RESEARCH ARTICLE

A method to reconstruct anguilliform fishes from partially digested items

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Abstract Estimating the amounts of resources consumed by individuals is important in many studies. For predators, allometric relationships can be used to extrapolate the size of preys from undigested remains found in the stomach and in the faeces. However, such equations are available for a limited number of species. Based on a large sample size gathered in New Caledonia on both predators (sea kraits) and their preys (anguilliform fishes), we provide the first allometric relationships that allow estimating accurately the mass and the size of various anguilliform fish species.

Introduction

Characterising the diet and estimating the amount of resources consumed by individuals is of central importance in most ecological studies. For many predators, the number and the size of the preys eaten per unit of time are among the key parameters required to address a wide array of biological problems such as those related to the prey-predator relationships, energy budget, intra- or inter-specific competition for instance. Unfortunately, under field conditions, it is often diffi-

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cult to accurately estimate the dimensions (e.g. body length, body mass) of preys. Indeed, it is almost impossible to measure the food items of predators before they are consumed; afterwards, they are often dilacerated and digested. However, different predators (birds, fishes, reptiles...) swallow their preys as a whole. The use of the allometric relationship that links the size and the mass of the prey items can be used to extrapolate their initial dimensions from uneaten fragments, or from undigested remains (e.g. cephalopod beaks, fish otoliths...) found in the stomach and in the faeces (Clarke 1986; Härkönen 1986). Although such relationships are species-specific and require a good knowledge of allometric links for the species of interest, this method has been validated and successfully employed in several vertebrate species, notably in seabirds and marine mammals (Duffy and Jackson 1986; Cherel et al. 2000 and references therein). Extending this approach to poorly studied taxonomic groups would be useful to better understand the diversity of the prey/predators systems.

Typical examples of scantly known assemblages are represented by the fish (preys) and the sea snakes (predators) (Heatwole 1999). Sea kraits (*Laticauda sp.*) forage in the coral reef ecosystems, and are highly specialized on anguilliform fish (mostly moray, conger and snake eels; Reed et al. 2002; Ineich et al. 2007; Brischoux and Bonnet 2007). In many areas, these fish are extremely secretive and escape classical sampling methods (underwater visual census or rotenone poisoning, Ackerman and Bellwood 2000; Willis 2001; Ineich et al. 2007). The snakes swallow their preys as a whole, and it is often possible to obtain their stomach content through forced regurgitation; thus, they offer a unique opportunity to study anguil-

liform fish (Reed et al. 2002; Brischoux and Bonnet 2007; Ineich et al. 2007). However, detailed diet studies are available for four species only, all from the same genus (sea kraits: *Laticauda colubrina*, Heatwole 1999; *L. saintgironsi* and *L. laticaudata*, Brischoux and Bonnet 2007; Ineich et al. 2007; *L. semifasciata*, Su et al. 2005). Consequently, there is an almost complete lack of information on these fish communities, several species preyed by the snakes being known from less than ten specimens (Brischoux and Bonnet 2007; Ineich et al. 2007). This deficiency of knowledge also means that it is currently impossible to estimate the biomass of the meals of the sea snakes, the fish being partly digested most of the time. Based on a large sample size gathered in New Caledonia, and using a wide range of both intact and partly digested fish, the aim of this study was to provide the first allometric equations that allow estimating the mass and the size of various anguilliform fish species. In the absence of comparable study, one of our first goals was to determine which morphological traits easily available from the stomach remains were the best predictors of the mass (or the size) of the fish.

Material and methods

The study was carried out from 2002 to 2005 on ten sites located in the southwest lagoon of New Caledonia (nine islets: Ténia, Mba, Signal, Larégnère, Amédée, Porc-Epic, Nouaré, Bayonnaise, Brosse; and a wreck: ever-prosperity). Sea kraits are mostly visible when they cross the shore, moving between the sea and the land. The perimeter of each islet was regularly surveyed, both during the day and at night. Most of the snakes were captured by hand and kept in cotton bags until processed. At first capture, the snakes were individually marked by scale clipping (Brischoux and Bonnet 2007). They were released after completion of the measurements 1–24 h after capture (Brischoux and Bonnet 2007). Pooling captures and recaptures, we examined more than 7,000 snakes (>4,000 individually marked; >3,000 recaptures). The abdomen of each snake was carefully palpated to detect the presence of a meal. The prey items were obtained by a gentle massage of the abdomen. Sea-kraits feed mostly on non-spiny fishes and it was easy to force them to regurgitate without risk of injury for the snake. Overall, we obtained 1,039 regurgitated prey items belonging to 49 species (37 non-anguilliform fish from 3 species were discarded from the analyses). Stomach contents fell into three broad categories: intact preys (i.e. the skin was undigested), partly digested preys (at least the

Table 1 List of the intact specimens used as references

Family	Sub-family	Species	N
Congridae	Congrinae	*Conger* sp.	16
Muraenidae	Muraeninae	*Cirrimaxilla formosa*	3
Muraenidae	Muraeninae	*Echidna unicolor*	1
Muraenidae	Muraeninae	*Enchelycore pardalis*	5
Muraenidae	Muraeninae	*Gymnothorax albimarginatus*	1
Muraenidae	Muraeninae	*Gymnothorax chilospilus*	67
Muraenidae	Muraeninae	*Gymnothorax eurostus*	10
Muraenidae	Muraeninae	*Gymnothorax fimbriatus*	8
Muraenidae	Muraeninae	*Gymnothorax formosus*	1
Muraenidae	Muraeninae	*Gymnothorax fuscomaculatus*	2
Muraenidae	Muraeninae	*Gymnothorax margaritoforus*	7
Muraenidae	Muraeninae	*Gymnothorax moluccensis*	5
Muraenidae	Muraeninae	*Gymnothorax pindae*	7
Muraenidae	Muraeninae	*Gymnothorax reticularis*	1
Muraenidae	Muraeninae	*Gymnothorax richardsonii*	2
Muraenidae	Muraeninae	*Gymnothorax* sp.	1
Muraenidae	Muraeninae	*Gymnothorax undulatus*	5
Muraenidae	Muraeninae	*Gymnothorax zonipectis*	2
Muraenidae	Uropterygiinae	*Anarchias allardicei*	2
Muraenidae	Uropterygiinae	*Anarchias cantonensis*	2
Muraenidae	Uropterygiinae	*Anarchias seychellensis*	1
Muraenidae	Uropterygiinae	*Anarchias* sp.	1
Muraenidae	Uropterygiinae	*Scuticaria tigrina*	3
Muraenidae	Uropterygiinae	*Uropterygius alboguttatus*	1
Muraenidae	Uropterygiinae	*Uropterygius concolor*	1
Muraenidae	Uropterygiinae	*Uropterygius macrocephalus*	1
Muraenidae	Uropterygiinae	*Uropterygius* sp.	1
Muraenidae	Unidentified	Unidentified	4
Ophichthidae	Myrophinae	*Muraenichthys* sp.	5
Ophichthidae	Myrophinae	*Myrophis microchir*	1
Ophichthidae	Ophichthinae	*Myrichtys maculosus*	1

head and/or the entire tail was intact, depending upon which extremity was swallowed first), and much-digested items (e.g. fragments of the tail). One hundred and sixty-eight preys (16% of the total, list of the species in Table 1), probably captured by the snakes shortly before forced regurgitation, were in excellent condition. On these complete fish, we immediately recorded seven easily accessible morphological traits: body mass (with an electronic scale: ±1 g); plus six other body-size traits (with a digital calliper: ±0.5 cm), (1) total length, (2) snout-vent length (SVL), (3) tail length from the cloaca to the tip of the tail, (4) body diameter recorded at the level of the head, mid-body, vent, and averaged, (5) eye diameter, and (6) jaw length from the tip of the snout to the most posterior join. Six hundred and twenty-five prey items (60%) were partly digested, but at least one of the above measurements was possible. Two hundred and forty-six (24%) of the remains were too digested and were considered as useless for our purpose. All the prey items were kept in a 90% alcohol solution, and were later identified to the nearest taxonomy level in the lab.

Table 2 Stepwise multiple regressions between total length or body mass as dependent variables and all other morphological traits as the predictive variables (see text)

Family	Sub-family	Dependant variable	Predictive variable	r^2	F	beta	P
All		Length	SVL	0.94	1475.81	0.97	<0.0001
		Mass	$\varnothing_{mean}$	0.91	981.87	0.96	<0.0001
Congridae		Length	Tail L	0.99	1014.26	0.99	<0.0001
		Mass	$\varnothing_{mid}$	0.99	3081.83	0.99	<0.0001
Muraenidae	All	Length	SVL	0.96	2548.79	0.98	<0.0001
		Mass	$\varnothing_{mean}$	0.92	1070.43	0.96	<0.0001
	Muraeninae	Length	SVL	0.96	1856.19	0.98	<0.0001
		Mass	$\varnothing_{mean}$	0.94	1179.30	0.97	<0.0001
	Uropterigiinae	Length	SVL	0.97	2548.79	0.98	<0.0001
		Mass	$\varnothing_{vent}$	0.98	389.31	0.99	<0.0001

By including the other variables (e.g. diameter), the proportion of explained variance never increased by more than 2%, we thus displayed in this table only the first significant predictor. All the variables were Ln-transformed to meet linearity

SVL snout-vent length, *Tail L* tail length, $\varnothing_{mid}$ diameter of the body recorded at mid-body, $\varnothing_{vent}$ diameter of the body recorded at the vent, $\varnothing_{mean}$ averaged body diameters

Results

Estimating the length of the preys

In order to identify the best predictor of total length, we performed stepwise multiple regressions (forward versus backward regressions led to similar results) with the total length of the prey as the dependent variable, and the six other traits as the independent variables. We first run the analyses by including all fish species. Then, we made a distinction between the two main taxonomic groups (the Ophichthidae sample size was too small to perform the tests separately). Table 2 summarises the results. Snout vent length was the best predictor in most cases, whilst tail length was the first trait retained for the Congridae. Overall, using snout vent length and/or tail length it was possible to estimate accurately the total size of the fish (all the models had $0.94 < r^2 < 0.99$). However, one of these traits was available for only 34% of the partly digested fish. Therefore, we examined if the other morphological traits sampled on more digested items (i.e. when greater than half of the fish was digested) could be employed confidently. Simple regressions (either all the fish pooled or grouped by family) suggested that even well digested prey items could be used to estimate the total length of the fish as the error remained acceptable using body diameter ($0.70 < r^2 < 0.96$), and jaw length ($0.69 < r^2 < 0.86$). The diameter of the eye, poorly correlated to the length of the fish, was useless ($0.49 < r^2 < 0.52$) (all $P < 0.001$).

Estimating the mass of the preys

As above, we used stepwise/simple regressions procedure with the mass of the prey as the dependent variable. The body diameter of the prey was the best predictor in most cases (Table 2). Fortunately, in the partly digested preys, this parameter was available in most cases (68%). We also examined if the other traits could be used when the body diameter was unavailable. SVL ($0.83 < r^2 < 0.97$), tail length ($0.72 < r^2 < 0.97$), total body length ($0.91 < r^2 < 0.98$), jaw length ($0.89 < r^2 < 0.95$) and the eye diameter ($0.52 < r^2 < 0.87$) were all useful characters to estimate the mass of the fish (all $P < 0.001$).

The accuracy to estimate the mass of the prey is a key parameter, notably in terms of energy budget. Therefore, we further tested the validity of our method. First, we randomly divided our sample of intact preys in two sub-samples of 84 intact fish each. We used the first set to calculate the equation that links body diameter (independent variable) and body mass (dependant variable). The terms of the regression were Ln body mass = 2.5353*Ln mean diameter -3.3953 ($F = 1020.62$, $df = 1, 84$, $r^2 = 0.93$, $P < 0.0001$). Then, we applied the equation to the second data set in order to compare the estimated mass with the actual mass of these fish: there was no significant difference between the predicted and the measured body mass (paired t-test, $t = -1.33$, $df = 83$, $P = 0.18$). Finally, we re-calculated the terms of the equation using the whole data set of intact preys ($N = 168$), and calculated the mean error when predicting the mass of the fish (difference between predicted and observed values expressed as 100 (measured body mass−predicted body mass)/maximum (measured body mass; predicted body mass)). On average, our error was small: $7.73 \pm 8.89\%$. A more focused analysis restricted to the Muraeninae, revealed an equivalent average error ($6.58 \pm 7.76\%$).

Conclusion

The anguilliform fish/sea kraits system is somewhat unique for several reasons. The large spectrum of poorly known fish (49 species) and the availability of undigested preys provided an ideal reference set to reconstruct partly digested items. Therefore, it was possible to use the sea kraits as powerful sampling tools to gather the main morphological characteristics of yet neglected fish communities (Ineich et al. 2007). Many of the anguilliform fish found in the stomach of the snakes contained eggs or secondary preys (e.g. remains of crustaceans). Reconstructing the size of the preys is crucial to assess questions related to their reproduction (e.g. size at maturity), and diet. Similarly, the length-size relationships we provide (Appendix) could be valuable for studies that deal with the biomass of reef fish. Notably, there is yet little information for tropical anguilliform fish (Kulbicki et al. 2005). On the other hand, thanks to the allometric equations derived from the intact preys, the dimensions of the snake preys could be accurately estimated in 76% of the cases. Sea kraits, as most snake species, are gape-limited predators and swallow their prey whole (Greene 1983). Obtaining accurate data on both the size and the mass of their prey is essential to address a wide array of biological problems such as those related to the prey-predator relationships, energy budget, intra- or inter-specific competition for instance.

Overall our simple method enables to fill up a gap of knowledge that concerns both the anguilliform fish and sea snake ecology. The method and equations presented at the end of this paper (Appendix) offer two advantages: they are accurate and simple to use.

Appendix

Table 3.

Table 3 Length-mass, mass-mean diameter and length-mean diameter relationships for new caledonian anguilliform fishes

Family	Sub-family	Species	Equation	F	r^2	P value	N
All			Ln(BM) = 2.87*Ln(TL) − 6.36	1173.67	0.88	<0.0001	168
			Ln(BM) = 2.60*Ln(MD) − 3.56	1893.37	0.92	<0.0001	161
			Ln(TL) = 0.73*Ln(MD) + 1.40	384.05	0.70	<0.0001	163
Congridae	Congrinae	*Conger sp.*	Ln(BM) = 3.37*Ln(TL) − 8.21	841.82	0.99	<0.0001	12
			Ln(BM) = 2.99*Ln(MD) − 4.55	582.97	0.98	<0.0001	14
			Ln(TL) = 0.86*Ln(MD) + 1.17	223.23	0.95	<0.0001	13
Muraenidae	All		Ln(BM) = 2.90*Ln(TL) − 6.40	1312.40	0.90	<0.0001	140
			Ln(BM) = 2.60*Ln(MD) − 3.60	1821.32	0.93	<0.0001	142
			Ln(TL) = 0.78*Ln(MD) + 1.26	490.35	0.78	<0.0001	143
	Muraeninae		Ln(BM) = 3.05*Ln(TL) − 6.87	1255.89	0.91	<0.0001	128
			Ln(BM) = 2.53*Ln(MD) − 3.45	2197.76	0.94	<0.0001	130
			Ln(TL) = 0.74*Ln(MD) + 1.32	700.46	0.84	<0.0001	130
		G. chilospilus	Ln(BM) = 3.31*Ln(TL) − 7.71	1007.88	0.94	<0.0001	63
			Ln(BM) = 2.46*Ln(MD) − 3.25	893.31	0.93	<0.0001	65
			Ln(TL) = 0.71*Ln(MD) + 1.43	645.64	0.91	<0.0001	65
		G. eurostus	Ln(BM) = 3.42*Ln(TL) − 7.87	440.94	0.98	<0.0001	10
			Ln(BM) = 2.29*Ln(MD) − 2.74	193.75	0.96	<0.0001	10
			Ln(TL) = 0.56*Ln(MD) + 1.53	139.02	0.94	<0.0001	10
		G. fimbriatus	Ln(BM) = 3.32*Ln(TL) − 7.86	308.79	0.98	<0.0001	8
			Ln(BM) = 2.55*Ln(MD) − 3.52	393.65	0.98	<0.0001	8
			Ln(TL) = 0.75*Ln(MD) + 1.34	230.27	0.97	<0.0001	8
		G. margaritophorus	Ln(BM) = 1.61*Ln(TL) − 1.86	19.42	0.79	<0.007	7
			Ln(BM) = 2.56*Ln(MD) − 3.52	87.79	0.94	<0.0003	7
			Ln(TL) = 1.31*Ln(MD) − 0.27	20.73	0.80	<0.007	7
	Uropterigiinae		Ln(BM) = 2.61*Ln(TL) − 5.86	342.54	0.97	<0.0001	12
			Ln(BM) = 3.21*Ln(MD) − 4.67	200.39	0.95	<0.0001	12
			Ln(TL) = 1.16*Ln(MD) + 0.61	76.86	0.87	<0.0001	13

For each taxonomic level, the equation, the regression parameters (F, r^2 and P value) and the number of specimens used (N) are given
BM, *TL* and *MD* stand for body mass, total length and mean diameter, respectively

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