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Carbon stable isotopes suggest that hippopotamus-vectored nutrients subsidize aquatic consumers in an East African river

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Abstract. The common hippopotamus, *Hippopotamus amphibius*, transports millions of tons of organic matter annually from its terrestrial feeding grounds into aquatic habitats. We evaluated whether carbon stable isotopes ($\delta^{13}C$) can be used as tracers for determining whether *H. amphibius*-vectored allochthonous material is utilized by aquatic consumers. Two approaches were employed to make this determination: (1) lab-based feeding trials where omnivorous river fish were fed a *H. amphibius* dung diet and (2) field sampling of fish and aquatic insects in pools with and without *H. amphibius*. Lab trials revealed that fish fed exclusively *H. amphibius* dung exhibited significantly more positive $\delta^{13}C$ values than fish not fed dung. Fish and aquatic insects sampled in a river pool used for decades by *H. amphibius* also exhibited more positive $\delta^{13}C$ values at the end of the dry season than fish and insects sampled from an upstream *H. amphibius*-free reference pool. Fish sampled in these same pools at the end of the wet season (high flow) showed no significant differences in $\delta^{13}C$ values, suggesting that higher flows reduced retention and use of *H. amphibius* subsidies. These data provide preliminary evidence that $\delta^{13}C$ values may be useful, in certain contexts, for quantifying the importance *H. amphibius* organic matter.

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Fig. 1. A single common hippopotamus, *Hippopotamus amphibius*, consumes greater than 10 tons of terrestrial organic matter annually during nightly nocturnal foraging forays onto land. Much of this terrestrial material is transported into the aquatic ecosystems where *H. amphibius* takes refuge each day. Photo credit: Tim Hearn.

INTRODUCTION

Stable isotopes of carbon, nitrogen, and hydrogen are commonly employed to measure how freshwater consumers use terrestrially generated allochthonous organic matter (Finlay 2001, Doucett et al. 2007, Finlay and Kendall 2008). The ecological importance of a wide range of such terrigenous allochthonous subsidies have been considered in lakes and rivers including litterfall, fruitfall, organic rich soil runoff, and terrestrial insect input (Polis et al. 1997, Nakano et al. 1999, Wantzen et al. 2002, Caraco et al. 2010, Roach 2013).

One potentially important but little studied route of terrestrial to aquatic organic matter subsidization in African watersheds may be maintained by the common hippopotamus, *Hippopotamus amphibius*. This herbivorous semi-aquatic mega-consumer forages widely on land

at night consuming largely terrestrial C4 grasses and some browse (Eltringham 1999, Grey and Harper 2002, Cerling et al. 2008). Individual *H. amphibius* consume approximately 40–50 kg (wet mass) of terrestrial organic matter per night (Lewison and Carter 2004) and then spend all or most of the daylight hours in aquatic refuges where a large proportion of this nutrient rich terrestrial intake is excreted (Fig. 1; Appendix A; Subalusky et al. 2014).

The potential ecological importance of *H. amphibius* as a vector of terrestrial organic matter subsidies to watersheds has been hypothesized by other researchers (Naiman and Rogers 1997, Grey and Harper 2002, Jacobs et al. 2007, Mosepele et al. 2009, Jackson et al. 2012, Subalusky et al. 2014). In this study we use carbon stable isotope ($\delta^{13}C$) measurements to evaluate whether the organic matter that *H. amphibius* excrete into riverine ecosystems is utilized by aquatic consumers. To this end, we

carried out feeding trials of captive river fish fed exclusively *H. amphibius* dung and conducted field sampling of aquatic vertebrates and invertebrates in parts of a river in central Kenya that did and did not harbor *H. amphibius*.

METHODS

Site description

Field sampling was conducted in Ewaso Ng'iro River in Laikipia District, Kenya (36°54′ E, 0°19′ N). Rainfall in the region is weakly trimodal with peak rainfall occurring in April–May, July–August, and October–November. The Ewaso Ng'iro has extremely high sediment loads, amongst the highest measured in Kenya (Gichuki 2002). This loading suppresses light penetration and severely inhibits in situ algal growth. The hydrologically dynamic nature of the Ewaso Ng'iro largely prevents the establishment of large stands of marginal plants or floating aquatic vegetation.

Field sampling was concentrated at two focal pools in the Ewaso Ng'iro: one pool that has been identified by local experts as a long-term occupancy site for an aggregation of *H. amphibius* (hereafter "*H. amphibius* pool") and a second pool, 1.8 km upstream, where resident *H. amphibius* were not seen (hereafter "reference pool"). The lower boundary of the reference pool is intersected by a public bridge and the disturbance from this crossing is in part presumed to deter use by *H. amphibius*. Sampling was conducted in a 185×40 m (mean depth $= 2.9$ m) region of the *H. amphibius* pool and in a 150×25 m (mean depth $= 2.7$ m) section of the reference pool.

Hippopotamus amphibius surveys

The presence or absence of *H. amphibius* at the *H. amphibius* pool and the reference pool were monitored visually throughout the study. During daylight hours observers counted numbers of *H. amphibius* present in the pool or on the bank. The presence and abundance of *H. amphibius* were also estimated at the *H. amphibius* pool using camera traps (Reconyx). The presence/absence and maximum number of *H. amphibius* observed at any point between 0700 and 1855 hours were recorded each day. To estimate patterns of long-term *H. amphibius* use of the *H. amphibius* and

reference pools, we interviewed nine persons with >10 years of permanent or intermittent residency in this region. In February 2012, we surveyed approximately 125 km of the Ewaso Ng'iro watershed near to our study site from fixed wing aircraft, recording the number and location of *H. amphibius*.

Hydrological monitoring

Daily records of river discharge were collected by a stream gauging station at Hulmes Junction on the Ewaso Ng'iro River, located 30 km upstream of the *H. amphibius* pool. These data were obtained from the Water Resources Management Authority (WRMA) and used to determine the seasonal variation in river discharge during this period of study. Any gaps in these discharge measurements were estimated using daily regional rainfall records from the Tropical Rainfall Measurement Mission (TRMM) and discharge/rainfall correlations.

Feeding trials

To determine, in a controlled environment, how consumption of *H. amphibius* dung may influence the isotopic composition of river consumer tissue, we monitored the isotopic composition of wild-caught guppies, *Poecilia reticulata*, fed exclusively *H. amphibius* dung. For these trials fresh *H. amphibius* dung was collected from four wild *H. amphibius* individuals at our study site, homogenized, frozen, and fed to *P. reticulata* throughout the duration of the experiment. *P. reticulata* are an introduced species in the Ewaso Ng'iro that have a broadly omnivorous diet. All *P. reticulata* used in these trials were originally captured from the reference pool lacking *H. amphibius*. Fifteen of these *P. reticulata* were lethally sampled immediately upon collection from the river (i.e., not held in captivity) to provide baseline values for isotopic comparison ("control *P. reticulata*"). Remaining captive *P. reticulata* were fed *H. amphibius* dung daily to satiation. Dung fed *P. reticulata* were starved for 24 h to completely clear their gut prior to collection (Potts 1998). *Poecilia reticulata* fed the *H. amphibius* dung diet were collected three months ($n = 11$) and six months ($n = 12$) after their switch to a dung diet ("dung fed" *P. reticulata*). All *P. reticulata* were measured (total length; TL), frozen, air-dried, ground whole, and
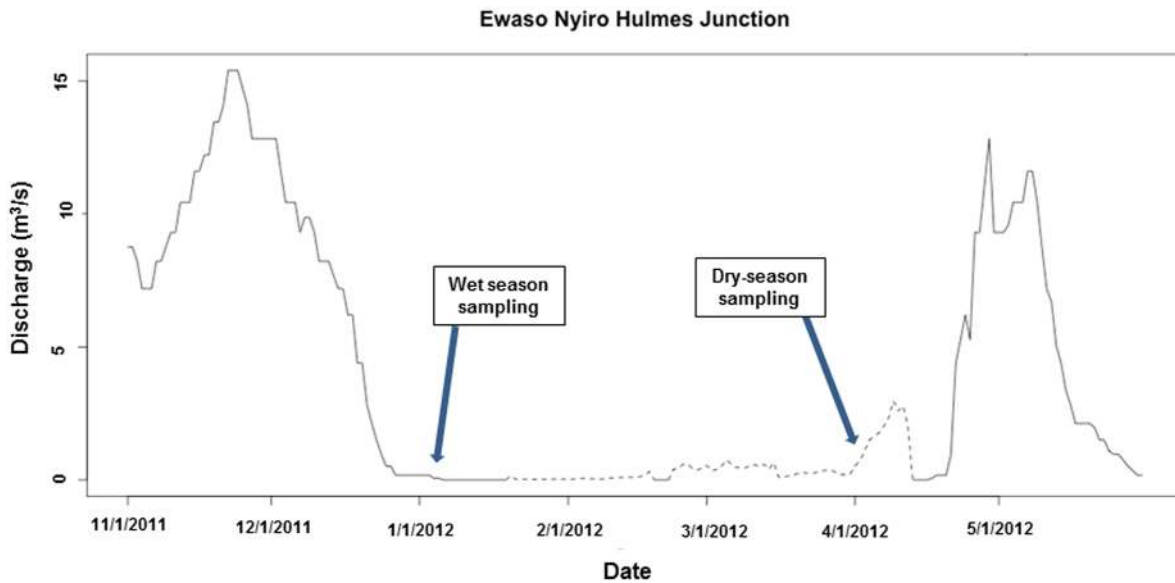
Ewaso Nyiro Hulmes Junction

Fig. 2. Actual (solid) and simulated (dotted) discharge in the Ewaso Ng'iro River at the Hulmes Junction gauge station. Markers indicate the timing of in situ stable isotope sampling conducted at the end of the wet and dry seasons.

analyzed for $\delta^{13}C$ and $\delta^{15}N$ values as described below. Because ultimately no isotopic differences were observed between dung fed *P. reticulata* sampled at three and six months (Appendix B), these groups were pooled for analysis. To determine if tissue compositional changes (e.g., changes in lipid concentration) influenced the isotopic values measured in dung-fed and control *P. reticulata* populations, we compared the C:N values (atomic) of both dung fed and control populations as well as the C:N values of dung fed *P. reticulata* sampled at month three and month six of the experiment.

In situ sampling

We collected and measured the isotopic composition of three putatively important allochthonous terrestrial organics sources in the Ewaso Ng'iro River: *H. amphibius* dung (n = 11); leaves of the C4 grass *Cynodon plectostachyus* (n = 8); and the abundant C3 riparian tree *Acacia xanthophloea* (n = 9). Samples were collected periodically over the course of the study. *C. plectostachyus*, like most of the grasses at our study site, employ the C4 photosynthetic pathway (Tieszen et al. 1979). *Acacia xanthophloea* is a deciduous/semi-deciduous C3 tree that often forms monodominant

stands along rivers in East Africa (Young and Lindsay 1988). We also sampled the isotopic composition of particulate organic matter (POM) in the *H. amphibius* pool (n = 9) and reference pool (n = 9) by filtering 25 ml of river water pumped 75 cm from the bottom of these pools onto pre-combusted glass fiber filters (Whatman 0.7 μm).

To examine potential patterns of use of these allochthonous sources by aquatic residents, we sampled in situ two abundant and ecologically important river consumers: the omnivorous cyprinid fish *Labeobarbus oxyrhynchus* (maximum length ~ 50 cm TL) and larvae of the dragonfly *Trithemis* spp. Both *Labeobarbus oxyrhynchus* and *Trithemis* spp. were collected from *H. amphibius* pool and the reference pool. *Labeobarbus oxyrhynchus* were sampled at two different times: once at the end of a particularly pronounced wet season (n = 9 *H. amphibius* pool; n = 10 reference pool) in January 2012 and once at the conclusion of the prolonged dry season in April 2012 (n = 8 both pools) (Fig. 2). All *L. oxyrhynchus* were measured (TL), fin clipped, and a sample of whole blood was drawn. *Trithemis* spp. were sampled only during the dry season in April 2012 (n = 8 *H. amphibius* pool; n = 7 reference pool). All

Trithemis spp. collected were taken by net, weighed, and ground whole. Isotopic turnover in whole blood and whole insects can vary but has been estimated to occur on the order of approximately one month (Hobson and Clark 1992, Buchheister and Latour 2010) and several weeks respectively (Gratton and Forbes 2006). C:N values of *Trithemis* spp. were compared between the *H. amphibius* pool and reference pools as a means of assaying potential differences in their lipid concentrations. All isotope samples were air dried at 45°C, ground, and analyzed for carbon and nitrogen stable isotopes at the UC Berkeley Center for Stable Isotope Biogeochemistry using a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer. All samples were run in bulk form without the extraction or isolation of lipids or other compounds. Only $\delta^{13}C$ values were measured in POM samples.

Statistics

Data were compared using either Welch's t-tests (when parametric assumptions were met) or Wilcoxon tests (i.e., $\delta^{13}C$ values of *L. oxyrhynchus* wet season; $\delta^{15}N$ values, TL, and C:N values of *P. reticulata*; mass of *Trithemis* spp.; and $\delta^{13}C$ values of *A. xanthophloea*). Error is reported throughout as standard deviation (SD). As a complement to direct observations of isotopic differences in consumers, we used Bayesian isotope mixing models to estimate the relative contribution of *H. amphibius* dung to *L. oxyrhynchus* and *Trithemis* spp. sampled in *H. amphibius* and reference pools. A two isotope ($\delta^{13}C$, $\delta^{15}N$), two source (*H. amphibius* dung/C4 grass and C3 riparian tree material) mixing model was implemented in R using MixSIAR (Parnell et al. 2013, Stock and Semmens 2013). Additional details of mixing model construction are listed in Appendix C. All statistics were run in R (R Core Team 2014).

Results

Hippopotamus amphibius surveys

A total of 38 visual surveys split evenly between the *H. amphibius* pool and reference pool were conducted in January and April 2012. *H. amphibius* were present in 100% of the surveys at the *H. amphibius* pool and were never detected at the reference pool. From the camera traps, we analyzed 13,103 images representing 91 consecutive days of monitoring. *H. amphibius* were present during 100% of these monitored days at the *H. amphibius* pool. The average daily maximum number of *H. amphibius* recorded at the *H. amphibius* pool was 19 (±3.7 SD) individuals and their numbers varied little across the wet and dry season (Appendix D).

All interview respondents stated that a *H. amphibius* pod had been continuously resident at the *H. amphibius* pool since their arrival to the region (i.e., >10 years ago). Based on these reports, *H. amphibius* have been largely resident at *H. amphibius* pool since at least 1947. All but one respondent reported having never observed resident *H. amphibius* at the reference pool (the single observation was of a mother and calf pair that used the reference pool briefly in 2005). Aerial surveys indicated that the nearest pod of consistently resident *H. amphibius* (~5 animals) was located 43 km upstream of our study reference pool.

Hydrological monitoring

Discharge data for the Ewaso Ng'iro River during the period of November 1, 2011 to May 31, 2012 at the Hulmes Junction station are shown in Fig. 2. Discharge in the Ewaso Ng'iro declined sharply before the January 2012 (high flow) in situ isotope sampling period and increased shortly after the April 2012 (low flow) in situ isotope sampling period.

Feeding trials

The $\delta^{13}C$ values of captive, dung fed *P. reticulata* were significantly more positive than $\delta^{13}C$ values of control *P. reticulata* collected from the control pool (lacking hippos) and not fed a dung diet ($\delta^{13}C = -19.77 \pm 1.31$ vs. $\delta^{13}C = -21.20 \pm 1.41$; $t = 3.2$, $p < 0.01$). This shift in the $\delta^{13}C$ values of dung fed *P. reticulata* was in the direction of the more positive C4 plant values measured for *H. amphibius* dung (Fig. 3). No difference was observed in the $\delta^{15}N$ values of dung fed and control *P. reticulata* ($\delta^{15}N = 11.07 \pm 0.45$ vs. $\delta^{15}N = 10.81 \pm 0.47$; $W = 209$, $p = 0.28$). There was no difference in the total length of dung fed and control *P. reticulata* ($W = 175$; $p = 0.94$). C:N values of dung fed and control *P. reticulata* were not significantly different ($W = 138$; $p = 0.31$). C:N values of dung fed *P. reticulata*
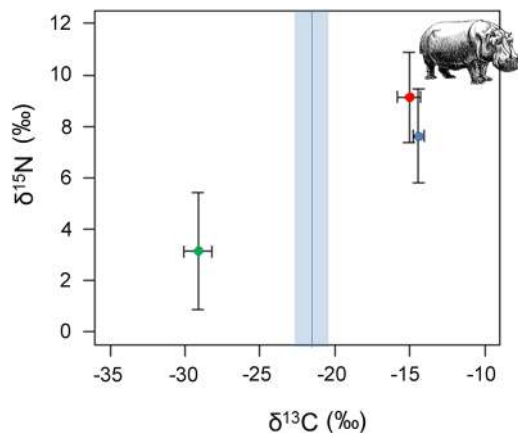
Fig. 3. Carbon and nitrogen stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$ values; mean ($\pm$SD)) of three potential sources of allochthonous organic matter to the Ewaso Ng'iro River: *Hippopotamus amphibius* dung (red circle), the near-river C4 grass *Cynodon plectostachyus* (blue circle), and the C3 riparian tree *Acacia xanthophloea* (green circle). The $\delta^{13}C$ values of particulate organic carbon (POM) sampled in the Ewaso Ng'iro are also plotted (mean blue line with $\pm$SD plotted in light blue); nitrogen concentrations were too low to measure the $\delta^{15}N$ values of POM. The $\delta^{13}C$ and $\delta^{15}N$ values were measured relative to the standards V-PDB and air, respectively.

Fig. 4. Carbon stable isotope values ($\delta^{13}C$; mean $\pm$ SD) of river fish *Labeobarbus oxyrhynchus* (circles) and aquatic larvae of the dragonfly *Trithemis* spp. (triangle) sampled in an Ewaso Ng'iro River pool hosting a resident pod of *Hippopotamus amphibius* (black) and a nearby reference pool lacking *H. amphibius* (white). *Labeobarbus oxyrhynchus* were sampled in both pools at the end of the wet and dry seasons. *Trithemis* spp. were sampled only after the dry season. More positive $\delta^{13}C$ values suggest an increased affinity to the observed heavier $\delta^{13}C$ values of *H. amphibius* dung. An asterisk indicates statistically significant differences between pools. The $\delta^{13}C$ and $\delta^{15}N$ values were measured relative to the standards V-PDB and air, respectively.

harvested and sampled at three months were not significantly different from dung fed *P. reticulata* harvested at six months ($W = 84$; $p = 0.29$).

In situ sampling

Hippopotamus amphibius dung and the dominant C4 grass *C. plectostachyus* differed in $\delta^{13}C$ values ($t = 2.5$, $p = 0.04$), but not in $\delta^{15}N$ values ($t = -0.55$, $p = 0.59$; Fig. 3). Dung was more positive with respect to both $\delta^{13}C$ values ($W = 99$, $p < 0.001$) and $\delta^{15}N$ values ($t = 6.5$, $p < 0.001$) than the C3 riparian tree *A. xanthophloea* (Fig. 3). The $\delta^{13}C$ values of river POM in the *H. amphibius* pool were not significantly different from the $\delta^{13}C$ values of POM measured in the reference pool ($\delta^{13}C = -21.17 \pm 0.66$ vs $\delta^{13}C = -22.17 \pm 1.09$; $t = -1.4$, $p = 0.24$; Fig. 3).

The $\delta^{13}C$ values of the river fish *L. oxyrhynchus* were not significantly different between the *H. amphibius* and reference pools during wet season sampling ($W = 33$, $p = 0.36$; Fig. 4). During the dry season, however, $\delta^{13}C$ values of *L. oxy-*

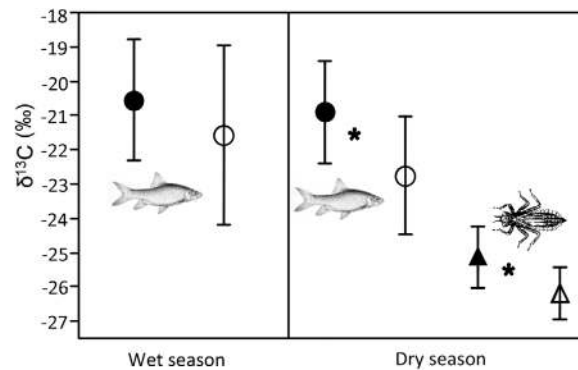*rhynchus* sampled in the *H. amphibius* pool were significantly more positive than $\delta^{13}C$ values of fish sampled in the reference pool lacking *H. amphibius* ($t = -2.3$, $p = 0.04$). There was no significant difference between $\delta^{15}N$ values of *L. oxyrhynchus* in the *H. amphibius* and reference pools during either the wet or the dry season (wet: $t = 0.57$, $p = 0.58$; dry: $t = 1.8$, $p = 0.10$). No difference was observed between the mean length of the fish *L. oxyrhynchus* collected in the *H. amphibius* and reference pools during either sampling period (wet: $t = -0.30$, $p = 0.77$; dry: $t = -1.9$, $p = 0.08$).

Dragonfly larvae *Trithemis* spp. sampled in the *H. amphibius* pool during the dry season also had significantly more positive $\delta^{13}C$ values than *Trithemis* spp. sampled in the reference pool ($t = -2.46$, $p = 0.03$; Fig. 4). No difference in $\delta^{15}N$ values ($t = -0.92$, $p = 0.37$) or C:N values ($t = -1.0$, $p = 0.32$) was observed between pools for *Trithemis* spp. No difference was observed between the mean dry weight of *Trithemis* spp. collected in the *H. amphibius* and reference pools

($W = 15.5$, $p = 0.16$).

Median values from the posterior distributions of Bayesian isotope mixing models suggested that intake of *H. amphibius* dung/C4 grass was higher in the *H. amphibius* pool than in the reference pool for both *L. oxyrhynchus* and *Trithemis* spp. (Appendix C: Table C1). The difference between the median estimated contribution of *H. amphibius* dung/C4 grass to *L. oxyrhynchus* in the *H. amphibius* pool and the reference pool was much higher during the dry season.

DISCUSSION

Results from laboratory feeding trials and field sampling preliminarily suggest that fish and aquatic invertebrates in Kenya's Ewaso Ng'iro River make use of organic matter vectored into the river by resident *H. amphibius*. Field monitoring of our Ewaso Ng'iro River pool study sites confirmed that *H. amphibius* are common at our *H. amphibius* pool and absent at the reference pool, and that this difference has likely been consistent for a minimum of six decades. Controlled dung feeding trials provided some indication of the magnitude and direction of isotopic shift that could be expected for an aquatic consumer that becomes heavily reliant upon *H. amphibius* dung. The $\delta^{13}C$ values of *P. reticulata* guppies fed exclusively on dung shifted towards the more positive C4 $\delta^{13}C$ values measured in *H. amphibius* dung. These differences were similar to those observed among field sampled aquatic consumers measured in the *H. amphibius* and reference pools of the Ewaso Ng'iro River. The $\delta^{13}C$ values of one of the most abundant large consumers in this watershed, the fish *L. oxyrhynchus*, were more positive in the *H. amphibius* pool (Fig. 4). This difference, however, was only significant for *L. oxyrhynchus* during the dry season. The $\delta^{13}C$ values of the predatory aquatic insect *Trithemis* spp. were also found to be significantly more positive in the *H. amphibius* pool during the dry season (the only season in which it was sampled; Fig. 4).

Results from isotope mixing models mirrored patterns exhibited in direct comparisons of $\delta^{13}C$ value differences. Median values of the posterior distributions generated by the mixing models (Appendix C: Table C1; indicative of the most probable estimates of source contribution) indicated a higher contribution of *H. amphibius* dung/C4 grass to both *L. oxyrhynchus* and *Trithemis* spp. sampled in the *H. amphibius* pool. This between pool difference was five times more pronounced for *L. oxyrhynchus* during the dry season than the wet season.

The observation that differences in the $\delta^{13}C$ values for the river fish *L. oxyrhynchus* were only significantly different during the dry season (low flow period) and that estimated dung contributions were higher during this period suggests that river hydrology may influence consumer use of *H. amphibius* derived subsidies. Increased river flow during wet periods may dilute and flush away organic material that *H. amphibius* import to sites like the *H. amphibius* pool. Conversely dry/low flow periods may concentrate these subsidies and facilitate increased ecological utilization of *H. amphibius* derived organic matter. River flow rates are known to regulate the ecological impacts of allochthonous subsidies in other contexts, although increased flow rates are often associated with increased delivery rather than increased removal of allochthonous materials (Huryn et al. 2001, Abrantes and Sheaves 2010, Roach 2013).

Our conclusions assume that the sourcing dynamics of non-*H. amphibius* organic carbon are largely the same in these two hydrologically similar river pools. Inter-site variation in the dynamics of this delivery was not apparent and this lack of difference is partially supported by the observed lack of difference in the $\delta^{13}C$ values of POM between study pools. Consequently, we provisionally suggest that the tens of thousands of kilograms of dung (Subalusky et al. 2014) produced by the aggregation of *H. amphibius* resident year round in this relatively small (~0.75 ha surface area) river pool presents a more parsimonious explanation for the recorded shifts in river consumer $\delta^{13}C$ values.

Determining whether *H. amphibius*-derived nutrient subsidies are important to river consumers is a broadly important question. For example, *Labeobarbus*, the genus of fish studied in this work, is a commercially important group of fishes in East Africa and hundreds of tons of *Labeobarbus* are harvested annually (Lake Fisheries Development Program (LFDP) 1997, Dadebo et al. 2013). This harvest is particularly important

in protein deficient regions (de Graaf et al. 2006, Dadebo et al. 2013). If, as these results suggest, *Labeobarbus* draws directly or indirectly upon *H. amphibius*-vectored subsidies in ecologically important ways, then these findings provide provisional support for the hypothesized links between *H. amphibius* and fisheries productivity (Mosepele et al. 2009). Such connections should be considered when evaluating the broader ecological significance of historical and contemporary reductions in the abundance and range of *H. amphibius* (Manlius 2000, Van Kolfschoten 2000, Lewison et al. 2008). Firmly establishing the ecological importance of *H. amphibius* subsidies will require further study carried out at more comprehensive spatial and temporal scales.

It is likely that δ^{13}C values will not be useful for tracing utilization of *H. amphibius* vectored subsidies in all contexts. A careful review of the isoscape of any particular study region will be required to evaluate the local utility of carbon stable isotopes for identifying potential use of *H. amphibius* excreta. For example, δ^{13}C values would likely be a less powerful diagnostic tool for studying *H. amphibius* subsidies in systems where C4 marginal plants (e.g., plants that have δ^{13}C values similar to *H. amphibius* dung), such as papyrus, are abundant and make a substantial contribution to river detrital pools (Grey and Harper 2002). The overall degree of landscape-watershed coupling must also be considered. In watersheds where the physical transport of C4 derived organic matter into rivers is continuously high or spatially heterogeneous, it will be challenging to discern *H. amphibius* contributions to aquatic food webs. Further research will help to better clarify how these issues of context shape the global utility of carbon stable isotopes for tracking and contextualizing the importance of *H. amphibius* subsidies to freshwater ecosystems.

ACKNOWLEDGMENTS

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SUPPLEMENTAL MATERIAL

APPENDIX A

Underwater video of Hippopotamus amphibius dunging

Video of *Hippopotamus amphibius* defecating while taking refuge in its diurnal aquatic refuge. Dung is rapidly consumed by resident fish *Labeo* sp. nov. 'Mzima.' This video footage was collected from Mzima Springs in Kenya, a site with uniquely high water clarity which permits observation of interactions between *H. amphibius* and river consumers. Video © Deeble and Stone Productions (markdeeble.wordpress.com); used with permission. doi: http://dx.doi.org/10.1890/ES14-00514.2
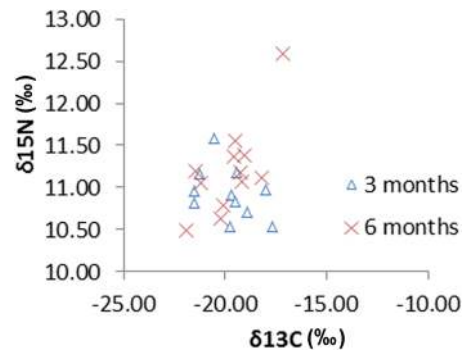
APPENDIX B

Fig. B1. Time partitioned results of lab-based *Hippopotamus amphibius* dung feeding trials. Stable isotope composition (δ^{13}C and δ^{15}N) of guppies *Poecilia reticulata* fed exclusively *Hippopotamus amphibius* dung in the laboratory for three months and six months. δ^{13}C and δ^{15}N values were measured relative to the standards V-PDB and air, respectively. There were no significant differences between dung fed *P. reticulata* sampled at three months and six months (δ^{13}C: $t = -1.51$, $p = 0.15$; δ^{15}N: $W = 63$, $p = 0.88$), suggesting that the majority of the isotopic transitioning in these *P. reticulata* occurred in less than three months.

APPENDIX C

Isotope mixing model results

A two isotope (δ^{13}C, δ^{15}N), two source Bayesian isotope mixing model was used to estimate potential differences in utilization of *Hippopotamus amphibius* dung by aquatic consumers sampled in *H. amphibius* and reference pools. The sources utilized in this model were *H. amphibius* dung/C4 grass (represented by *H. amphibius* dung (n = 11)) and C3 riparian tree material (represented by leaves of the abundant C3 riparian tree *Acacia xanthophloea* (n = 9)). Published fractionation values for aquatic consumers used in all models were taken from McCutchan et al. (2003): $\Delta\delta^{13}$C: $+0.4 \pm 0.2$ (mean $\pm$ SD); $\Delta\delta^{15}$N: $+2.3 \pm 0.3$. These fractionation values were applied in the case of both *Labeobarbus oxyrhynchus* and *Trithemis* spp. consumers.

Table C1. Median values of the posterior distributions generated from stable isotope mixing models predicting reliance of consumers *L. oxyrhynchus* (fish) and *Trithemis* spp. (dragonfly larvae) on the two sources examined. The difference between predicted contributions of *H. amphibius* dung/C4 grass and C3 riparian tree material in *H. amphibius* and reference pools are reported. The disparity between the estimated contribution of *H. amphibius* dung/C4 grass to *L. oxyrhynchus* sampled in the *H. amphibius* pool and those sampled in the reference pool was much greater during the low flow dry season.

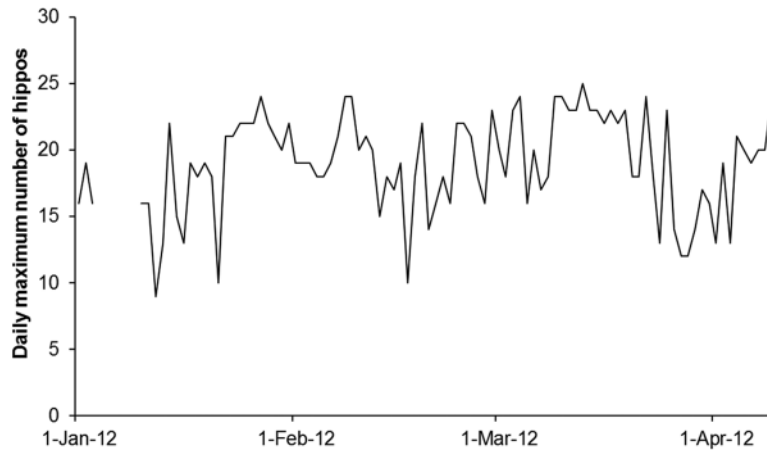|	Wet season			Dry season		
Carbon source	*H. amphibius*	Reference	Difference	*H. amphibius*	Reference	Difference
Labeobarbus						
Dung *H. amphibius*/C4 grass	0.62	0.60	0.02	0.59	0.49	0.10
C3 riparian tree	0.38	0.40	−0.02	0.41	0.51	−0.10
Trithemis						
Dung *H. amphibius*/C4 grass	NA	NA	NA	0.27	0.21	0.07
C3 riparian tree	NA	NA	NA	0.73	0.80	−0.07

APPENDIX D

Fig. D1. *Hippopotamus amphibius* abundance as measured at *H. amphibius* pool site. Plot of daily maximum counts of *Hippopotamus amphibius* individuals recorded at the *H. amphibius* pool via camera trap images taken at 5-min intervals during daylight hours over the study period.