

USING STABLE ISOTOPE ANALYSES TO IDENTIFY ALLOCHTHONOUS INPUTS TO LAKE NAIVASHA MEDIATED VIA THE HIPPOPOTAMUS GUT*

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The hippopotamus grazes nocturnally on land and resides in water during the day. Much of the ingested material must therefore be defecated directly into the aquatic system and can thus be considered an allochthonous resource available to aquatic consumers. The utility of stable isotope analyses of carbon and nitrogen to distinguish hippo faecal matter from other potential basal resources was tested at Lake Naivasha, Kenya. Hippopotami proved faithful to a short grass diet although supplementary grazing of aquatic macrophytes was observed. The typical isotopic ratios of C4 grasses ingested were not altered substantially by gut processes, and were clearly distinct from algal and aquatic macrophyte isotopic ratios. However, marginal plants such as *Cyperus papyrus* exhibit C4 ratios, and so the technique is suitable only for use in localities where 'contamination' from such sources is negligible.

Keywords: Allochthonous; C4 plants; Carbon 13; Hippopotamus; Lake Naivasha; Natural variations; Nitrogen 15

INTRODUCTION

The hippopotamus (*Hippopotamus amphibious* – hereafter hippo) is a large, amphibious mammal and common resident in lakes and rivers bordered by grassland throughout sub-Saharan Africa. It is typically herbivorous; adults are capable of ingesting approximately 40 kg of short sward grasses during nocturnal forays of the catchment [1]. Although there have been reports of carnivory in hippos [2], it is relatively rare and likely fulfilling a nutritional need since vegetation often lacks essential nutrients or trace elements [1]. Studies of the dietary composition of hippos are few, the most detailed being those of Field [3–6]; grass species were dominant with some dicotyledons ingested. From comparison of gut content versus sward abundance, Field [6] suggested that hippos ingested grass species selectively.

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Since grazing occurs mainly on land, and much of the day is spent partially submerged to avoid overheating and dehydration, a large proportion of ingested food is defecated directly into the water. Hippo dung can therefore be considered as allochthonous organic matter mediated via the hippo gut. Previous studies have examined effects of hippos on surrounding water quality (*e.g.* [7]) but few, if any, have investigated direct use of the particulate matter by aquatic consumers. Hippos are pseudo-ruminants; they possess a chambered stomach suggesting ruminant-like digestion, but still have to ingest large quantities of low quality food and process it relatively quickly [3]. Egested material is partially fermented and thus an ideal substrate for further microbial activity. Many terrestrial insects such as members of the Scarabaeidae are known to utilise dung either directly for food or as a nutritional source for their developing offspring. This behaviour is relatively easy to observe and assess on land, but considerably more difficult in aquatic systems.

It was hypothesised that stable isotope analyses (SIA) of carbon and nitrogen could be used to differentiate between hippo dung, other allochthonous and autochthonous basal resources, and thus potentially be used to trace contributions from hippo dung to aquatic consumers directly (*c.f.* [8]). Prior to an evaluation of contributions to the food-web, a preliminary study was necessary to determine whether the isotopic signature of hippo dung was distinct relative to other putative basal resources. The study was reliant on various assumptions requiring investigation. First, hippos were selective in their feeding of particular grass species as suggested by Field [6]. Second, the isotopic composition of faecal material would reflect that of the ingested material although digestion and assimilation are acknowledged to have subsequent effects [9]. Third, the ingested grass species were using the C4 photosynthetic pathway, and thus likely to exhibit a distinct signature compared to aquatic basal resources. Fourth, that C4 derived allochthonous material was not introduced to the study site via alternative vectoring to invalidate the use of SIA.

METHODS

Observations were made and samples collected from Lake Naivasha, a shallow, productive freshwater lake in the western rift of Kenya, E. Africa during July and August 2001. The hippo population fluctuates around 500 individuals [10]. Samples were collected from a Kenya Wildlife Services training ground (Hippo Camp) in the riparian zone on the eastern side of the lake. Hippo activity in the water was monitored at dawn and dusk. Foraging range in the riparian zone was determined by tracking and monitoring grazing disturbance on the grass swards. Terrestrial plant cover was determined along transects (sample points every 100 m) from the waterline to the point most distant at which hippo activity was detected. Shoots and leaves of abundant plant species (pooled from >10 plants) were collected for determination of elemental and stable isotope composition. Aquatic macrophytes and sediment samples from the littoral zone close to hippo resting areas were collected by grapnel and Ekman grab respectively. Samples of a deteriorating phytoplankton bloom were scooped from surface scum. All plant samples were rinsed copiously and oven dried at ~60 °C for 48 hours. Dung samples were collected on land as a proxy for those deposited in water. Fresh faecal matter from the previous night was sampled on dawn forays. Sub-samples were taken for wet (fresh) and dry (oven dried at ~60 °C) weight, and for examination of plant or molluscan remains. Dried samples were stored in aluminium foil packets prior to cryogenic homogenisation (Glen Creston, UK) and subsequent stable isotopic analyses. To control for macrophyte ingestion, dung and forage grasses were collected from the Marula Estate, where hippos reside in the Malewa river (no macrophytes). Triplicate samples

weighed into tin cups were combusted in a Carlo Erba NA1500 elemental analyser, interfaced with a Micromass IsoPrime IRMS. Isotope ratios are expressed conventionally in per mil (‰) relative to secondary standards of known relation to Vienna PDB for carbon, and atmospheric nitrogen. Repeat analysis ($n = 120$) of an internal standard showed precision of $<0.2\text{‰}$ for both isotope ratios.

RESULTS AND DISCUSSION

Three groups of hippos were observed during daylight. At dusk, 10–70% of individuals in each group were observed to graze on aquatic vegetation prior to emergence on land. Such grazing activity was observed less frequently at dawn. Hippos ventured a maximum of 1.4 km distant from the lake-shore. From patterns of dunging and grazing disturbance, hippos appeared to concentrate their grazing on *Pennisetum clandestinum* swards at 0.5, 0.8 and 1.2–1.4 km. At these locations, mean percentage ground cover by plant species comprising $>5\%$ was calculated from all transects; *P. clandestinum* comprised 50–100% (median 84.8%). Only stems of *P. clandestinum* were identifiable in dung samples, contributing 64–69% wet weight. This is comparable to 40% non-stem material in dung samples reported by Owen-Smith [11]. There was no evidence that would suggest grazing of aquatic macrophytes, such as recalcitrant remains of molluscs or other macroinvertebrates. Therefore, despite observations of ingestion of aquatic macrophytes, the limited time of grazing on these resources and the dominance of *P. clandestinum* in the faecal material suggests hippos were mostly consuming a C4 grass diet.

Dunging rate was estimated at one per individual per night on land. Maximum dung wet weight was approximately 8 kg. If it is crudely assumed that a hippo ingests 40 kg per night, excretes 8 kg on the catchment and the remainder in the lake, then the Naivasha population of hippos introduce 5840 tonnes of dung into the lake annually (370 tonnes of carbon and 15 tonnes of nitrogen based upon dung wet weight = $5.8 \times$ dry weight; mean dung composition: 37% carbon; 1.5% nitrogen).

Stable isotope ratios of hippo dung closely reflected those of *P. clandestinum* collected from along the transects; mean dung ratios were approximately 2‰ ^{13}C -depleted and 1‰ ^{15}N -enriched (Tab. I). Only mean values were compared since no inference could be made as to which dung sample related to a particular area of grass grazed. There was considerable variability in the $\delta^{15}\text{N}$ of *P. clandestinum* which may partly be explained by grazer activity (Fig. 1). Intense grazing depicted by reduced sward depth exhibited a positive relationship to the grass $\delta^{15}\text{N}$ values suggesting some feedback of urinary products into the soil. Hippo dung isotope ratios would not be expected to match those of *P. clandestinum* exactly due to some assimilation in the gut and subsequent addition of waste products prior to excretion [9].

A further source of variation would be the inclusion of a relatively small proportion of aquatic macrophyte material (as observed). However, the relationship between dung and grass isotopic values at Marula Estate (where hippos cannot ingest macrophytes) was similar to that at Hippo Camp (Tab. I) and so the impact of aquatics on dung isotopic signature appears negligible.

Terrestrial plants from amongst the *Pennisetum* sward, marginal plants and aquatic autochthonous production also were analysed for stable isotope ratios (Tab. I). Each group contained plants exhibiting typical C3-pathway carbon isotope values (around -25‰), clearly distinct from those utilising the C4-pathway, and comparable to other Kenyan sites [12]. Two submerged aquatic species, *Najas horrida* and *Potamogeton pectinatus* exhibited extremely ^{13}C -enriched values. Since all plant samples were rinsed and acidified, these values

TABLE I Carbon and Nitrogen Stable Isotope Ratios (Mean \pm 1 SD‰) from Hippo Dung and Terrestrial, Marginal and Aquatic Plants, (Including Phytoplankton and Sediment) from Lake Naivasha. n = Replicates.

Species	n	$\delta^{13}C$	$\delta^{15}N$
Hippo dung	25	-12.2 ± 1.8	5.0 ± 1.2
Terrestrial			
<i>Pennisetum clandestinum</i>	10	-10.3 ± 1.0	3.9 ± 3.1
<i>Juncus tenuis</i>	5	-8.88 ± 0.1	3.2 ± 0.5
<i>Aristida siberana</i>	3	-12.4 ± 0.1	4.0 ± 0.4
<i>Oxalis corniculata</i>	3	-26.6 ± 0.1	-3.8 ± 0.2
Clover spp	3	-27.5 ± 0.4	-1.9 ± 0.1
Marginal			
<i>Helichrysum</i> spp.	3	-26.4 ± 0.1	-1.1 ± 0.3
<i>Polygonum amphibium</i>	5	-26.6 ± 1.1	2.0 ± 2.6
<i>Cyperus papyrus</i>	5	-11.7 ± 0.2	2.6 ± 0.2
<i>Papyrus typha</i>	3	-8.2 ± 0.1	-2.4 ± 0.3
Sedge spp	3	-8.4 ± 0.2	6.9 ± 1.3
Aquatics			
<i>Salvinia molesta</i>	5	-27.7 ± 0.3	8.4 ± 0.4
<i>Eichhornia crassipes</i>	5	-24.5 ± 0.2	3.3 ± 0.3
<i>Nymphaea caerulea</i>	5	-22.9 ± 0.1	-0.2 ± 0.2
<i>Najas horrida</i>	5	-2.0 ± 0.6	-0.8 ± 0.7
<i>Potamogeton pectinatus</i>	5	-2.3 ± 0.0	0.3 ± 0.3
<i>Aulacoseira</i> dominated bloom	5	-15.0 ± 0.4	13.4 ± 0.3
Sediment	5	-17.5 ± 0.6	2.5 ± 0.4
Marula Estate			
Hippo dung	8	-11.5 ± 0.7	7.3 ± 1.0
<i>P. clandestinum</i>	5	-10.2 ± 0.2	5.8 ± 1.4

must reflect the dissolved inorganic carbon environment surrounding the plants. The phytoplankton bloom was likely to have elevated pH, and a corresponding increase in relative contribution from HCO_3^- to the DIC pool may be expected. Phytoplankton $\delta^{13}C$ values also suggested an enriched DIC source. However, bloom conditions and localised nutrient

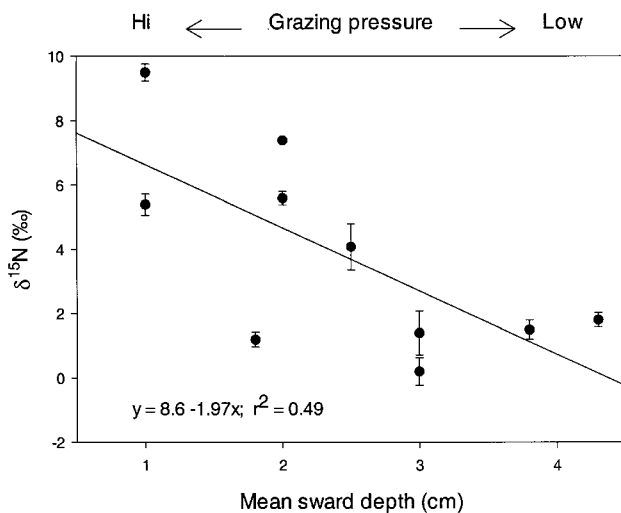


FIGURE 1 Relationship between mean sward depth (as an indicator of grazing pressure) and $\delta^{15}N$ of *Pennisetum clandestinum* at 'Hippo Camp', Lake Naivasha. Fitted line is a linear regression; error bars are 1 SD from triplicate analyses.

enrichment, coupled with rapid turnover times are reported to cause short term shifts in algal isotopic values [13], but under stable conditions, algal signatures in Lake Naivasha would be more depleted in both ^{13}C and ^{15}N and therefore even more distinct from the hippo dung isotope values. Primary production ($100\text{--}150\text{ mg C m}^{-3}\text{ hr}^{-1}$) is the main contributor to sedimentation in Lake Naivasha [14] and this is reflected in the similarity of the algal and sediment $\delta^{13}\text{C}$ values (Tab. I).

Transport of alternative allochthonous resources into Lake Naivasha is linked to wind and rainfall events. Much of the catchment, and in some places the riparian zone is under horticulture [10] and thus mostly C3 vegetation. The notable C4 exception, maize is grown on higher ground in the river valleys. Inputs from the Malewa river ($\sim 80\%$ of inflow) appear restricted to within 1 km of the river mouth [15] and unlikely to reach the study site [16]. Although this material generally had an organic matter content of $>50\%$ [15], it is of a lower content, and by inference quality, compared to fresh dung. Aerial deposition from wind erosion in the catchment has not been quantified, or qualified from an isotopic perspective. It does not appear to contribute greatly to sediment composition [16] relative to algal production [14] and the refractory nature of aerially-derived material again suggests a lower quality resource for aquatic consumers compared to hippo dung. Marginal plants may contribute detrital material, especially *C. papyrus* (C4) which forms substantial stands around parts of the riparian zone. However, C4 marginal plants were scarce at the study site compared to the dense submerged stands of *Najas* and *Potamogeton*. These extended 40–50 m from the shore, likely preventing shoreline erosion from introducing further allochthonous material.

In summary, the current study suggests that hippos were selectively ingesting particular grass species and that the hippo dung isotope values reflected those of the ingested material. The resultant C4-derived dung signature was distinct compared to the dominant autochthonous basal resources sampled from the lake, and vectoring from other sources which might exhibit similar signatures appeared negligible at the site. Caution is advised when applying SIA to other sites, since the occurrence of C4 marginal plants, and temporal variability in algal isotopic signatures may obscure dung isotope ratios and preclude the use of the method.

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References

- [1] Eltringham, S. K. (1999). *The Hippos*. Academic Press, London, p. 184.
- [2] Dudley, J. P. (1998). Reports of carnivory by the common hippo *Hippopotamus Amphibius*. *S. Afr. J. Wild. Res.*, **28**, 58–59.
- [3] Field, C. R. (1968). The food habits of some wild ungulates in relation to land use and management. *E. Afr. Agric. For. J.*, **33**, 159–162.
- [4] Field, C. R. (1968). Methods of studying the food habits of some wild ungulates in Uganda. *Proc. Nutr. Soc.*, **27**, 172–177.
- [5] Field, C. R. (1970). A study of the feeding habits of the hippopotamus (*Hippopotamus amphibious* Linn.) in the Queen Elizabeth National Park, Uganda, with some management implications. *Zool. Afr.*, **5**, 71–86.
- [6] Field, C. R. (1972). The food habits of wild ungulates in Uganda by analyses of stomach contents. *E. Afr. Wildlife J.*, **10**, 17–42.

- [7] Wolanski, E. and Gereta, E. (2001). Water quantity and quality as the factors driving the Serengeti ecosystem, Tanzania. *Hydrobiol.*, **458**, 169–180.
- [8] Grey, J., Jones, R. I. and Sleep, D. (2001). Seasonal changes in the importance of allochthonous organic matter to the diet of zooplankton in Loch Ness indicated by stable isotope analysis. *Limnol. Oceanogr.*, **46**, 505–513.
- [9] Minagawa, M. and Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta*, **48**, 1135–1140.
- [10] Earthwatch Scientific Report (1997–1999). Research at Lake Naivasha on permit, *OP/13/001/12C 46*.
- [11] Owen-Smith, R. N. (1988). Megaherbivores. *The Influence of Very Large Body Size on Ecology*. Cambridge University Press, p. 384.
- [12] Gichuki, J., Triest, L. and Dehairs, F. (2001). The use of stable carbon isotopes as tracers of ecosystem functioning in contrasting wetland ecosystems of Lake Victoria, Kenya. *Hydrobiol.*, **458**, 91–97.
- [13] O'Reilly, C. M., Hecky, R. E., Cohen, A. S. and Plisnier, P.-D. (2002). Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnol. Oceanogr.*, **47**, 306–309.
- [14] Hubble, D. S. and Harper, D. M. (2001). Impact of light regime and self shading by algal cells on primary productivity in the water column of a shallow tropical lake (Lake Naivasha, Kenya). *Lakes and Reservoirs: Research and management*, **6**, 143–150.
- [15] Kitaka, N., Harper, D. M. and Mavuti, K. M. (2003). Phosphorus inputs to Lake Naivasha from its catchment and the trophic state of the lake. *Hydrobiol.* (in press).
- [16] Tarras-Wahlberg, H., Everard, M. and Harper, D. M. (2003). Geochemical and physical characteristics of river and lake sediments at Naivasha, Kenya. *Hydrobiol.* (in press).