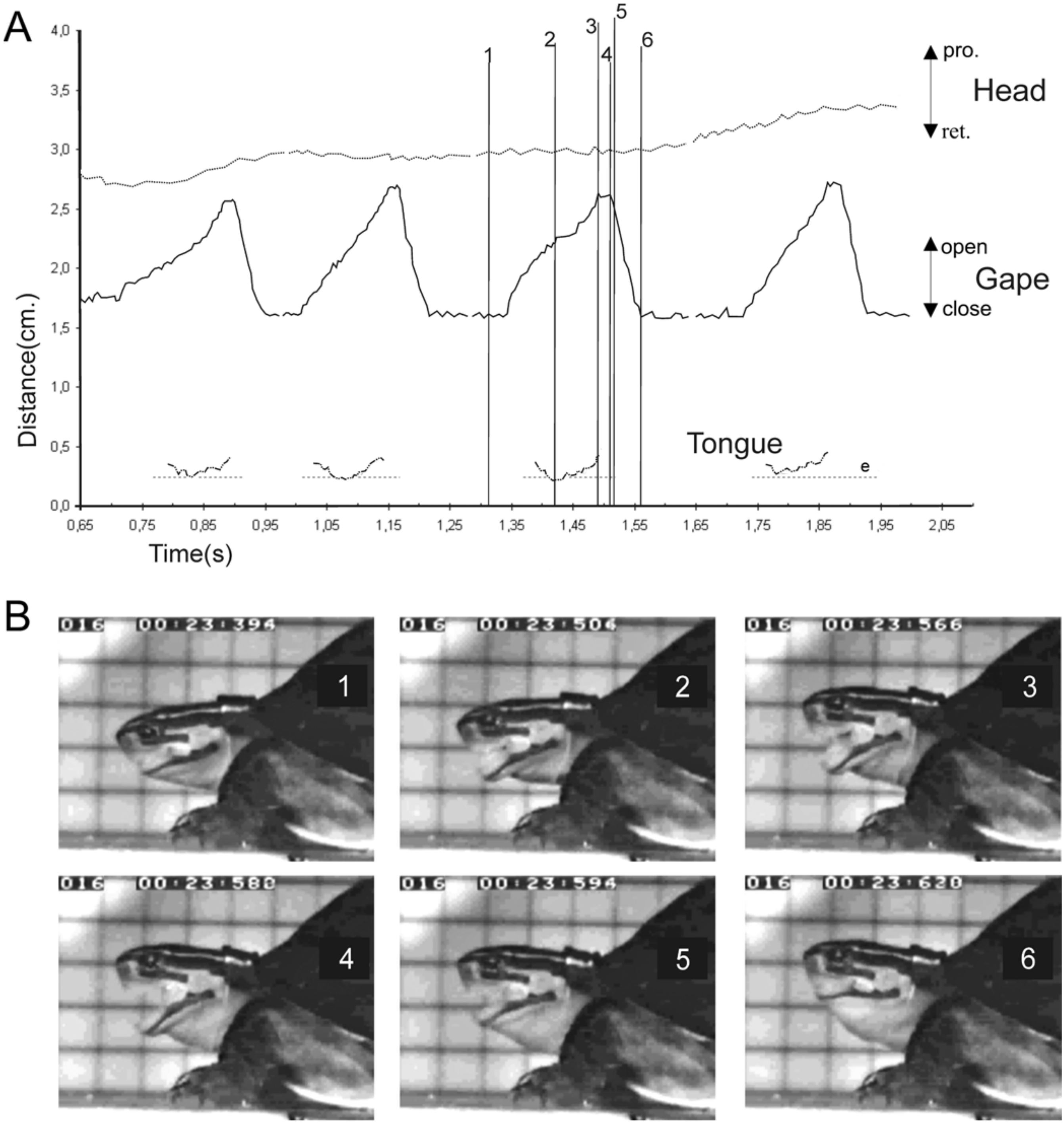

A
B
Distance(cm.)
Time(s)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
0,65
0,75
0,85
0,95
1,05
1,15
1,25
1,35
1,45
1,55
1,65
1,75
1,85
1,95
2,05
1
2
3
4
5
6
▲pro.
Head
▼ret.
▲open
Gape
▼close
Tongue
e
016 00:23:394
016 00:23:506
016 00:23:566
016 00:23:580
016 00:23:594
016 00:23:620

Fig. 8

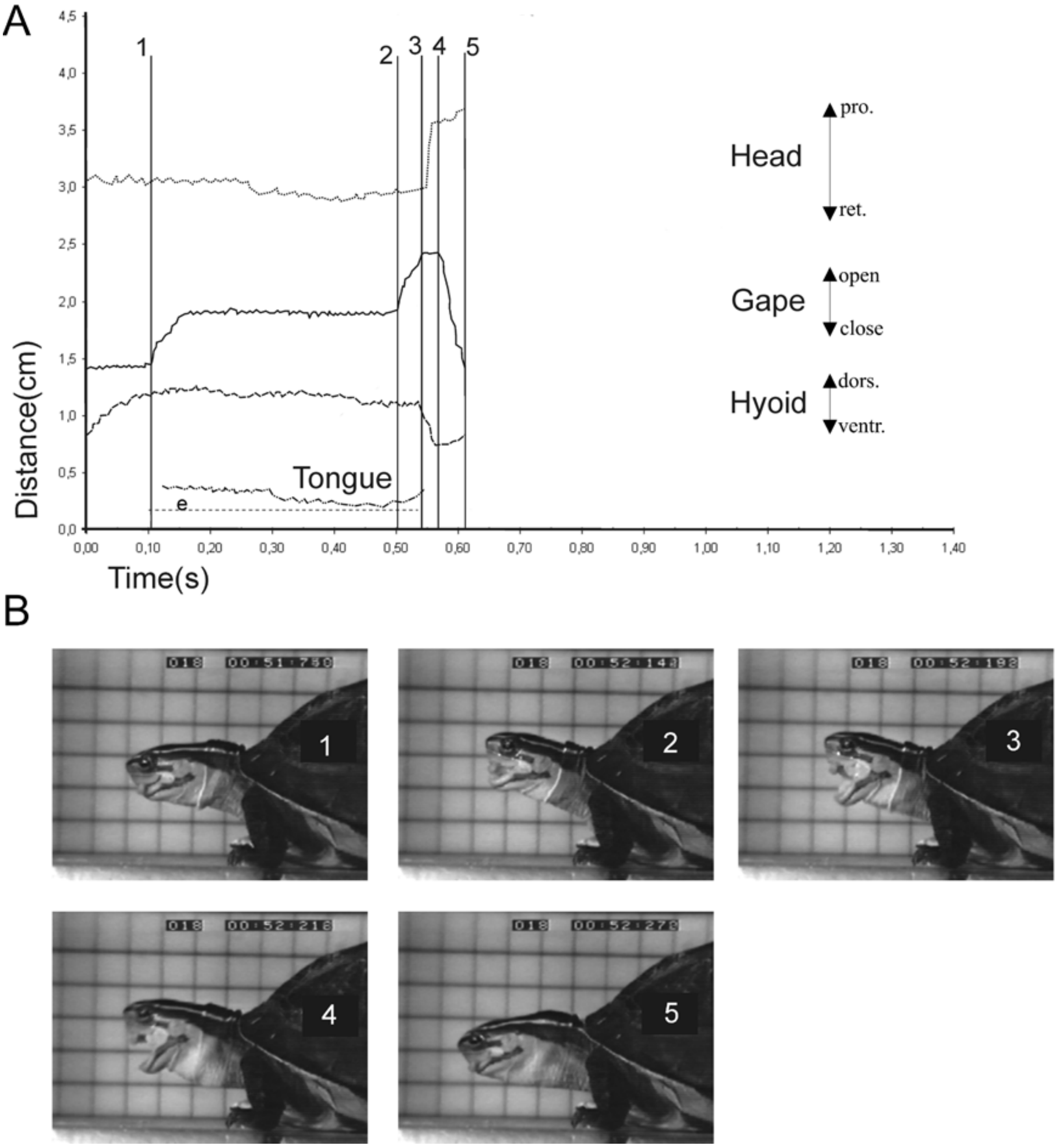

A
1
2
3
4
5
Head
pro.
ret.
Gape
open
close
Hyoid
dors.
ventr.
Tongue
e
Distance(cm)
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0
4,5
Time(s)
0,00
0,10
0,20
0,30
0,40
0,50
0,60
0,70
0,80
0,90
1,00
1,10
1,20
1,30
1,40
B
018 00:51:760
1
018 00:52:148
2
018 00:52:198
3
018 00:52:218
4
018 00:52:270
5

Fig. 9

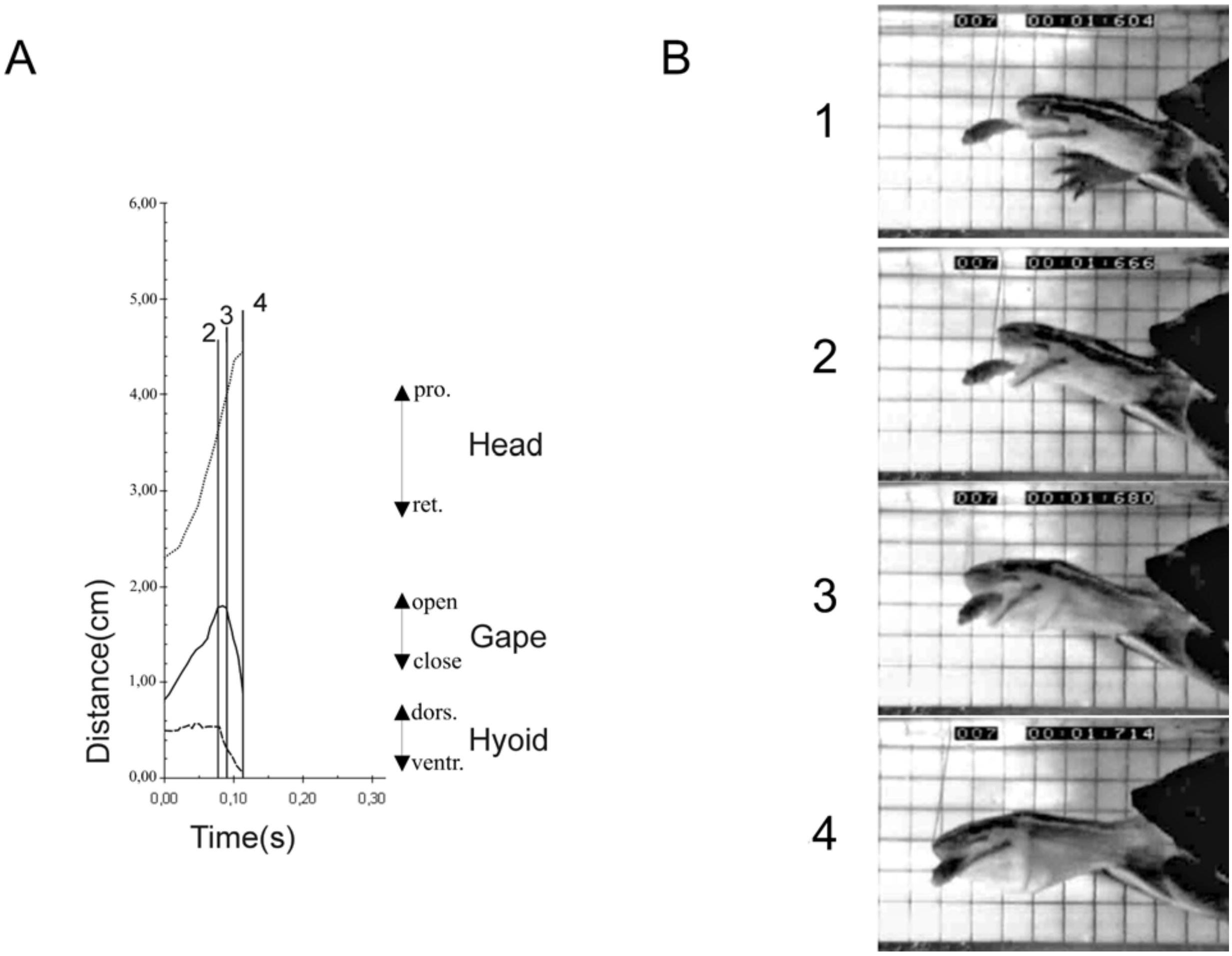
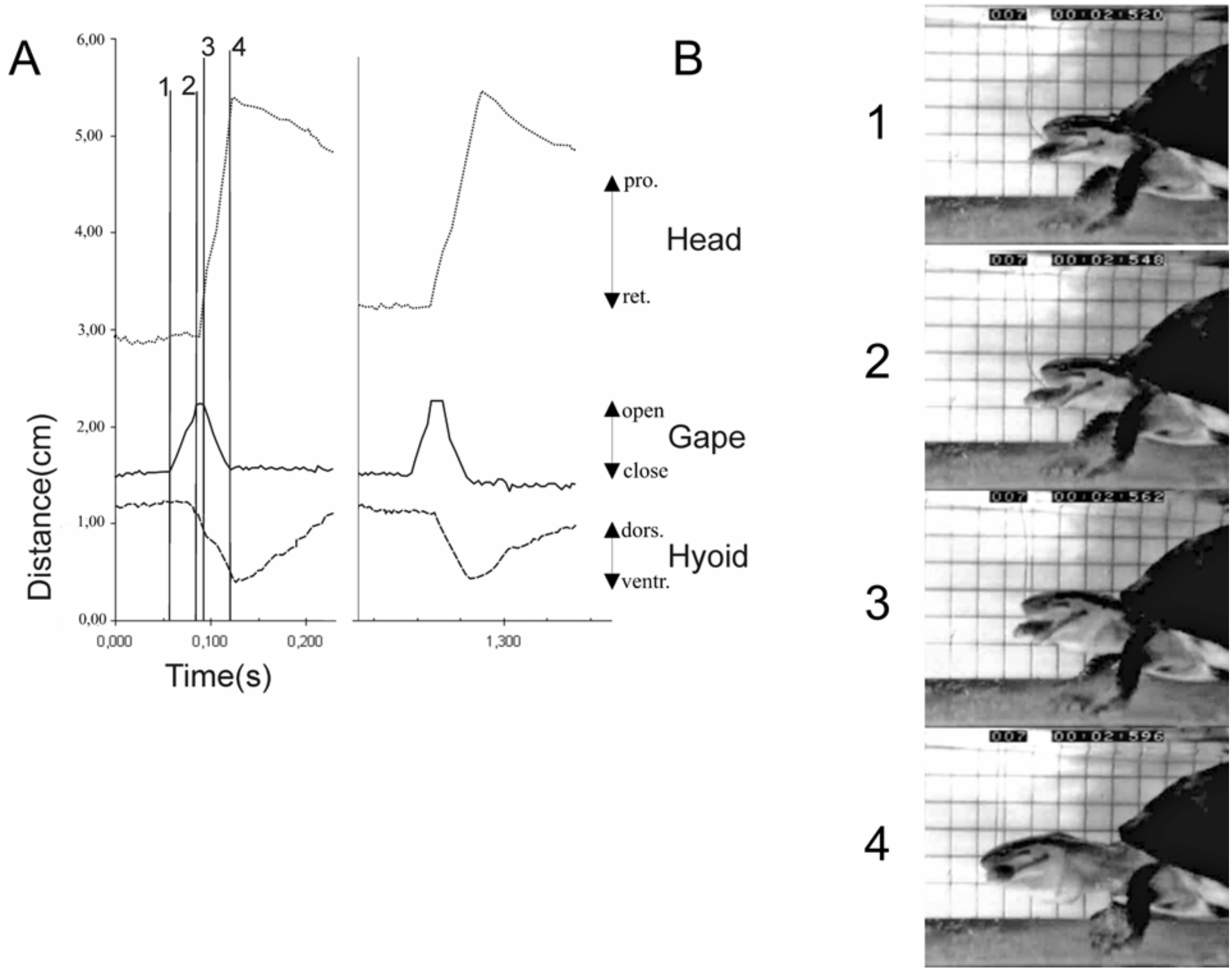

A
B
6,00
5,00
4,00
3,00
2,00
1,00
0,00
Distance(cm)
0,00
0,10
0,20
0,30
Time(s)
2
3
4
pro.
Head
ret.
open
Gape
close
dors.
Hyoid
ventr.
1
007 00:01:604
2
007 00:01:666
3
007 00:01:680
4
007 00:01:714

Fig. 10


A
B
6,00
5,00
4,00
3,00
2,00
1,00
0,00
Distance(cm)
0,000
0,100
0,200
1,300
Time(s)
1
2
3
4
pro.
Head
ret.
open
Gape
close
dors.
Hyoid
ventr.
007 00:02:520
007 00:02:548
007 00:02:562
007 00:02:596

II. Eingereichte Arbeiten:

II.2. Aquatic feeding in a terrestrial turtle: a functional-morphological study of the feeding apparatus in the Indochinese box turtle *Cuora galbinifrons* (Chelonia, Geoemydidae). Zoomorphology (submitted).

Nikolay Natchev, Patrick Lemell, Egon Heiss, Christian Beisser, Josef Weisgram

Aquatic feeding in a terrestrial turtle: a functional-morphological study of the feeding apparatus in the Indochinese box turtle *Cuora galbinifrons* (Chelonia, Geoemydidae).

Nikolay Natchev, Patrick Lemell, Egon Heiss, Christian Beisser and Josef Weisgram

Department of Theoretical Biology

University of Vienna

Althanstrasse 14

A-1090 Vienna, Austria

Corresponding author:
Josef Weisgram, Dept. Theor. Biology
Althanstrasse 14, A-1090 Wien, Austria
josef.weisgram@univie.ac.at

key words: Turtle, feeding, kinematics, morphology, *Cuora galbinifrons*;

Abstract

In the IUCN Red List of Threatened Species (2007), the Indochinese box turtle *Cuora galbinifrons* is classified as a “critically endangered” species. Very little is known about the biology of this geoemydid chelonian. *Cuora galbinifrons* is regarded as a purely terrestrial species, but our results demonstrate that it can feed both on land and in water. The inverse relationship between the relative development of the hyoid apparatus and the tongue found in the most investigated chelonians is not valid in the Indochinese box turtle. Our morphological analysis of the feeding apparatus reveals that the palate shape and the design of the tongue are consistent with terrestrial feeders, but the construction of the hyoid complex is more characteristic of aquatic feeders. Previous studies have demonstrated that tongue enlargement negatively impacts the capacity of the turtles to suction feed. The present study focuses on the aquatic intraoral prey transport kinematic patterns. Our analysis is based on high-speed films with 250 fr/s and high-speed cineradiography with 50 fr/s. The aquatic intraoral food transport mechanisms differ depending on prey size: small items are transported predominantly by “inertial suction”, whereas larger items are moved by the tongue – normally a clear terrestrial strategy. As the genus *Cuora* is primitively aquatic, the use of lingual food transport in the aquatic environment is presumably an aberrant modus typical only for the most terrestrial among the Asian box turtles.

Introduction:

According to Bramble (1973), feeding in turtles is media dependent. Thus, several chelonians can capture the food on land, but to continue the feeding process they have to go under water (Bramble and Wake, 1985; Weisgram, 1985; Lemell et al., 1997). Within the largest recent turtle superfamily – Testudinoidea – one can distinguish fully aquatic and completely terrestrial forms, as well as species with semi-terrestrial habitats. Some emydids and geoemydids can handle the different requirements connected to the high density and viscosity of water compared to air (Gans, 1969); they can complete the whole feeding process both on land and in water (Summers et al., 1998; Natchev et al., 2008 in press). The Indochinese box turtle, *Cuora galbinifrons*, is a terrestrial geoemydid (Richter et al., 2007) and is apparently the least aquatic species within the genus *Cuora* (see Stuart and Parham, 2004). Our experiments have demonstrated that this turtle is able to feed in both media. An important aim of the present study is to investigate the bauplan of the feeding apparatus in *C. galbinifrons* and to analyse how the opportunistic "feeding ecology" of the species is reflected by the head design. Within the superfamily Testudinoidea the skull form is closely correlated with ecological factors (Claude et al., 2004). As *C. galbinifrons* is a highly terrestrial turtle (see Pritchard, 1979; Ernst and Barbour, 1989), we can expect the head morphology to exhibit features similar to those found in tortoises (see Bramble, 1973; Wocheländer et al., 1999).

All turtles studied to date (Bramble, 1978; Weisgram, 1985; Bels and Renous, 1992; Lauder and Prendergast, 1992; Lemell and Weisgram, 1997; Van Damme and Aerts, 1997; Bels et al., 1998; Summers et al., 1998 Aerts et al., 2001; Lemell et al., 2000; Lemell et al., 2002, Bels et al., 2008; Natchev et al., 2008 in press) use hydrodynamic mechanisms for aquatic food uptake (see Van Damme and Aerts, 1997; Aerts et al., 2001). Under water the tongue is not used in food prehension.

Aquatic prey transport in turtles is poorly analysed (see Bels et al., 2008 for overview). According to Aerts et al. (2001) this transport involves a combination of "compensatory suction" and "inertial suction". Nonetheless, some purely aquatic turtles like *Malaclemys terrapin* and *Dermochelys coriacea* transport food by the tongue (Bels et al., 1998). The gape kinematical patterns of the aquatic food ingestion and the transport cycles in these two species adhered exactly to the kinematical patterns proposed in the "generalized cyclic model" of Bramble and Wake (1985). The movements of the hyoid complex were not analysed (Bels et al., 1998). Transport mechanisms in which suction plays the dominant role are termed according to Bels et al. (2008) "intraoral-aquatic hyoid transport" (here abbreviated IAHT) and the lingual-based transport is termed "intraoral-aquatic lingual transport" (here abbreviated IALT). The present study applies high-speed cinematography (250 fr/s) to investigate whether the Indochinese box turtle uses suction or tongue-based transport when feeding under water. The genus *Cuora* is ancestrally aquatic (see Claude et al., 2003), so IAHT can be predicted for these geoemydids. For *C. galbinifrons*, as the most terrestrial species within this genus, we predict – as typical for all highly terrestrial turtles (see Winokur, 1988) – a large and movable tongue. Tongue enlargement and greater lingual mobility negatively impacts the capacity to suction feed (see Lemell et al., 2000). To obtain information on the exact movement of food items within the oropharyngeal cavity, we used high-speed cineradiography with 50 fr/s. We hypothesise that the Indochinese box turtle is able to use IALT, but that the kinematical pattern will show differences to *M. terrapin* and *D. coriacea* because aquatic lingual transport has evolved de novo independently within *Cuora* sp.

Material and methods:

Cuora galbinifrons galbinifrons BOURRET, 1939 (the Indochinese box turtle) is known from small areas including provinces in southern and northern Vietnam as well as eastern-central Laos (Stuart and Parham, 2004). Very little is known about its biology (Pritchard, 1979). Ernst and Barbour (1989) reported that the species inhabits bushy woodlands and even forests at high elevations. It can withstand great temperature differences and an average relative humidity of 83% (Rogner, 1995). Rogner (1995) also reports that *C. galbinifrons* does enter the water briefly, but spends most of its time on land buried in moss. This box turtle is primarily carnivorous but also feeds on fruits and vegetables.

The animals used for our experiments were kept in a terrarium (120 x 70 x 70 cm) with a pool of water at 25 °C constant temperature and a 12/12 h light/dark cycle. They were fed fruits and vegetables (bananas, cucumbers, apples, tomatoes, etc.), small invertebrates (mealworms, snails and earthworms) and food pellets. Our turtles frequently entered the water pool and spent prolonged periods in water, especially when food items were offered.

Anatomy

The anatomical investigations were conducted on four *C. galbinifrons* specimens – two females (obtained commercially; carapace length 14.4 and 12.9 cm) and two males (carapace length 16.9 and 17.6 cm). The female animals were euthanized by abdominal injection of a lethal dose of sodium pentobarbital (Nembutal). The two adult male specimens were preparations belonging to the collection of the Museum of Natural History in Vienna, where they were stored in 70 % alcohol. The morphology of the skeletal elements and the jaw, hyoidal and cervical muscles were examined using dissection microscopes (WILD M5A and WILD 420). The terminology of Schumacher (1973) was used to describe the musculature and the tendon systems.

For scanning electron microscopy (SEM), one tongue was fixed overnight in modified Karnovsky solution (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer; Karnovsky, 1965). After rinsing in 0.5% cacodylate buffer, the sample was postfixed in buffered 1% osmium tetroxide at 37 °C for 2 h and treated with 25% HCl at 60 °C for 30 min in order to remove the mucus from the surface. This procedure was followed by dehydration in a graded ethanol series, HMDS (hexamethyldisilazane) drying and gold coating in the Sputtercoater AGAR B7340. The specimen was observed in a Philips XL-20 scanning electron microscope.

Film recordings

For filming aquatic feeding events, the aquarium was filled with 15 cm water: the food items were offered at the bottom in front of the animals. The turtles were fed *Zophobas* larvae (body length: 38-49 mm). Three subadult *C. galbinifrons* individuals (carapace length 14.9, 14.1 and 12.9 cm) were filmed in lateral view (10 films per specimen) using a NAC-1000 high-speed video system at 250 fr/s. The first transport cycles in 24 videos were selected for further analyses.

The horizontal and vertical coordinates of each landmark (Fig. 1) were digitized frame by frame using an AVI-digitiser (© P. Snelderwaard). Based on the landmarks' displacement in the bi-directional level, we calculated: a) the gape amplitude –

distance between the tips of the upper and the lower jaw; b) ventral hyoid movement – dorso-ventral height of the head at the level of the origin of the Ceratobranchiale II (CB II); c) the anterior-posterior movements of the tongue – the distance between the tip of the tongue and the imaginary line connecting the upper and lower jaw's tips; d) the extension and retraction of the neck – the distance between the point "P" on the parietal cranial surface and the anterior tip of the carapace. These data enabled us to measure and represent graphically the duration of the different feeding stages, the time required to reach peak gape, the duration of hyoid retraction, the duration of jaw closing, the time required to pro- and retract the head, and the duration of the total cycles.

For radiographic experiments we used a U-matic video recorder Sony VO-5800 PS at 50 fr/s. A wire-grid (square size 7 x 7 mm) was used as a background. The turtle were fed mealworms (body length: 15-19 mm) and *Zophobas* larvae (body length: 38-49 mm.). The food items were marked with contrast medium (Gastrografin) to demonstrate the movement of the food inside the oropharyngeal cavity and the oesophagus. One day prior to filming, lead markers were glued to the skull on the points used as relevant markers (see Fig. 1). A total of 12 aquatic feeding events were analysed. To calculate kinematic variables, only data derived from NAC video sequences were used.

Results:

Anatomy

The skull in *C. galbinifrons* is relatively high and narrow, with well-defined ventral and dorsal emarginations of the temporal roof. Nonetheless, a slender temporal arch (see Fig. 2a and 2c), formed by the postorbital, quadratojugal and quadrate, is present (contrary to Wermuth & Martens, 1961). There is no direct connection between jugal and quadratojugal bones. Characteristic for *C. galbinifrons* are the concave prefrontals. The palatines are thin and transparent bony plates. The whole dorsal palate region is strongly vaulted. The vertical plates of the processus pterigoideus externus are small and rounded. The supraoccipital is slightly enlarged caudally, forming a short and fragile median supraoccipital ridge (Crista supraoccipitalis).

The hyoid apparatus consists of the hyoid body, a pair of rudimental cornu hyale, two pairs of hyoid horns and a hypoglossum. The hyoid body is mainly cartilaginous, caudally elongated and ventrally arched (see Fig. 2b). The lingual process is long, narrow and elastic. Ventro-rostrally to the lingual process is a cartilaginous hypoglossum. The branchial horns I (CB I) are caudo-dorsally elongated and ossified. Epibranchialia I remain cartilaginous. The branchial horns II are flattened, shorter, but wider than CB I, and their caudal ends are distinctly divided laterally. The posterior hyoid horns attach to the ventro-lateral wall of the anterior oesophagus: they function as dilatators during hyoid depression and retraction. The tongue of *C. galbinifrons* is a large, triangular, beefy and highly movable organ. The dorsal surface shows a high number of densely packed lingual papillae. These lingual papillae are large, columnar and homogeneously distributed, but fuse together in the posterolateral region, forming relatively smooth, fleshy “horns” (hindtongue) ambilateral to the glottis (Fig. 3).

The main jaw and hyobranchial muscles (see Schwenk and Rubega, 2005) are shown in Fig. 2a. The M. adductor mandibulae externus fills the whole upper temporal fossa. The M. add. mand. ext. pars supeficialis and pars profunda are less voluminous in *C. galbinifrons* compared to other box turtles. The external tendon system has a myovector-changing function in turtles (see Schumacher, 1956, 1973; Bramble, 1974; Lordanskii, 1990). In *C. galbinifrons* no transiliens cartilage was present. The trochlear process is a tiny, rough-surfaced area on the quadrate (see Fig. 2c).

Aquatic food transport

Our sequences show that *C. galbinifrons* needs up to 14 transport cycles when feeding on large prey (*Zophobas* larvae). The neck is in retracted position when the initial hyolingual protraction starts. The gape opens slowly as the tongue slides under the prey, fixing it against the upper jaw. According to the terminology of Bramble and Wake (1985), we termed this phase SO I. At a certain point the gape opening stops. That marks the beginning of the SO II-phase. The durations of SO I and especially of SO II vary strongly influencing the duration of the whole feeding cycle (see Tab. 1). During the fast opening of the gape (FO-phase), the prey loses contact with the palate region, sticking on the dorsal lingual surface (Fig. 4c). Maximum gape lasts 0.043 ± 0.008 s (termed here “Maximum Gape”- MG phase). During the MG-phase, the tongue is rapidly retracted, dragging the prey further into the mouth. The movement of the prey corresponds exactly to the movement of the hyolingual complex (Fig. 4c, d). With the jaw closing (FC phase), the prey is fixed in its new position inside the oral cavity.

Smaller prey (mealworms) require fewer transport cycles (mean = 2.3 ± 0.9). The head and neck remain static. As the pharynx expands, the prey is carried deep within it (Fig. 5c, d). The kinematic patterns of the hyoid do not correlate with food movements inside the oropharyngeal cavity. Shortly prior to jaw closing, the prey is at the level of the second branchial horns (Fig. 5d). During the second cycle, the prey is attached by the hindtongue to the pharyngeal roof prior to peak gape (Fig. 5f). During hyoid retraction the prey loses contact with the tongue surface and floats with the swallowed water into the anterior oesophagus (Fig. 5g, h). During hyoid elevation the food item once again moves rostrally with the outstreaming water, but remains behind the tongue (Fig. 5i). The prey is swallowed by peristaltic contractions of the intermandibular and constrictor musculature, supported by neck extension and head elevation (Fig. 5j to m).

Discussion:

The structural differences in the feeding apparatus of terrestrial and aquatic tetrapods can be explained by the specific physical properties of the two fluids – water and air (Bramble and Wake, 1985). The aquatic testudinoids have a flattened skull with elongated squamosal and posterior cranial parts – compared to the maxillar region (see Claude et al., 2004). The dorsal vault of the palates is lacking, which helps prevent swirls in the water flow during suction (Bramble, 1973). In herbivorous terrestrial testudinids, e.g. *Testudo* sp., *Gopherus* sp., the skull is higher and the cranial flexure is more prominent (see Bramble, 1974; Wocheslӓnder et al., 1999; Claude et al., 2004). In purely aquatic turtles, the massively developed hypobranchial musculature correlates with a large, well-ossified hyoid apparatus (see Van Damme and Aerts, 1997; Lemell et al., 2002). The hypoglossum is large and can even ossify. In terrestrial testudinoids, the hyoid corpus and hypoglossal plates are small, mainly cartilaginous and more flexible than in aquatic forms. The transiliens cartilage is large and can even ossify to an Os transiliens (Bramble, 1974). The tongue morphology in turtles can vary significantly (see Beisser et al., 1995, 1998, 2001, 2004; Iwasaki, 1992; Weisgram et al., 1989; Winokur, 1973, 1988). Purely aquatic turtles such as *Acanthochelys pallidipectorys* and *Chelus fimbriatus* have small tongues with a simple surface topography (Beisser et al., 1995; Lemell et al., 2002). Terrestrial testudinids possess a highly mobile, large and beefy tongue equipped with abundant high and slender lingual papillae (Winokur, 1988).

The head morphology in *C. galbinifrons* does not fit completely into the schema described above. The vaulted palate is necessary to provide space for the large and movable tongue. The dorsal tongue surface with numerous columnar lingual papillae in *C. galbinifrons* is structured quite similarly to testudinids. This resemblance apparently reflects convergent adaptation to a predominantly terrestrial lifestyle. The inverse relationship between the relative development of the hyoid apparatus and the tongue found in the most investigated chelonians (see Lemell et al., 2000) is not valid in the Indochinese box turtle. In older specimens, the hyoid complex can be well ossified (see Richter et al., 2007). The elongated form of the hyoid body and the elasticity of the cartilaginous lingual processes allow tongue protrusion even outside the mouth cavity in terrestrial feeding. The large hyoid body and the broad hypoglossum promote the pharyngeal expansion during underwater feeding. A distensible anterior oesophagus is predicted in all vertebrates confronted with bidirectional waterflow (Lauder and Shaffer, 1986). In *C. galbinifrons*, the remarkably spread CB II horns are attached laterally to the highly expandable anterior oesophagus wall; this ensures large dilation during hyoid retraction (Fig. 4e).

In most sequences of transport of small mealworms, the neurocranium remains fully static during hyoid retraction. The prey is drawn far into the pharynx by the water flow. We propose that this hydraulic mechanism is similar to the “inertial suction” mechanism (sensu Van Damme and Aerts, 1997) found in ingestion in *Chelodina longicollis*. This transport mode is highly efficient: within a few cycles the prey can be positioned behind the tongue. The large tongue acts as a barrier holding the prey inside the pharyngeal area of the oral cavity when the swallowed water is expelled.

In *C. galbinifrons*, the kinematic patterns of pharyngeal packing differ from those of transport and swallowing. During pharyngeal packing, hyoid protraction is modest but its elevation is distinct (see Fig. 5f). We hypothesise that the dorsal shift of the hyoid is effected mainly by activating the intermandibular muscles. One possible mechanism of muscle activity is proposed here: first, the most anterior part of the M. intermandibularis contracts. The gape amplitude increases constantly as the caudal parts of the M. intermandibularis are activated. The tongue remains elevated and the combined activity of the intrinsic lingual muscles and the intermandibular muscles forces the food item caudally into the pharynx and anterior oesophagus. The tongue in *C. galbinifrons* has a well-developed hindtongue (Fig. 3), which makes pharyngeal packing highly effective (see Schwenk, 2000). This explains the lack of a discrete “pharyngeal compression phase” (see Smith, 1984; Herrel et al., 1996). A characteristic feature is the food storage in the posterior third of the oesophagus – here termed “esophageal packing” (Fig. 6). The reason for this behaviour remains unclear. Histological investigation of the oesophageal epithelium in *C. galbinifrons* reveals no pepsin-secreting regions (Scheidl, 2002). Nonetheless, pre-digestion cannot be completely excluded because gastric digestive enzymes may be present in the most posterior oesophageal segments.

The hydrodynamic effects typically used by aquatic tetrapods for food transport apparently play a subordinate role when *C. galbinifrons* feeds on larger prey. The food item does not lose contact with the tongue surface, and the posterior movement of the prey correlates exactly with the magnitude of the hyoid retraction. On land the connection between the tongue and prey is due to wet adhesion and the interlocking effects (McDowell, 1972; Schwenk, 2000). Under water, only surface interlocking seems to be relevant in large prey transport in *C. galbinifrons*. Using the terminology of the general feeding cyclic model (Bramble & Wake, 1985), we can recognise a separation of the jaw opening cycles into SOI, SOII and FO in IALT cycles in *C. galbinifrons*. SOI and SOII prior to FO gape phases are found in terrestrial food transport in *Testudo hermanii* (Wocheländer et al., 2000) and *Terrapene carolina*

(Bels et al., 1997), but also in the IALT cycles in *M. terrapin* and *D. coriacea* (Bels et al., 1998). Bels et al. (2008) propose that the intraoral transport cycles are similar for all terrestrial turtles and for turtles using IALT. In that hypothetical generalized cycle, tongue protraction starts early in the SO stage and retraction occurs at the beginning of the FO phase. Our kinematical analyses do not support that theory for *C. galbinifrons*. When feeding on *Zophobas* larvae, hyoid retraction started during a static gape phase (here termed MG-phase) and correlated with the initiation of neck extension. We propose that neck extension improves control over the direction of the prey being moved toward the pharynx.

The ancestor of the genus *Cuora* was a purely aquatic form (for an overview see Claude et al., 2003; Joyce and Gauthier, 2004). In *C. aboinensis* the tongue is used only for prey fixation underwater (Natchev et al., 2008 in press). We speculate that aquatic tongue-based food transport is typical only for the most land-related species within the Asian box turtles – *C. galbinifrons* and possibly *C. flavomarginata*.

Acknowledgements

We are very grateful for the uncomplicated material support by Richard Gemel, NHM Wien. We would also like to thank Rebecca German and Ken Kardong for discussing and commenting on our work. Michael Stachowitsch improved our English. This study was supported by the FWF 20094-B17 Austrian research grant.

References:

Aerts P, Van Damme J, Herrel A (2001) Intrisic Mechanics and Control of Fast Cranio – Cervical Movements in Aquatic Feeding Turtles. Amer Zool 41: 1299-1310

Beisser CJ, Weisgram J, Splechtna H (1995) The dorsal lingual epithelium of *Platemys pallidipectoris* (Pleurodira, Chelidae). J Morphol 226: 267-276

Beisser CJ, Weisgram J, Hilgers H, Splechtna H (1998) Fine structure of the dorsal lingual epithelium of *Trachemys scripta elegans* (Chelonia: Emydidae). Anat Rec Part A 250: 127-135

Beisser CJ, Lemell P, Weisgram J (2001) Light and electron microscopy of the tongue of *Pelusios castaneus* (Chelonia; Pleurodira). Tissue & Cell 33 1: 63-71

Beisser CJ, Lemell P, Weisgram J (2004) The dorsal lingual epithelium of *Rhinoclemmys pulcherrima incisia* (Chelonia, Cryptodira). Anat. Rec. Part A 277: 227-235

Bels VL, Renous S (1992) Kinematics of feeding in two marine turtles (*Chelonia mydas* and *Dermochelys coriacea*). Proc of the 6th Ordinary Meeting of the Soc Eur Herp Hung Nat Hist Mus: 73-78

Bels VL, Davenport J, Delheusy V (1997) Kinematic analysis of the feeding behaviour in the box turtle *Terrapene carolina* (L.), (Reptilia: Emydidae). J Exp Zool 277: 198-212

Bels VL, Davenport J, Renous S (1998) Food Ingestion in the Estuarine Turtle *Malaclemys terrapin*: Comparison with the Marine Leatherback Turtle *Dermochelys coriacea*. Mar Biol Ass UK 78: 1-20

Bels VL, Baussart S, Davenport J, Shorten M, O`Riordan RM, Renous S, Davenport J (2008) Functional Evolution of feeding behaviour in turtles. In: Wyneken J, Godfrey MH, Bels V (eds) Biology of turtles 8. CRC Press Taylor & Francis Group, pp 189-212

Bramble DM (1973) Media dependent feeding in turtles. Amer Zool 13: 1342

Bramble DM (1974) Occurrence and Significance of the Os transiliens in Gopher Tortoises. Copeia 74: 102-108

Bramble DM (1978) Functional analysis of underwater feeding in the snapping turtle. Am Zool 18: 623

Bramble DM, Wake DB (1985) Feeding Mechanisms of Lower Tetrapods. In: Hildebrand M, Bramble DM, Liem KF, Wake DB (eds) Functional Vertebrate Morphology. 13**.** Harvard University Press Cambridge, Massachusetts and London, England, pp 230-261

Claude J, Paradis E, Tong H, Auffray JC (2003) A geometric morphometric assessment of the effects of the environment and cladogenesis on the evolution of the turtle shell. Biol J Linn Soc 79: 585-501

Claude J, Pritchard PCH, Tong H, Paradis E, Auffray JC (2004) Ecological correlates and evolutionary divergence in the skull of turtles: a geometric morphometric assessment. Syst Biol 53: 933-948

Ernst CH, Barbour RW (1989) Turtles of the World. USA Smithsonian Inst, Washington DC, London

Gans C (1996) Comments on inertial Feeding. Copeia 4: 855-857

Herrel A, Cleuren J, De Vree F (1996) Kinematics of feeding in the lizard *Agama stelio*. J Exp Biol 199: 1727-1742

IUCN 2007. 2007 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 05 June 2008.

Iordanskii N (1990) Evolucija komplexnaich adaptacii: Chelustnoi aparat amfibii I reptilii. Izdatelstvo Nauka

Iwasaki S (1992) Fine structure of the dorsal epithelium of the tongue of the freshwater turtle, *Geoclemys reevesii* (Chelonia, Emydinae). J Morphol 211: 125-135

Joyce W, Gauthier J (2004) Paleoecology of Triassic stem turtles sheds new light on turtle origins. Proc Roy Soc Lond B 271:1-5

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27: 137

Lauder GV, Schaffer HB (1986) Functional design of the feeding mechanism in lower vertebrates: unidirectional and bidirectional flow systems in the tiger salamander. Zool J Linn Soc 88: 277-290

Lauder GV, Prendergast T (1992) Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. J Exp Biol 164: 55-78

Lemell P, Weisgram J (1997) Feeding Patterns of *Pelusios castaneus* (Chelonia: Pleurodira). Neth J Zool 47: 429-441

Lemell P, Beisser CJ, Weisgram J (2000) Morphology and Function of the Feeding Apparatus of *Pelusios castaneus* (Chelonia; Pleurodira). J Morphol 244:127-135

Lemell P, Lemell C, Snelderward P, Gumpenberger M, Wocheslander R, Weisgram J (2002). Feeding Patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). J Exp Biol 205: 1495-1506

McDowell SB (1972). The evolution of the tongue in snakes and its bearing on snake origins. Evol Biol 6: 191-273

Natchev N, Heiss E, Lemell P, Stratev D, Weisgram J (2008). Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. Zoology in press

Pritchard PCH (1979). Encyclopedia of Turtles. TFH Publications, Inc, Neptune NJ

Richter S, Auer M, Fritz U (2007).Variation of hyoid morphology in geoemydid terrapins. Amphibia-Reptilia 28: 148-153

Rogner M (1995). Schildkröten I. Heidi – Rogner – Verlag

Scheidel E (2002) Histologie and histochemie des Oesophagusepithels der Schildkroete *Cuora galbinifrons* Bourret, 1939 (Emydidae, Chelonia). Unpublished diploma thesis. Univ. Vienna

Schumacher G (1956) Morphologische Studie zum Gleitmechanismus des M. adductor mandibulae externus bei Schildkröten. Anat Anzeiger 103: 1-12

Schumacher G (1973) The Head Muscles and Hyolaryngeal Skeleton of Turtles and Croccodilians. In: Gans C, Parsons TS (eds) Biology of the Reptilia 4. Academic Press, New York, pp 101-199

Schwenk K (2000) Feeding: Form, Function and Evolution in tetrapod Vertebrates. Academic Press, San Diego-San Francisco-New York-Boston-London-Sydney-Tokyo

Schwenk K, Rubega M (2005) Diversity of Vertebrate feeding Systems. In: Stark M, Wang T (eds) Physiological and Ecological Adaptations to Feeding in Vertebrates. Science Publishers , Enfield, New Hampshire, pp 1-41

Smith KK (1984) The use of the tongue and hyoid apparatus during feeding in lizards (*Ctenosauria similes* and *Tupinambus nigropunctatus*). J Zool Lond 202:115-143

Stuart BL, Parham JF (2004) Molecular phylogeny of critically endangered indochinese box turtle (*Cuora galbinifrons*). Mol Phyl Evol 31: 164-177

Summers AP, Darouian KF, Richmond AM, Brainerd EL (1998) Kinematics of Aquatic and Terrestrial Prey Capture in *Terrapene carolina*, With Implications for the Evolution of Feeding in Cryptodire Turtles. J Exp Zool 281: 280-287

Van Damme J, Aerts P (1997) Kinematics and functional morphology of aquatic feeding in Australian snake-necked turtles (Pleurodira; Chelodina). J Morphol 233: 113-125

Weisgram J (1985) Feeding Mechanics of *Claudius angustatus* COPE 1865. Fortschritte der Zoologie 30: 257-260

Weisgram J, Ditrich H, Splechtna H (1989) Comparative functional study of the oral cavity in two turtle species. Plzen lek Sborn Supp l59: 117-122

Wermuth H, Martens R (1961) Schildkröten, Krokodile, Brückenechsen. Jena: VEB Gustav Fischer – Verlag

Winokur BM (1973) Adaptive modifications of the buccal mucosae in turtles. Amer Zool 13: 1347-1348

Winokur BM (1988) The buccopharyngeal mucosa of the turtles (Testudines). J Morphol 196: 33-52

Wocheslӓnder R, Hilgers H, Weisgram J (1999) Feeding Mechanism of *Testudo hermanni boettigeri* (Chelonia, Cryptodira). Neth J Zool 49: 1-13

Wocheslӓnder R, Gumpenberger M, Weisgram J (2000) Intraoral food transport in *Testudo hermanni* (Chelonia, Cryptodira) – a radiographic video analysis. Neth J Zool 50: 445-454

Cuora galbinifrons aquatic feeding patterns	
Variable	Transport of *Zophobas* larvae (38-49 mm) N=24
Duration of the gape cycle (s)	0.564 ± 0.102
Time to peak gape (s)	0.462 ± 0.126
Duration of "SO I" stage (s)	0.093 ± 0.028
Duration of "SO II" stage (s)	0.277 ± 0.098
Duration of "FO" stage (s)	0.060 ± 0.007
Duration of the MG stage (s)	0.043 ± 0.008
Duration of the "FC" stage (s)	0.059 ± 0.009
Duration of the hyoid retraction (s)	0.072 ± 0.01

Tab. 1
Mean ± standard deviation for eight kinematic variables in aquatic feeding events in *C. galbinifrons*; SO I = slow open I, SO II = slow open II, FO = fast open, MG = maximal gape, FC = fast close.

Figure legends

Fig. 1: Points used for kinematical analyses of feeding cycles of *Cuora galbinifrons*. U – anterior tip of upper jaw; L - anterior tip of lower jaw; H – hyoid; P – parietal bone; C – anterior tip of carapace; T – anterior tip of tongue; e – imaginary line connecting U and L;

The distance between U and L is referred to as gape; the distance between H and P indicates the dorso-vertical movements of the hyobranchial apparatus; the distance between P and C shows the head extension rate.

Fig. 2: *C. galbinifrons*. Schematic illustration of head morphology. a. Osteology and myology of the skull; b. Hyoid and hypoglossum, lateral view; c. Skull in dorsal view (right adductor complex removed; left jugal bar removed); d. Hyoid and hypoglossum, dorsal view; Ame - M. add. mand. externus; Amem - M. add. mand. externus pars media; Bmd - M. branchiomandibularis; CbI - Ceratobranchiale I; CbII - Ceratobranchiale II; Ch - Corpus hyoidei; Chy - Cornu chyale; Chd - M. coracohyoideus Cso - Crista supraoccipitalis; Dm - M. depressor mandibulae; Eo - Os exoccipitale; EpI - Epibranchiale I; F - Os frontale Fs - foramen stapedius; Hg - Hypoglossum; Im - M. intermandibularis; J - Os jugale; Md - Mandibula; Mx - Os maxillare; Op - Os opistoticum; P - Os parietale; Pf - Os praefrontale; Pl - Os palatinum; Po - Os postorbitale; Pr - Os prooticum; Pl - Processus lingualis hyoidei; Q - Os quadratum; Qj - Os quadratojugale; Rm - Ramphotheca; S - Os squamosum; Ty – Tympanum.

Fig. 3: Scanning electron micrograph showing total view of the tongue of *C. galbinifrons*. The high and slender lingual papillae (arrows) are distributed throughout

the dorsal tongue surface, but are fused together in the area of the “hindtongue” (ht); ap – lingual apex; gl – glottis.

Fig. 4: *C. galbinifrons*: Aquatic food transport of a large prey item (*Zophoba* sp. larva): kinematical pattern diagram and frame sequence from a high-speed video (250 fr/s); K - kinematics of neck extension; L - gape; M - dorso-ventral hyoid movement; O - anterio-posterior movement of tongue; n - imaginary line connecting the jaw tips.

Fig. 5: *C. galbinifrons*: Aquatic food transport (a - e); pharyngeal packing (f - i) and swallowing (j - m) of a small prey item (mealworm); time in s; based on x-ray film sequence (50 fr/s).

Fig. 6: “Oesophageal packing” event in *C. galbinifrons*; frame selected from x-ray film sequence (50 fr/s). P – prey.

Fig. 1

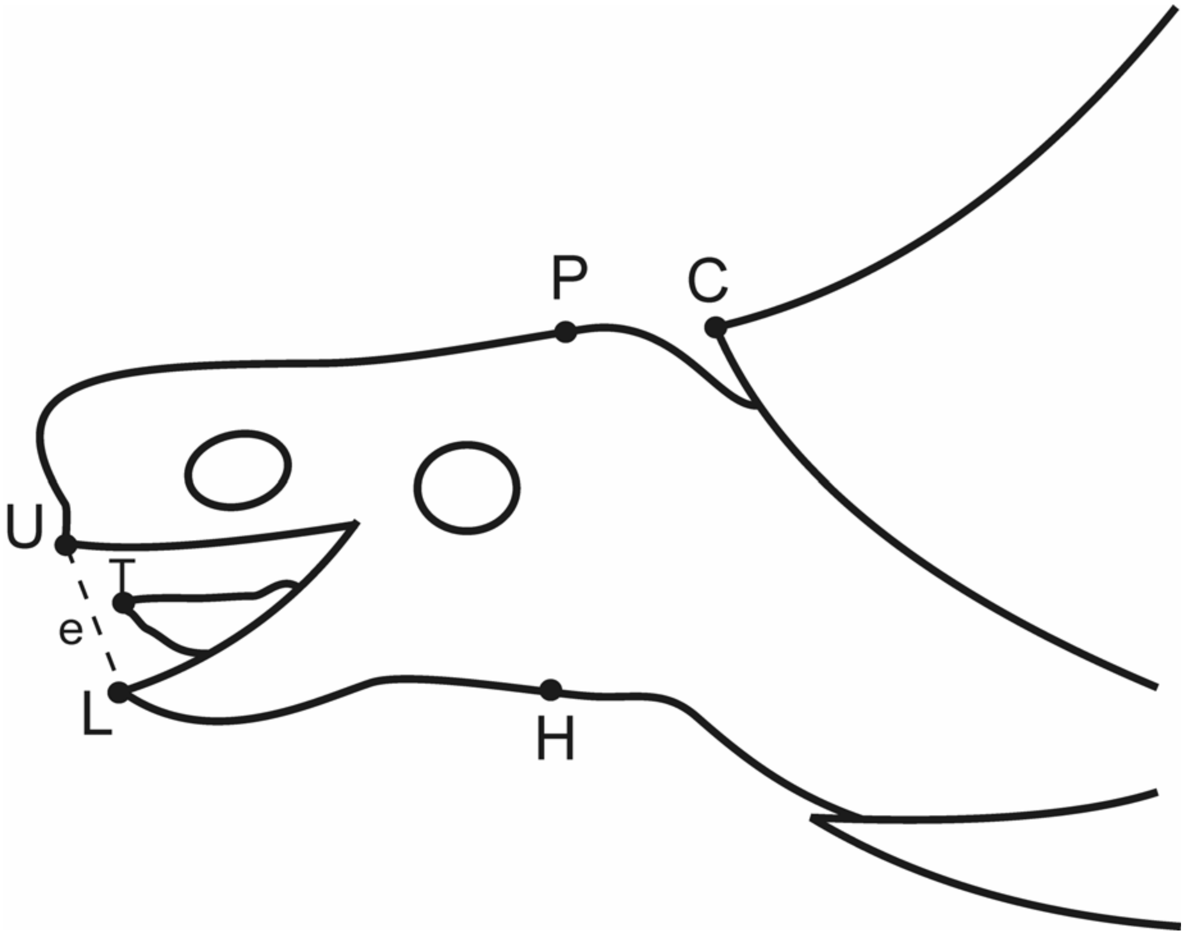


Fig. 2

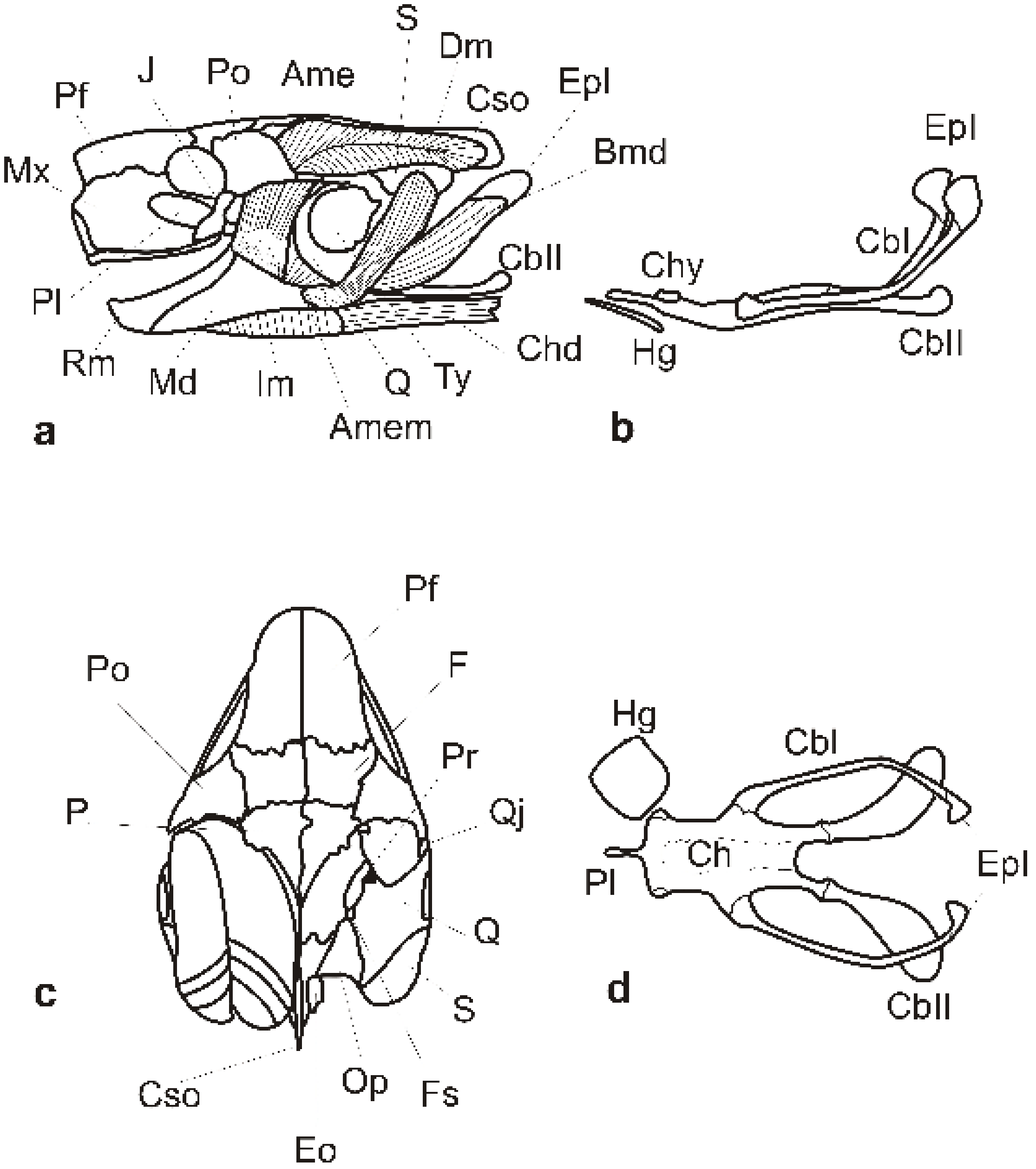

S
Dm
J
Po
Ame
Cso
EpI
Pf
Bmd
Mx
CbII
Pl
Chd
Rm
Md
Im
Q
Ty
Amem
a
EpI
CbI
Chy
Hg
CbII
b
Pf
Po
F
Pr
P
Qj
Q
S
Cso
Op
Fs
Eo
c
Hg
CbI
Ch
Pl
EpI
CbII
d

Fig. 3

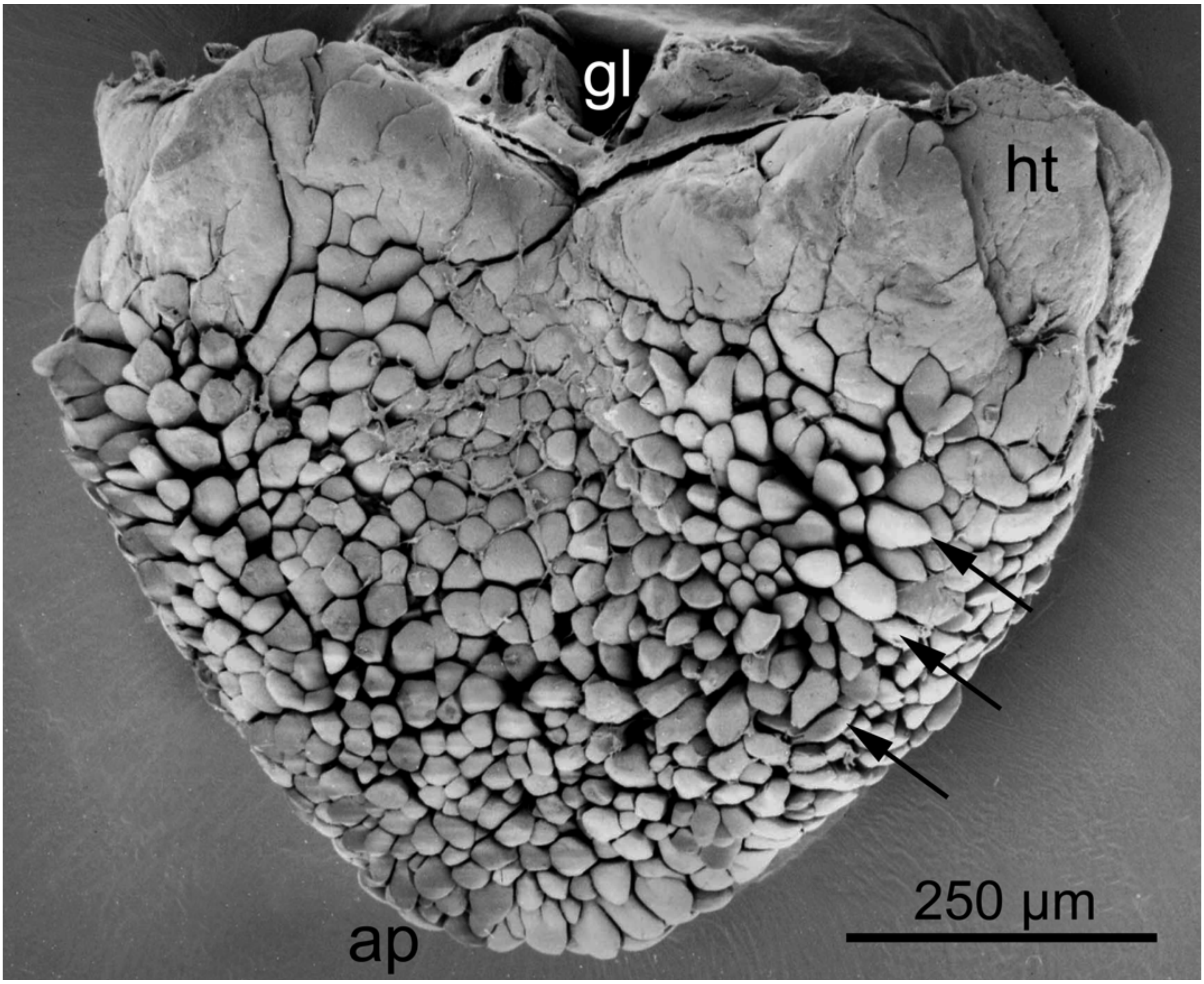


Fig. 4

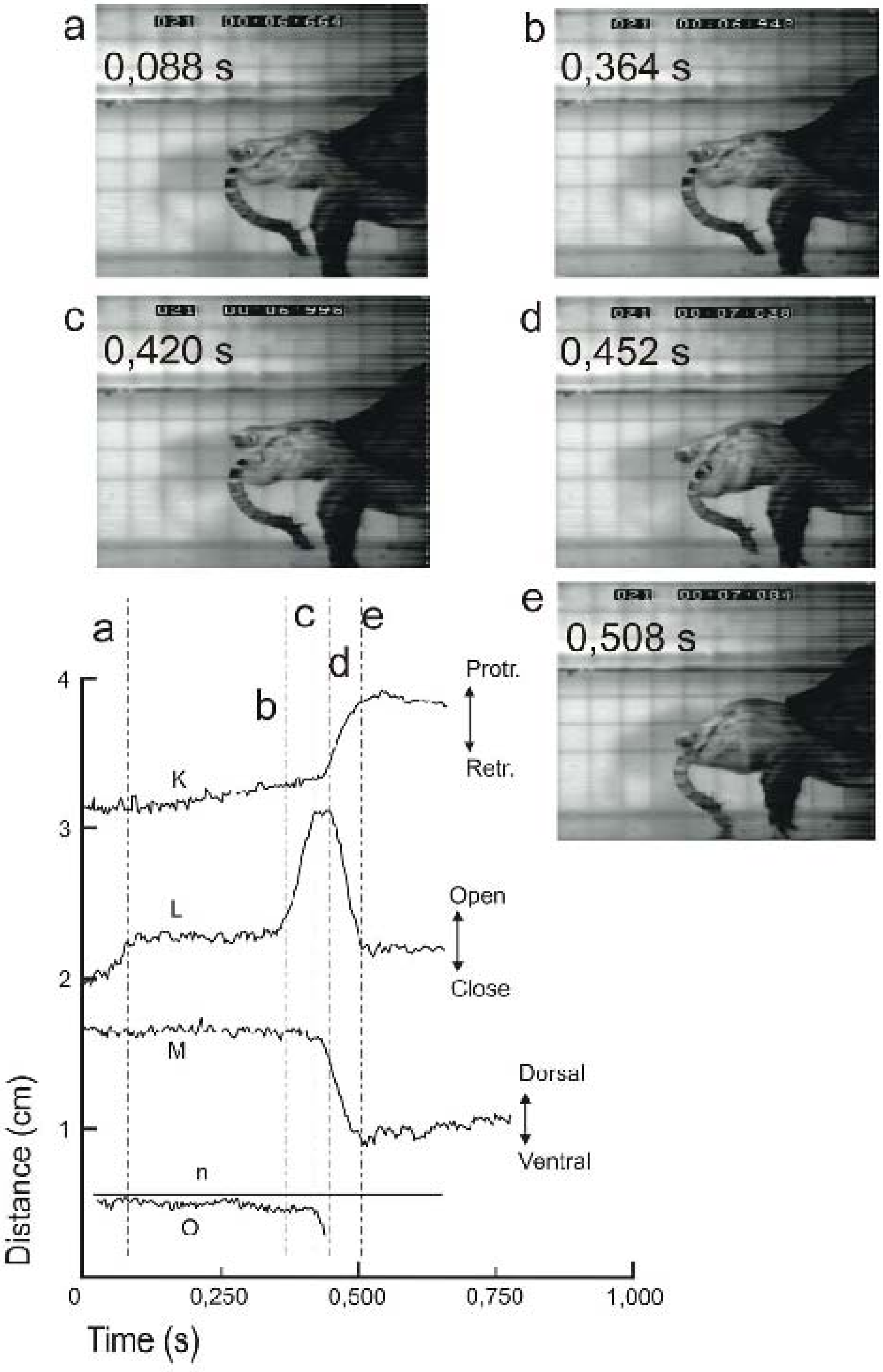

a
0,088 s
b
0,364 s
c
0,420 s
d
0,452 s
e
0,508 s
a
b
c
d
e
K
L
M
n
O
Protr.
Retr.
Open
Close
Dorsal
Ventral
Distance (cm)
0
1
2
3
4
0,250
0,500
0,750
1,000
Time (s)

Fig. 5

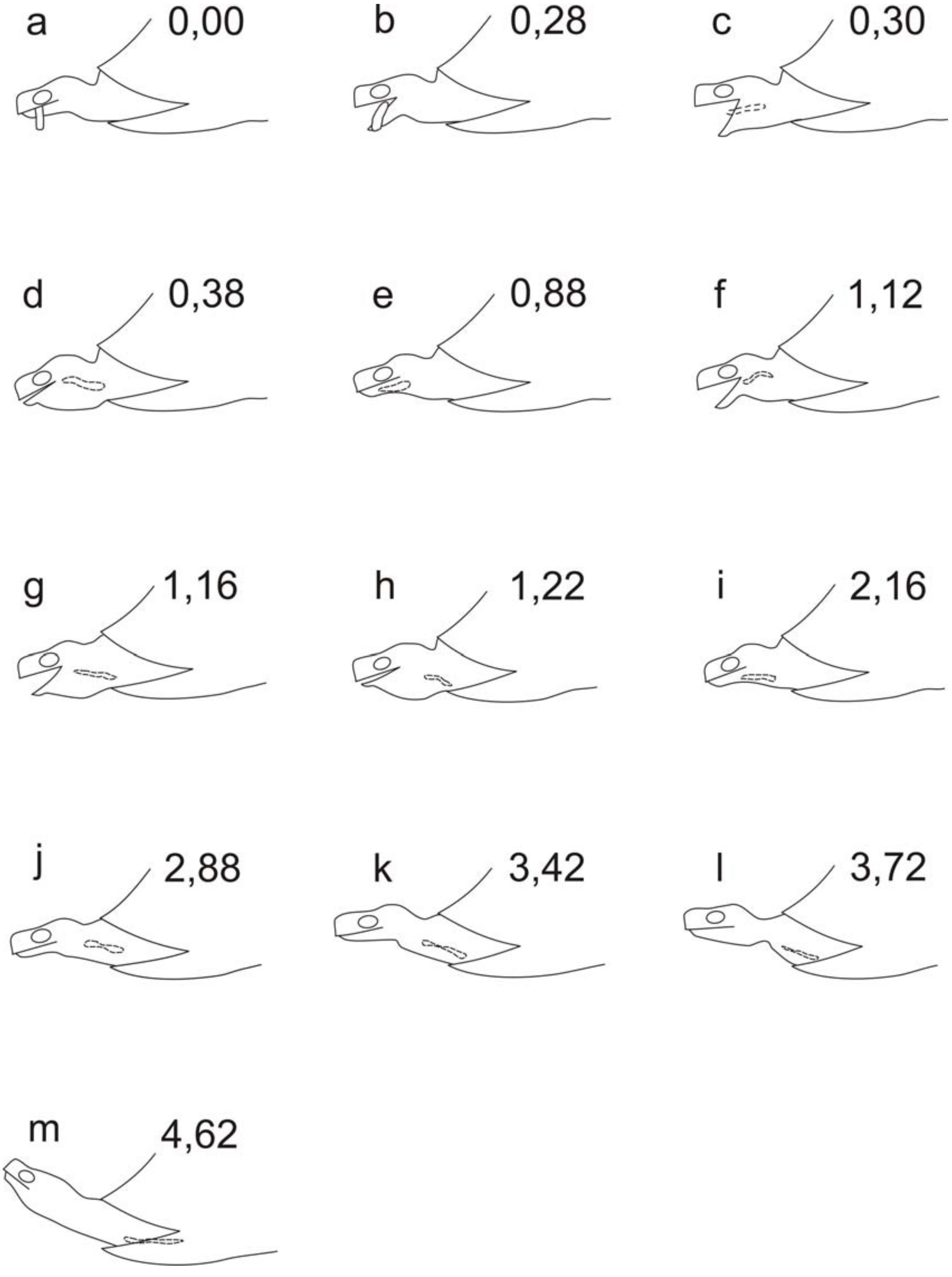
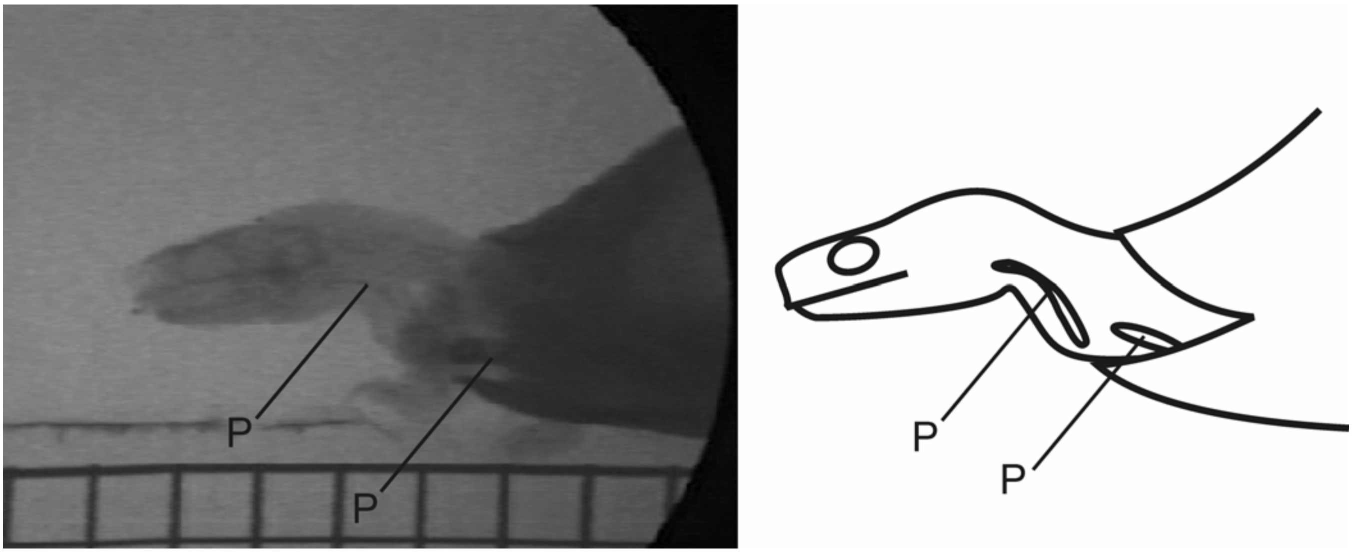

a
0,00
b
0,28
c
0,30
d
0,38
e
0,88
f
1,12
g
1,16
h
1,22
i
2,16
j
2,88
k
3,42
l
3,72
m
4,62

Fig. 6



II. Eingereichte Arbeiten:

II.3. Feeding and breathing: The bifunctionality of the oropharynx in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). Journal of experimental biology (sumitted).

Egon Heiss, Nikolay Natchev, Christian Beisser, Patrick Lemell, Josef Weisgram

Feeding and breathing: The bifunctionality of the oropharynx in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae)

Egon Heiss, Nikolay Natchev, Christian Beisser, Patrick Lemell, Josef Weisgram

Abstract

In tetrapods, the oropharyngeal cavity and its anatomical structures are mainly responsible for the uptake and intraoral transport of food. In this study we propose a second function of the oropharynx in the North American common musk turtle, *Sternotherus odoratus*. We describe the bifunctionality of the oropharynx in feeding and aquatic gas exchange, analogous to the trade-off found in the closely related soft-shelled turtles.

Using high-speed video, we demonstrate experimentally that *S. odoratus* practices hydrodynamic feeding underwater but is unable – despite all efforts – to complete the feeding process on land. Scanning electron microscopy and light microscopy reveal that the reason for this inability is the small and weak tongue. Although small, the tongue bears a variety of lobe-like papillae, which could be interpreted incorrectly as an adaptation for terrestrial food uptake. These papillae also largely cover the whole oropharynx, are highly vascularized and apparently play an essential role in aquatic gas exchange underwater.

Introduction

Morphological investigations on the oropharyngeal mucosa in chelonians have demonstrated the correlation between the design of the oropharyngeal cavity and the feeding mode (Beisser et al., 1995; 1998; 2001; 2004; Iwasaki, 2002; Heiss et al., 2008, Natchev et al., 2009). Tortoises have beefy tongues with numerous tall and slender lingual papillae (Winokur, 1988; Wocheslӓnder et al., 1999; 2000; Beisser et al., 2004). This increase of the total dorsal tongue surface promotes the interlocking effect in lingual food prehension and food transport. Aquatic turtles ingest and transport food by using hydrodynamic feeding mechanisms, in which the tongue plays a subordinate role and the lingual papillae are moderately sized (Beisser et al., 2001) or completely absent (Beisser et al., 1995; Lemell et al., 2002).

Kinosternids are reported to be exclusively aquatic feeders (Ernst and Barbour, 1989; Rogner, 1996; Schilde, 2004), although they occasionally emerge on land. The kinosternid *Sternotherus odoratus* almost permanently lives in the water as an adult, but juveniles spend time on land, inter alia searching for food. Therefore, one hypothesis of the present study is that (at least juvenile) musk turtles are able to feed on land. We test this hypothesis experimentally. The capacity to feed on land is highly contingent on oropharyngeal morphological adaptations (see Heiss et al., 2008 and Natchev et al., 2009). To date no information is available on the morphology of the oropharyngeal mucosa in kinosternids. We therefore test how the bauplan of that organ correlates with feeding behavior.

Another goal of this study is to search for oropharyngeal organs potentially responsible for aquatic gas exchange by using scanning electron microscopy and histological methods. The capability of *S. odoratus* to remain submerged for prolonged periods has been the object of many physiological studies (see Saunders et al., 2000 for overview). According to Pritchard (1979) and Stone et al. (1992), the common musk turtles use their papillous skin for oxygen uptake while submerged. Bagatto et al. (1997), however, demonstrated that the cutaneous surface area may not be the main factor behind aquatic breathing in kinosternids. A highly vascularized oropharyngeal mucosa is known to be the key organ responsible for aquatic gas exchange in the sistergroup of kinosternids (according to Gaffney and Meylan, 1988): the soft-shelled turtles, or Trionychidae (Gage and Gage, 1886; Dunson, 1960; Girgis, 1961; Wang et al., 1989; Yokosuka et al., 2000). We expect that a similar organ enables gas exchange in submerged *S. odoratus*. The main aim of the present study is therefore to test whether the oropharyngeal specializations of *S. odoratus* exhibit a bifunctionality combining both feeding (on land and under water) and aquatic breathing.

Material and Methods

Sternotherus odoratus (Latreille, 1802), the stinkpot or common musk turtle, is a small-sized but abundant species ranging from southern Canada to the eastern half of the USA. This species inhabits a wide range of aquatic habitats: rivers, lakes, swamps, cattle tanks, canals – and even fast-flowing creeks with rocky bottoms (Pritchard, 1979; Rogner, 1996; Schilde, 2004; Bonin et al., 2006). *S. odoratus* are reported to be omnivorous with a strong tendency to carnivory, feeding in the wild on various plants, worms, molluscs, crayfish, insects, tadpoles, fishes and their eggs; they can also take bites of flesh from dead animals (Ernst and Barbour, 1989; Schilde, 2004).

For the present study, five juvenile, four subadult and three adult *S. odoratus* ranging in size (straight carapace length) from 25.6 mm to 37.2 mm (juveniles), 61.5 mm to 69.3 mm (subadult) and 93.4 mm to 114 mm (adult) were used. The turtles were obtained commercially and kept in a 360 liter tank with 20 % land and 80 % water, and a 12 h dark/12 h light cycle. The animals were fed with earthworms, fish pieces and turtle-food pellets from the pet trade.

For filming aquatic and terrestrial feeding, food items were offered in front of the animals on the bottom of a glass aquarium (19 x 7 x 19 cm) with 5 cm water level or without water, respectively. For experiments, all turtles were fed with small fish pieces (apparently their preferred food) measuring approximately 4 x 4 x 6 mm. They were filmed in lateral view with the digital high-speed camera Photron Fastcam-X 1024 PCI at 500 fr/s (aquatic feeding) or at 250 fr/s (terrestrial feeding), with a reference grid (1 x 1 cm) as a background.

For morphological investigations, the animals were anesthetized by intraperitoneal injection of sodium pentobarbital and, after deep narcosis, decapitated. The heads were immersed immediately in fixation solution. For scanning electron microscopy (SEM), two heads of juvenile turtles were immersed for 24 h at room temperature in modified Karnovsky solution (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer; Karnovsky, 1965). After rinsing in 0.1 M cacodylate buffer, the lower jaw with all the ventral oropharyngeal structures was removed from the head in order to better view both the ventral and dorsal surfaces of the oropharyngeal cavity. Then, samples were postfixed in 0.5% osmium tetroxide for 2 h at 37 °C, washed in distilled water, and treated with 25% HCl at 40 °C for 15 min to remove the mucus from the surface. After repeated washing in distilled water, the samples were dehydrated in a graded ethanol and acetone series and dried in a critical point drying machine (Polaron: Watford, England). The dried samples were then coated with gold in an AGAR B7340 Sputtercoater and observed in a Philips XL-20 scanning electron microscope.

For paraffin-based histology, two juvenile and two subadult turtles were used. The heads and two biopsies of the dorsal and ventral neck were immersed in Bouin-fixative (Romeis, 1989) for 30 days, changing the solution twice a week. After complete fixation and decalcification, the upper jaw was removed from the rest of the head and the cornified rhamphothecae were cut off. Then, the samples were dehydrated in a graded ethanol-isopropanol series and embedded in paraffin. After polymerization, 7-µm-thin serial-sections were made on a Reichert-Jung 2030 rotation microtome. The sections were mounted on glass slides and, after removing the paraffin, stained with Haematoxylin (H) - Eosin (E), periodic acid Schiff (PAS) - Haematoxylin and Alcian blue (AB) - Haematoxylin (after Romeis, 1989; Kiernan, 2003). The preparations were documented by digital photography under a Nikon Eclipse 800 light microscope.

For semi-thin sectioning, one head of a juvenile turtle was fixed in the above- (for SEM) described modified Karnovsky solution for 48 h, washed tree times in 0.1 M cacodylate buffer, postfixed for 2 h at room temperature in buffered 0.5% osmium tetroxide, and decalcified in EDTA (ethylenediaminetetraacetic acid) for 30 days. Afterwards, the lower jaw was removed from the rest of the head and the rhamphothecae were cut off. This procedure was followed by dehydration in a graded ethanol and acetone series and embedding in Agar 100 Resin (Agar Scientific). After polymerization at 65 °C for 15 h, semi-thin (1 µm) sections were made on a Reichert Ultracut S microtome (Leica) using histo diamond knives (Diatome). The sections were mounted on glass slides, stained with Toluidine blue (TB) and documented as described above for histological sections.

For morpho-functional comparision, sections of oropharyngeal papillae of the European pond turtle, *Emys orbicularis,* were kindly purchased by Mr. Stefan Kummer (Univ. of Vienna). *E. orbicularis* is highly aquatic and inhabits similar environments as *S. odoratus* – but in Europe. The tissue preparation, staining and digital imaging were the same as described above for the paraffin-based histology of *S. odoratus*.

Results

a. Terrestrial and aquatic feeding behavior

In 286 cases where food items were offered on the land part of the aquarium, the prey was captured by young (230 times; 80.4%) or subadult (56 times; 19.6%) individuals and brought immediately to water for further transport, manipulation and swallowing. Adults showed no interest in the food items presented on land. Behavioral observations, documented cinematographically, showed that *S. odoratus* employed (fig. 1: 1a-6a) hydrodynamic mechanisms to feed underwater. Prey capture on land involved jaw prehension. When access to water was hindered, none of the tested animals was able to transport the food through the oropharyngeal cavity, despite repeated efforts (fig. 1: 1b-6b).

b. Morphology

SEM

Scanning electron microscopy (SEM) of juvenile *S. odoratus* revealed the blunt, massive and highly keratinized rhamphothecae (fig. 2A). The surface of the palatal mucosa is relatively flat, in contrast to the ventral side of the oral cavity, which shows a multiplicity of structures. The posterior part of the ventral rhamphotheca passes into the almost unkeratinized and triangular floor of the mouth (fig. 2A). The floor of the mouth itself is hidden mesoposteriorly by the tongue. The tongue of *S. odoratus* is small with a flannel-like appearance and is studded with relatively large, flattened, lobe-like and depressed lingual papillae (fig. 2A, B). Posteriorly adjacent to the tongue lies a narrow and small groove, the glottis. The glottis itself is surrounded by oropharyngeal papillae, which closely resemble the lingual papillae (fig. 2A).

Posterior to the glottis, the papillae increase in number and length, often overlap, resembling a blunt, rocky landscape (fig. 2C). In this region, similar structures are also present in the dorsal part of the oropharyngeal cavity. The pharyngeal papillae are oriented longitudinally relative to body axis (fig. 2A, C).

LIGHT MICROSCOPY

In overview cross sections, the splanchnocranial components and the hypoglossum of the hyolingual complex are cartilaginous and the intrinsic musculature of the tongue is poorly developed (fig. 3A, B, C).

The lingual papillae, which are extensions of the lingual mucosa, are expanded in histological cross sections (fig. 3A) on the anterior tongue part, becoming more slender posteriorly (fig. 3B, C; 4A). These slender and lobe-like papillae sometimes cover the glottal slot (fig. 3B). On the root of the tongue and behind the tongue, the papillae become more numerous and elongated. The highest density occurs posterior to the glottis, in the pharyngeal cavity. These papillae are relatively short and simple in juvenile turtles (fig. 3C) but tall and branched in subadults (fig. 4A).

Higher magnification showed the high degree of vascularization of the oral mucosa in *S. odoratus* (fig. 4B, C). In the deeper lamina propria, large blood vessels run parallel to the surface, giving rise to vessels to the superficial layer, where they form an extensive capillary network (fig. 4B, C). These capillary vessels run immediately subjacent to the basement membrane and are most dense in the pharyngeal papillae (fig. 4C).

The oropharyngeal mucosa consists mostly – if not completely – of a non-keratinized stratified cuboidal to columnar epithelium and an underlying connective tissue containing loosely (superiorly) to densely (in deeper regions) packed collagen fibers (fig. 3A). Keratinization occurs exclusively on the dorsal and ventral interfaces to the rhamphothecae. The oropharyngeal epithelium consists of 2 to 5 cell layers and the appearance of the cells varies according to their function. While the oral epithelium (palate, floor, tongue) contains many columnar goblet (mucous) cells, those cells are scattered in the pharyngeal epithelium, where cuboidal cells are prevalent. The thickness of the oropharyngeal epithelial layer varies between 10 and 35 µm. No multicellular glands were found in the oropharyngeal mucosa of the examined *S. odoratus*.

In contrast to the oropharyngeal mucosa, the superficial layer of the dermis of the outer skin seldom contains blood vessels (compare fig. 4C and fig. 5A), although larger veins and arteries are present in the deeper dermis. The epithelium of the outer skin consists of 2-3 basal cell layers plus at least 2-4 flattened superficial keratinocytes (fig. 5A), which are eosinophilic (data not shown) and PAS-positive (fig. 5A). The whole width of the epithelium of the outer skin varies between 20 and 50 µm.

The oropharyngeal papillae of the European pond turtle *E. orbicularis* are, compared with those of *S. odoratus*, flat and rare, and their capillarization is scarce (fig. 5B).

Discussion

The common musk turtle, *S. odoratus*, is highly aquatic, although occasionally found on banks of its home waters (Pritchard, 1979; Ernst and Barbour, 1989; Rogner, 1996; Schilde, 2004). Our experiments on feeding behavior support those observations. When food was offered in the water, all – young, subadult, and adult – individuals immediately grabbed and swallowed it. Prey capture and transport occurred, as in certain other aquatic cryptodirans, via hydrodynamic mechanisms (see Bramble and Wake, 1985; Lauder and Prendergast, 1992; Aerts et al., 2001; Summers et al., 1998; Natchev et al., 2009). As young animals sometimes climbed out onto the land part of the aquarium, we tested the hypothesis that they may also be able to feed on land – in contrast to subadult and adult individuals that rarely left the water. Food items offered on land were immediately grasped by young animals and brought to the water. Subadults sometimes showed a similar behavior, but adults never did. When access to water was hindered, the young individuals captured the prey successfully but failed in all cases to transport it toward the esophagus. All those turtles studied so far that feed exclusively (terrestrial) or occasionally (semiaquatic) on land use their tongue for terrestrial food transport (Weisgram et al., 1989; Wocheslånder et al., 1999, 2000; Natchev et al., 2009); their tongues are beefy and papillated with abundant mucous glands (Nalavade and Varute, 1976; Iwasaki, 1992; Iwasaki et al., 1992, 1996; Beisser et al., 2004). In contrast, exclusive aquatic feeders have a small and flat tongue with sparse glandular tissue (Winokur, 1988; Iwasaki, 1992; Iwasaki et al., 1992, 1996; Weisgram et al., 1989; Beisser et al., 1995, 1998, 2001; Lemell et al., 2000; 2002).

Interestingly, the common musk turtle does not fit into that schema, as the morphological investigations revealed a weak and small tongue (typical for aquatic feeders) but with numerous floppy papillae (expected for terrestrial feeders). All animals tested lost the food item when fed on land because they were unable to fix the food to the palate with their tiny tongue during the jaw opening of the first transport cycle. Therefore, the presence of lingual papillae in *S. odoratus* cannot be explained as an adaptation for occasional terrestrial feeding. Their orientation would not promote the interlocking effect between tongue and prey.

Papillae similar to the lingual ones, but branched and larger, are present posterior to the lingual root, around the glottis and throughout the pharyngeal cavity. Similar amplifications of the unkeratinized oropharyngeal mucosa are described for the sistergroup (according to Gaffney and Meylan, 1988) of the Kinosternidae: the Trionichidae or soft-shelled turtles. Trionychidae practise gas exchange underwater through skin and pharynx while hibernating and diving (see Gage and Gage, 1886; Dunson, 1960; Girgis, 1961; Wang et al., 1989; Yokosuka et al., 2000). Interestingly, physiological investigations revealed that *S. odoratus* oxygenates its blood under water like soft-shelled turtles do. The common musk turtles can remain underwater at 10 °C for more than 100 days (Ultsch et al., 1984; Jackson et al., 1984) and at 3 °C for at least 150 days (Ultsch, 1985, 1988; Ultsch and Wasser, 1990; Ultsch and Cochran, 1994; Ultsch and Jackson, 1995) without discernible damage. While submerged, the turtles remain aerobic as evidenced by the relatively small increases in plasma lactate (Ultsch and Cochran, 1994; Ultsch and Jackson, 1995). Three organs are predicted to be involved in aquatic gas exchange in chelonians: the cloacal bursae, the skin and the oropharyngeal mucosa. Cloacal gas exchange has been demonstrated in some pleurodiran turtles (see King and Heatwole, 1994; Gordos and Franklin, 2002; Clark et al., 2008). All kinosternids, however, lack cloacal bursae (Dunson, 1960; Peterson and Greenshields, 2001) and their skin is thick, strongly keratinized (especially plastron and carapace) and lacks an extensive capillary network (see fig. 5A). This excludes those two modes of gas exchange for *S. odoratus.* In contrast to the latter example, the surface-amplifying oropharyngeal papillae are highly vascularized. Histologically, those structures are very similar to the viliform oropharyngeal papillae described for the soft-shelled turtle *Trionyx sinensis japonicus*; its papillae definitely play a central role in gas exchange underwater (Yokosuka et al., 2000). The oropharyngeal papillae of *T. sinensis japonicus* are slender, tall and branched. In contrast to this, the papillae of *S. odoratus* are lobe-like and oriented longitudinally relative to the body axis. Such an orientation provides a maximum of contact area between papillar surface and water when water is pumped in and out through the mouth. Large oropharyngeal papillae probably negatively impact suction feeding in turtles because they prevent a smooth and therefore fast water flow into the oral cavity (for overview see Lemell et al., 2002; Beisser et al., 2004). The depressed and longitudinally oriented oropharyngeal papillae in *S. odoratus* probably minimize their negative impact on hydrodynamic feeding mechanisms.

A moderate to extensive capillarization, coupled with cutaneous surface amplification, is a strong indicator for cutaneous respiration in vertebrates (according to Feder and Burggren, 1985). Within tetrapods, cutaneous gas exchange contributes significantly to tissue respiration in nearly all amphibians, some reptiles and certain mammals. In *S. odoratus* the density of capillaries immediately beneath the thin oropharyngeal epithelium is comparable (if not even higher) to that of lungless salamanders (Plethodontidae), which exclusively rely on cutaneous respiration (see Feder and Burggren, 1985). Lungless salamanders can cover their demand for gas exchange lifelong this way. Cutaneous respiration in trionychids and kinosternids can ensure survival while diving at a decreased activity level (Dunson, 1960) or during hibernation (Ultsch and Jackson, 1995; Yokosuka et al., 2000). At high metabolic rates, these animals can no longer cover their oxygen demand in this manner and die if prevented from reaching the water surface to breath (Dunson, 1960).

The high degree of vascularization and capillarization in the oropharyngeal papillae of *S. odoratus* becomes more apparent if compared with that of *E. orbicularis*. The ecology and feeding behavior of this aquatic European turtle are similar to those of the common stinkpot. *E. orbicularis* has a prolonged hibernation, but it has a far lower capacity for lengthy submergence than *S. odoratus*. *E. orbicularis* must periodically seek the water surface to breath during hibernation (Bonin et al., 2006) and *S. odoratus* does not (Ultsch and Cochran, 1994; Ultsch and Jackson, 1995). The oropharyngeal surface in *E. orbicularis* is flat with rare and small papillae that contain some blood vessels but lack a well-developed capillary network. Such a design minimizes the potential for gas exchange through the oropharyngeal mucosa.

We assume that the oropharyngeal papillae of *S. odoratus* are morpho-functional adaptations for gas exchange underwater. Their design should not significantly affect the potential of this species to suction feed. The oropharynx in this turtle, therefore, exhibits a bifunctionality concerning feeding and breathing underwater. Future studies will examine the feeding behavior and oropharyngeal structures of other kinosternids to determine whether such a bifunctionality found in *S. odoratus* represents a unique trade-off within that group.

Acknowledgments

The authors acknowledge Katharina Singer and Stefan Kummer (both Univ. of Vienna) for assistance in histology, Richard Gemel (Natural History Museum Vienna) for comments on the ecological framework, and Michael Stachowitsch (Univ. of Vienna) for improving our English. This study was supported by the Austrian Science Fund FWF P20094-B17.

References

Aerts, P., Van Damme, J., Herrel, A. (2001). Intrinsic mechanics and control of fast cranio-cervical movements in aquatic feeding turtles. *Amer. Zool.* **41**, 1299-1310.

Bagatto, B., Guyer, C., Hauge, B., Henry, R. P. (1997). Bimodal Respiration in Two Species of Central American Turtles. *Copeia.* **1997(4)**, 834-839.

Beisser, C. J., Weisgram, J., Splechtna, H. (1995). The dorsal lingual epithelium of *Platemys pallidipectoris* (Pleurodira, Chelidae). *J. Morphol.* **226**, 267-276.

Beisser, C. J., Weisgram J., Hilgers H., Splechtna, H. (1998). Fine structure of the dorsal lingual epithelium of *Trachemys scripta elegans* (Chelonia: Emydidae). *Anat. Rec.* **250**, 127-135.

Beisser, C. J., Lemell, P., Weisgram, J. (2001). Light and transmission electron microscopy of the dorsal lingual epithelium of *Pelusios castaneus* (Pleurodira: Chelidae) with special respect to its feeding mechanics. *Tissue Cell* **33**, 63-71.

Beisser, C. J., Lemell, P., Weisgram J. (2004). The dorsal lingual epithelium of *Rhinoclemmys pulcherrima incisa* (Chelonia, Cryptodira). *Anat. Rec.* **277A**, 227-235.

Bonin, F., Devaux, B., Dupre, A. (2006). *Turtles of the world.* Paris, London: A & C Black Publishers Ltd.

Bramble, D. M. and Wake, D. B. (1985). Feeding mechanisms of lower tetrapods. In: *Functional Vertebrate Morphology* (ed. M. Hildebrand, D. M. Bramble, K. F. Liem, D. B. Wake), pp. 230-261. Cambridge, MA: Harvard Univ. Press.

Clark, N. J., Gordos, M. A., Franklin, C. E. (2008). Diving behaviour, aquatic respiration and blood respiratory properties: a comparison of hatchling and juvenile Australian turtles. *J. Zool.* **275**, 399-406.

Dunson, W. A. (1960). Aquatic respiration in *Trionyx spinifer asper*. *Herpetologica.* **16**, 277-283.

Ernst, C. H. and Barbour, R. W. (1989). *Turtles of the world*. Washington, DC: Smithsonian Institution Press.

Feder, M. E. and Burggren, W. W. (1985), Cutaneous gas exchange in vertebrates: Design, patterns, control and implications. *Biol. Rev.* **60**, 1-45.

Gaffney, E. S. and Meylan, P. A. (1988). A Phylogeny of Turtles. In: *The Philogeny and Classification of the Tetrapods*. *Vol. 1, Amphibians, Reptilies, Birds.* (ed. J. Benton), pp 157-219. Oxford: Claredon Press.

Gage, S. H. and Gage, S. P. (1886). Aquatic respiration in soft-shelled turtles: a contribution to the physiology of respiration in vertebrates. *Amer. Natur.* **20**, 233-236.

Girgis, S. (1961). Aquatic respiration in the common Nile turtle, *Trionyx triunguis* (Forskal). *Comp. Biochem. Physiol.* **3**, 206-217.

Gordos, M. and Franklin, C. E. (2002). Diving behaviour of two Australian bimodally respiring turtles, *Rheodytes leukops* and *Emydura macquarii*, in a natural setting. *J. Zool.* **258**, 335-342.

Heiss, E., Plenk, H. JR., Weisgram, J. (2008). Microanatomy of the Palatal Mucosa of the Semiaquatic Malayan Box Turtle, *Cuora amboinensis*, and Functional Implications. *Anat. Rec.* **291**, 876-885.

Iwasaki, S. (1992). Fine structure of the dorsal epithelium of the tongue of the freshwater turtle, *Geoclemys reevesii* (Chelonia, Emydinae). *J. Morphol*. **211**, 125-135.

Iwasaki, S., Asami, T., Asami, Y., Kobayashi, K. (1992). Fine structure of the dorsal epithelium of the tongue of the Japanese terrapin, *Clemmys japonica* (Chelonia, Emydinae). *Arch. Histol. Cytol.* **55**, 295-305.

Iwasaki, S., Wanichanon, C., Asami, T. (1996). Ultrastructural study of the dorsal lingual epithelium of the Asian snail-eating turtle, *Malayemys subtrijuga*. *Ann. Anat.* **178**, 145-152.

Iwasaki, S. (2002). Evolution of the structure and function of the vertebrate tongue. *J. Anat.* **201**, 1-13.

Jackson, D. C., Herbert, C. V., Ultsch, G. R. (1984). The comparative physiology of diving in North American freshwater turtles. II. Plasma ion balance during prolonged anoxia. *Physiol. Zool.* **57**, 632–640.

Karnovsky, M. J. (1965). A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J. Cell. Biol.* **27**, 137A.

Kiernan, J. A. (2003). *Histological and histochemical methods: Theory and practice.* Third edition. New York: Oxford University Press.

King, P. and Heatwole, H. (1994). Non-pulmonary respiratory surfaces of the chelid turtle *Elseya latisternum*. *Herpetologica.* **50**, 262-265.

Lauder, G. V. and Prendergast, T. (1992). Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. *J. Exp. Biol.* **164**, 55-78.

Lemell, P., Beisser, C. J., Weisgram, J. (2000). Morphology and function of the feeding apparatus of *Pelusios castaneus* (Chelonia; Pleurodira). *J. Morphol.* **244**, 127-135.

Lemell P, Lemell C, Snelderwaard P, Gumpenberger M, Wocheslânder R, Weisgram J. (2002). Feeding patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). *J. Exp. Biol.* **205**, 1495-1506.

Nalavade, M. N., Varute, A. T. (1976). Histochemical studies on the mucins of the vertebrate tongues. VIII. Histochemical analysis of mucosubstances in the tongue of the turtle. *Folia Histochem. Cytochem.* **14**, 123-134.

Natchev, N., Heiss, E., Lemell, P., Stratev, D., Weisgram, J. (2009). Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. *Zoology*. **112**, 113-127.

Peterson, C. C. and Greenshields, D. (2001). Negative test for cloacal drinking in a semi-aquatic turtle (*Trachemys scripta*), with comments on the functions of cloacal bursae. *J. Exp. Zool.* **290**, 247-254.

Pritchard, P. C. H. (1979). *Encyclopedia of turtles*. Neptune, NJ: TFH Publications, Inc.

Rogner, M. (1996). *Schildkröten 2.* Minden: Heidi-Rogner-Verlag.

Romeis B. (1989). *Mikroskopische Technik.* 17th ed. (ed. P. Böck). p. 97. München, Wien, Baltimore: Urban u. Schwarzenberg.

Saunders, K., Roberts, A. C., Ultsch, G.R. (2000). Blood viscosity and hematological changes during prolonged submergence of northern and southern musk turtles (*Sternotherus odoratus*). *J. Exp. Zool.* **287**, 459–466.

Schilde, M. (2004). *Die Moschusschildkröte Sternotherus odoratus.* Münster: Natur und Tier – Verlag GmbH.

Stone, P. A., Dobie, J. L., Henry, R. P. (1992). Cutaneous surface area and bimodal respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). *Physiol. Zool.* **65**, 311–330.

Summers, A. P., Darouian, K. F., Richmond, A. M., Brainerd, E. L. (1998). Kinematics of aquatic and terrestrial prey capture in *Terrapene carolina*, with implications for the evolution of feeding in cryptodire turtles. *J. Exp. Zool.* **281**, 280-287.

Ultsch, G. R., Herbert, C. V., Jackson, D. C. (1984). The comparative physiology of diving in North American freshwater turtles. I. Submergence tolerance, gas exchange, and acid-base balance. *Physiol. Zool.* **57(6)**, 620-631.

Ultsch, G. R. (1985). The viability of nearctic freshwater turtles submerged in anoxia and normoxia at 3 and 10°C. *Comp. Biochem. Physiol.* **A81**, 607-611.

Ultsch, G. R. (1988). Blood gases, hematocrit, plasma ion concentrations, and acid–base status of musk turtles (*Sternotherus odoratus*) during simulated hibernation. *Physiol. Zool.* **61**, 78–94.

Ultsch, G. R. and Wasser, J. S. (1990). Plasma ion balance of North American freshwater turtles during prolonged submergence in normoxic water. *Comp. Biochem. Physiol.* **97A**, 505-512.

Ultsch, G. R. and Cochran, B. M. (1994). Physiology of northern and southern musk turtles (*Sternotherus odoratus*) during simulated hibernation. *Physiol. Zool.* **67**, 263–281.

Ultsch, G. R. and Jackson, D. C. (1995). Acid–base status and ion balance during simulated hibernation in freshwater turtles from the northern portions of their ranges. *J. Exp. Zool.* **273**, 482–493.

Wang, Z. X., Sun, N. Z., Sheng, W. F. (1989). Aquatic respiration in soft-shelled turtles, *Trionyx sinensis*. *Comp. Biochem. Physiol.* **92A**, 593-598.

Weisgram, J., Ditrich, H., Splechtna, H. (1989). Comparative functional anatomical study of the oral cavity in two turtle species. *Plzen. Lek. Sborn. Suppl.* **59**, 117-122.

Winokur, R. M. (1988). The buccopharyngeal mucosa of the turtles (Testudines). *J. Morphol.* **196**, 33-52.

Wocheslünder, R., Hilgers, H., Weisgram, J. (1999). Feeding Mechanism of *Testudo hermanni boettgeri* (Chelonia; Cryptodira). *Neth. J. Zool.* **49**, 1-13.

Wocheslünder, R., Gumpenberger, M., Weisgram, J. (2000). Intraoral food transport in *Testudo hermanni* (Chelonia, Cryptodira) – A radiographic video analysis. *Neth. J. Zool.* **50**, 445-454.

Yokosuka, H., Ishiyama, M., Yoshie, S., Fujita, T. (2000). Villiform processes in the pharynx of the soft shelled turtle, *Trionyx sinensis japonicus*, functioning as a respiratory and presumably salt uptaking organ in the water. *Arch. Histol. Cytol.* **63**, 181-192.

Figure legends:

Fig. 1. Selected video frames showing prey capture and transport by a juvenile *S. odoratus* underwater (1a-6a, recorded at 500 fr/s) and on land (1b-6b, recorded at 250 fr/s). The animals use hydrodynamic feeding mechanisms underwater: the prey (P) is sucked into the mouth by a fast opening of the jaws (2a) and hyolingual depression (3a, 4a). On land, the animals grab the prey item (P) with the jaws (2b, 3b) but fail to transport it through the oral cavity (4b-6b).

Fig. 2. Scanning electron micrographs showing the ventral surface of the mouth of a young *S. odoratus* individual. A Overview, showing the massively keratinized rhamphotheca (Rh), which presents the ventral part of the “beak”, the lightly keratinized floor of the mouth (F), the small tongue (T), the glottis (indicated by arrowhead) and the pharynx (Ph). Note that the tongue (details shown in B) and the pharynx (details shown in C) are studded with flattened, floppy papillae (arrows). The white, vertical lines in micrograph A indicate where the histological sections (see Fig. 3 and Fig. 4) were taken. Scale bars: A 1 mm; B and C 200 µm.

Fig. 3. Light micrographs of cross sections of the ventral oral cavity of a young *S. odoratus* individual. For a better orientation, the white, vertical lines in the scanning electron micrograph of Fig. 2A indicate where the sections were taken. A Anterior section showing the tongue with some flannel-like papillae (arrows) and the floor of the mouth (F). Note the thin epithelium (ep) and the scarcely developed intrinsic musculature (M). B Slender floppy papillae (arrows) can cover the glottal slot (G). The hyolingual skeleton is well developed and cartilaginous. C Floppy papillae are also abundant posterior to the glottis: in the pharynx. Chy, Corpus hyoidei; Hy,

hypoglossum; lp, lamina propria; Orc, oral cavity; Phc, pharyngeal cavity; Pl, processus lingualis; Tr, trachea. Scale bars: A and B 200 µm; C 500 µm.

Fig. 4. Light micrographs of cross sections of the pharynx of subadult *S. odoratus*. A Overview showing the large and sometimes branched pharyngeal papillae (arrows). The arrowheads point to branched papillae. The asterisks mark some large blood vessels that supply the capillaries. B Larger blood vessel (bv) branches into a papilla (arrow). C Numerous small capillaries run immediately subjacent to the epithelium (arrowheads). ep, epithelium; Phc, pharyngeal cavity; Tr, trachea. Scale bars: A 1 mm; B and C 50 µm.

Fig. 5. Light micrographs of (A) longitudinal section of the skin of the neck of a subadult *S. odoratus* and (B) of a transverse section of two postglottal papillae of *E. orbicularis*. A Note the superficial keratin-layer (k) and the absence of capillaries in the skin of *S. odoratus*. B The small and rare papillae in the pharynx of *E. orbicularis* do not show a well-developed capillary network, in contrast to those of *S. odoratus* (see Fig. 4). Both cutaneous surfaces shown here are not suitable for life-supporting cutaneous respiration. de, dermis; ep, epithelium; Ex, external space; Phc, pharyngeal cavity. Scale bars: A 20 µm, B 50 µm.

Fig. 1

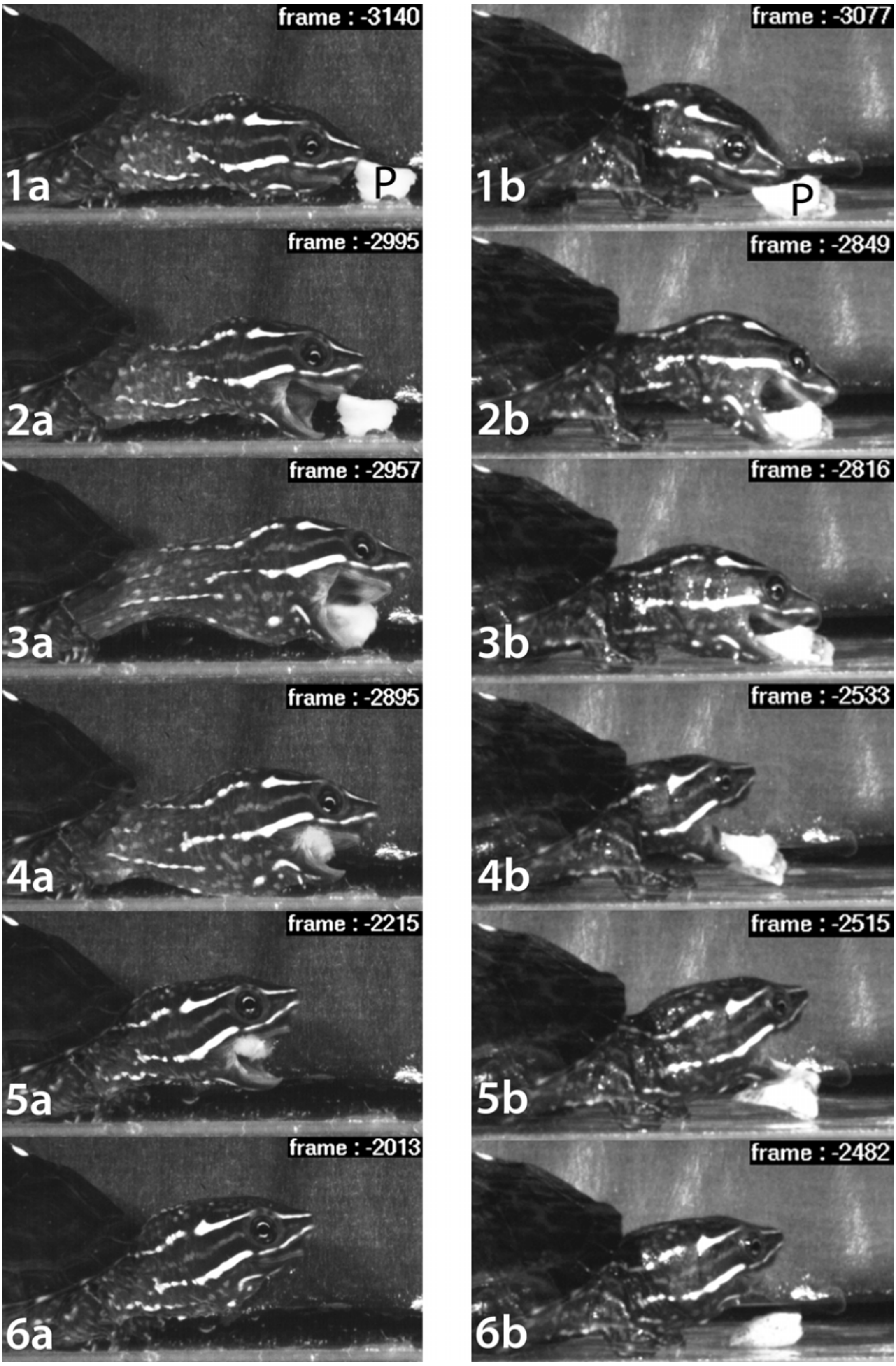

frame : -3140
1a
P
frame : -3077
1b
P
frame : -2995
2a
frame : -2849
2b
frame : -2957
3a
frame : -2816
3b
frame : -2895
4a
frame : -2533
4b
frame : -2215
5a
frame : -2515
5b
frame : -2013
6a
frame : -2482
6b

Fig. 2

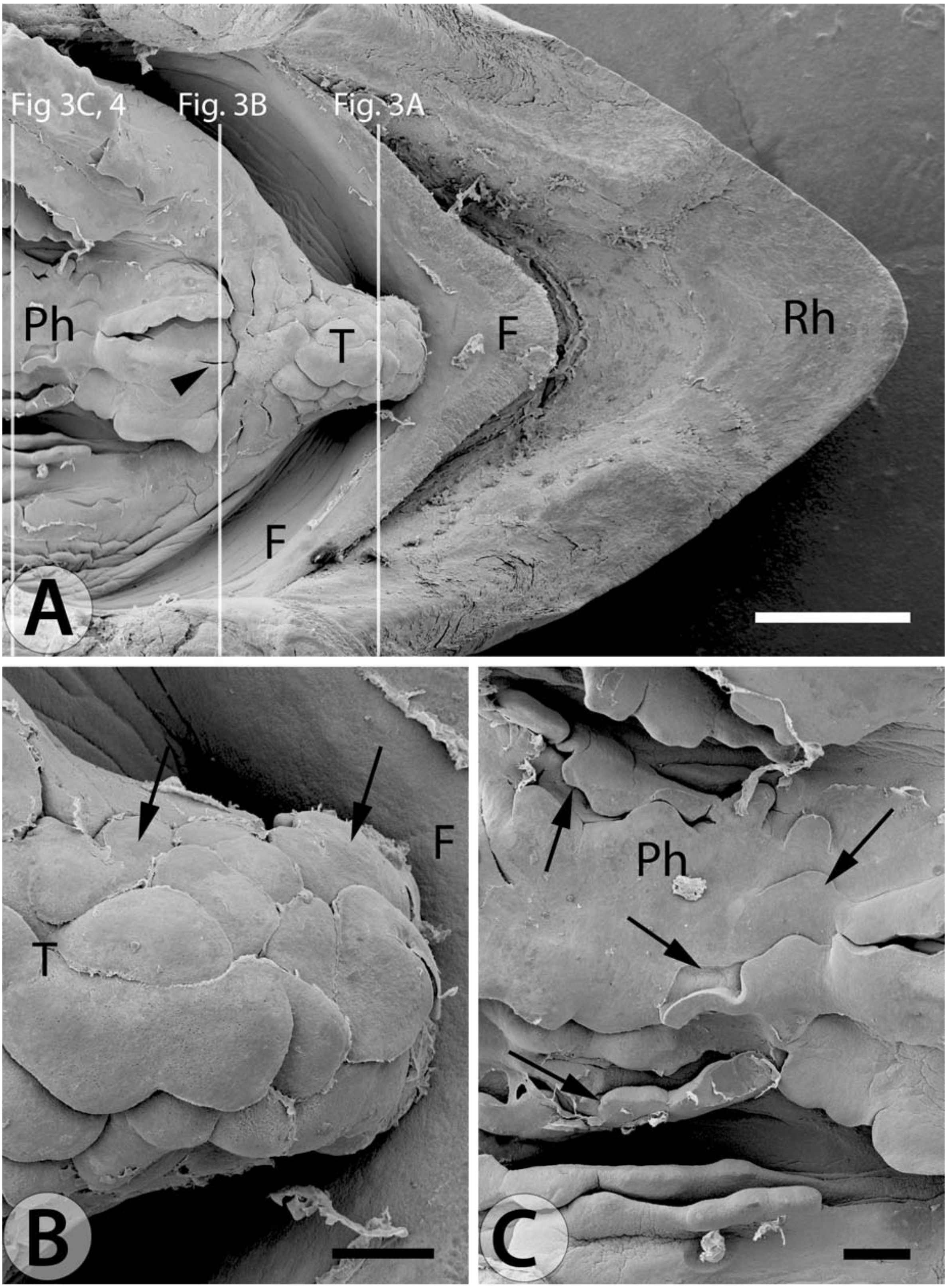


Fig. 3

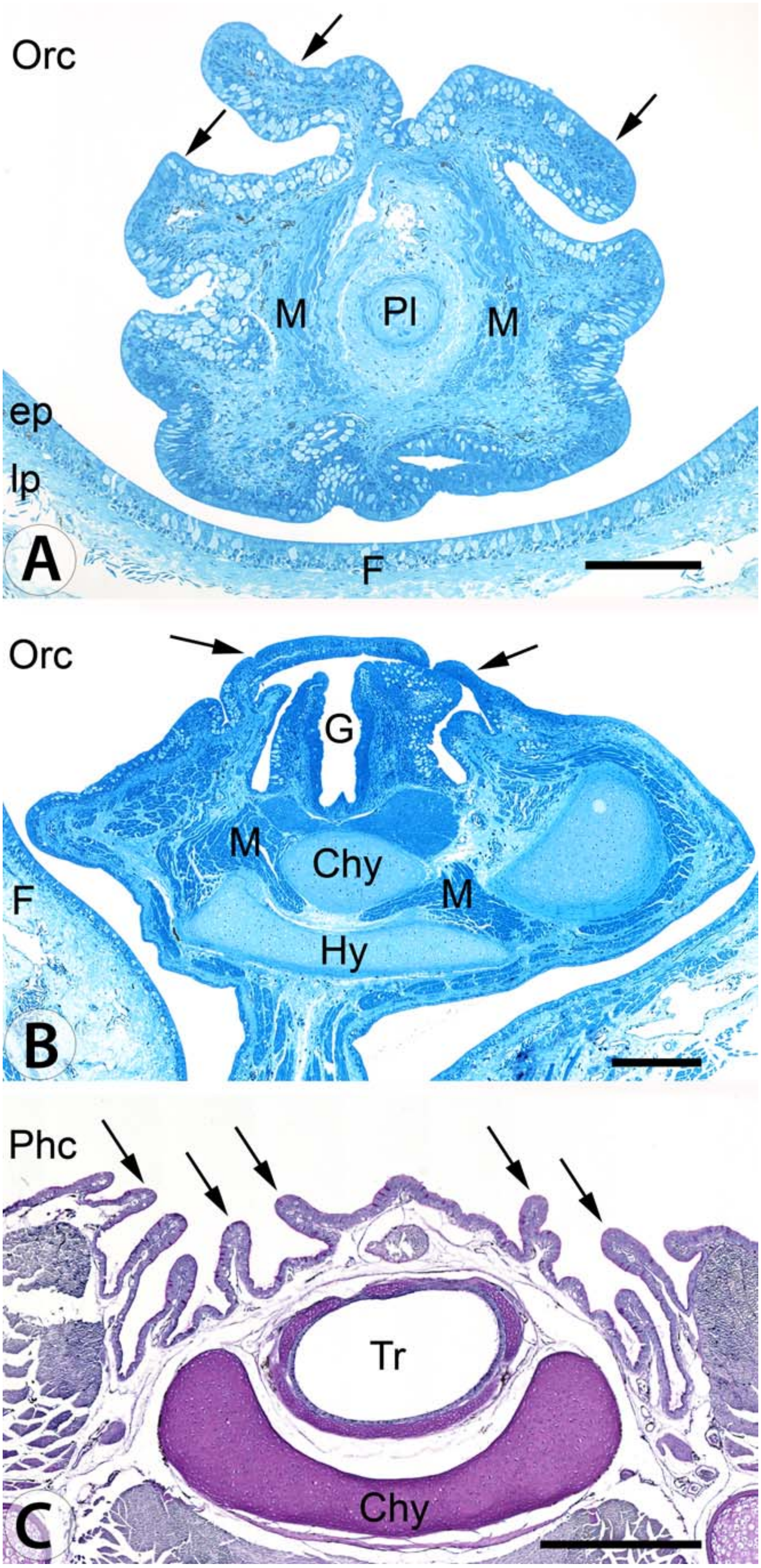


Fig. 4

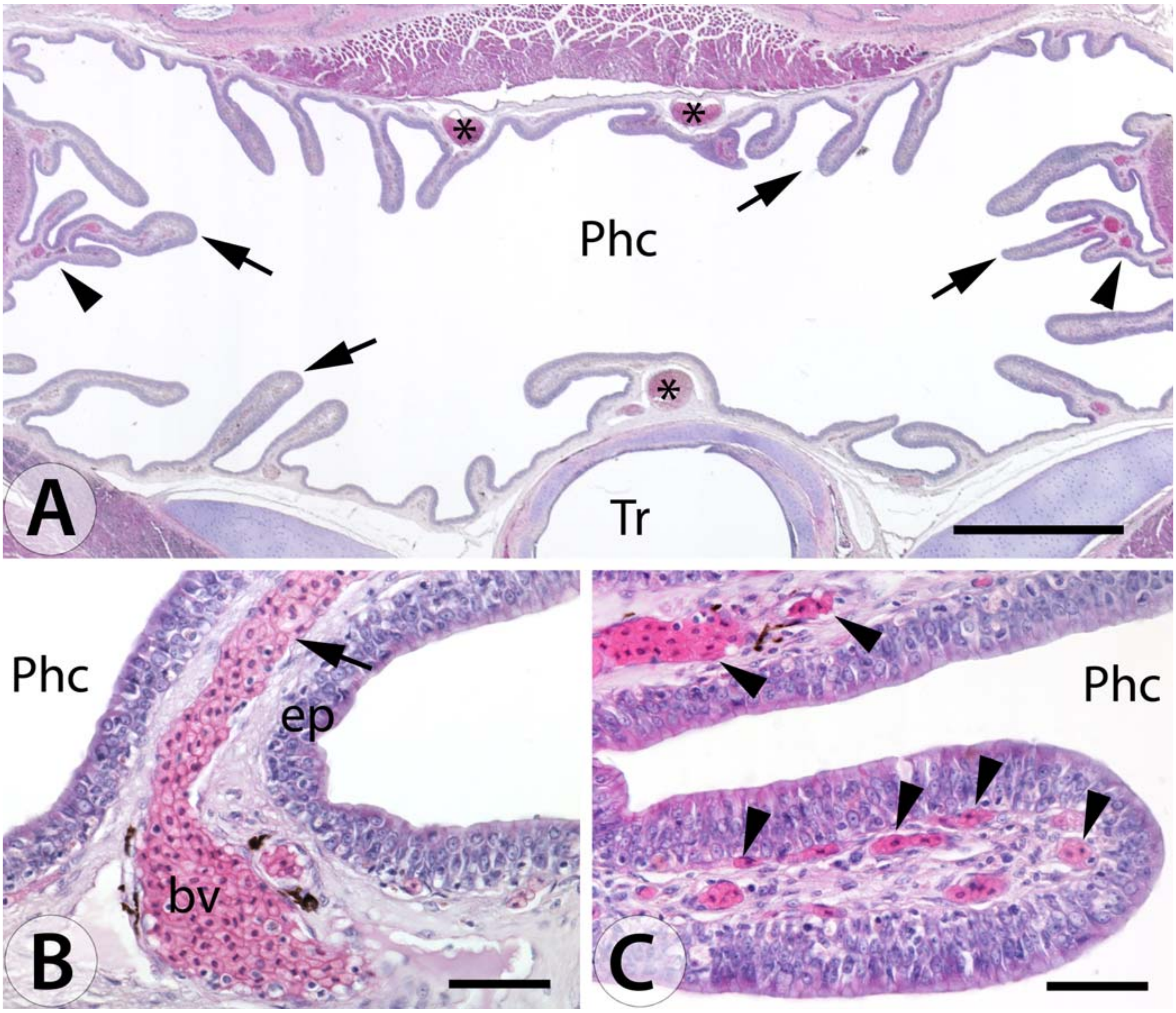
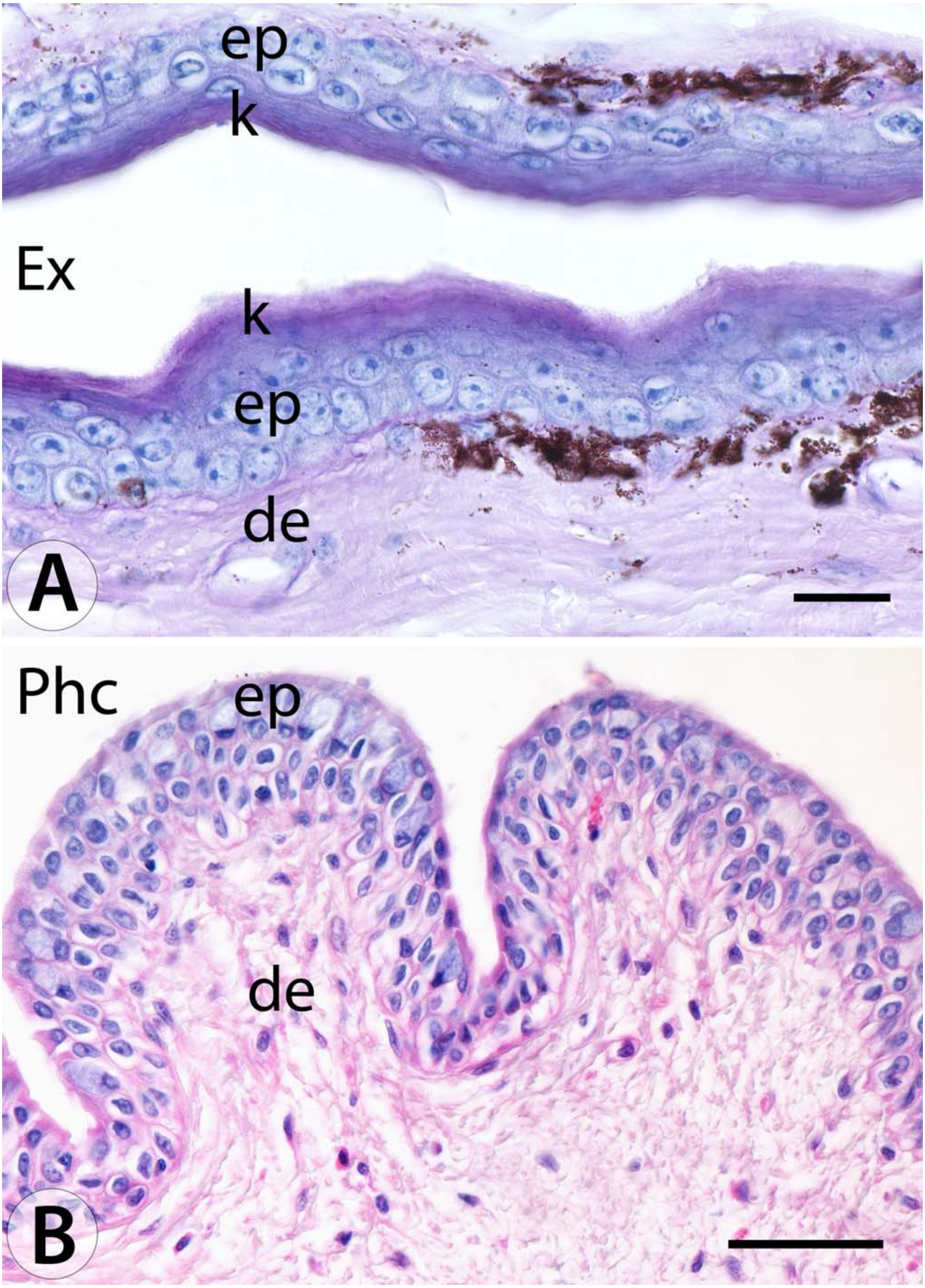


Fig. 5



II. Eingereichte Arbeiten:

II.4. Kinematic analysis of aquatic prey capture, prey transport and swallowing in the Common Musk Turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). Journal of experimental zoology (submitted).

Nikolay Natchev, Egon Heiss, Katharina Singer, Stefan Kummer, Dietmar Salaberger, Josef Weisgram

Kinematic analysis of aquatic prey capture, prey transport and swallowing in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae)

Nikolay Natchev[a], Egon Heiss[a], Katharina Singer[a], Stefan Kummer[a], Dietmar Salaberger[b], Josef Weisgram[a] *

[a] *University of Vienna, Department of Theoretical Biology, Althanstrasse 14, A-1090 Vienna, Austria*

[b] *FH OÖ Forschungs- & Entwicklungs GmbH, Fakultät für Technik und Umweltwissenschaften, Stelzhamerstraße 23, 4600 Wels, Austria*

*Author for correspondence: *E-mail address:* josef.weisgram@univie.ac.at (J. Weisgram).

Abstract

The present study examines the kinematic patterns of prey capture, prey transport, pharyngeal packing and swallowing in the common musk turtle *Sternotherus odoratus*. These data are supplemented by morphological descriptions of the skull and the hyolingual complex. Although the hyoid is mainly cartilaginous, *S. odoratus* use exclusively hydrodynamic mechanisms in prey capture and prey transport. The tongue is relatively small, with weakly developed intrinsic musculature. We propose that the elasticity of the hypoglossum and the hyoid body reduces the capability of *S. odoratus* to suction feed, but allows these turtles to effectively reposition food items within the oropharyngeal cavity during transport, manipulation and pharyngeal packing. We maintained equal conditions in all feeding experiments by using food items (pieces of fish) of the same size, always offered at the same position at the bottom of the aquarium. Nonetheless, the measured values showed large ranges. The duration of prey capture and prey transport cycles is relatively long in *S. odoratus* compared to other chelonians. These turtles can modulate the onset of jaw opening relative to the initiation of hyoid retraction both in prey capture and in prey transport cycles. We propose that the numerous taste buds distributed over the entire oropharyngeal mucosa, play an important role for the sensorimotor feedback control of food transport kinematics. In *S. odoratus* the jaw, hyoid and cervical movements are apparently widely decoupled and the motor functions of the feeding apparatus are adjusted to every single feeding situation.

Introduction

In tetrapods the feeding process consists of four main phases: “prey capture” or “ingestion”; “food transport”; “pharyngeal packing”; and “swallowing” or “mammalian deglutition” (see Smith, 1992; Schwenk, 2000a). In turtles, aquatic prey capture has been studied in pleurodirans (Van Damme and Aerts, 1997; Lemell and Weisgram, 1997; Lemell et al., 2002) and also in marine (including one estuarine) cryptodirans (Bels and Renous, 1992; Bels et al., 1998). To date, food uptake kinematics have been analysed in only three freshwater cryptodirans – *Chelydra serpentina* (Lauder and Prendergast, 1992), *Terrapene carolina* (Summers et al., 1998) and *Cuora amboinensis* (Natchev et al., 2009). Kinematic analysis of prey transport in turtles is relatively scarce (see Bels et al., 2008). Information on feeding kinematics in Trionichoidea (Gaffeney and Meylan, 1988) is completely lacking.

The oldest known chelonians were probably aquatic (see Li et al., 2008), other stem turtles had a terrestrial paleoecology (Joyce and Gautier, 2004; Scheyer and Sander, 2007). The feeding apparatus of recent chelonians is secondarily adapted to aquatic feeding (Lauder and Prendergast, 1992); turtles have developed aquatic feeding convergent with anamniote feeding systems. According to Lauder and Prendergast (1992), there are kinematic similarities in the prey capture modes of some bony fishes, salamanders and *C. serpentina*. These authors explain the analogy in the underwater food uptake motor functions by hydrodynamic constrains placed on prey capture due to the physical properties of water as a feeding medium. The ingestion mechanism of *C. serpentina* is termed “ram feeding” by Lauder and Prendergast (1992) – the prey is not sucked up into the oral cavity, but engulfed by the jaws in a rush forward strike of the cervicocranial complex. In those tetrapods utilising "bidirectional feeding" (see Reilly and Lauder, 1992), any oropharyngeal volume expansion involves an internal pressure decrease. Van Damme and Aerts (1997) introduced the terms "compensatory suction" and "inertial suction" for turtles, retaining the term "ram feeding" only for feeding systems with unrestrained water through-flow. According to Summers et al. (1998) the term "compensatory suction" exactly describes the prey capture mode in cryptodirans.

The "neuromotor program" of the underwater food transport is predicted to be conserved in the evolution of gnathostome feeding systems (see Reilly and Lauder, 1990). In the cyclic model proposed by these authors for anamniotes, the jaw cycle is divided in a "fast open" and "fast close" phase - hyoid retraction starts simultaneously to the onset of jaw opening. The coincidence between hyoid retraction and jaw opening is regarded as a uniform pattern throughout tetrapods. This hypothesis is not always supported for turtles (see Lemell and Weisgram, 1997; Natchev et al., 2009). Their transport modes seem to be extraordinarily variable. According to Aerts et al. (2001) the underwater transport in chelonians involves a combination of "compensatory suction" and "inertial suction" (both mechanisms are termed "intraoral-aquatic hyoid transport" – Bels et al., 2008). Still, some chelonians (even completely aquatic species) use a tongue-based transport (termed according to Bels et al. (2008), "intraoral-aquatic lingual transport").

Our study describes the kinematics of the head, jaws and hyoid complex based on high-speed cinematography, during the whole aquatic feeding process in the kinosternid *S. odoratus*. These data are supplemented by morphological descriptions of the skull and the hyolingual complex. We propose that *S. odoratus* use exclusively hydrodynamic mechanisms when feeding underwater. We discuss our results on prey capture and prey transport in the context of the "hydrodynamic constraints" hypothesis and the hypothesis of the conservation of the feeding motor patterns in vertebrate evolution (for an overview see Lauder and Prendergast, 1992; Smith, 1994; Alfaro and Herrel, 2001; Deban et al., 2001).

Material and Methods:

The 25 recent kinosternid species are divided into four genera: *Claudius*, *Staurotypus*, *Kinosternon* and *Sternotherus* (Pritchard, 1979). The common musk turtle, or stinkpot – *Sternotherus odoratus* (synonym *Kinosternon odoratum* (see Bonin et al., 2006)) – is an entirely aquatic species, widely distributed in the eastern USA, south to the Mexican border and in north up to Canada (Rogner, 1995; Ernst and Barbour, 1989). Common musk turtles forage at the substratum of rivers, lakes and swamps. The diet of *S. odoratus* is mainly carnivorous and includes carrion, insects, molluscs, crayfish and fish (Pritchard, 1979; Bonin et al., 2006; Schilde, 2007). For a *S. odoratus* population in Lancaster County, Pennsylvania, Ernst (1986) reported that the turtles were active approximately 200 days per year. Feeding did not start until the water temperature reached 18 °C. The maximum observed annual feeding period is 150 days. No subspecies of *S. odoratus* have been described (Pritchard, 1979).

Our study animals were obtained commercially and kept in a 360 l tank with 20 % land and 80 % water, and a 12 h dark/12 h light cycle. The turtles were fed fish, pieces of liver, hard and soft pellets and occasionally small snails.

For morphological analysis, the turtles were anesthetised by intraperitoneally injecting sodium pentobarbital and, after deep narcosis, decapitated. The heads were immersed immediately in fixation solution.

For computer tomography (ct), the heads of one subadult (carapace length: 69.3 mm) and one adult animal (carapace length: 114 mm) were immersed in 4% formaldehyde for two weeks prior to storage in 70% ethanol. The 3D data were generated using industrial X-ray Computer Tomography. During measurement projection, images were obtained using an a-Si matrix detector at several angular positions. Depending on the density, the atomic number and the irradiation length, different gray values occurred in these 2D images. A full 360 degree rotation typically generated 720 images. Tab. 1 lists the parameters used for the CT-scans. Surface and volume reconstructions were made using Amira 4.1 (Mercury Computer Systems).

For scanning electron microscopy (SEM), two heads of juvenile turtles were immersed for 24 h at room temperature in modified Karnovsky solution (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer; Karnovsky, 1965). After rinsing in 0.1 M cacodylate buffer, the lower jaw was removed. Then, samples were postfixed in 0.5% osmium tetroxide for 2 h at 37 °C, washed in distilled water, and treated with 25% HCl at 40 °C for 15 min to remove the mucus from the surface. After repeated washing in distilled water, the samples were dehydrated in a graded ethanol and acetone series and dried in a critical point drying machine (Polaron: Watford, England). The dried samples were then coated with gold in an AGAR B7340 Sputtercoater and observed in a Philips XL-20 scanning electron microscope.

For histological analysis, two juvenile and two subadult turtles were used. The heads were immersed in bouin-fixative (Romeis, 1989) for 30 days, changing the solution twice a week. After complete fixation and decalcification, the upper jaw was removed from the rest of the head and the cornified rhamphothecae were cut off. Then, the samples were dehydrated in a graded ethanol-isopropanol series and embedded in paraffin. After polymerisation, 7 µm thin serial-sections were made on a Reichert-Jung 2030 rotation microtome. The sections were mounted on glass slides and, after removing the paraffin, stained with Haematoxylin (H) – Eosin (E), periodic acid Schiff (PAS) –Haematoxylin and Alcian blue (AB) – Haematoxylin (after Romeis, 1989;

Kiernan, 2003). a A Nikon Eclipse 800 light microscope was used for digital photographic documentation.

For filming aquatic feeding, food items were offered in front of the animals on the bottom of a glass aquarium (19 x 7 x19 cm) with a water depth of 12 cm. The food items were pieces of epaxial musculature of fish. Their size was individually calibrated to correspond to the distance between the tip of the lower jaw and point "A" (Fig. 1) at the jaw articulation of every single turtle used in our experiments. The type and size of the food were chosen to enable the whole feeding process to be recorded – from food uptake to swallowing. When feeding on hard pellets or snail for example, the stinkpot exhibit many combined "manipulation-transport" cycles, and the duration of the whole process was over the time our filming system is able to store. Three subadult speciments (carapace length: 61.5 - 69.3 mm) were filmed in strict lateral view with a reference grid (1 x 1 cm) as background, using a "Photron Fastcam-X 1024 PCI" camera with 500 f/s. The frame rate of 500 f/s was chosen as the lowest possible rate at which no blurs by fast landmark displacements occur on the images. For a total of 24 films (8 sequences pro specimen), the horizontal (on the X-axis) and vertical (Y-axis) coordinates of each landmark indicated on Fig. 1 were recorded frame by frame using "SIMI-MatchiX" (copyright (c) by SIMI Reality Motion Systems, Unterschleisheim, Germany). Based on landmark displacement in the bi-directional level, we calculated: a) the gape amplitude – distance between the tips of the upper and the lower jaw; b) ventral hyoid movement – distance between point "S" on the squamosal and point "H" at the origin of the Ceratobranchiale II; c) the extension and retraction of the neck – the distance between point "S" on the squamosal and the anterior tip of the carapace; d) the movements of the food item.

Results

The skull in *S. odoratus* is relatively flat and elongated with a prominent supraoccipital ridge and high temporal arches (see also Bever, 2008 in press). The ramphothekas are well developed and typical for the most kinosternids; the edges are blunt. The palatins form no dorsal flexure (Fig. 2c). The tongue is relatively small with weakly developed intrinsic musculature (Fig. 2e). In subadults the hypoglossum, hyoid body, epibranchials I and the second hyoid horn are completely cartilaginous. Only the ceratobranchials I are ossified. Our ct experiments demonstrate that even in older specimens the hyoid complex remains mainly cartilaginous (Fig. 2b). There are only two pairs of ossifications on the hyoid corpus (at the basis of ceratobranchials I and II).

Scanning electron microscopy focused on the occurrence and distribution of taste buds (tb), which were identified by their typical taste pore with large microvillae in the center (Fig. 2d,f). Tbs were found throughout the oropharyngeal cavity: from the well-keratinised anteriormost part of the mouth to the posteriormost part of the pharynx. Most tbs in juvenile and subadult turtles were present on the anterior palate (anterior to the choanae) and on the anterior floor of the mouth. Tbs are onion- to barrel-shaped and consist of 30 to 60 specialised, slender epithelial cells. The nuclei of the tb-cells are located mainly in the basal half to two-thirds of the cells. While the descending cell processes contact the basement membrane, the slender cytoplasmatic processes of the apical region extend freely with their microvilli into the tastepore (Fig. 2d,f). Light microscopy revealed that the dorsal surface of the tongue is enlarged due to the formation of floppy papillae (Fig. 2e).

When feeding on small pieces of fish, the feeding process in *S. odoratus* includes the prey capture cycle, zero to five (average 2.4 ± 1.5) transport cycles, and two to six (average 4.1 ± 1.3) pharyngeal packing cycles, followed by swallowing. For our marker tracking, "time zero" was taken as the moment of the first detectable hyoid elevation prior to "jaw opening". The food items were offered at the bottom of the aquarium in front of the animals. The turtles swam slowly toward the prey and stopped their forward locomotion when the tip of the lower jaw was 0.49 ± 0.17 cm from the fish. Prey capture started with hyoid elevation followed by jaw opening. The body was almost motionless. In most of our sequences (16 from a total of 24) we were able to detect a separation of "slow jaw open (SO)" and "fast jaw open (FO)" phases (see Fig. 4b). In 8 films, the gape increased gradually and no discrete phases were recognised prior to reaching "peak gape". During jaw opening the head rotated ventrally, as the rotation was convoyed (except in 3 cases) by neck extension. In 6 of our sequences the hyoid retraction started prior to reaching peak gape (Fig. 4a). In 18 cycles the first detectable retraction of the hyoid complex started during a static gape phase in which the jaw amplitude remained at its maximum for a period of time (here termed MG-phase). The MG-phase (present in all 24 "prey capture" events) was followed by fast jaw closing. We were able to detect that the food item was actively sucked up into the mouth due to the pharyngeal expansion, but often was pushed slightly forwards during jaw closing (Fig. 3). In 4 of 24 prey capture events, the prey was ingested entirely within the oropharyngeal cavity and the animals closed their jaws completely.

Prey transport cycles started with hyoid protraction followed by jaw opening. The tongue was positioned derby under the prey. In 25 of a total of 47 transports, we detected a fast extension of the neck during jaw opening. Retraction of the hyolingual complex started simultaneously to the initiation of head protraction (Fig. 3). The magnitude and velocity of hyoid retraction was variable, but in all sequences the process started prior to reaching peak gape (Figs. 3, 4). The MG-phase was detected in 8 of our sequences. In the transport cycle in which the prey was positioned almost entirely within the mouth, the neurocranium remained motionless or was ventrally rotated rather than protracted.

During pharyngeal packing, the prey was not visible. The jaw and hyoid displacement amplitudes were small (sometimes < 1 mm). In most cases the jaws remained fully closed (Fig. 3). In contrast to prey capture and transport, in pharyngeal packing the hyolingual complex was slightly elevated rather then protracted at the beginning of every cycle. The contractions of the subcutaneous pharyngeal compressors were clearly recognisable. Muscle relaxation let the hyoid slowly sink to its position at the beginning of the cycle. During pharyngeal packing, the head remained permanently retracted. Pharyngeal packing was followed by swallowing. Swallowing was clearly delimitable from the pharyngeal packing cycle because the head was always elevated and the contraction of the constrictor muscles induce a very typical “bend” visible on the ventral side of the oesophagus.

Discussion:

According to Bever (2007; 2009 in press) those skull features in *S. odoratus* associated with the feeding apparatus are the most variable cranial structures, both with regard to continuous and discrete characters. One explanation for the postnatal morphological variations in the continuous characters could be an ontogenetic dietary shift (see Pritchard, 1979; Bonin et al., 2006; Bever, 2007, 2009 in press). Our morphological investigations based on ct and histological analysis show little change in the design of the hyoid complex during ontogeny. The hyoid corpus remains mainly cartilaginous even in adult musk turtles. Completely ossified hypoglossum and hyoid body are predicted in all turtles using predominantly suction feeding under water (Bramble, 1973; Van Damme and Aerts, 1997; Lemell et al., 2000; Lemell et al., 2002). A cartilaginous hyoid in chelonians is associated with a greater involvement of the tongue in feeding (Wochesländer et al., 1999; Natchev et al., 2009). *S. odoratus* utilise exclusively hydrodynamic mechanisms in prey capture and prey transport, so that one can expect a more rigid hyoid complex construction in this species. We propose that the elasticity of the hyoid body allows the common stinkpot to increase the mobility of its relatively weakly developed tongue and to ensure the efficiency of aquatic food transport and pharyngeal packing. Turtles with large and ossified hyoids can generate strong suction forces during prey ingestion, but the food transport in such species involves so-called “slow suction”. The time between two successive transport cycles is prolonged by up to half a minute and the transport gape cycles durations are up to 10 times longer than in prey capture (see Lemell et al., 2002). Some of our prey capture sequences show that the turtles actively suck up food items by expanding the oropharyngeal cavity. In two of our films the food uptake can even be described as pure “inertial suction” (sensu Van Damme and Aerts, 1997) – the neurocranium remains fully static as the prey moves into the mouth. As the gape kinematic patterns, the neck extension duration and neck extension velocity vary dramatically, we propose that *S. odoratus* adjusts its prey capture behaviour to every single feeding situation. This depends on the prey position, which the turtle mainly determines by olfactory feedback. The fish pieces used in our experiments remain under the jaws prior to initial jaw opening, indicating that the animals do not directly see the food items.

In contrast to most cryptodirans studied to date (Lauder and Prendergast, 1997; Bels et al., 1998; Summers et al., 1998; Bels et al., 2008), hyoid retraction in the common musk turtle starts shortly before or even after reaching peak gape during prey capture. This behaviour may reflect a relatively low capacity of *S. odoratus* to suction feed. The abrupt retraction of the relatively small and elastic hyoid complex cannot provide extreme suction forces like in *C. fimbriatus* for example. The common stinkpot lacks the skinny "cheeks" to each side of the mouth opening, as found in some specialised suction feeders (see Lemell et al., 2002). Accordingly, the water flow cannot be directed as precisely toward the oropharynx. The largest possible gape during the start of head protraction and the initiation of suction may increase this turtle's chances to successfully grasp the prey.

When feeding on pieces of fish, *S. odoratus* transported the food items via "intraoral-aquatic hyoid transport" (sensu Bels et al., 2008). In 53.2 % of our sequences, prey was transported by a combination of "compensatory suction" and "inertial suction" (sensu Aerts et al., 2001). The onset of hyoid retraction is coordinated with the onset of neck extension. In 46.8 % of our experiments, the turtles used pure "inertial suction". The beginning of pharyngeal expansion does not correspond to the start of the jaw open phase as predicted by Reilly and Lauder's (1990) model. Even in sequences where the slow open phase of the jaws is absent, variability is evident in the delay between hyoid retraction initiation and the start of gape increase (see Tab. 2; Figs. 3, 4). In some turtles that feed under water and possess relatively well-developed tongues, the hyoid retraction starts shortly prior to or even after reaching peak gape (see Natchev et al., 2009). The amboina box turtle *Cuora amboinensis* uses its tongue to fix the prey against the palatins during jaw opening. This enables it to hold the food items within the oral cavity even at maximal gape. The common musk turtle has a relatively weak intrinsic lingual musculature and the tongue cannot fix the prey. *S. odoratus* rely exclusively on hydrodynamic mechanisms for prey transport, so the start of hyoid retraction has to be initiated after the prey is released from the ramphothekas and before it has an opportunity to escape or float out of the oral cavity. Based on the many taste buds found on all oropharyngeal surfaces (Fig. 2 d,f), we propose that chemosensory feedback – beyond the information provided by the mechanoreceptor system – plays an important role in coordinating movements of the feeding apparatus during prey capture and transport. The highest tb concentration occurs in the anterior palate and floor of the mouth. This anterior concentration of tbs enables rapid motoneural responses ("eat it or leave it"; Heiss et al, 2008) because this is where the first contact with the prey takes place. In the natural environment, a negative response (rejection) may be crucial; the biological benefit of avoiding harmful food is self-evident (Schwenk, 1985; Berkhoudt, 1985; Berkhoudt et al., 2001, Heiss et al., 2008).

In recent years, the common musk turtle has become an increasingly popular pet in Europe (see Schilde, 2007). As this species hibernates in its natural North American habitats, these turtles would probably be able to withstand the cold periods of the central European climate and to reproduce if released into the wild. *S. odoratus* has a large offspring rate, low juvenile mortality and extremely early maturity – with 1.5 years (Mitchel, 1988). The common musk turtle is an opportunistic species with a very plastic feeding behaviour. It could very well become invasive in Europe, as was the case in *Trachemys script* in the past decades (see Arvy and Servan, 1998; Kleevein and Wöss, 2008 in press).

Acknowledgements

Dr. Christan Beisser, Richard Gemel and Dr. Patrick Lemell are acknowledged for helpful comments on the manuscript and material support. Stephan Handschuh enquired the ct -reconstructions. We would like to thank Prof. Vincent Bels and Stephan Montuelle for discussion of our work. We thank Dr. Michael Stachowitsch for scientific English proofreading. Supported by FWF, Project P20094-B17.

References:

Aerts P, Van Damme J, Herrel A. 2001. Intrinsic mechanics and control of fast cranio-cervical movements in aquatic feeding turtles. Am. Zool. 41: 1299–1310.

Alfaro ME, Herrel A. 2001. Introduction: major issues of feeding motor control in vertebrates. Am. Zool. 41:1243-1247.

Arvy C, Servan J. 1998. Imminent competition between *Trachemy scripta* and *Emys orbicularis* in France. In Fritz U (ed): Proceedings of the EMYS Symposium Dresden 96.-Martensiella, 10:33-40.

Bels VL, Renous S. 1992. Kinematics of feeding in two marine turtles (*Chelonia mydas* and *Dermochelys coriacea*). In Proc. Sixt Ord. Gen. Meet. S.E.H., Budapest I (ed Z. Korsos, J. Kiss):73-78.

Bels VL, Davenport J, Renous S. 1998. Food Ingestion in the Estuarine Turtle *Malaclemys terrapin*: Comparison with the Marine Leatherback Turtle *Dermochelys coriacea*. Mar. Biol. Ass. U.K. 78:1-20.

Bels VL, Baussart S, Davenport J, Shorten M, O`Riordan RM, Renous S, Davenport J. 2008. Functional Evolution of feeding behaviour in turtles. In Biology of turtles (ed J. Wyneken, M.H. Godfrey, V. Bels), 189-212 (chapter 8). CRC Press Taylor & Francis Group.

Berkhoudt H. 1985. Structure and function of avian taste receptors. In: King AS, McLelland J, editors. Form and Function in Birds. Vol. 3. London: Academic Press. p 463-496.

Berkhoudt H, Wilson P, Young B. 2001. Taste buds in the palatal mucosa of snakes. African. Zool. 36:185-188.

Bever GS. 2007. Comparative growth in the postnatal skull of the extant North American turtle *Pseudemys texana* (Testudinoidea: Emydidae). Acta Zoologica Stockholm 89:107–131.

Bever G S. (in press) Postnatal ontogeny of the skull in the extant North American turtle *Sternotherus odoratus* (Cryptodira; Kinosternidae). Bulletin of the American Museum of Natural History: 188 pp manuscript.

Bonin F, Devaux B, Dupre A. 2006. Turtles of the world. A& C Black London.

Bramble DM.1973. Media dependent feeding in turtles. Am. Zool.13:1342.

Deban SM, O'Reilly JC, Nishikawa KC. 2001. The evolution of the motor control of feeding in amphibians. Am. Zool. 41:1280-1298.

Ernst CH. 1986. Ecology of the Turtle, *Sternotherus odoratus*, in Southeastern Pennsylvania J. Herpetol. 20 (3):341-352.

Ernst CH, Barbour RW. 1989. Turtles of the World. USA Smithsonian Inst. Washington D.C. – London.

Heiss E, Plenk H, Weisgram J. 2008. Microanatomy of the Palatal Mucosa of the Semiaquatic Malayan Box Turtle, *Cuora amboinensis*, and Functional Implications. Anat. Rec. 291:876-885.

Gaffney ES, Meylan PA. 1988. A Phylogeny of Turtles. In The Phylogeny and Classification of the Tetrapods. Vol. 1, Amphibians, Reptilies, Birds. (ed J Benton), 157-219 Syst. Assoc. ,spec. vol. Oxford: Claredon Press.

Joyce W, Gauthier J. 2004. Paleoecology of Triassic stem turtles sheds new light on turtle origins. Proc. Roy. Soc. Lond. 271:1-5.

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol. 27:137A.

Kiernan J. 2003. Histological and histochemical methods: Theory and practice. Third edition. New York: Oxford University Press.

Kleewein A, Wöss G. 2008. Das Vorkommen von allochtonen Wasserschildkröten in Wien. ÖGH-Aktuel in print.

Lauder, GV, Prendergast T. 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. J. Exp. Biol. 164:55-78.

Lemell P, Weisgram J. 1997. Feeding Patterns of *Pelusios castaneus* (Chelonia: Pleurodira). Neth. J. Zool 47 (4):429-441.

Lemell P, Beisser CJ, Weisgram J. 2000. Morphology and Function of the Feeding Apparatus of *Pelusios castaneus* (Chelonia; Pleurodira). J. Morphol. 244:127-135.

Lemell P, Lemell C, Snelderward P, Gumpenberger M, Wocheslánder R, Weisgram J. 2002. Feeding Patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). J. Exp. Biol. 205:1495-1506.

Li C, Wu XC, Rieppel O, Wang L-T, Zhao LJ. 2008. An ancestral turtle from the Late Triassic of southwestern China. Nature 456:497-501.

Mitchell JC. 1988. Population Ecology and Life Histories of the Freshwater Turtles *Chrysemys picta* and *Sternotherus odoratus* in an Urban Lake. Herpetol. Monogr. 2:40-61.

Natchev N, Heiss E, Lemell P, Stratev D, Weisgram J. 2009. Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. Zoology.

Pitchard PCH. 1979. Encyclopedia of Turtles. TFH Publications, Inc, Neptune NJ.895 p.

Reilly SM, Lauder GV. 1990. The Evolution of the Tetrapoda Feeding Behaviour: Kinematic Homologies in Prey Transport. Evolution 44(6):1542-1557.

Rogner M. 1995. Schildkröten I. Heidi-Rogner-Verlag.

Romeis B. 1989. Mikroskopische Technik. 17th ed. (ed Böck P.). p. 97. München, Wien, Baltimore: Urban u. Schwarzenberg.

Scheyer TM, Sander PM. 2007. Terrestrial palaeoecology for basal turtles indicated by

shell bone histology. Proc. Roy. Soc. Lond. B 274: 1885–1893.

Schilde M. 2007. Die Muschus-Schildkröte Sternotherus odoratus. 2004 Natur und Tier –Verlag Gmbh.

Schwenk K. 1985. Occurrence, distribution and functional significance of taste buds in lizards. Copeia 1985:91-101.

Schwenk K 2000: Feeding: form, function and evolution in tetrapod vertebrates. Academic Press, San Diego-San Francisco-New York-Boston-London-Sydney-Tokyo.

Smith KK. 1992. The evolution of the mammalian pharynx. Zool. J. Linn. Soc. 104:313-349.

Smith KK. 1994. Are neuromotor systems conserved in evolution? Brain. Behav. Evolut. 43:293-305.

Summers AP, Darouian KF, Richmond AM, Brainerd EL. 1998. Kinematics of Aquatic and Terrestrial Prey Capture in *Terrapene carolina*, With Implications for the Evolution of Feeding in Cryptodire Turtles. J. Exp. Zool. Part A 281:280-287.

Van Damme J, Aerts P. 1997. Kinematics and functional morphology of aquatic feeding in Australian snake-necked turtles (Pleurodira; Chelodina). J. Morphol. 233:113-125.

Wocheslánder R, Hilgers H, Weisgram J. 1999. Feeding Mechanism of *Testudo hermanni boettigeri* (Chelonia, Cryptodira). Neth. J. Zool. 49 (1):1-13.

Tab. 1

Unit	kV		µm	min	
Parameter	Voltage	Projections	Voxelsize	Duration	Filter
Value	120	990	27	60	none

Table 1. Parameters used for the ct- scans. Measurements conducted at Upper Austrian University of Applied Sciences, Wels Campus using the Microfocus-tube of the device RayScan 250 XE by RayScan Technologies GmbH (Meersburg, Germany).

Tab. 2

variable	behaviour	n	AV	SD	MAX	MIN
total cycle duration (s)	capture	24	0.475	0.255	1.016	0.216
	transport	47	0.104	0.042	0.248	0.050
SO phase duration (s)	capture	18	0.362	0.247	0.846	0.126
	transport	18	0.182	0.108	0.406	0.062
FO phase duration (s)	capture	18	0.057	0.021	0.114	0.030
	transport	47	0.041	0.014	0.068	0.018
FC phase duration (s)	capture	24	0.052	0.022	0.096	0.026
	transport	47	0.059	0.032	0.192	0.022
hyoid retraction duration (s)	capture	24	0.035	0.011	0.060	0.016
	transport	47	0.037	0.017	0.084	0.014
neck extension duration (s)	capture	21	0.081	0.037	0.180	0.038
	transport	25	0.051	0.023	0.122	0.006
MG phase duration (s)	capture	24	0.023	0.011	0.040	0.008
	transport	8	0.021	0.012	0.046	0.014
hyoid retraction delay to begin of FO phase (s)	capture	18	0.061	0.025	0.134	0.022
	transport	47	0.021	0.008	0.036	0.010
hyoid retraction delay to end FO phase (s)	capture	24	0.004	0.008	0.020	-0.012
	transport	47	-0.018	0.008	-0.008	-0.042
fast jaw opening velocity (cm/s)	capture	18	4.899	1.888	10.173	2.786
	transport	47	9.276	5.548	25.329	0.483
fast jaw closing velocity (cm/s)	capture	24	10.808	4.308	21.154	5.147
	transport	47	8.384	4.268	17.425	3.467
hyoid retraction velocity (cm/s)	capture	24	9.172	3.385	18.535	4.189
	transport	47	7.874	6.397	28.000	0.600
neck extension velocity (cm/s)	capture	21	5.440	2.074	10.675	2.317
	transport	25	4.425	2.798	9.517	0.426

Table 2. Mean ± standard deviation for 26 kinematic variables from aquatic feeding events of *Sternotherus odoratus*. SO, slow open phase; FO, fast open phase; MG, maximal gape phase; FC, fast close phase; n, number of measurements; AV,

average; SD, standard deviation: MAX, maximum in the measured variable; MIN, minimum in the measured variable.

Figure legends:

Fig. 1. Points used for kinematic analyses of feeding cycles in *Sternotherus odoratus*. A, ventral margin of tympanum (jaw articulation); C, anterior tip of carapace; F, centre of mass of feeding items; H, basis of CB II on hyoid; L, anterior tip of lower jaw; N, point “zero” on the measurement board; S, dorsal margin of tympanum (dorsal-most point of squamosal); U, anterior tip of upper jaw.

Fig. 2. *Sternotherus odoratus*, morphology of head. a, ct-scan after Amira 4.1 reconstruction from ventral position with schematic illustration of hyoid complex (subadult specimens): cbI, ceratobranchiale I; cb II, ceratobranchiale II, ch, corpus hyoidei; hg, hypoglossum; lj, lower jaw; uj, upper jaw; b, ct-scan after Amira 4.1 reconstruction from ventral position (adult specimens): cbI, ceratobranchiale I; oss I, island of ossification at basis of cb I; oss II, island of ossification at basis of cb II; c, ct-scan after Amira 4.1 reconstruction, sagital section (subadult specimens); d, Scanning electron micrograph at medium magnification showing three adjoining taste buds (tb). tbs are recognized by their taste pores containing large microvillae (indicated by arrows); e, Light micrograph cross-section of tongue of a subadult *S. odoratus* slighly anterior to the glottis. Note the cartilaginous hyoidal “support” elements and the weakly developed intrinsic musculature: hg, hypoglossum; pl, processus lingualis; tim, tongue intrinsic musculature; f, Light micrograph showing in detail a cross-section of a typical taste bud (tb) of *S. odoratus* from the anterior floor of the mouth.

Fig. 3. Kinematic profiles from an aquatic feeing event in *S. odoratus*, including one prey capture, three prey transport, six pharyngeal packing and two swallowing cycles (based on cinematography – 500 fr/s): protr., protraction; retr., retraction; dors., dorsal; ventr., ventral.

Fig. 4. Kinematic profiles from aquatic feeing events in *S. odoratus* based on cinematography (500 fr/s), representing the time difference between the initiation of hyoid retraction and reaching peak gape: a, prey capture - the hyoid retraction starts prior to reaching peak gape; b, prey capture - hyoid retraction starts almost simultaneously with reaching peak gape; c, prey capture event - hyoid retraction starts during maximal gape phase (MG-phase); d, prey transport - minimal difference between onset of hyoid retraction and peak gape; e, prey transport - average time difference between onset of hyoid retraction and peak gape; f., prey transport - maximal time difference between onset of hyoid retraction and peak gape.

Fig. 1

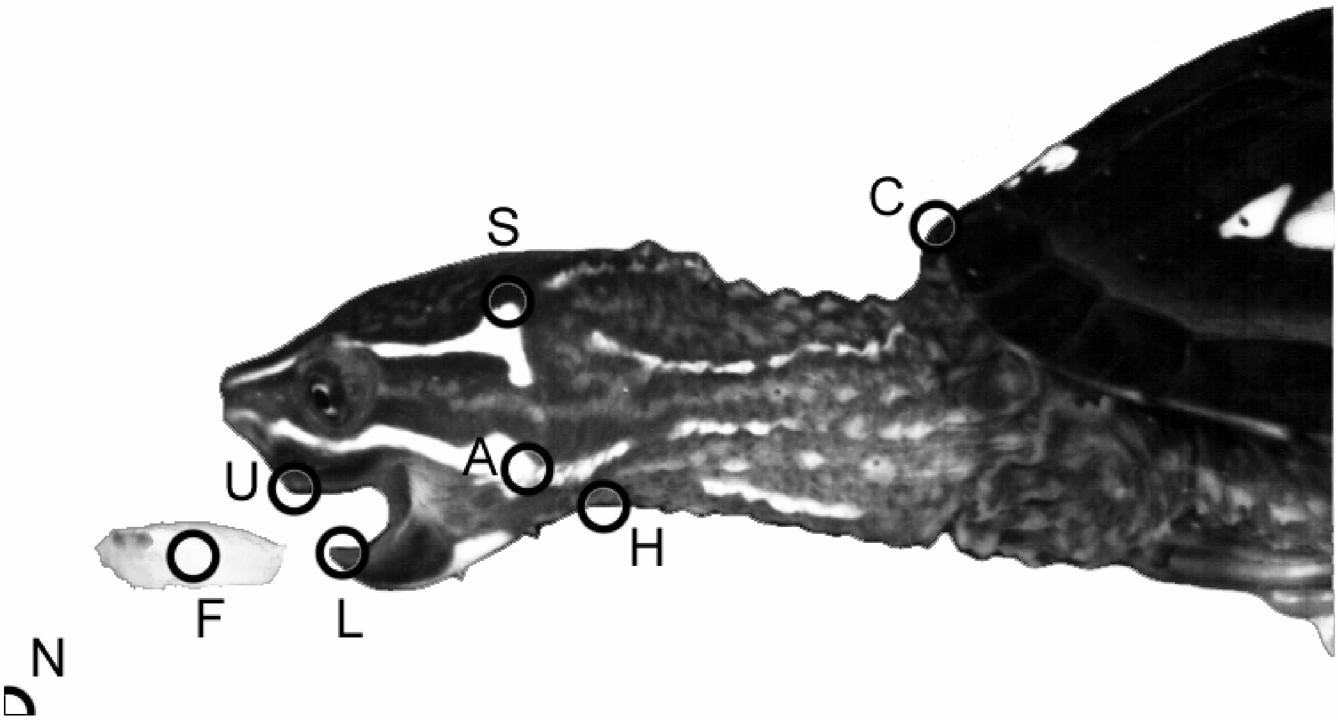


Fig. 2

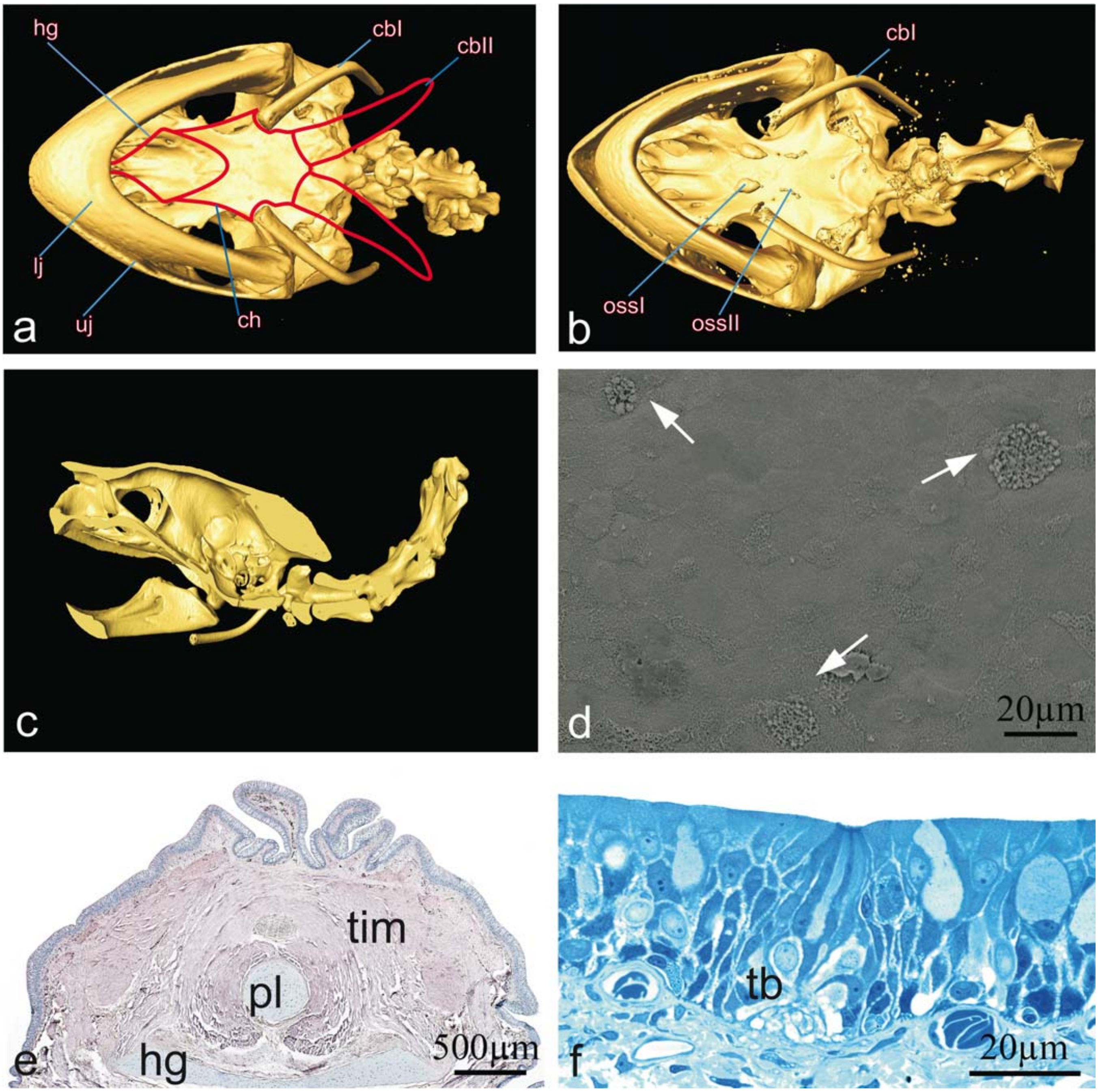

hg
cbI
cbII
lj
uj
ch
a
cbI
ossI
ossII
b
c
20µm
d
tim
pl
hg
500µm
e
tb
20µm
f

Fig. 3

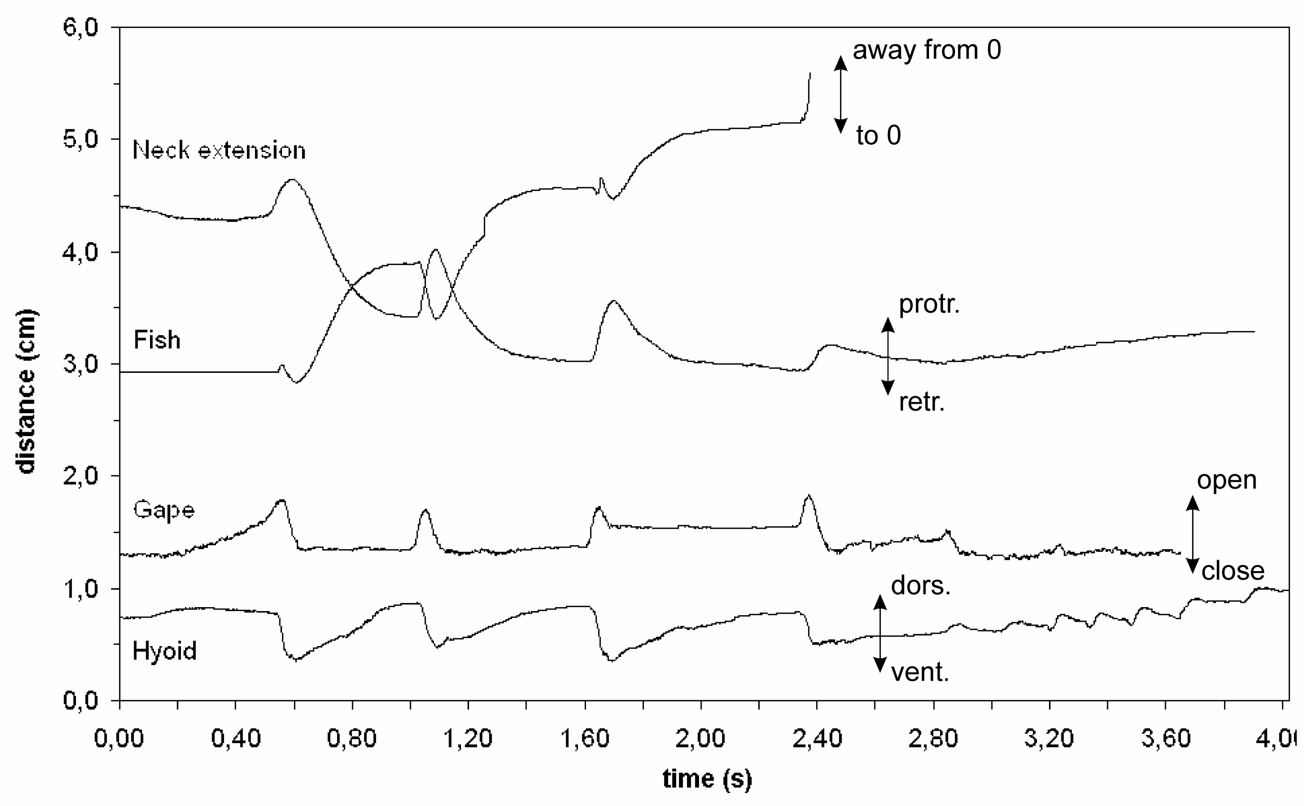
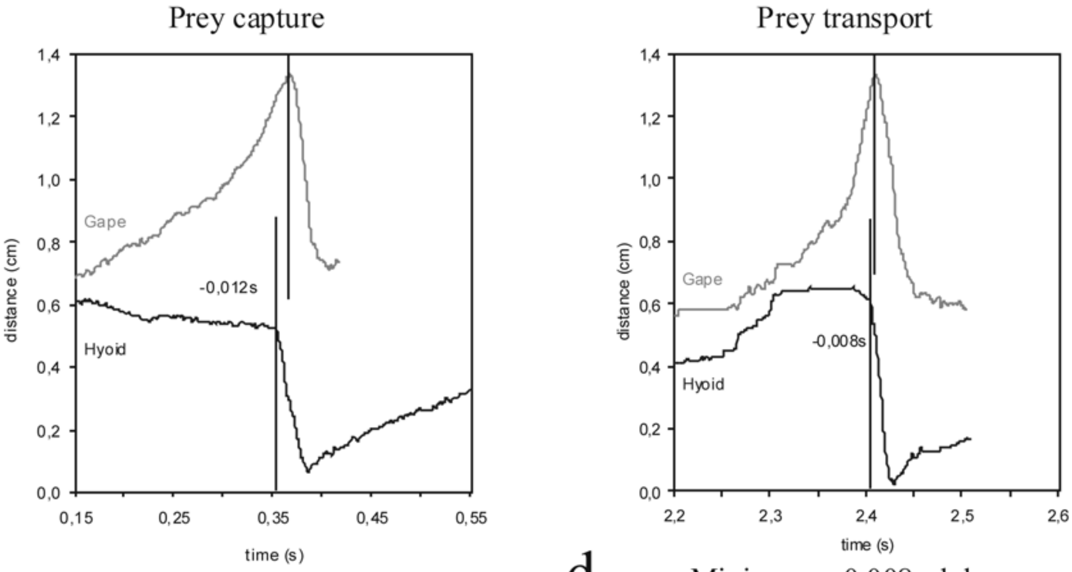
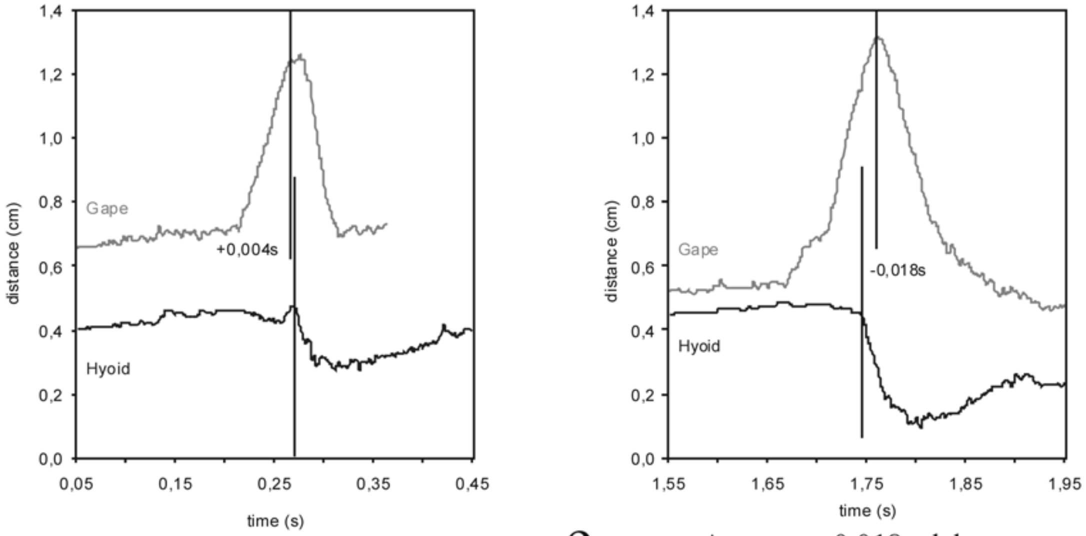

6,0
5,0
4,0
3,0
2,0
1,0
0,0
distance (cm)
0,00
0,40
0,80
1,20
1,60
2,00
2,40
2,80
3,20
3,60
4,0
time (s)
Neck extension
Fish
Gape
Hyoid
away from 0
to 0
protr.
retr.
open
close
dors.
vent.

Fig. 4



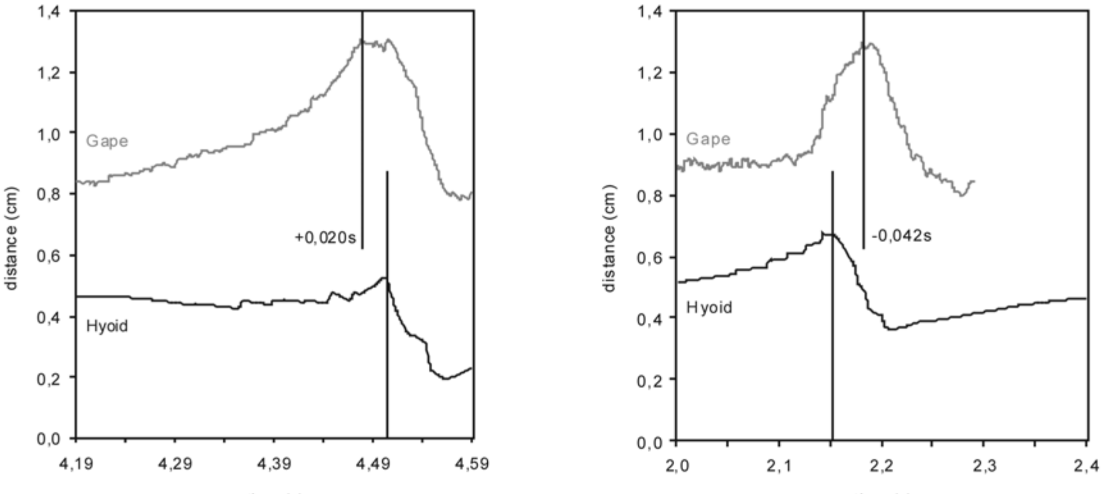
a Minimum: -0,012s delay

d Minimum: -0,008s delay



b Average: 0,004s delay

e Average: -0,018s delay



c Maximum: 0,020s delay

f Maximum: -0,042s delay

III. Schlussfolgerung

III .1. Die Morphologie des Fressapparates in *Cuora sp.*

„Egal wie elegant, kompliziert und zuverlässig ein Experiment über Nahrungsaufnahme bei Tetrapoden konzipiert wird, es sollte immer von einer sorgfältigen anatomischen Untersuchung begleitet sein“ (De Vree und Gans,1989). Die meisten Studien die sich mit „feeding“ in Schildkröten beschäftigen beinhalten anatomische Deskriptionen wie auch kinematische Analysen (Van Damme & Aerts, 1997; Lemell & Weisgram, 1997; Wocheländer et al., 1999; Lemell et al., 2000; Lemell et al., 2002). Diese Arbeiten liefern Informationen über Bau und Funktion, der beim Fressen beteiligten Kopfelemente. Die einzigen zwei Publikationen über Nahrungserwerb von Schildkröten, die in beiden Medien fressen können (Bels et al., 1997; Summers et al., 1998) beinhalten leider keine morphologische Beschreibungen, oder Analysen über Form und Funktion.

Lemell et al. (2000) postulieren, dass die relative Entwicklung der Zunge und die Entwicklung des Hyoid – Apparates bei Schildkröten sich in einer inversen Relation zueinander befinden. Für hoch spezialisierte Arten ist diese Regel eigentlich immer gültig. Die morphologischen Untersuchungen bei *C. flavomarginata*, *C. amboinensis* und *C. galbinifrons* haben gezeigt, dass dies bei Generalisten nicht immer der Fall ist. Der Bau des Fressapparates korreliert eng mit der Ökologie. *C. flavomarginata* besitzt eine gut entwickelte Zunge und einen relativ kleinen und fast vollständig knorpeligen Hyoidkörper. Der terrestrische Nahrungstransport ist bei dieser Schildkröte sehr effizient im Gegensatz zu *C. amboinensis,* bei der die Zunge etwas kleiner, aber das Hyoid kräftiger gebildet ist. Trotz ihrer amphibischen Ernährungsweise, ist die Amboina Schildkröte sehr gut an das aquatische Fressen angepasst. „Prey capture“ und „prey transport“ unter Wasser sind bei dieser Art schneller als bei den meisten rein aquatischen Cryptodiren. Das Fressverhalten in *C. amboinensis* ist ein einzigartiges Beispiel dafür, dass die Entwicklung von Merkmalen die den Nahrungserwerb an Land begünstigt, nicht unbedingt die aquatische „feeding performance“ einschränken muss. Das ist aber nicht der Fall bei *C. galbinifrons*. Diese, überwiegend an terrestrisches Leben angepasste Art, hat die Fähigkeit unter Wasser zu fressen im Laufe ihrer Entwicklung zum Landtier nicht verloren. Die Zunge ist besser entwickelt als bei *C. flavomarginata*, doch die Hyoidkörperkostruktion ähnelt der von *C. amboinensis*. Aquatischer Transport grosser Beute erfolgt nicht mehr durch hydraulische Mechanismen und ist im Vergleich zu *C. amboinensis* verlangsamt und erschwert.

Die funktionell-morphologische Analyse bei hier untersuchten *Cuora* Arten demonstriert, dass relativ kleine Unterschiede in der Form des Fressapparates, fundamentale Auswirkungen auf dessen Funktion haben können.

III.2. Analyse der kinematischen Daten

Viele Untersuchungen (zur Übersicht siehe Smith, 1994; Schwenk, 2000; Alfaro & Herrel, 2001; Schaerleaken et al., 2007, 2008; Bels et al., 2008) über „prey capture“ und „prey transport“ basieren auf der Vermutung, dass die Kinematik dieser Prozesse von der Basis her stereotyp und uniform ist. Die kinematischen Muster des Beuteerwerbs und des Transportes werden bei vielen funktionellen und evolutionären Studien über Tetrapoden als „Homologie-Indikatoren“ benutzt (siehe Bramble & Wake, 1985; Reilly & Lauder, 1989; Wainwright et al., 1989; Bels & Goose, 1990; Reilly & Lauder, 1990; Reilly & Lauder, 1991; Bels & Delheusy, 1992; Lauder & Reilly, 1994; Gillis & Lauder, 1994; Reilly, 1995; Reilly, 1996; Bels et al., 1997; Bels et al., 1998; McBrayer & Reilly, 2002; Bels et al., 2008). Laut Reilly & Lauder (1990) bleiben die verschiedenen Phasen des Fresszyklus so stabil in der Evolution erhalten, dass sie bei verschiedenen Tiergruppen direkt verglichen werden können. In einem verallgemeinerten Modell homologisieren diese Autoren die sogenannte „recovery“ und „ preperatory“ Phasen zu den „slow open I und II“ (SO I und SO II) des GFC Models entwickelt von Bramble und Wake (1985).

Die Annahme, dass die Fresskinematik vorprogrammiert ist, erlaubt Rückschlusse über die Evolution von ganzen „Hauptphasen“ wie „prey capture“ und „prey transport“. In seiner Studie über *Salamandra salamandra*, leitet Reilly (1996) die Fresszyklen von einander in folgender Reihenfolge ab: terrestrischer „prey transport“ ist von aquatischem Fressverhalten evoluiert und ausgehend von diesem terrestrischen „prey transport“ hat sich die terrestrische „prey capture“ entwickelt. Die Hypothese, dass die Neuromotorik des Fressapparates evolutionär konservativ erhalten wird, ist untermauert durch die extreme Uniformität der Dauer- und Abstands- Messungen in vielen Studien. Extrem klein Zeitunterschiede werden z. B. von Reilly (1995) für *S. salamandra* berichtet: für N = 40, „gape cycle time“ = 41.0 ± 1.1 ms; „time to maximum hyoid depression“ = 24.7 ± 0.7 etc. Diese Zahlen hinterlassen den Eindruck, dass die Nahrungsaufnahme der Tetrapoden ein sich immer gleich wiederholender, nicht variabler Prozess ist.

Durch statistische Analysen ist auch der Einfluss verschiedener Faktoren (wie z.B. „Individualismus“, „Lebensalter“, „prey type“ etc.) getestet worden. Die Ergebnisse solcher Analysen sind oft sehr überraschend, Reilly & Lauder (1989) berichten, dass die Fresskinematik in *Ambystoma mexicanum* hoch stereotyp sei und sich bezüglich verschiedener Beuteobjekte nicht ändert. Die verschiedenen Individuen zeigen im Vergleich zueinander verschiedene Motorik, aber diese Motorik bleibt beim Fressen von verschiedenen Beutetypen (z.B. Guppies und Regenwürmer) immer genau gleich. Reilly (1995) faßt zusammen, dass Fressen bei Salamandern generell hoch stereotyp (“hard-wired”) ist und die Kinematik durch die ontogenetische Entwicklung unverändert bleibt. Laut Reilly & Lauder (1990) ist die Kinematik von Transport und “ingestion” bei aquatischen Salamandern sehr ähnlich zueinander und auch ähnlich zu den entsprechenden Vorgängen bei Actinopterygia und Lungenfischen. Auf dieser Basis ziehen diese Autoren den Schluss, dass manche Elemente des Fressvorgangs bei allen Tetrapoda-Gruppen unverändert bleiben. Aus so einer Feststellungskaskade, könnte man am Ende schließen, dass der Fressmechanismus der Schildkröten gleich dem Fressmechanismus der z.B. Lungenfische funktioniert und diese Ähnlichkeit durch die extrem rigide Methodik der Varianzanalyse fest bewiesen ist. Rein morphologisch betrachtet ist aber eine „Kinematikhomologisierung“ zwischen verschiedenen Tetrapodenklassen überhaupt nicht möglich, da nicht immer die gleichen anatomischen Strukturen im Fressvorgang involviert sind.

Trotzdem ist die Frage sehr interessant, wieso die Fresskinematik innerhalb der Tetrapoden so uniform zu sein scheint. Eine mögliche Erklärung könnte die enge Artenauswahl bei vielen Untersuchungen liefern (siehe Deban et al., 2001). Ökologisch hoch spezialisierte Arten, wie auch Tiere die sehr schnelle Fresszyklen aufweisen, wie z.B. *Chelydra serpentina* (siehe Lauder und Prendergast, 1992), verwenden wahrscheinlich „feed – forward“ kontrollierte Fressmechanismen die nicht sehr variabel sind. Also auf der Basis von Messungen an „spezialisierten“ Tieren, werden Schlussfolgerungen über gesamte Tierklassen gezogen, obwohl die meisten Vertreter dieser Klassen eigentlich eine opportunistische Lebensweise mit großer Vielfalt haben.

Auch methodisch sind viele Analysen von schnellem Fressverhalten nicht frei von Fehlerquellen. Die meisten Untersuchungen an Urodelen und manche

Untersuchungen an Echsen und Schildkröten z.B. (für Publikationsliste siehe oben), wurden anhand von Videos bei 200 fr/s gemacht. In *S. salamandra* wurde eine „gape cycle time“ von 41.0 ± 1.1 ms (Reilly 1995) gemessen. Laut Reilly & Lauder (1990) ist der Fresszyklus der niederen Tetrapoden in 4 (oder 5) Phasen unterteilt. Das bedeutet praktisch, dass von jeder dieser Phasen nicht einmal zwei Bilder am Film zu sehen sein werden. Das macht die Markierung der Phasengrenzen hoch subjektiv, extrem schwierig und beeinflusst die Genauigkeit der Messergebnisse äußerst negativ. Die Bildfolgen bei 200 f/s sind in 5 ms Schritten - dass macht die Messung der Dauer sehr kurzer Phasen wie z.B. „fast open“ und „fast close“ sehr ungenau. Für eine Phase die unter 20 ms dauert ist der Messfehler über 50%.

Bei den Transportkinematik-Analysen von McBreyer & Reilly (2002) ist jedes zweite Bild der Filmsequenzen mit 200 fr/s digitalisiert, also in Schritten von 10 ms. Die Erklärung von McBreyer & Reilly (2002) ist, dass innerhalb von 20 ms keine aktive Bewegung möglich ist. Auch wenn diese Behauptung axiomatisch akzeptiert werden könnte, bliebe die Frage wie man dann die Grenzen zwischen den verschiedenen Phasen bestimmen kann und ob die gewonnenen Messergebnisse für eine Varianzanalyse überhaupt korrekt genug sind.

In Rahmen diese Dissertation wurden high-speed Filme mit 250 fr/s und 500 fr/s analysiert, wobei die Digitalisierung aller gemessenen Punkte Bild für Bild erfolgte. Da sehr kurze Phasen, wie z.B. die „maximum gape (MG) – Phasen“, eine Dauer von lediglich 12ms bis 77ms haben, war eine statistische Bearbeitung der gewonnenen Daten nur begrenzt möglich. Ein weiterer Grund für die Vernachlässigung statistischer Methoden ist die teilweise hohe Inhomogenität der gemessenen Werte und die Höhe der Standardabweichungen. Die motorische Variabilität bei den hier untersuchten Schildkröten kann durch ihre opportunistische Fressökologie erklärt werden. Bei allen vier Arten ist die Nahrungsaufnahme und der Transport permanent unter sensomotorischer „feed - back“ Kontrolle (siehe Deban et al., 2001). Die erhöhte Modulationskapazität der Fresssysteme der untersuchten Schildkröten, macht sie ungeeignet zum Testen der verallgemeinerten kinematischen Modelle von Bramble & Wake (1985) und von Reilly & Lauder (1990). Die funktionell - morphologische Analyse der Fressmechanismen der drei *Cuora* - Arten und dem von *S. odoratus* liefert aber wichtige Information über die Evolution der Habitatpräferenzen innerhalb der cryptodiren Schildkröten.

III.3. Hypothesen über die Evolution terrestrischen Fressens bei Cheloniern

Unsere Ergebnisse demonstrieren, dass terrestrische Nahrungsaufnahme mindestens dreimal „de novo“ unabhängig innerhalb der rezenten Chelonier entstanden ist. Die terrestrische Fresskinematik der Geoemydiden, Emydiden und Testudiniden zeigt gravierende Unterschiede. Alle bis jetzt untersuchten Geoemydiden und Emydiden benutzen „jaw prehension“ an Land. Nachdem der Ancestor der Testudiniden aquatisch war (siehe Joyce und Gauthier, 2004) ist für den basalen Vertreter dieser Unterfamilie „jaw prehension“ zu vermuten. „Lingual prehension“ und Herbivorie sind sekundär innerhalb dieses Taxons entstanden. Zukünftige kinematische Untersuchung an den primitivsten rezenten Vertretern der Testudinden, *Manauria sp.* und *Rhynoclemys sp*. (siehe Le et al., 2006 für phylogenetische Übersicht) werden diese Theorie überprüfen.

Die morphologischen und kinematischen Untersuchungen an *S. odoratus* demonstrieren, dass diese aquatischen Schildkröten problemlos an Land ihre Nahrung schnappen können, die kritische Fressphase ist die des erste

Transportzyklus. Die Zunge ist zu klein und nicht beweglich genug um die Beute während des „jaw opening“ gegen den Gaumen zu fixieren. Die Unfähigkeit zum terrestrischen Nahrungstransport ist eine wichtige Einschränkung dieser Tiere um von den aquatischen Lebensräumen unabhängig zu werden.

Bis jetzt ist keine Information vorhanden, ob Testudinidae überhaupt versuchen unter Wasser zu fressen. Innerhalb der rezenten Schildkröten existieren keine Arten die sekundär aquatischen Nahrungserwerb entwickelt haben. In diesem Kontext ist zu vermuten, dass die Schildkröten, die rein terrestrische Lebensweise aufweisen, nicht in der Lage sind ihre Nahrung unter Wasser zu erwerben, da die Vergrößerung des Zungenvolumes eine negative Auswirkung auf die Saugfähigkeit hat (Lemell et al., 2000). Es ist zu erwarten, dass manche aquatische Schildkröten es im Rahmen ihrer evolutionären Entwicklung geschafft haben an Land den Fressvorgang zu vollenden. Rein terrestrische Testudiniden hingegen haben offensichtlich den "point of no return" zu einer aquatischen Lebensweise bereits überschritten. Die Evolution terrestrischer Nahrungsaufnahme ist demzufolge eine Einbahnstrasse.

C. galbinifrons weist, was die Morphologie der Zunge betrifft, viele gemeinsame Merkmale mit den Testudiniden auf. Unter Wasser, erfolgt der Transport von kleineren Beuteobjekten durch Verwendung hydrodynamischer Mechanismen, doch große, schwere Zophobaslarven werden mit Hilfe der voluminösen Zunge transportiert (eigentlich eine terrestrische Strategie). Interessanterweise ist die Verwendung der Zunge für aquatischen Nahrungstransport auch bei anderen Schildkröten nachgewiesen. Bels et al. (1998) beschreiben „tongue based transport“ unter Wasser bei *Dermochelys coriacea* und *Malaclemys terrapin*. Wenn die Vorfahren der Schildkröten terrestrisch lebten kann vermutet werden, dass die Beteiligung der Zunge am Nahrungstransport (aquatisch oder terrestrisch) unter den rezente Gruppen ein altes „Erbe“ von den gemeinsamen Ancestoren darstellt. Bels et al. (2008) haben ein Zungentransport-Modell vorgeschlagen, das allgemein für alle rezente Schildkröten gültig sein soll. Laut diesem Modell, ist der Fresszyklus in vier Phasen geteilt: „slow open I (SO I)“, slow open II (SO II)“, „fast open (FO)“ und „fast close (FC)“. Die Hyoidprotraktion soll früh während "SO I" erfolgen und die Retraktion startet am Ende von „SO II“. Dieses Modell entspricht genau dem „generalysed feeding cyclic model (GFCM)“ von Bramble & Wake (1985). Somit versetzen uns kinematische Analysen des Nahrungstransportes rezenter Schildkröten in die Lage, Rückschlüsse über den Nahrungs-Transport des gemeinsamen Schildkröten-Ancestors (oder sogar über die Transportkinematik des Ancestors aller Tetrapoden – so es einen solchen je gab) zu ziehen. Unsere Untersuchungen widerlegen diese Vorstellung. Da *D. coriacea* (Dermochelidae), *M. terrapin* (Emydidae), alle Testudinidae und *C. galbinifrons* (Geoemydidae) keine eng verwandten Arten sind, erscheint die „Verteilung“ des Zungentransportes innerhalb der rezenten Chelonia sehr „lückenhaft“. Innerhalb der Pleurodira gibt es keine Beteiligung der Zunge beim Transport der Nahrung (Van Damme & Aerts, 1997; Lemell & Weisgram, 1997; Lemell et al., 2002). Nachdem die Gattung *Cuora sp.* ancestral aquatisch ist (siehe Einleitung I), wird im Rahmen diese Studie vermutet, dass die Beteiligung der Zunge beim Unterwasserfressen in *C. galbinifrons* eine aberrante und isolierte Situation repräsentiert.

Bleibt die Frage offen, wieso manche Schildkröten, die nicht an Land fressen, doch gut entwickelte Zungen besitzen. Eine mögliche Erklärung wäre, dass die Zunge bei den meisten Cheloniern für aquatische „prey capture“ und „prey transport“ keine wichtige Rolle spielt, für „pharyngeal packing“ und „swallowing“ aber eingesetzt werden kann. Bei Arten, die nur linguale Rudimente besitzen sind diese wichtigen Fressphasen offensichtlich erschwert. Bei *C. fimbriatus* wird die Rolle der Zunge bei „pharyngeal packing“ vom ganzen Hyoidkomplex übernommen. Die Matamata braucht um 80 ms für „prey capture“, aber mehrere Minuten für „swallowing“ (siehe Lemell et al., 2002).

Interessanterweise ist die „ingestion“ Kinematik in *C. fimbriatus* hochgradig stereotyp, die „prey transport“ Motorik ist hingegen sehr variabel (siehe Lemell et al., 2002). Es gibt leider keine Information über den „prey transport“ bei *C. serpentina*, doch der „strike“ bei diesem „ram-feeder“ ist auch sehr schnell und die Kinematik ist extrem uniform (Lauder und Prendergast, 1992). Für Beutefang mit dieser Geschwindigkeit, egal ob durch „compensatory suction“ oder „inertial suction“, sind hoch koordinierte Kiefer- und Zungenbeinbewegungen nötig. Angesicht der kurzen Dauer ist es möglich, dass bei *C. fimbriatus* und *C. serpentina* der Beutefang unter neuromotorischer „feed forward“ Kontrolle steht. Doch ist das Motorprogramm bei den beiden Arten nicht „homolog“, da die kinematischen Muster sich grundsätzlich voneinander unterscheiden.

Der aquatische Beuteerwerb in *C. amboinensis* und besonders in *C. galbinifrons* ist langsamer als bei den beiden oben erwähnten, hoch spezialisierten, räuberischen Arten. Die Kinematik ist ziemlich variabel, da „sensorimotor feedback“ entscheidende Rolle bei der Motorkontrolle spielt. Der evolutionäre „shift“ innerhalb der Gattung *Cuora sp.* (oder zu mindest für die, im Rahmen dieser Studie untersuchten Arten), scheint von spezialisierten aquatischen Tieren zum semi-aquatischen „generalist“ gelaufen zu sein. Der ancestrale Nahrungsaufnahmemodus dieser Gattung war wahrscheinlich „compensatory suction“ unter „feed forward“ Motorkontrolle. Der Nahrungserwerbsapparat hat sich von einem „optimisierten“ aquatischem zu einem „opportunistischen“ aqua-terrestrischem System entwickelt, bei welchem sehr variable Nahrungsaufnahmemethoden angewandt werden können.

IV. Literatur

Aerts P, Van Damme J, Herrel A. 2001. Intrinsic mechanics and control of fast cranio-cervical movements in aquatic feeding turtles. Am. Zool. 41: 1299–1310.

Ahlberg P, Clack J, Blom H. 2005. The axial skeleton of the Devonian tetrapod *Ichthyostega* : The chimpanzee genome. Nature. 437:137-140.

Alexander McN. 1970. Mechanics of the feeding action of various teleost fishes. J. Zool. London 162:145156.

Alfaro ME, Herrel A. 2001. Introduction: major issues of feeding motor control in vertebrates. Am. Zool. 41:1243-1247.

Alfaro M. 2002. Forward attack modes of aquatic feeding garter snakes. Funct. Ecol. 16:204-215.

Alfaro M. 2003. Sweeping and striking: a kinematic study of the trunk during prey capture in three thamnophiine snakes. J. Exp. Biol. 206:2381-2392

Anderson C. 1993. The modulation of feeding behaviorin response to prey type in the frog *Rana pipiens*. J. Exp. Biol. 179:1-12.

Arvy C, Servan J, 1998. Imminent competition between *Trachemy scripta* and *Emys orbicularis* in France. In Fritz U (ed): Proceedings of the EMYS Symposium Dresden 96.-Martensiella, 10:33-40.

Bagatto B, Guyer C, Hauge B, Henry R. 1997. Bimodal Respiration in Two Species of Central American Turtles. Copeia. 4:834-839.

Beisser C, Weisgram J, Splechtna H. 1995. The dorsal lingual epithelium of *Platemys pallidipectoris* (Pleurodira, Chelidae). J. Morph. 226:267-276.

Beisser C, Weisgram J, Hilgers H, Splechtna H. 1998. Fine structure of the dorsal lingual epithelium of *Trachemys scripta elegans* (Chelonia: Emydidae). Anat. Rec. 250:127-135

Beisser C, Weisgram J. 2001. Dorsal tongue morphology and lingual glands in Chelonians. J. Morph. 248:205 (abstract).

Beisser C, Lemell P, Weisgram J. 2001. Light and electron microscopy of the tongue of *Pelusios castaneus* (Chelonia: Pleurodira). Tissue and Cell 33:63-71.

Beisser C, Lemell P, Weisgram J, 2004. The dorsal lingual epithelium of *Rhinoclemmys pulcherrima incisia* (Chelonia, Cryptodira). Anat. Rec. 277A:227-235.

Bell D. 1990. Kinematics of prey capture in the chameleon. Zool. Jb. Anat. 119:313-336.

Bels V, Baltus I. 1988. The influence of food items on the feeding cycle in *Anolis equestris* (Reptilia: Iguanidae). Copeia 2:479-481.

Bels V. 1990. Quantitative analysis of prey-capture kinematics in *Anolis equestris* (Reptilia: Iguanidae). Can. J. Zool. 68:2192-2198.

Bels VL, Renous S. 1992. Kinematics of feeding in two marine turtles (*Chelonia mydas* and *Dermochelys coriacea*). In Proc. Sixt Ord. Gen. Meet. S.E.H., Budapest I (ed Z. Korsos, J. Kiss):73-78.

Bels V, Goosse V. 1990. Comparative kinematic analysis of prey capture in *Anolis carolinensis* (Iguania) and *Lacerta viridis* (Scleroglossa). J. Exp. Zool. 255:120-124.

Bels V, Renous S. 1992. Kinematics of feeding in two marine turtles (*Chelonia mydas* and *Dermochelys coriacea*). Proc of the 6th Ordinary Meeting of the Soc. Eur. Herp. Hung. Nat. Hist. Mus. pp 73-78.

Bels V, Delheusy V. 1992. Kinematics of prey capture iniguanid lizards: comparison between *Anolis equestris* (Anolinae) and *Oplurus cuvieri* (Oplurinae). Belg. J. Zool.122:223-234.

Bels V, Davenport J, Delheusy V. 1997. Kinematic analysis of the feeding behaviour in the box turtle *Terrapene carolina* (L.), (Reptilia: Emydidae). J. Exp. Zool. 277:198–212.

Bels VL, Davenport J, Renous S. 1998. Food Ingestion in the Estuarine Turtle *Malaclemys terrapin*: Comparison with the Marine Leatherback Turtle *Dermochelys coriacea*. Mar. Biol. Ass. U.K. 78:1-20.

Bels VL, Baussart S, Davenport J, Shorten M, O`Riordan RM, Renous S, Davenport J. 2008. Functional Evolution of feeding behaviour in turtles. In Biology of turtles (ed J. Wyneken, M.H. Godfrey, V. Bels), 189-212 (chapter 8). CRC Press Taylor & Francis Group.

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

Benton M. 2006. Vertebrate palaeontology. Publisher Blackwell Publishing, Oxford.

Berkhoudt H. 1985. Structure and function of avian taste receptors. In: King AS, McLelland J, editors. Form and Function in Birds. Vol. 3. London: Academic Press. p 463-496.

Berkhoudt H, Wilson P, Young B. 2001. Taste buds in the palatal mucosa of snakes. African Zool 36:185-188.

Bever GS. 2007. Comparative growth in the postnatal skull of the extant North American turtle *Pseudemys texana* (Testudinoidea: Emydidae). Acta Zoologica Stockholm 89:107–131.

Bever G S. (in press) Postnatal ontogeny of the skull in the exfant North American turtle *Sternotherus odoratus* (Cryptodira; Kinosternidae). Bulletin of the American Museum of Natural History: 188 pp manuscript.

Bonin F, Devaux B, Dupre A. 2006. Turtles of the world. A& C Black London.

Bramble DM. 1973. Media dependent feeding in turtles. Am. Zool.13:1342.

Bramble DM. 1974. Occurrence and Significance of the Os transiliens in Gopher Tortoises. Copeia 74:102-108.

Bramble D. 1978. Functional analysis of underwater feeding in the snapping turtle. Am. Zool. 18:623.

Bramble D, Wake D. 1985. Feeding Mechanisms of Lower Tetrapods. In (eds Hildebrand M, Bramble D, Liem K, Wake D.) Functional Vertebrate Morphology. Belknap Press of Harvard University, Cambridge, Massachusetts, pp 230–261.

Clark N, Gordos M, Franklin C. 2008. Diving behaviour, aquatic respiration and blood respiratory properties: a comparison of hatchling and juvenile Australian turtles. J. Zool. 275: 399-406.

Claude J, Paradis E, Tong H, Auffray J. 2003. A geometric morphometric assessment of the effects of the environment and cladogenesis on the evolution of the turtle shell. Biol. J. Linn. Soc. 79:585–501.

Claude J, Pritchard P, Tong H, Paradis E, Auffray C. 2004. Ecological correlates and evolutionary divergence in the skull of turtles: a geometric morphometric assessment. Syst. Biol. 53:933–948.

Condon K. 1987. A kinematic analysis of mesokinesis in the Nile monitor (*Varanus niloticus*). Exp. Biol. 47:73-87.

Cundall D, Greene, H. 2000. Feeding in snakes. In (ed. Schwenk K.) Feeding: Form, function, and evolution in tetrapod vertebrates, pp 293-333. San Diego: Academic Press.

Daghfous G, Libourel P, Reveret L, Bels V. 2008. Best of both worlds: Prey capture behaviour in banded watersnake (*Nerodia fasciata*). Comp. Bioch. Physiol. Part A 150:96 (abstract).

Daeschler E, Shubin N, Jenkins, F. 2006. A Devonian tetrapod-like fish and the evolution of the tetrapod body plan. Nature 404:758-764.

De Vree F, Gans C. 1989. Funktional morphology of the feeding mechanisms in lower tetrapods. Fortschritte der Zoologie 35:115-127.

Deban S.1997. Modulation of prey-capture behaviorin the plethodontid salamander *Ensatina eschscholtzii*. J. Exp. Biol. 200:1951–1964.

Deban SM, O'Reilly JC, Nishikawa KC. 2001. The evolution of the motor control of feeding in amphibians. Am. Zool. 41:1280-1298.

Deban S, Olson W. 2002. Suction feeding by a tiny predatory tadpole. Nature 420:41-42.

De Braga M, Rieppel O. 1997. Reptile phylogeny and the interrelationships of turtles. Zool. J. Linn. Soc. 120: 281-354.

Dunson W. 1960. Aquatic Respiration in *Trionyx spinifer asper*. Herpetologica. 16:277-283.

Elias J, McBrayer L, Reilly M. 2000. Prey transport kinematics in *Tupinambis teguixin* and *Varanus exanthematicus*: conservation of feeding behaviour in “chemosensory-tongued” lizards. J. Exp. Biol. 203:791-801.

Ernst CH. 1986. Ecology of the Turtle, *Sternotherus odoratus*, in Southeastern Pennsylvania J. Herpetol. 20 (3):341-352.

Ernst CH, Barbour RW. 1989. Turtles of the World. USA Smithsonian Inst. Washington D.C. – London.

Gaffney ES, Meylan PA. 1988. A Phylogeny of Turtles. In The Philogeny and Classification of the Tetrapods. Vol. 1, Amphibians, Reptilies, Birds. (ed Benton J), 157-219 Syst. Assoc. ,spec. vol. Oxford: Claredon Press.

Gaffney E. 1990. The comparative osteology of the Triassic turtle *Proganochelys*. Bulletin of the American museum of natural history 194 New York.

Gaffney E, Tong H, Meylan P. 2006. Evolution of the side-necked turtles: the families Bothremydidae, Euraxemydidae, and Araripemydidae. Bull. Am. Mus. Nat. Hist. 300:1-698.

Gage SH, Gage SP. 1886. Aquatic respiration in soft-shelled turtles: a contribution to the physiology of respiration in vertebrates. Amer. Natur. 20:233-236.

Gans C. 1969. Comments on inertial feeding. Copeia 1969:855–857.

Gillis G, Lauder G. 1994. Aquatic prey transport and the comparative kinematics of *Ambystoma tigrinum* feeding behaviors. J. Exp. Biol. 187:159-179.

Girgis S. 1961. Aquatic respiration in the common Nile turtle, *Trionyx triunguis* (Forskal). Comp. Biochem. Physiol. 3:206-217.

Goosse V, Bels V. 1992. Kinematic and functional analysis of feeding behaviour in *Lacerta viridis* (Reptilia: Lacertidae). Zool. Jb. Anat. 122:187-202.

Gordos M, Franklin C. 2002. Diving behaviour of two Australian bimodally respiring turtles, *Rheodytes leukops* and *Emydura macquarii*, in a natural setting. J. Zool. 258:335-342.

Heiss E, Plenk H, Weisgram J. 2008. Microanatomy of the Palatal Mucosa of the Semiaquatic Malayan Box Turtle, *Cuora amboinensis*, and Functional Implications. Anat. Rec. 291:876-885.

Hedges S, Poling L. 1999. A molecular phylogeny of reptiles. Science 283:998-1001.

Herrel A, Cleuren J, De Vree F. 1995. Prey capture in the lizard *Agama stellio*. J. Morphol. 224:313-329.

Herrel A, Cleuren J, De Vree F. 1996. Kinematics of feeding in the lizard *Agama stelio*. J. Exp. Biol. 199:1727-1742.

Herrel A, De Vree F. 1999. Kinematics of intraoral transport and swallowing in the herbivorous lizard *Uromastix acanthinurus*. J. Exp. Biol. 202:1127-1137.

Herrel A, Meyers J, Nishikawa K, De Vree F. 2001a. The evolution of feeding motor patterns in lizards: modulatory complexity and constraints. Am. Zool. 4:1311-1320.

Herrel A, Meyers J, Nishikawa K, De Vree F. 2001b. Morphology and histochemistry of the hyolingual apparatus in chameleons. J. Morphol. 249:154-170.

Herrel A, Meyers J, Aerts P, Nishikawa K. 2001c. Functional implications of supercontracting muscle in the chameleon tongue retractors. J. Exp. Biol. 204:3621-3627.

IUCN 2007. 2007 IUCN Red List of Threatened Species. <www.iucnredlist.org>. Downloaded on 05 June 2008.

Iwasaki S. 1992. Fine structure of the dorsal epithelium of the tongue of the freshwater turtle, *Geoclemys reevesii* (Chelonia, Emydinae). J. Morph. 211:125-135.

Iwasaki S, Asami T, Asami Y, Kobayashi K. 1992. Fine structure of the dorsal epithelium of the tongue of the Japanese terrapin, *Clemmys japonica* (Chelonian, Emydidae). Arch.Histol.Cytol. 55:295-305.

Iwasaki S, Asami T, Wanichanon C. 1996. Fine structure of the dorsal lingual epithelium of the juvenile Hawksbill turtle, *Eretmochelys imbricate bissa*. Anat. Rec. 244:437-443.

Jackson D, Herbert C, Ultsch G. 1984. The comparative physiology of diving in North American freshwater turtles. II. Plasma ion balance during prolonged anoxia. Physiol. Zool. 57:632–640.

Jackson D, Wasser J, Silver R. 1988. Effect of induced hypercapnia on anaerobic metabolic rate of anoxic musk turtles. Am. J. Physiol. Regul. Integr. Comp. Physiol. 254:944-948.

Jordanskii N. 1990. Evolucija kompleksnaich adaptacii: chelustnoi aparat amfibii i reptilii. Izdatelstvo Nauka, Moskva.

Joyce W. 2007. A phylogeny of Mesozoic turtles. Bul. Peabody Mus. Nat. Hist. 48:3-102.

Joyce W, Gauthier J. 2004. Paleoecology of Triassic stem turtles sheds new light on turtle origins. Proc. Roy. Soc. Lond. 271:1-5.

Kardong K, Berkhoudt H. 1998. Intraoral transport of prey in the reticulated python: tests of a general tetrapod feeding model. Zoology 101:7–23.

Karnovsky M. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol. 27:137.

Kiernan J. 2003. Histological and histochemical methods: Theory and practice. Third edition. New York: Oxford University Press.

King P, Heatwole H. 1994. Non-pulmonary respiratory surfaces on the chelid turtle *Elseya latisternum*. Herpetologica. 50:262-265.

Kardong K. 1982. Comparative study of changes in prey capture behaviour in the cottonmouth (*Agkistrodon piscivorus*) and Egyptian cobra (*Naja haje*). Copeia. 1982:337-343.

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell. Biol. 27:137A.

Kiernan, J. A. (2003). Histological and histochemical methods: Theory and practice. Third edition. New York: Oxford University Press.

Kleewein A, Wöss G. 2008. Das Vorkommen von allochtonen Wasserschildkröten in Wien. ÖGH-Aktuel in print.

Kraklau D. 1991. Kinematics of prey capture andchewing in the lizard *Agama agama* (Squamata: Agamidae). J. Morphol. 210:195–212.

Lappin A, German M. 2005. Feeding behavior modulation in the leopard lizard (*Gambelia wislizenii*): effects of noxious versus innocuous prey. Zoology 4:287-295.

Lauder G. 1985. Aquatic feeding in lower vertebrates. In (eds Hildebrand M, Bramble D, Wake, B) 12:211-229.

Lauder G, Schaffer H. 1986. Functional design of the feeding mechanism in lower vertebrates: unidirectional and bidirectional flow systems in the tiger salamander. Zool J Linn Soc 88:277-290.

Lauder G, Prendergast T. 1992. Kinematics of aquatic prey capture in the snapping turtle *Chelydra serpentina*. J. Exp. Biol. 164:55-78.

Lauder G, Shaffer H. 1993. Design of feeding systems in aquatic vertebrates: major patterns and their evolutionary interpretations. In: The Scull 3. (eds. Hanken J. and Hall B.) 113-149. Chicago: University of Chicago Press.

Le M, Raxworthy C, McCord W, Mertz L. 2006. A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. Mol. Phyl. Evol. 40:517-531.

Lemell P, Weisgram J. 1997. Feeding Patterns of *Pelusios castaneus* (Chelonia: Pleurodira). Neth. J. Zool 47 (4):429-441.

Lemell P, Beisser CJ, Weisgram J. 2000. Morphology and Function of the Feeding Apparatus of *Pelusios castaneus* (Chelonia; Pleurodira). J. Morphol. 244:127-135.

Lemell P, Lemell C, Snelderward P, Gumpenberger M, Wochesländer R, Weisgram J. 2002. Feeding Patterns of *Chelus fimbriatus* (Pleurodira: Chelidae). J. Exp. Biol. 205:1495-1506.

Li C, Wu XC, Rieppel O, Wang L-T, Zhao LJ. 2008. An ancestral turtle from the Late Triassic of southwestern China. Nature 456:497-501.

Liem K. 1970. Comparative functional anatomy of the Nandidae (Pisces; Teleostei). Fieldiana Zool. 56:1-166.

Liem K. 1980. Adaptive significance of intra- and interspecific differences in the feeding repertoire of cichlid fishes. Amer. Zool. 20:295-314.

McBrayer L, Reilly SM. 2002. Testing amniote modelsof prey transport kinematics: a quantitative analysis of mouth opening patterns in lizards. Zoologica 105:71–81.

McDowell SB. 1972. The evolution of the tongue in snakes and its bearing on snake Origins. Evol Biol 6:191-273.

Meyers J. Nishikawa K. 2000. Comparative study of tongue protrusion in three iguanian lizards: *Sceloporus undulatus*, *Pseudotrapelus sinaitus* and *Chamaeleo jacksoni*. J. Exp. Biol. 203:2833-2849.

Meyers J, Herrel A.2005. Prey capture kinematics of ant eating lizards. J. Exp. Biol. 208:113-127.

Mehta R, Wainwright P. 2007a. Raptorial jaws in the throat help moray eels swallow large prey. Nature 449:79-82.

Mehta R, Wainwright P. 2007b. Biting releases constraints on moray eel feeding kinematics. J. Exp. Biol. 210:495-504.

Miller M, Osse J, Verhagen J. 1982. A quantitative hydrodynamical model of suction feeding in fish. J. Theor. Biol. 95:49-79.

Miller M, Osse J. 1984. Hydrodynamics of suction feeding in fish. Trans. Zool. Soc. London. 37:51-135.

Miller M, Van Leeuwen J. 1985. The flow in front of the mouth of a prey-capturing fish. Fortschritte der Zoologie 30:223-227.

Mitchell JC. 1988. Population Ecology and Life Histories of the Freshwater Turtles *Chrysemys picta* and *Sternotherus odoratus* in an Urban Lake. Herpetol. Monogr. 2:40-61.

Montuelle S, Daghfous G, BELS V. 2008. Effect of Locomotor Approach on Feeding Kinematics in the Green Anole (*Anolis carolinensis*). J. Exp. Zool. 309A:563-567.

Nalavade M, Varute A. 1976. Histochemical studies on the mucins of the vertebrate tongues. VIII. Histochemical analysis of mucosubstances in the tongue of the turtle. Folia Histochem. Cytochem. 14:123-134.

Natchev N, Heiss E, Lemell P, Stratev D, Weisgram J. 2008. Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora* *flavomarginata* (Chelonia, Geoemydidae), with emphasis on terrestrial feeding patterns. Zoology. (published online).

Nishikawa, K. 2000. Feeding in frogs. In (ed Schwenk K) Feeding: Form, function, and evolution intetrapod vertebrates, pp 117–147. San Diego: Academic Press

Norton S, Brainerd E. 1993. Convergence in the feeding machanics of ecomorphologically similar species in the Centrachidae and Cichlidae. J. Exp. Biol. 176:11-29.

O'Reilly J. 2000. Feeding in Caecilians. In Schwenk K. (ed). Feeding: Form, function and evolutionin tetrapod vertebrates, pp 149–166. Academic Press, San Diego.

Osse J, Muller M, Van Leeuwen J. 1985. The analysis of suction feeding in fish. In (Eds Duncker H, Fleischer G.) Fortschritte der Zoologie, Vol. 30. Gustav Fischer Verlag, Stuttgart, pp 250–255.

Peterson C, Greenshields D. 2001. Negative test for cloacal drinking in a semi-aquatic turtle (*Trachemys scripta*), with comments on the functions of cloacal bursae. J. Exp. Zool. 290: 247-254.

Pitchard PCH. 1979. Encyclopedia of Turtles. TFH Publications, Inc, Neptune NJ.895 p. Reilly SM, Lauder G. 1989. Physiological bases of feeding behavior in salamanders: do motor patterns vary with prey type?. J. Exp. Biol. 141:343-358.

Reilly SM, Lauder GV. 1990. The Evolution of the Tetrapoda Feeding Behaviour: Kinematic Homologies in Prey Transport. Evolution 44(6): 1542-1557.

Reilly SM, Lauder G. 1991. Experimental morphology of the feeding mechanism in salamanders. J. Morph. 210:33-44.

Reilly SM, Lauder G. 1992. Morphology behaviour and evolution: Comparative kinematics of aquatic feeding in salamanders. Brain Beh. Evol. 40:182–196.

Reilly SM, Lauder G. 1994. Amphibian feeding behavior: comparative biomechanics and evolution. In (ed Gilles R) Advances in comparative and environmental physiology, Vol. 18. pp 163-195. Springer Verlag.

Reilly SM. 1995. The ontogeny of aquatic feeding behavior in *Salamandra salamandra*: stereotypy and isometry in feeding kinematics. J. Exp. Biol. 198:701-708.

Reilly SM. 1996. The metamorphosis of feeding kinematics in *Salamandra salamandra* and the evolution of terrestrial feeding behavior. J. Exp. Biol. 199:1219-1227.

Rest J, Ast J, Austin C, Waddell P, Tibbetts E, Hay J, Mindell D. 2003. Molecular systematics of primary reptilian lineages and the tuatara mitochondrial genome. Molecular Phylogenetics and Evolution 29 (2): 289-297.

Rieppel O, de Braga M. 1996. Turtles as diapsid reptiles. Nature 384: 453-455.Rieppel O. 2000. Turtles as diapsid reptiles. Zool. Scr. 29: 199-212.

Rogner M. 1995. Schildkröten I. Heidi – Rogner – Verlag.

Romeis B. 1989. Mikroskopische Technik. 17th ed. (ed Böck P) p 97. München, Wien, Baltimore: Urban u. Schwarzenberg.

Ross C, Eckhardt A, Herrel A, Hylander W, Metzger K, Schaerlaeken V, Washington R, Williams S, 2007. Modulation of intra-oral processing in mammals and lepidosaurs. Integr. Comp. Biol. 47:118-136.

Saunders K, Roberts A, Ultsch G. 2000. Blood viscosity and hematological changes during prolonged submergence of northern and southern musk turtles (*Sternotherus odoratus*). J. Exp. Zool. 287:459-466.

Schaerlaeken V, Meyers J, Herrel A. 2007. Modulation of prey capture kinematics and the role of lingual sensory feedback in the lizard *Pogona vitticeps*. Zoology 110:127-138.

Schaerlaeken V, Herrel A, Meyers J. 2008. Modulation, individual variation, and the role of lingual sensory afferents in the control of prey transport in the lizard *Pogona vitticeps*. J. Exp. Biol. 211:2071-2078.

Scheidel E. 2002. Histologie and histochemie des Oesophagusepithels der Schildkroete *Cuora galbinifrons* Bourret, 1939 (Emydidae, Chelonia). Unpublished diploma thesis. Univ. Vienna

Scheyer TM, Sander PM. 2007. Terrestrial palaeoecology for basal turtles indicated by shell bone histology. Proc. Roy. Soc. Lond. B 274: 1885–1893.

Schilde M. 2007. Die Muschus-Schildkröte *Sternotherus odoratus*. 2004 Natur und Tier –Verlag Gmbh.

Schumacher G. 1956. Morphologische Studie zum Gleitmechanismus des M. adductor mandibulae externus bei Schildkröten. Anat. Anz. 103:1-12.

Schumacher G. 1973. The head muscles and hyolaryngeal skeleton of turtles and crocodilians. In (eds Gans C, Parsons T.) Biology of the Reptilia, Vol. 4. Academic Press, New York, pp 101-199.

Schwenk K. 1994. Why do snakes have forked tongues? Science 263, 1573-1577.

Schwenk K. 1985. Occurence, distribution and functional significance of taste buds in lizards. Copeia 1985:91-101.

Schwenk K. 2000a. An introduction to tetrapoda feeding. In (ed Schwenk K) Feeding: form, function and evolution in tetrapod vertebrates. San Diego: Academic Press.

Schwenk K. 2000b. Feeding in lepidosaurs. In (ed Schwenk K) Feeding: form, function and evolution in tetrapod vertebrates. San Diego: Academic Press.

Schwenk K, Throckmorton G. 1989. Functional and evolutionary morphology of lingual feeding in squamate reptiles: phylogenetics and kinematics. J. Zool. London 219:153-175.

Schwenk K, Rubega M. 2005. Diversity of Vertebrate feeding Systems. In (eds Stark M, Wang T) Physiological and Ecological Adaptations to Feeding in Vertebrates. Science Publishers , Enfield, New Harpshire, pp 1-41.

Smith KK. 1984. The use of the tongue and hyoid apparatus during feeding in lizards (*Ctenosaura similis* and *Tupinambis nigropunctatus*). J. Morphol. 202:115-143.

Smith KK. 1986. Morphology and function of the tongue and hyoid apparatus in varanus (Varanidae, Lacertilia). J. Morph. 187:261-287.

Smith KK. 1992. The evolution of the mammalian pharynx. Zool. J. Linn. Soc. 104:313-349.

Smith KK. 1994. Are neuromotor systems conserved in evolution? Brain. Behav. Evolut. 43:293-305.

Smith T, Kardong K, Bels V. 1999. Prey capture behavior in the blue-tongued skink, *Tiliqua scincoides*. J. Herpetol. 33:362–369.

Smith T, Povel D, Kardong K. 2002. Predatory strike of the tentacled snake (*Erpeton tentaculatum*). J. Zool. Lond. 256:233 -242.

Sokol, O. M. 1962. The tadpole of *Hymenochirus boettgeri*. Copeia 1962:272–284.

Sponder D, Lauder G. 1980. Terrestrial feeding in the mudskipper Periophtalmus (Pisces: Teleostei): a cineradiographic analysis. J. Zool. Lond. 193:517–530.

Stone P, Dobie J, Henry R. 1992. Cutaneous surface area and bimodal respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). Physiol. Zool. 65:311–330.

Stuart B, Parham J. 2004. Molecular phylogeny of critically endangered Indochinese box turtle (*Cuora galbinifrons*). Mol. Phylogenet. Evol. 31:164-177.

Summers AP, Darouian KF, Richmond AM, Brainerd EL. 1998. Kinematics of Aquatic and Terrestrial Prey Capture in *Terrapene carolina*, With Implications for the Evolution of Feeding in Cryptodire Turtles. J. Exp. Zool. Part A 281:280-287.

Ultsch G, Herbert U, Jackson D. 1984. The comparative physiology of diving in North American freshwater turtles. I. Submergence tolerance, gas exchange, and acid-base balance. Physiol. Zool. 57:620-631.

Ultsch G. 1985. The viability of nearctic freshwater turtles submerged in anoxia and normoxia at 3° and 10°C. Comp. Biochem. Physiol. 81A:607-611.

Ultsch G. 1988. Blood gases, hematocrit, plasma ion concentrations, and acid–base status of musk turtles (*Sternotherus odoratus*) during simulated hibernation. Physiol. Zool. 61:78–94.

Ultsch G, Wasser J. 1990. Plasma ion balance of North American freshwater turtles during prolonged submerge in normoxic water. Comp. Biochem. Physiol. 97A:505-512.

Ultsch G, and Cochran B. 1994. Physiology of northern and southern musk turtles (*Sternotherus odoratus*) during simulated hibernation. Physiol. Zool. 67:263–281.

Ultsch G, Jackson D. 1995. Acid–base status and ion balance during simulated hibernation in freshwater turtles from the northern portions of their ranges. J. Exp. Zool. 273:482–493.

Wang Z, Sun N, Sheng W. 1989. Aquatic respiration in soft-shelled turtles, *Trionyx sinensis.* Comp. Biochem. Physiol. 92A:593-598.

Urbani J, Bels V. 1995. Feeding behaviour in two scleroglossan lizards: *Lacerta viridis* (Lacertidae) and *Zonozaurus laticaudatus* (Cordylidae). J. Zool. London 236:265-290.

Urbani J, Bels V. 1999. Feeding behavior in two scleroglossan lizards: *Lacerta viridis* (Lacertidae) and Zonosaurus laticaudatus (Cordylidae). J. Zool. London 236:265–290.

Van Damme J, Aerts P. 1997. Kinematics and functional morphology of aquatic feeding in Australian snake-necked turtles (Pleurodira; Chelodina). J. Morphol. 233:113-125.

Van Wassenbergh S, Herrel A, Adriaens D, Huysentruyt F, Devaere S, Aerts P. 2006a. Evolution: a catfish that can strike its prey on land. Nature 440:881.

Van Wassenbergh S, Aerts P, Herrel A. 2006b. Hydrodynamic modelling of aquatic suction performance and intraoral pressure: limitations for comperative studies. J. R. Soc. Interface 3:507-514.

Van Wassenbergh S, Aerts P. 2008. Dynamics of suction feeding in fish: Insights from unsteady, rotationally symmetric CFD models. Comp. Bioch. Physiol. Part A 150: 84 (abstract).

Vincent S, Herrel A, Irschick D. 2004. Ontogeny of intersexual head shape and prey selection in the pitviper, *Agkistrodon piscivorus*. Biol. J. Linn. Soc. Lond. 81:151-159.

Vincent S, Moon B, Herrel A, Kley N. 2007. Are ontogenetic shifts in diet linked to shifts in feeding mechanics? Scaling of the feeding apparatus in the banded watersnake *Nerodia fasciata*. J. Exp. Biol. 210:2057-2069.

Wagner G, Schwenk K. 2000. Evolutionary stabile configurations: functional integration and the evolution of phenotypic stability. In (eds Hetch M, MacIntyre R, Clegg M). Evolutionary Biology 31:155-217.

Wainwright P, Kraklau D, Bennett F. 1991. Kinematics of tongue projection in *Chamaeleo oustaleti*. J. Exp. Biol. 159:109-133.

Wake D.1982. Functional and developmental constraints and opportunities in the evolution of feeding systems in urodeles. In (Mossakowski D, Roth G. (eds.) Environmental adaptation and evolution, pp 51-66. Gustav Fischer, Stuttgart, New York.

Wake, D. B. and S. M. Deban. 2000. Terrestrial feeding in salamanders. In (ed. Schwenk K.) Feeding: Form, function and evolution in tetrapod vertebrates, pp 95-116. San Diego: Academic Press.

Wassersug R, Hoff K. 1979. A Comparativestudy of the buccal pumping mechanism of tadpoles. Biol. J. Linn. Soc. 12:225–259.

Wainwright P, Sandford C, Reilly S, Lauder G. 1989. Evolution of Motor Patterns: Aquatic Feeding in Salamanders and Ray-Finned Fishes. Brain Bah. Evol. 34:329-341.

Wilkinson M, Nussbaum R. 1999. Evolutionary relationships of the lungless caecilian *Atretochoana eiselti* (Amphibia: Gymnophiona: Typhlonectidae). Zool. J. Linn. Soc. 126:191-223.

Weisgram J. 1985. Feeding mechanics of Claudius angustatus Cope 1865. In (Eds Duncker H, Fleischer G.) Fortschritte der Zoologie, Vol. 30. Gustav Fischer Verlag, Stuttgart, pp 257–260.

Weisgram J, Dittrich H, Splechtna H. 1989. Comparative functional study of the oral cavity in two turtle species. Plzen. Lek. Sborn., Suppl. 59:117-122.

Wilkinson M. 1991. Adult tooth crown morphology in the Typhlonectidae (Amphibia: Gymnophiona): A reinterpretation of variation and its significance. Z. Zool. Syst. Evolut.—Forsch. 29:304-311.

Wermuth H, Mertens R. 1961. Schildkröten, Krokodile, Brückenechsen. VEB Gustav Fischer Verlag, Jena.

Winokur B. 1973. Adaptive modifications of the buccal mucosae in turtles. Amer. Zool. 13: 1347-1348.

Winokur B. 1988. The buccopharyngeal mucosa of the turtles (Testudines). J. Morph. 196: 33-52.

Wocheslånder R, Hilgers H, Weisgram J. 1999. Feeding Mechanism of *Testudo hermanni boettigeri* (Chelonia, Cryptodira). Neth. J. Zool. 49 (1):1-13.

Wocheslånder R., Gumpenberger M, Weisgram J. 2000. Intraoral food transport in *Testudo hermanni* (Chelonia, Cryptodira) – a radiographic video analysis. Neth. J. Zool. 50:445–454.

Yokosuka H, Ishiyama M, Yoshie S, Fujita T. 2000. Villiform processes in the pharynx of the soft shelled turtle, Trionyx sinensis japonicus, functioning as a respiratory and presumably salt uptaking organ in the water. Arch. Histol. Cytol. 63:181-192.

Zardoya R, Meyer A (1998) Complete mitochondrial genome suggests diapsid affinities of turtles. Proc Natl Acad Sci USA 95:14226-14231.

V. Abstrakt

In Gegensatz zur aquatischen Nahrungsaufnahme, deren Kinematik relativ gut untersucht ist, ist der Mechanismus des terrestrischen Fressens der Schildkröten nahezu unbekannt. Im Rahmen dieser Dissertation wird eine detaillierte, vergleichend- und funktionsmorphologische Analyse des Fressvorgangs unter Wasser und an Land bei drei Arten von Geoemydidae und einer Spezies der Kinosternidae durchgeführt. Die Anatomie des Craniums und des Hyolingualkomplexes von *Cuora galbinifrons*, *Cuors amboinensis*, *Cuora flavomarginata* und *Sternotherus odoratus* werden beschrieben. Bezüglich der Kopfmorphologie der drei Geoemydidae Arten der Gattung *Cuora sp.*, konnten einige Ungenauigkeiten der bisherigen Beschreibungen korrigiert werden. Die gewonnene Information über die Konstruktion des Neurocraniums, Kiefer- und Hyolingualapparates wird funktionell - morphologisch ausgewertet um Korrelationen zwischen „Bauplan“ und Habitatpräferenzen besser verstehen zu können. Eine vergleichend-morphologische Untersuchung beantwortet die Frage, wie die Strukturen des Fressapparates bei eng verwandten Arten mit alternierenden Habitatpräferenzen ausgebildet sind und welche Funktion sie besitzen. Die vorliegenden Studien beinhalten die erste kinematische Analyse des Fressvorganges bei Vertretern der größten rezenten Schildkrötengruppe Geoemydidae. Die vorliegende Arbeit beinhaltet auch die erste, auf high-speed Videos (500 fr/s) basierende, Untersuchung der Nahrungsaufnahme von einem Vertreter der Kinosternidae (*S. odoratus*). Die funktionell-morphologische Analyse bei hier untersuchten *Cuora* Arten demonstriert, dass relativ kleine Unterschiede in der Form des Fressapparates, fundamentale Auswirkungen auf dessen Funktion haben können. Unsere Ergebnisse demonstrieren, dass terrestrische Nahrungsaufnahme

mindestens dreimal „de novo“ unabhängig innerhalb der rezenten Chelonier entstanden ist. Die morphologischen und kinematischen Untersuchungen an *S. odoratus* demonstrieren, dass diese aquatischen Schildkröten problemlos an Land ihre Nahrung schnappen können, die kritische Fressphase ist die des ersten Transportzyklus. Es ist zu erwarten, dass manche aquatische Schildkröten es im Rahmen ihrer evolutionären Entwicklung geschafft haben an Land den Fressvorgang zu vollenden. Rein terrestrische Testudiniden hingegen haben offensichtlich den "point of no return" zu einer aquatischen Lebensweise bereits überschritten. Die Evolution terrestrischer Nahrungsaufnahme ist demzufolge eine „Einbahnstrasse“.

VI. Curriculum vitae

PERSONAL INFORMATION

Name	MAG. NATCHEV NIKOLAY DOBRINOV
Address	WIEN 1040 MARGARETENSTRASSE 30/30 AUSTRIA
Telephone	00436766318081
E-mail	natchev@gmx.at
Nationality	Bulgarian
Date of birth	07.11.1975

Education:

1994: English language school “N. Vapzarov”: Profile English and French languages; School leaving examination with Protocol Num: 15 from 24.06.1994; Shumen, Bulgaria;

1994 to 1998: University of Shumen “K. Preslavski”: Study in Fak: Biology; Profile Biology and Chemistry;

1998 to 1999: University of Vienna: Vorstudienlehrgang (Profile German language);

2004: University of Vienna: diploma thesis; advisor: Josef Weisgram; thesis title: Zur Form und Funktion des Kiefer-Zungenbeinapparates bei *Cuora flavomarginata* (Chelonia; Cryptodyra);

2004 - present: University of Vienna: doctoral thesis; advisor: Josef Weisgram; primary thesis title: Analysis of the feeding kinematics in Cryptodiran turtles;

Positions:

2004 - present: University of Vienna: lectorship of Vertebrate morphology;

2007 – present: University of Vienna: research assistant FWF (Project P20094-B17);

Publications:

Weisgram, J., Beisser, C., Lemell, P., Natchev, N., Wocheslander, R., (2006) Different food ingestion modes in turtles - a comparison. Annual of Konstantin Preslavsky University, Shumen vol. XVI B 4; pp. 193-214. Faculty of Natural Sciences.

Natchev, N., Weisgram, J., (2007) Die viscerale Muskulatur bei *Cuora flavomarginata* (Chelonia; Cryptodira). Annual of Konstantin Preslavsky University, Shumen, Vol. XVIII B 3, Faculty of Natural Sciences.

Natchev, N., Heiss, E., Weisgram, J., (2008) On the Hyobranchial musculature in *Cuora amboinensis* (Daudin, 1802): Chelonia, Geoemydidae. Annual of Konstantin Preslavsky University, Shumen Vol. XVIII B 6, Faculty of Natural Sciences.

Natchev, N., Heiss, E., Lemell, P., Stratev, D., Weisgram, J., (2009) Analysis of prey capture and food transport kinematics in two Asian box turtles, *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae). Zoology.

Heiss, E., Natchev, N., Rabanser, A., Weisgram, J., Hilgers, H., (2009) Three types of cutaneous glands in the skin of the salamandrid *Pleurodeles waltl*. A histological and ultrastructural study. Journal of morphology (in press).

Natchev, N., Heiss, E., Lemell, P., Kummer, S., Schwacha, T., Weisgram J., (2009) Kinematical analysis of animal behaviour - the challenge to increase the frame rate in digital high - speed cinematography. Biotechnology and Biotechnological equipment Journal (in press).

Abstracts:

Natchev, N., Lemell, P., Weisgram, J., (2007) Feeding Patterns of Asian Box Turtles – A Comparative Study on *Cuora amboinensis* and *Cuora flavomarginata* (Chelonia, Geoemydidae). Journal of Morphology vol. 268; pp. 1110 (abstract).

Natchev, N., Lemell, P., Weisgram, J., (2007) Feeding Patterns in *Cuora galbinifrons* (Bouret, 1939). Journal of Morphology vol. 268; pp. 1110 (abstract).

Natchev, N., Heiss, E., Lemell, P., Weisgram, J., (2008) Kinematic analysis of prey capture, prey transport and swallowing in the Common Musk Turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). Comperative Biochemistry and Physiology, Part A vol. 150; pp. 95 (abstract).

Heiss, E., Natchev, N., Weisgram, J., (2008) On the biochemical and bizarre mechanical defensive strategies of the salamandrid *Pleurodeles waltl*. Comperative Biochemistry and Physiology, Part A vol. 150; pp. 95 (abstract).

Natchev, N, Heiss, E., Lemell, P., Weisgram, J., (2009) Diversity in terrestrial food uptake strategies within testudinoids (Chelonia; Cryptodira). (abstract submitted)

Publications submitted:

Natchev, N., Lemell, P., Heiss, E., Beisser, C., Weisgram J., (2008) Aquatic feeding in a terrestrial turtle: a functional-morphological study of the feeding apparatus in the Indochinese box turtle *Cuora galbinifrons* (Chelonia, Geoemydidae). Zoomorphology (submitted).

Heiss, E., Natchev, N., Beisser, C., Lemell, P., Weisgram, J., (2008) Feeding and breathing: The bifunctionality of the oropharynx in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). Journal of experimental biology (submitted).

Natchev, N., Heiss, E., Singer, K., Kummer, S., Salaberger, D.,Weisgram, J., (2009) Kinematic analysis of aquatic prey capture, prey transport and swallowing in the common musk turtle *Sternotherus odoratus* (Chelonia, Kinosternidae). Journal of experimental zoology (submitted).

Heiss., E., Natchev., N, Salaberger, D., Gumpenberger., M, Weisgram, J., (2009) On spears and poison: The bizzare but powerful defensive mechanism of the salamandrid *Pleurodeles waltl* against predators. Journal of experimental zoology (submitted).

Scientific contributions:

Heiss, E., Plenk, H., Weisgram, J., (2008) Microanatomy of the palatal mucosa of the semiaquatic Malayan box turtle, *Cuora amboinensis*, and functional implications. The anatomical record vol. 291; pp. 876-885.

Congresses:

V NATIONAL CONFERENCE “NATURAL SCIENCES 2006”. September 2006, Varna, Bulgaria.

VIII-th Congress of Vertebrate Morphology. July 2007. Paris, France.

VI NATIONAL CONFERENCE “NATURAL SCIENCES 2007” September 2007. Varna, Bulgaria.

SEB at 2008. July 2008. Marseille, France.

VII NATIONAL CONFERENCE “NATURAL SCIENCES 2008” September 2008. Varna, Bulgaria.

XI ANNIVERSARY SCIENTIFIC CONFERENCE. May 2009. Sofia, Bulgaria.

SEB at 2009 June 2009. Glasgow, UK.

VII. Danksagung

Ich danke allen meinen Freunden und Lehrern!!!

Die Arbeit wurde unterstützt vom Fonds zur Förderung der wissenschaftlichen Forschung; P 20094-B17.