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# Northern map turtles (*Graptemys geographica*) derive energy from the pelagic pathway through predation on zebra mussels (*Dreissena polymorpha*)

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## SUMMARY

1. Zebra mussels (*Dreissena polymorpha*) derive their energy from the pelagic energy pathway by filtering plankton. Because zebra mussels occur in high densities in littoral habitats, they potentially constitute an important trophic link between littoral consumers and pelagic energy sources. Northern map turtles (*Graptemys geographica*) are widespread in North America and consume zebra mussels.
2. We used stable isotopes analyses to quantify the flow of energy from the pelagic pathway to northern map turtles and to infer the contribution of zebra mussels to map turtle biomass. We then built a bioenergetic model to estimate the annual intake of zebra mussels by northern map turtles in Lake Opinicon, Ontario, Canada.
3. Stable isotopes analyses indicated that zebra mussels constitute between 0% and 14% of the diet of males and between 4% and 36% of the diet of females. Assuming that zebra mussels account for all of the pelagic contribution, we estimated that map turtles consume 3200 kg of zebra mussels annually. Because female map turtles are much larger than males and consume more zebra mussels, they are responsible for 95% of the zebra mussel biomass ingested annually.
4. The pelagic pathway supports an important part of the standing crop biomass of map turtles in Lake Opinicon. We highlight the importance of freshwater turtles in lake ecosystems. Unravelling the trophic interactions mediated by freshwater turtles will lead to a more integrated picture of lake ecosystems.

*Keywords:* energy flow, *Graptemys geographica*, pelagic pathway, stable isotopes, zebra mussels

## Introduction

Consumers can potentially exploit resources linked to different energy pathways (Polis, Anderson & Holt, 1997; Vander Zanden & Vadeboncoeur, 2002). The quantity of energy transferred from primary producers to consumers in a given pathway affects the productivity of consumers, which in turn affects their impact on the ecosystem (Polis & Hurd, 1995; Polis *et al.*, 1997). Thus, identifying the ultimate energy sources of consumers is a central theme in ecosystem

studies (Vander Zanden & Vadeboncoeur, 2002). The energy flow between primary producers and consumers in a given pathway ultimately depends on the availability of resources linked to that pathway. Two major energy pathways dominate lake ecosystems (Vadeboncoeur, Vander Zanden & Lodge, 2002): the pelagic pathway and the benthic pathway. The pelagic pathway is associated with open water where phytoplankton are the primary producers. The benthic pathway is associated with the bottom and the littoral where benthic algae (periphyton) are the primary producers.

Invasive species can shift the energy flow between producers and consumers by modifying the availability of resources associated with each pathway (Strayer

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*et al.*, 1999). The introduction of zebra mussels (*Dreissena polymorpha* Pallas) to North America and Europe has altered the energy flow in freshwater ecosystems (Strayer *et al.*, 1999; Macguire & Grey, 2006), resulting in dramatic modifications of invertebrate and fish communities (Ricciardi, Whoriskey & Rasmussen, 1997; Strayer, 1999; Strayer, Hattala & Kahnle, 2004; Strayer & Malcom, 2007). Zebra mussels can consume most of the annual pelagic primary productivity (Stoeckmann & Garton, 1997; Strayer *et al.*, 1999) and typically reach higher biomasses than most other benthic aquatic organisms (Strayer, 1999). Although zebra mussels derive their energy from the pelagic pathway, they mostly occupy littoral habitats (Dermott & Munawar, 1993; Jones & Ricciardi, 2005). For consumers associated with littoral habitats that possess the morphological capacity to consume hard prey, zebra mussels can be an important source of pelagic energy that was previously unavailable. Despite the numerous studies documenting predation on zebra mussels by aquatic consumers (reviewed by Molloy *et al.*, 1997), we are unaware of any study that has quantified the extent of trophic energy transfer from the pelagic pathway to predators of zebra mussels. Measuring the energy flow between zebra mussels and benthic consumers will help understand the ecosystem-level consequences of invasive zebra mussels. Quantifying the contribution of zebra mussels to the biomass of consumers will also provide insights into the demographical impacts of this new prey on its predators.

Freshwater turtles are especially abundant in eastern North America and they mostly inhabit littoral areas of lakes and rivers (Ernst, Lovich & Barbour, 1994). A recent stable isotopes analysis of lake Jackson (Florida) food web revealed that benthic algae almost entirely support a freshwater turtle community composed of six species (Aresco & James, 2005). Although freshwater turtle communities are not as diverse as fish communities, some turtles can reach biomasses comparable to, or greater than, those attained by fishes (Iverson, 1982; Congdon, Greene & Gibbons, 1986). Freshwater turtles therefore have the potential to play an important role as consumers in freshwater ecosystems (Bury, 1979; Moll & Moll, 2004). The recent emphasis on the importance of the benthic energy pathway stresses the need to adopt a whole-ecosystem perspective of lakes (Schindler & Scheuerell, 2002; Vander Zanden & Vadeboncoeur, 2002). Thus,

traditionally overlooked consumers such as aquatic turtles should be studied in an ecosystem context. Quantifying the energy sources of aquatic turtles and their interaction with invasive species that change food web structure will provide important insights into the role turtles play in lake ecosystems.

Northern map turtles (*Graptemys geographica* LeSueur) are widespread in central and eastern North America (Ernst *et al.*, 1994) and their range broadly overlaps the current range of invasive zebra mussels and quagga mussels (*Dreissena bugensis* Andrusov). Northern map turtles are primarily molluscivorous, but also consume other invertebrates (Vogt, 1981; Lindeman, 2006a). Diet studies conducted prior to zebra mussel invasion indicated that native bivalves were rare in the diet of northern map turtles and that map turtles derived most of their energy from the benthic pathway through the consumption of snails, caddisfly larvae and crayfish (Vogt, 1981). In contrast, a recent study performed after the invasion of zebra mussels showed that northern map turtles, especially females, consume large quantities of invasive dreissenid mussels (Lindeman, 2006a). However, the magnitude of the utilization of this new source of energy relative to the benthic pathway has not been quantified. In this study, we used stable isotopes analyses to quantify the energy flow from the pelagic pathway to northern map turtles. We then constructed an individual-based bioenergetic model to estimate the intake of zebra mussels by northern map turtles and the contribution of zebra mussels to map turtle biomass.

## Methods

### *Study site and turtle biomass*

We conducted this study in Lake Opinicon at the Queen's University Biological Station, 100 km south of Ottawa, Ontario, Canada (Fig. 1). Lake Opinicon is a small (788 ha) and shallow (mean depth 4.9 m) mesotrophic lake. The littoral zone of lake Opinicon constitutes 69% of the surface of the lake and 80% of the bottom is covered by macrophytes (Karst & Smol, 2000). This lake has been in a clear-water state since its development (>11 000 years BP) indicating relatively low pelagic primary productivity (Karst & Smol, 2000). Zebra mussels became noticeable in lake Opinicon during the mid-1990s (G. Blouin-Demers,

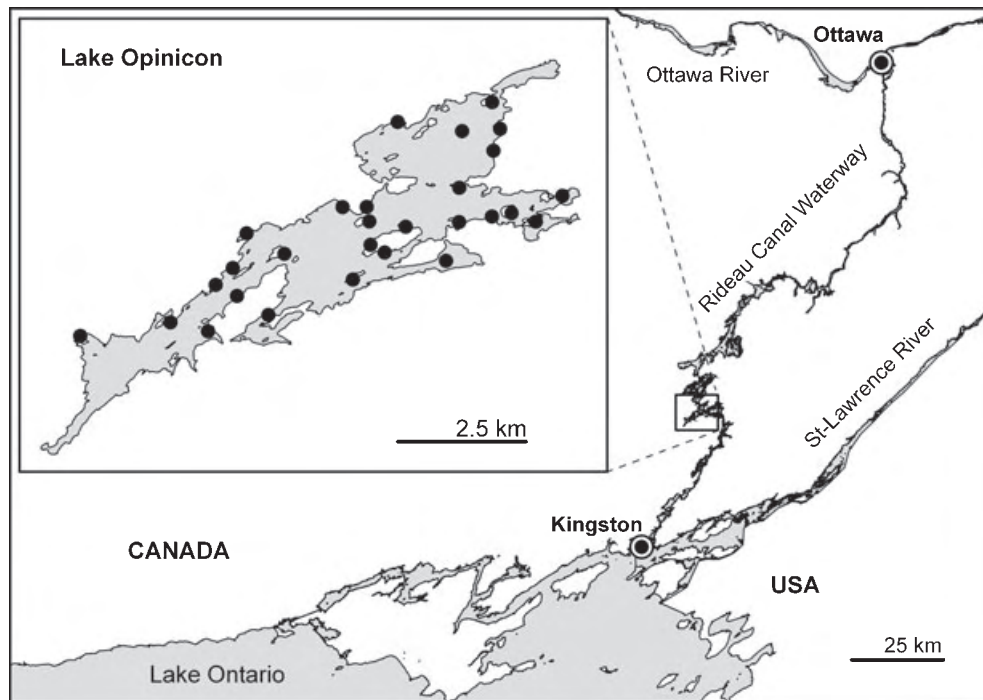


Fig. 1 Lake Opinicon in southeastern Ontario, Canada. Circles on the inset map indicate capture locations of map turtles within Lake Opinicon.

pers. obs.) and have now reached a mean density of 2962 individuals  $m^{-2}$  (range: 16–6912,  $n = 9$  sites) in the littoral zone (G. Bulté, unpubl. data).

We sampled map turtles in lake Opinicon between 2003 and 2006 with basking traps and by snorkelling. Every captured individual was measured, weighed and given a unique mark by drilling small holes in the marginal scutes. We used the software CAPTURE (Rexstad & Burham, 1991) to estimate population size. We used a sampling interval of 1 year and counted one capture per year for individuals that were captured multiple times in the same year. We estimated standing crop biomass with the following equation:

$$\Sigma(N_i \times W_i)$$

where  $N_i$  is the number of individuals in mass class  $i$  and  $W_i$  is the midpoint of the mass class  $i$ .

#### Stable isotopes analyses

In lakes, pelagic primary producers (phytoplankton) are depleted in  $^{13}C$  (more negative  $\delta^{13}C$ ) compared to littoral primary producers (periphyton). The boundary layer present around the periphyton impedes the

diffusion of dissolved inorganic carbon, resulting in a smaller isotopic fractionation by benthic primary producers compared to pelagic primary producers (Hecky & Hesslein, 1995). Those isotopic differences at the base of the food web are maintained across trophic levels due to limited trophic fractionation of carbon isotopes (France, 1996) and the differences can thus be used to track the proportion of each energy source contributing to the biomass of a predator (Post, 2002; Vander Zanden & Vadeboncoeur, 2002). Zebra mussels consume phytoplankton and thus integrate a more negative  $\delta^{13}C$  ratio than benthic grazers such as snails (Post, 2002). Dreissenid mussels are the only pelagic consumer reported to be frequently consumed by northern map turtles (Lindeman, 2006a) and faeces analyses in our population support this observation (G. Bulté, unpubl. data). Other prey items complementing the diet of map turtles in our population are putative benthic consumers: caddisfly larvae and viviparid snails (G. Bulté, unpubl. data).

From May to August 2005, we collected blood (0.05 mL) from the caudal vein of males ( $n = 20$ ) and females ( $n = 39$ ) for stable isotopes analyses. Sampled individuals were chosen to represent the size distribution of our study population. We also

sampled at three sites specimens of the three prey (trichoptera, zebra mussels and trap-door snails) most commonly consumed by map turtles in our study population (G. Bulté, unpubl. data). For each prey type, we measured the isotopic ratio on composite samples composed of at least 10 individuals from each site.

Samples from turtles and prey were freeze-dried and isotope ratios were measured on a mass spectrometer at the Hatch Isotope Laboratory at the University of Ottawa. Stable isotope values are reported in the  $\delta$  notation where  $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}/{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}) - 1] \times 1000$ . Mean standard deviation for replicates was 0.19‰. Turtle  $\delta^{13}\text{C}$  values were converted into proportions of pelagic (zebra mussels) and littoral (snails and trichoptera) prey with a two end-member mixing model using the software ISOERROR 1.04 (Phillips & Gregg, 2001). When calculating mixing models, ISOERROR takes into account the variability in the  $\delta^{13}\text{C}$  of both the sources (prey) and the mixture (turtle) and provides 95% confidence intervals (CI) around the estimated proportions. For the mixing model, we divided the turtles into three groups: males, small females overlapping in size with males [plastron length (PL) <126 mm] and large females (PL >126 mm). Predators tend to be slightly enriched in  $^{13}\text{C}$  relative to their prey (Post, 2002). To account for this trophic fractionation, we added 0.23‰ to the  $\delta^{13}\text{C}$  of the prey. This value was measured between the food and the claws of captive *Trachemys scripta* Schoepff (Aresco & James, 2005), a species closely related to map turtles.

Lipids tend to be depleted in  $^{13}\text{C}$  relative to carbohydrates and proteins, which can introduce a bias in mixing models (Kiljunen *et al.*, 2006; Post, 2007). When lipids constitute an important proportion of the tissue analysed, lipid extraction or mathematical normalization has been recommended (Post *et al.*, 2007; Kiljunen *et al.*, 2006). Studies on marine birds, however, have shown that lipid extraction does not result in meaningful difference in  $\delta^{13}\text{C}$  because of the very low lipid content of avian blood (Bearhop *et al.*, 2003; Cherel *et al.*, 2005). Freshwater turtle blood has roughly half the lipid concentration of marine bird blood (Chaikoff & Entenman, 1946). Therefore, we did not extract lipids from our blood samples or mathematically normalize blood  $\delta^{13}\text{C}$  prior to analysis because it makes no difference for turtle blood.

### Bioenergetics

Standard metabolic rate (SMR) is the minimum energy cost for an ectotherm and is often referred to as the cost of living. We estimated SMR by measuring oxygen consumption ( $\text{VO}_2$ ) on resting, post-absorptive northern map turtles (see Standard metabolic rate). Basing energy budgets on SMR underestimates the energy intake because SMR does not incorporate the energy allocated to activity and reproduction. For our purpose, however, SMR allows calculation of a conservative estimate of the contribution of the pelagic pathway to map turtle annual energy budget. Field metabolic rate (FMR) is the energy cost of the daily activities of an animal. Unfortunately, FMR cannot be measured with doubly-labelled water in freshwater turtles because of high water turnover rates (Booth, 2002). FMR of lizards (estimated using doubly-labelled water) is typically 1.3–2.5 times the SMR (McNab, 2002). We thus estimated FMR of northern map turtles by multiplying their SMR by a factor of 2.5. We chose the upper limit to obtain a maximum estimate of their field energy expenditure.

To quantify the flow of energy from the pelagic pathway, we estimated the total energy allocated to SMR and FMR for the entire active season (15 April–15 October, 183 days). We first calculated SMR for each 10 g class for males and 40 g class for females with the predictive equations from the  $\text{VO}_2$  measurements (see Standard metabolic rate). We estimated the amount of energy allocated to SMR at 5 °C intervals between 7.5 and 37.5 °C for each size class assuming a conversion factor of 19.67 J mL<sup>-1</sup> of O<sub>2</sub> consumed (Gessaman & Nagy, 1988). The energy allocated to SMR at each temperature class was then multiplied by the proportion of time spent at that temperature (obtained from the temperature loggers, see Measurement of body temperature) during the active season. The total annual energy allocated to SMR for each size class was obtained by summing the energy allocated to SMR at each body temperature class. We then multiplied the annual energy allocated to SMR by a factor of 2.5 to estimate FMR. We did not include energy expended during hibernation in our model. Northern map turtles typically hibernate in water near 0 °C (Crocker *et al.*, 2000) and undergo metabolic depression to reduce energy expenditure (Maginniss, Ekelund & Ulstch, 2004). Therefore, energy expenditure during hibernation probably

represents a trivial portion of the annual energy budget.

To determine how much of the energy allocated annually to SMR and FMR was fuelled by zebra mussels, we multiplied the annual energy allocation to SMR and FMR by the proportion of zebra mussels in the diet (obtained from the stable isotopes analyses). To estimate the biomass of zebra mussels consumed to sustain SMR and FMR, we back-calculated the dry weight of zebra mussels ingested (including the shell) from the amount of energy coming from zebra mussels assuming an energy density of  $1.52 \text{ KJ g}^{-1}$  of whole organism dry mass (mean value for summer, spring and autumn from Magoulick & Lewis, 2002). We then predicted whole organism wet mass using a regression between wet mass and dry mass obtained from zebra mussels from our population (wet mass =  $-0.016 + 2.69 \times$  dry mass,  $n = 89$ ,  $R^2 = 0.99$ ,  $P < 0.001$ ). We assumed a digestive efficiency for energy of 90%, a value typical of carnivorous turtles (Kepenik & McManus, 1974; Spencer, Thompson & Hume, 1998).

#### *Estimation of standard metabolic rate*

To estimate SMR, we measured  $\text{VO}_2$  using open-flow respirometry. Mass and temperature both affect SMR. Thus, we measured  $\text{VO}_2$  at 14, 20, 26, and 32 °C in 16 (six males and 10 females) post-absorptive northern map turtles ranging in mass from 50 to 2300 g. Turtles were maintained in outdoor basins filled with lake water for at least 2 days prior to measurements to allow gut clearance. We placed turtles for 2 h in a cooler filled with water adjusted at the experimental temperature to allow thermal equilibration. Turtles were then moved to an opaque respirometry chamber (volume 0.5–11 L depending on the size of the turtle) lined with a moist cloth to prevent desiccation. The chamber was placed in a temperature-controlled cabinet adjusted to the experimental temperature and turtles were left undisturbed with circulating air for 2 h prior to measurements. During  $\text{VO}_2$  measurements, fresh exterior air was pumped through a drierite® column to absorb water before entering the chamber. The flow of air entering the chamber was regulated by a flowmeter adjusted to 65, 100 or 200  $\text{mL min}^{-1}$  depending on the size of the turtle. A subsample of air exiting the chamber (50% of the flow entering the chamber) was desiccated through a

second drierite® column and sent to a gas analyzer (Sable Systems FC-1, Henderson, NV, U.S.A.). The concentration of  $\text{O}_2$  in the chamber was measured every 20 s for 220 min and baseline measurements were made at the beginning and at the end of each trial to account for drift. All  $\text{VO}_2$  measurements were made between 18:00 and 24:00 hours, during which period diurnal turtles are normally resting.

To eliminate bouts of activity from our estimate of SMR, we used only the lowest 25th percentiles of the data for each individual in the calculation of SMR (165/660 data points). This approach provides a good estimate of SMR in reptiles (Litzgus & Hopkins, 2003; Hopkins *et al.*, 2004). SMR was calculated from  $\text{VO}_2$  using the software Datacan (Sable Systems Datacan V, Henderson, NV, U.S.A.) according to Withers (1977).

#### *Measurement of body temperature*

Temperate turtles exhibit important diurnal and seasonal variations in body temperature (Edwards & Blouin-Demers, 2007) that affect their energy requirements. To incorporate these fluctuations in our energy budget, we measured body temperature ( $T_b$ ) in active turtles during their whole active season. We surgically implanted miniature temperature loggers (Thermochron iButton DS1921 and DS1922L, Dallas Semiconductor, Sunnyvale, CA, U.S.A.) in the body cavity of eight adult males, nine adult females and nine juvenile females following the methods of Edwards & Blouin-Demers (2007). Loggers recorded internal  $T_b$  every 26–110 min from May to October. Turtles implanted with temperature loggers were also equipped with radio-transmitters (model SI-2FT and SB-2T; Holohil Systems, Carp, ON, U.S.A.) bolted to the carapace, which allowed us to recapture the turtles the following spring and surgically remove the loggers to download the data. All our procedures were approved by the Animal Care Committee at the University of Ottawa (protocol BL-179).

#### *Demographical consequences*

The new energy source that zebra mussels represent for northern map turtles may positively affect the demography of the species. To investigate potential demographical effects of zebra mussel consumption, we compared the mean mass of hatchlings (controlling for body size) produced by females from Lake

Opinicon prior to the invasion of zebra mussels (Mathers, 1979) to the mean mass of hatchlings from 2005 (G. Bulté, unpubl. data).

## Results

### Population size and turtle biomass

Between 2003 and 2006, we marked 898 turtles (377 males and 521 females) in 1337 captures events. Using a closed population model incorporating individual heterogeneity in the capture probability ( $M_h$ ) (Otis *et al.*, 1978), we estimated the population to be 1569 individuals (95% CI: 1487–1662). The total biomass of northern map turtles in the lake was estimated to be 1130 kg or 1.43 kg ha<sup>-1</sup> (1.2 turtles ha<sup>-1</sup>). In our population, adult females are on average 10 times the mass of adult males and, consequently, females constitute 90% of the biomass.

### Stable isotopes

Zebra mussels had a  $\delta^{13}\text{C}$  of  $-29.9\text{‰}$  while benthic prey had a  $\delta^{13}\text{C}$  of  $-20.2\text{‰}$ . The  $\delta^{13}\text{C}$  of turtle blood (Table 1) was higher in males than in females, but did not differ between female size groups (ANOVA  $F_{(3,56)} = 10.6$ ,  $P < 0.001$  followed by a Tukey–Kramer HSD pair-wise comparison). The mixing model reflected those differences with the contribution of the pelagic energy pathway (Table 1) being less important in males (0–17%) than in both female groups (4–36%).

### Standard metabolic rate

We used multiple regression to determine the effect of mass ( $M$ ) and temperature ( $T$ ) on SMR in male and female map turtles. We  $\log_{10}$  transformed the data to linearize the relationships. For both sexes, there was

no  $\log_{10}M \times T$  interaction (females  $P = 0.18$ , males  $P = 0.41$ ) so we used a reduced model to predict the effect of both variables on SMR.  $\text{VO}_2$  increased with mass and temperature in females (model:  $R^2 = 0.83$ ,  $F = 82.51$ ,  $P < 0.0001$ ;  $\log_{10}M$ : partial  $R^2 = 0.43$ ,  $F = 85.31$ ,  $P < 0.0001$ ;  $T$ : partial  $R^2 = 0.40$ ,  $F = 79.44$ ,  $P < 0.0001$ ) and in males (model:  $R^2 = 0.79$ ,  $F = 40.36$ ,  $P < 0.0001$ ;  $\log_{10}M$ : partial  $R^2 = 0.08$ ,  $F = 8.07$ ,  $P < 0.009$ ;  $T$ : partial  $R^2 = 0.71$ ,  $F = 72.85$ ,  $P < 0.0001$ ). SMR was predicted with the following equations:

$$\text{Female: } \log_{10}\text{VO}_2 = -2.23 + 0.872 \times \log_{10}M + 0.055 \times T$$

$$\text{Males: } \log_{10}\text{VO}_2 = -1.77 + 0.544 \times \log_{10}M + 0.059 \times T$$

### Body temperature

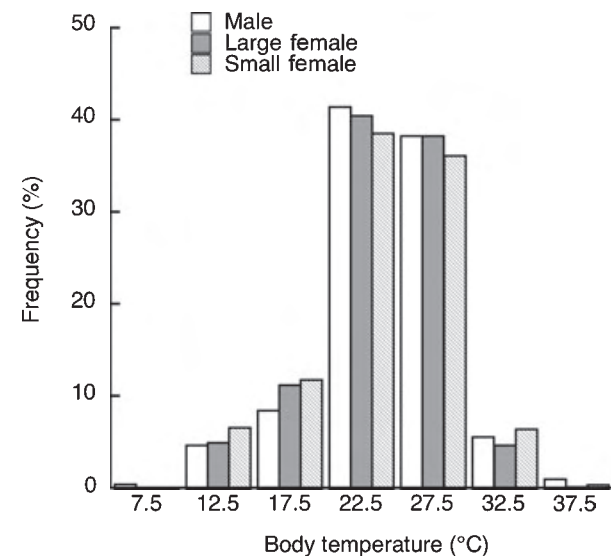
The distributions of body temperature of adult males, adult females and juvenile females were very similar (Fig. 2). Body temperatures during the whole active season ranged between 7 and 39 °C.

### Bioenergetics

At the individual level, we calculated that an average size male (mass = 166 g, length = 98 mm) needs 596 kJ year<sup>-1</sup> to sustain its SMR and 1490 kJ year<sup>-1</sup> to sustain its FMR. An average size mature female

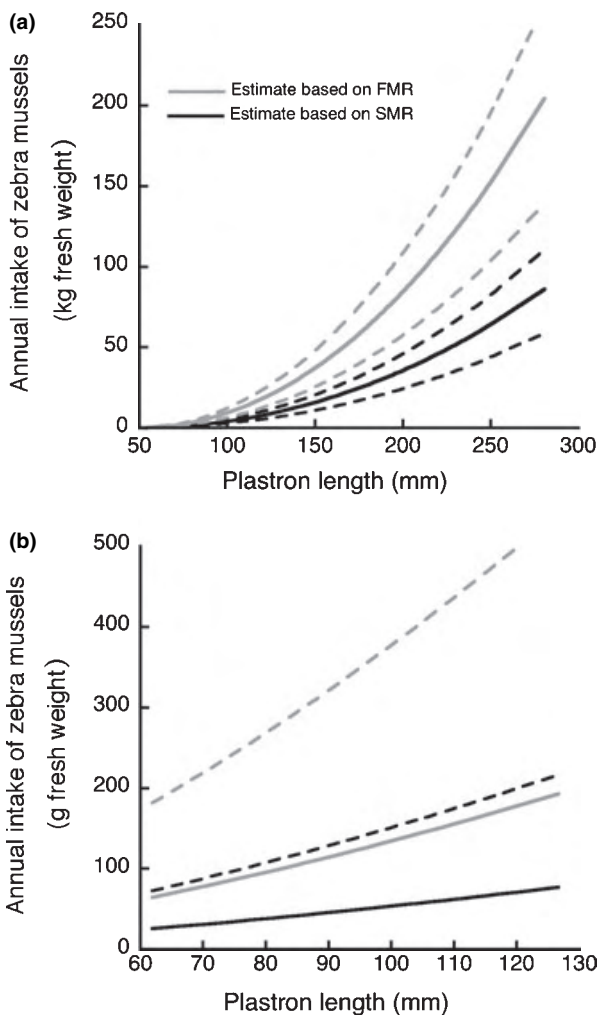
**Table 1**  $\delta^{13}\text{C}$  values of northern map turtle blood from Lake Opinicon and the proportion of zebra mussels in their diet calculated with a two-source mixing model

Group ( $n$ )	$\delta^{13}\text{C}$ (SD)	% Zebra mussels (95% CI)
Male (20)	-20.5 (1.9)	5 (0–14)
Small female (15)	-21.5 (1.6)	16 (5–27)
Large female (24)	-22.7 (1.3)	28 (19–36)



**Fig. 2** Body temperatures of adult males ( $n = 8$ ), juvenile females ( $n = 9$ ) and adult females ( $n = 9$ ) from Lake Opinicon from May to October 2005.

(mass = 1660 g; length = 224 mm) requires 8993 kJ year<sup>-1</sup> for its SMR and 22482 kJ year<sup>-1</sup> for its FMR. The annual intake of zebra mussels by the northern map turtle increased with body size in both sexes (Fig. 3). We estimated that an average size male ingests annually between 0.03 and 0.27 kg of zebra mussels (fresh weight), while an average size female ingests between 33 and 137 kg annually (Fig. 3). At the population level, northern map turtles in lake Opinicon ingest between 833 and 1680 kg year<sup>-1</sup> (best estimates = 1271 kg) of zebra mussels to sustain their SMR and between 2082 and 4199 kg year<sup>-1</sup> (best estimates = 3117 kg) to sustain their FMR (Table 2).



**Fig. 3** Estimated annual zebra mussel intake of female (a) and male (b) northern map turtles in Lake Opinicon. Dashed lines indicate intake estimates based on the 95% confidence limits of the proportion of zebra mussels in the diet calculated using a two end-members mixing model.

**Table 2** Summary of the map turtle population level energetics estimates

Group	Population size (95% CI)	Energy intake from zebra mussels (kJ year <sup>-1</sup> )		Biomass of zebra mussel ingested (kg year <sup>-1</sup> )	
		SMR	FMR	SMR	FMR
Males (all sizes)	659 (624–597)	19 457 (0–54 480)	48 642 (0–136 200)	19 (0–53)	47 (0–132)
Small females (PL < 126 mm)	275 (260–291)	46 330 (14 478–78 181)	115 825 (36 195–195 452)	44 (14–74)	110 (35–185)
Large females (PL > 126 mm)	635 (601–672)	1 282 242 (869 360–1 641 270)	3 205 605 (2 173 400–4 103 175)	1208 (819–1553)	3020 (2047–3882)

SMR, standard metabolic rate; FMR, field metabolic rate; PL is plastron length. Numbers in parentheses are estimates based on the upper and lower confidence limits of the proportion of zebra mussels in the diet.

Females ingest 95% of this biomass. Taking into account that females represent 90% of the biomass of map turtles in the lake, we estimated from the upper and lower bounds of the confidence limit of the mixing model that zebra mussels support 25–33% of the standing crop biomass of northern map turtles in Lake Opinicon.

#### *Demographical consequences*

ANCOVA indicated that the relationship between PL and mean hatchling mass was the same in 1978 and in 2005 ( $F = 10.54$ ,  $R^2 = 0.49$ ,  $n = 37$ , sources of variation: year,  $P = 0.26$ ; PL,  $P < 0.001$ ; year  $\times$  PL,  $P = 0.46$ ), suggesting that zebra mussels have not affected the reproductive output of northern map turtles in lake Opinicon.

### **Discussion**

#### *Zebra mussel predation and the integration of the pelagic pathway*

Zebra mussels recently became an important prey item for northern map turtles (Lindeman, 2006a), thus providing a trophic link between the pelagic pathway and map turtles. We estimated that the pelagic pathway currently supports between 24% and 33% of the standing crop biomass of northern map turtles from lake Opinicon. In our study population, the only pelagic consumers commonly found in the faeces of map turtles are zebra mussels (G. Bulté, unpubl. data). Similarly, Lindeman (2006a) found dreissenid mussels to be the only pelagic consumer in the faeces of northern map turtles from Lake Erie. Predation on zebra mussels therefore probably accounts for the entire flow of pelagic energy to map turtles in Lake Opinicon.

Two hypotheses could explain the present heavy reliance on zebra mussels by map turtles. First, zebra mussels could have reduced the density or diversity of native prey traditionally consumed by northern map turtles, making zebra mussels the only alternative prey. Secondly, zebra mussels may constitute a more readily available energy source than native prey. When given the choice, captive juvenile female map turtles prefer native snails over zebra mussels (Serrouya, Ricciardi & Whoriskey, 1995), suggesting that in nature they may avoid zebra mussels. However, the presence

of zebra mussels generally increases the density of benthic invertebrates (caddisfly larvae and gastropods) typically found in the diet of map turtles (Ricciardi *et al.*, 1997; Stewart *et al.*, 1999; Ward & Ricciardi, 2007). This suggests that northern map turtles are consuming zebra mussels by 'choice' rather than because of a lack of better energy sources. Although zebra mussels are not as nutritive as some native snails (Serrouya *et al.*, 1995), their very high abundance may outweigh their lower energy density. At our study site, zebra mussels are on average 100 times more abundant than viviparid snails, the most important native molluscs in the diet of map turtles in Lake Opinicon (G. Bulté, unpubl. data).

Zebra mussels are not the only invasive bivalve that has altered the diet of freshwater turtles. Lindeman (2006b) found that female Texas map turtles (*Graptemys versa* Stejneger) went from a diverse diet of benthic invertebrates and algae to a diet almost exclusively composed of Asian clams (*Corbicula* sp.) following the invasion of this species in the 1970s. Lindeman (2006b) also listed three other *Graptemys* sp., including *G. geographica*, that are now consuming Asian clams. Those prey shifts have presumably also altered the energy sources for these species.

#### *Demographical consequences of zebra mussels on northern map turtles*

We found no evidence that female northern map turtles are producing larger hatchlings since the invasion of zebra mussels. Freshwater turtles typically inhabit highly productive environments, and Congdon (1989) has suggested that their energy budget should be process-limited rather than resource-limited. Process limitation could explain why an increase in resource availability has not resulted in an increase in the reproductive output of map turtles. Being a novel energy source for map turtles, it is possible that zebra mussels have positively affected northern map turtles by increasing the carrying capacity of the ecosystem for the species. If this were the case, the density of map turtles should have increased following the invasion of zebra mussels. Unfortunately, our historical data are restricted to reproductive output of females. Like most turtles, however, northern map turtles have low recruitment rates and delayed maturity (10–14 years). Zebra mussels and map turtles have been sympatric in Lake

Opinicon for at most two map turtle generations, which is probably insufficient to produce a noticeable numerical response in the population.

#### *Bioenergetics and stable isotopes*

Estimating the energy budget of animals includes multiple sources of error and an important shortcoming of this approach is the "near impossibility of estimating the confidence limits of a summed budget" (McNab, 2002, p. 307). Our goal was to provide a realistic estimate of the biomass of zebra mussels ingested by northern map turtles. To provide a biologically meaningful interval of values, we estimated consumption from both SMR and FMR. Estimates based on SMR should thus be seen as conservative values while our estimates based on FMR should be seen as maximum values.

The estimation of population energetics is impeded by the errors associated with population size estimates and demographic parameters, and the error associated with microclimate measurements (McNab, 2002). To estimate population size, we used a closed population model. Turtles have low recruitment and high adult survivorship (Congdon, Dunham & Sels, 1994). Thus, over a 3-year period we can safely assume that recruitment and mortality were insufficient to bias our population estimate (demographic closure). In addition, lake Opinicon is part of the Rideau canal waterway (Fig. 1) and access to other waterbodies is restricted by locks (geographic closure). The satisfaction of the closure assumptions coupled with a high recapture rate (70%) insure small errors in our estimate of population size.

For ectotherms, SMR and FMR depend largely on body temperature. Consequently, errors associated with the estimation of  $T_b$  affect estimates of energy intake. Measurements of microclimatic variables may serve to infer  $T_b$  in reptiles. This approach is problematic for aquatic turtles, however, because they regularly move between water and land. Thus, turtle  $T_b$  may rarely be in thermal equilibrium with the environment, making the inference of  $T_b$  from microclimatic measurements prone to errors. Directly measuring  $T_b$  using bio-logging technology is an efficient way to circumvent this limitation. With this approach, we obtained  $T_b$  profiles with small errors ( $\pm 0.5$  °C) in free-ranging animals.

Diet analysis with stable isotopes has the advantage of directly measuring assimilated food (i.e. food converted into biomass; Peterson & Fry, 1987). However, one important assumption of this approach is that change in the isotopic ratio between predator and prey (i.e. trophic fractionation) is accurately accounted for. To correct for trophic fractionation, we used the trophic fractionation measured in claws of another species of freshwater turtle (Aresco & James, 2005). However, blood may discriminate carbon isotopes differently than claws or species-specific differences in trophic fractionation may exist. To evaluate the potential error caused by trophic fractionation, we investigated the sensitivity of the mixing model to variation in the fractionation factor given the differences we measured in the two sources (*ca.* 10‰). Our analysis showed that a 0.1 increment in the fractionation factor increased the contribution of zebra mussels by 1%. Post (2002) reviewed the trophic fractionation of carbon stable isotopes for several taxa and reported a mean fractionation of +0.39‰ (SD = 1.3). We used a smaller fractionation factor (0.23) than the average trophic fractionation reported by Post (2002). Therefore, if incorrect, our fractionation factor is more likely to underestimate than to overestimate the proportion of zebra mussels in the diet of the northern map turtle, thus making our estimates conservative. In addition, the sensitivity of a two-sources mixing model to fractionation factor is a function of the differences between the isotopic ratios of the two sources. In this study, we measured a large difference between the two sources relative to the average trophic fractionation expected for stable carbon isotopes, thus making our mixing model relatively insensitive to fractionation factor.

When estimating the trophic link between predator and prey, it is also important to sample tissues that will integrate isotopic variation at comparable temporal scales (i.e. having similar turnover rates) (Post, 2002). In temperate lakes, snails and zebra mussels integrate the variation in  $\delta^{13}\text{C}$  of primary producers over one growing season (Post, 2002). Turnover rate of blood is unknown in turtles. However, given that complete turnover of  $\delta^{13}\text{C}$  in claws, a tissue with relatively slow turnover, takes 12 months in juvenile turtles (Aresco & James, 2005), we assumed that blood was reflecting diet over a time period comprised within one active season.

Several recent studies have taken advantage of stable isotopes analyses to demonstrate that the benthic energetic pathway largely supports fishes typically assumed to be pelagic consumers (Hecky & Hesslein, 1995; Schindler & Scheuerell, 2002; Vadeboncoeur *et al.*, 2002; Vander Zanden & Vadeboncoeur, 2002; Karlsson & Bystrom, 2005). In contrast, our study demonstrates that the northern map turtle, a consumer associated with littoral habitats, derives an important part of its energy from the pelagic pathway because of its ability to consume zebra mussels. We are aware of only one other study (Aresco & James, 2005) that has used stable isotopes analyses to unravel energy sources of turtles in lake ecosystems. Despite the errors associated with bioenergetics and stable isotopes analyses, combining those tools is a powerful approach to study energy flow in ecosystems. Such tools should be employed more frequently if we are to understand better the trophic interactions mediated by freshwater turtles as well as other large mobile consumers and, thus, paint a more integrated picture of lake ecosystems.

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