

## Prediction of absorption efficiency from food nitrogen in amphibians

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**Abstract.** Gravimetric estimation of absorption efficiency in amphibians is a time-consuming process and still subject to technical errors. The need for the application of indirect methods requiring no quantitative recovery of feces is indicated. From 41 values reported for 11 amphibian species, it is observed that nitrogen content of food is significantly ( $P < 0.001$ ) and positively correlated ( $> 0.9$ ) with absorption efficiency. From the obtained regression equation (absorption efficiency =  $49.615 + 4.596$  nitrogen), the per cent efficiency can be predicted with 3.748 standard error.

**Keywords.** Absorption efficiency; methods of estimation; food nitrogen as index of Ae.

### 1. Introduction

Absorption is measured as the difference between ingestion ( $I$ ) and egestion ( $E$ ). Absorption efficiency (Ae) is an index of the proportion of  $I$  that is transferred from the gut lumen into the body of the animal. It is usually expressed as percentage. Hence the quantitative estimation of feces has become an important component in the study of amphibian energetics. Unlike many invertebrates (e.g. Crustacea: Lautenschlager *et al* 1978), most amphibians produce semi-solid fecal pellets, which are not bagged by a 'peritrophic membrane'; on being voided, the semi-solid fecal pellets (i) lose soluble materials immediately (Marian 1982), (ii) undergo decomposition (Robinson and Bailey 1981), (iii) may be reingested by the experimental animal (e.g. *Rana catesbeiana* tadpoles: Steinwascher 1978) and/or (iv) may be ingested by the offered prey organism (e.g. *Tubifex tubifex* ingesting feces of *Rana tigrina* tadpole: Marian 1982); hence, the recovery of feces in a given experimental situation may not be complete.

Moreover, the very process of collection of feces is cumbersome and time-consuming, and yet liable for errors. Hence, relatively few estimates of Ae of amphibians have been attempted (Seale 1985). The difficulties involved in the processes of recovery and quantification of feces call for identifying an easily measurable component of food, which could serve as a reliable index of Ae of amphibians. We have considered nitrogen, a non-inert moiety of food, as a possible index of Ae and have found that the nitrogen content of food is positively and significantly correlated with the Ae of fishes, reptiles, polychaetes and aquatic insects (Pandian and Marian 1985a,b,c,d). In the present paper, we have attempted to use this approach to estimate the absorption efficiency of amphibians.

### 2. Materials and methods

Using gravimetric procedure (Marian and Pandian 1985), few estimates of Ae were made in the tadpoles of *Bufo melanostictus* and *Rana cyanophlyctis* (table 1). However, most information for the present study was taken from pertinent

Table 1. Absorption efficiency (Ae) of amphibians fed feed containing different nitrogen levels.

Species, size and sex	Temperature (°C)	Ration (%)	Food	Nitrogen content (% dry wt)	Absorption efficiency (%)	Comments	References
<b>Caudates</b>							
<i>Plethodon cinereus</i>	10	ad lib	<i>Lasius americanus</i>	7.0 <sup>a</sup>	90	T affects Ae	Merchant (1970)
	15	ad lib	<i>L. americanus</i>	7.0 <sup>a</sup>	86		
	20	ad lib	<i>L. americanus</i>	7.0 <sup>a</sup>	80		
<i>P. cinereus</i>	10	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	90	T affects Ae	Bobka et al (1981)
♂ ♀	15	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	86	but no size effect	
	20	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	80		
<i>P. cinereus</i>	5, 20, 25	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	90	No effect of Wt and T	Crump (1979)
(0.09-0.68 g)							
<i>P. shenandoah</i>	10	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	91		
	20	ad lib	<i>Drosophila</i> sp.	8.1 <sup>a</sup>	80		
<i>Triturus helveticus</i>			<i>Enchytraeus albidus</i>	9.0 <sup>a</sup>	91		Avery (1971)
<i>T. vulgaris</i>							
<i>Eurycea bislineata</i>	5	ad lib	Chironomid larvae	9.1 <sup>a</sup>	86	No T effect	Fitzpatrick (1973a)
	10	ad lib	"	9.1 <sup>a</sup>	94		
	15	ad lib	"	9.1 <sup>a</sup>	94		
<b><i>Desmognathus ochrophaeus</i></b>							
Gravid ♀	15	ad lib	<i>Phormia</i> maggots	8.0	87		Fitzpatrick (1973b)
Nongravid ♀	15	ad lib	<i>Phormia</i> maggots	8.0	86		
Nongravid ♀	15	ad lib	<i>Phormia</i> maggots	8.0	88		
<b>Anurans</b>							
<i>Rana tigrina</i> tadpole	27	ad lib	<i>Wolffia</i>	4.3	79	No T effect	Marian (1982)
	27	ad lib	<i>Chironomus</i> sp.	9.1	91		
	27	ad lib	<i>Streptocephalus</i>	9.0	91		
	27	ad lib	<i>Tubifex tubifex</i>	9.6	92		

<i>R. tigrina</i> tadpole	22	<i>ad lib</i>	<i>T. tubifex</i>	9.6	93	No T effect	Marian and Pandian (1985)
	27	<i>ad lib</i>	<i>T. tubifex</i>	9.6	92		
	32	<i>ad lib</i>	<i>T. tubifex</i>	9.6	94		
	37	<i>ad lib</i>	<i>T. tubifex</i>	9.6	96		
<i>R. tigrina</i> tadpole	27	10-100	<i>T. tubifex</i>	9.6	94	No R effect	Marian (1982)
<i>R. tigrina</i> (1-16 tadpoles/aquarium)	27	<i>ad lib</i>	<i>T. tubifex</i>	9.6	94	No density effect	Marian (1982)
<i>R. tigrina</i> tadpole (aquarium depth)	27	<i>ad lib</i>	Cabbage	9.6	94	No aquarium depth effect	Pandian and Marian (1985c)
<i>Rana cyanophlyctis</i> tadpole 0.56 g	26	<i>ad lib</i>	<i>T. tubifex</i>	9.6	94		Present study
<i>R. cyanophlyctis</i> 0.2-1 g	26	<i>ad lib</i>	<i>Hydrilla</i> sp.	4.8	73		Present study
	26	<i>ad lib</i>	<i>Wolffia</i>	4.3	74		
<i>R. tigrina</i> tadpole		<i>ad lib</i>	mixed diet	5.2	66		Hota (1984)
<i>Bufo melanostictus</i> tadpole	29	<i>ad lib</i>	carrot tuber	0.6	54		Present study
			sweet potato	1.3	51		
			beetroot	2.2	59		
			moringa leaf	4.4	71		
<i>B. melanostictus</i> tadpole		<i>ad lib</i>	mixed diet	5.2	70		Hota (1984)
<i>B. terrestris</i> adult 5-100 g	20, 25	<i>ad lib</i>	cricket	6.2*	74	No T and Wt effect	Smith (1976)
<i>Rana tigrina</i> adult 2-25 gW	28	3-100	<i>T. tubifex</i>	9.6	94		Marian (1982)
		<i>ad lib</i>	earthworm	9.1	89		
		<i>ad lib</i>	insect	6.3	74		
		<i>ad lib</i>	slug	8.0	90		

T, temperature; r, ration; Wt, body weight.

\*Values from Campbell (1970), Nicol (1969) or estimation done in our laboratory.

publications. From a survey, about 50 publications were considered. Of them only 12 publications were finally selected; the others were not selected for following reasons: (i) lack of information of N content of the food (Altig and McDearman 1975) and (ii) reporting desired information for amphibians reared under stress conditions such as exposure to pesticide (Marian *et al* 1983). We have chosen publications reporting reliable information on total Ae (in terms of dry weight or energy) of amphibians fed on natural food under normal, healthy, experimental conditions. In some cases, it has been possible for us to secure nitrogen content of the food from other publications (e.g. *Enchytraeus*: Nicol 1969) or from our own estimation (e.g. *Drosophila*). We have made a definite effort to give due representation to herbivorous and carnivorous amphibians so that a wide range of food spectrum would be represented.

Information thus obtained has been analysed under the following headings (table 1): (i) species, body weight and sex (ii) temperature, (iii) ration, (iv) food, (v) nitrogen content of the food and (vi) Ae:

$$\text{Ae (\%)} = \frac{\text{Food absorbed}}{\text{Food consumed}} \times 100,$$

where food absorbed is  $= I - E$ ,  $I$  = food consumed,  $E$  = feces egested (in terms of total dry weight or energy).

### 3. Results

Table 1 presents information on Ae of the tested amphibians inhabiting temperate and tropical regions. Of 11 species, for which reliable information is available, 6 are caudates and 5 are anurans; most of these caudates are adult animals and were tested on animal diet alone; the next 3 species are anuran tadpoles and were tested on a range of feeds from the nitrogen-poor sweet potato tuber to animal diet; the remaining data pertain to 2 adult anurans. The body weight of these amphibians ranged from 0.09 (*Plethoden cinereus*; Crump 1979) to 100 g (*B. melanostictus*; Smith 1976) and the nitrogen content of food from 0.6 (sweet potato tuber) to 9.6% (*T. tubifex*). Some were given different rations (3–100% of *ad libitum*) and subjected to experiments at temperature as low as 5°C and as high as 37°C. Thus, it has been possible to summarize data on the Ae of a number of amphibians studied under a wide range of feeding regimes and experimental conditions.

Figure 1 illustrates the relationship between nitrogen content of food and Ae of the tested amphibians. Tadpoles of *B. melanostictus* fed on tuber containing 0.6% N exhibited 51% efficiency; from this low value, the efficiency rapidly increased to over 90% in several amphibians fed on diet containing > 9% nitrogen. Hence the efficiency of amphibians is positively related to the nitrogen content of food.

To test whether the observed relationship is statistically significant, regression analysis is made considering nitrogen (independent variable) against Ae (dependent variable). The nitrogen content of food is positively correlated ( $r = 0.950$ ) to the Ae of the amphibians (figure 1); the regression obtained ( $\text{Ae} = 49.615 + 4.596 \text{ N}$ ) is highly significant ( $F(1) 1, 39 = 354; p < 0.0005$ ). To describe the precision of the prediction, standard error of the estimate is computed following Zar (1974), which accounts 3.748 of the total estimate. If this relationship is generally applicable, the efficiency of amphibians can reliably and precisely be predicted from the nitrogen content of food.

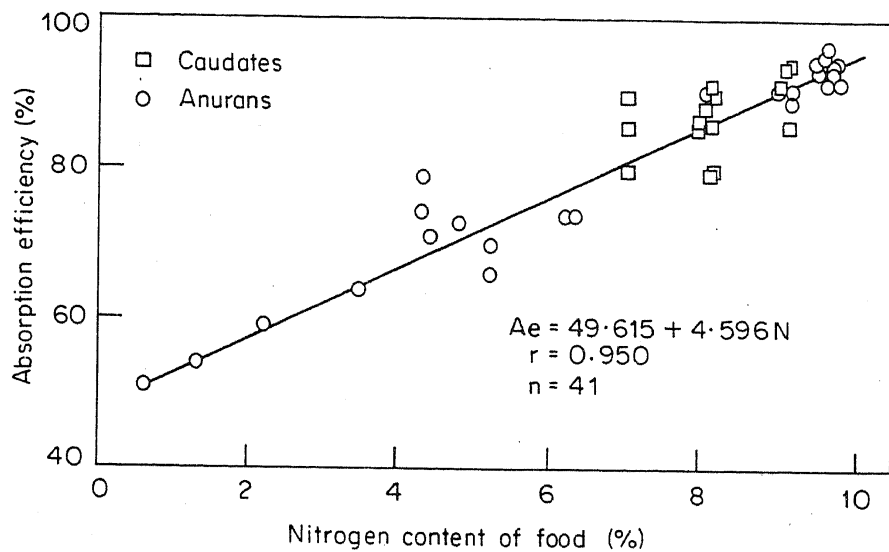


Figure 1. Relationship between the nitrogen content of food and Ae of amphibians.

#### 4. Discussion

The conclusion that food N can precisely predict Ae of amphibians based on 41 values estimated for 11 species. Although it is based on a very few estimations of Ae, the fact that a similar relation has been observed to hold good for fishes, polychaetes, aquatic insects, lepidopterans and reptiles (Pandian and Marian 1985a,b,c,d; Pandian T J and Marian M P, unpublished results) goes to support the conclusion.

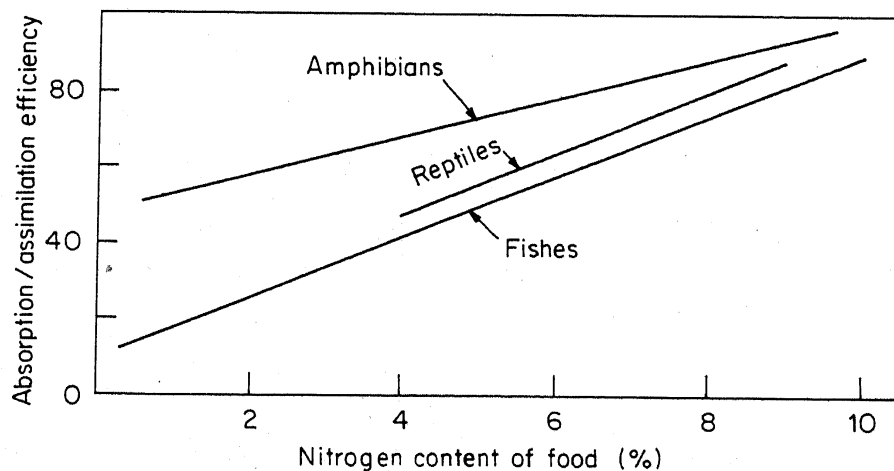
The laborious and time-consuming processes of feces recovery and quantification as well as the scope for introducing errors in the gravimetric procedure for absorption efficiency have led several investigators to defy attempts on the estimation of the efficiency of amphibians (Seale 1985). Although several direct and indirect methods (table 2) are available and frequently followed by fish biologists for estimation of Ae, amphibian biologists appear to be not aware of them. Although the radiotracer method was described by Calow and Fletcher (1972), which is relatively easier, and is based on the more sound principle, Bobka *et al* (1981) have chosen a different  $^{14}\text{C}$  technique for the estimation of absorption efficiency using a complicated procedure. Incidentally, among the indirect procedures thus far described, the food nitrogen index method, described by Pandian and Marian (1985a), requires no quantitative recovery of feces and yet is widely applicable (Pandian and Marian 1985b,c,d; Pandian T J and Marian M P, unpublished results).

Factors such as ration and body size do not significantly influence Ae of the tested amphibian. However, there are reports for and against temperature effect on the efficiency; the efficiency of the caudates *Plethodon* spp is inversely related to temperature (Merchant 1970; Boka *et al* 1981). On the other hand, a number of workers have reported that irrespective of changes in temperature, the efficiency remains unaffected in the caudate *Eurycea bislineata* (Fitzpatrick 1973a), the toad *Bufo terrestris* (Smith 1976) and the tadpole of *R. tigrina* (Marian and Pandian 1985). An important point to be noted here is that the efficiency was estimated in these animals under a wide range of aquatic, semi-aquatic and terrestrial situations; for instance,

Table 2. Available methods for the estimation of absorption efficiency in aquatic animals.

Method	Required estimates		Nature of method	Whether used for amphibians	Example
	Food	Feces			
1. Gravimetric	Consumption estimate required	Recovery and quantification required	Time consuming and laborious	Often used	Fitzpatrick (1972a, b)
2. Artificial markers					
i) Chromic oxide	Analysis required	Recovery not required but analysis required	Requires even distribution of $Cr_2O_3$ in food	No	Austreng (1978)
ii) Radiotracer					
a) $^{14}C$	Analysis required	Recovery not required but analysis required	Requires even distribution of $^{14}C$ in food	Rarely used	Bobka <i>et al</i> (1981)
b) $^{51}Cr$	Analysis required	Recovery not required but analysis required	Requires even distribution of $^{51}Cr$ in food	No	Calow and Fletcher (1972)
3. Natural markers					
i) Lignin	Analysis required	Recovery not required but analysis required	Time consuming	No	Klump and Nichols (1983)
ii) Ash	Analysis required	Recovery not required but analysis	Quickly done	No	Bowen (1981)
iii) Nitrogen	Analysis required	Neither recovery nor analysis required	Quickly done	No	Pandian and Marian (1985a, b, c, d)

Marian and Pandian (1985) estimated the efficiency of *R. tigrina* tadpoles kept in aquarium. Bobka *et al* (1981) made the estimate of *Plethodon* maintained in moist terrarium. It is apparent that when the efficiency of an amphibian is estimated in an aquarium, temperature fails to affect the efficiency but when it is estimated in a terrarium, the reverse is true. Therefore, water appears to have a modifying effect on the simple linear relationship observed between food-N and Ae. It is known that the simple linear relation between food-N and Ae is secondarily modified by water content of food in lepidopterans and other arthropods (Pandian T J and Marian M P, unpublished results). In general, terrestrial animals acquire water mainly from food, and gain or lose water through the body surface (Tracy 1976) and hence are forced to acquire more water by accelerating the alimentation process; such an acceleration will lower the efficiency (Ae). Pandian and Marian (unpublished results) have shown that the nitrogen is more important in the prediction of Ae, and water can serve as co-predictor to improve the accuracy of prediction of Ae from food-N. Basic data on the effect of water on Ae are urgently required for more amphibians, before it could be considered as a co-predictor. However, food-N, can precisely predict Ae of aquatic amphibians, in which feces recovery and quantification are difficult. In figure 2, a comparison of the trends obtained for food-N and Ae relation in fishes (Pandian and Marian 1985a), amphibians and reptiles (Pandian and Marian 1985b) are made; a positive and significant correlation observed between food-N and Ae of these animal groups renders it possible to predict Ae from food-N with more than 90% accuracy. It may be noted that the given diet is more efficiently digested and absorbed by amphibians than by fishes and reptiles; for instance, fed on food containing 6% N, the efficiency is 78% for amphibians in comparison to 57 and 63% for fishes and reptiles, respectively. The reason for this dissimilarity in the efficiency among these animal groups are not clear at present; further work and analysis are in progress to know the underlining physiological processes that is responsible for the differences.



**Figure 2.** Comparison of the trends obtained for food-N and absorption/assimilation efficiency relation in fishes, amphibians and reptiles. For fishes ( $\log Ae = 1.3706 + 0.5807 \log N$ ) and reptiles ( $Ae = 14.424 + 8.210 N$ ). Values are taken from Pandian and Marian (1985a, b).

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