# LINKING EXOTIC SNAILS TO CARBON CYCLING IN KELLY WARM SPRINGS, GRAND TETON NATIONAL PARK



# ✦ ABSTRACT

Biotic calcification has yet to be considered in most freshwater carbon budgets, despite previous calculations that suggest the importance of calcifying animals in altering inorganic carbon cycling. The freshwater snail, *Melanoides tuberculata*, has achieved a high abundance and a biomass of 34.2 g AFDM m<sup>-2</sup> after invading Kelly Warm Springs in Grand Teton National Park approximately five years ago. This high biomass suggests that introduced populations of *Melanoides* may alter ecosystem processes. We measured *Melanoides* growth rates and biomass to calculate the production of biomass, shell mass, and CO<sub>2</sub> for comparison with ecosystem carbon pools and fluxes.

*Melanoides* calcification in Kelly Warm Springs produced up to 10.4 mmol  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$ during summer months. Despite extremely high primary production and respiration in Kelly Warm Springs (-379 mmol  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$  and 445 mmol  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$ , respectively),  $CO_2$  produced from biotic calcification increased total  $CO_2$  production in Kelly Warm Springs from 65.9 to 76.3 mmol  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$ . This rate of  $CO_2$  production via biotic calcification is within the range of those previously calculated for freshwater systems and suggests the importance of considering the role of calcification in inorganic carbon budgets for areas dominated by calcifying organisms.

## **INTRODUCTION**

Most aquatic carbon dioxide  $(CO_2)$ budgets assume that any changes in total CO<sub>2</sub> concentrations are from primary production and community respiration, after accounting for groundwater inputs and evaporation rates (Wetzel 2001, Chauvaud et al. 2003). The ability of animals to alter elemental cycling has been well researched and reviewed (Vanni 2002) and there has been growing interest in the ecosystem-level consequences of exotic species invasions in the past several years (Simon and Townsend 2003). However, few studies have quantified the CO<sub>2</sub> production associated with the biological process of calcification (the formation and growth of calcium carbonate exoskeletons) to compare directly with whole ecosystem processes such as gross primary production and community respiration. When calcium carbonate  $(CaCO_3)$ exoskeletons are formed, nearly one mole of CO<sub>2</sub> is released for every mole of CaCO<sub>3</sub> fixed into shell material (Ware et al. 1991, Frankignoulle et al. 1994):

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + H_2O$$
.

The rising frequency of introductions and establishment of mollusks has the potential to increase the contributions of calcification to inorganic carbon budgets on a global scale (Robinson 1999, Chauvaud *et al.* 2003).

 $CO_2$  evasion from some aquatic ecosystems is an important source of CO<sub>2</sub> that may be missing from many regional carbon budgets (Cole et al. 1994, Richey et al. 2002, Cole et al. 2007). Until recently, many estimates of global carbon sources and sinks did not include contributions from freshwater ecosystems. Many streams and rivers in the United States are supersaturated with CO<sub>2</sub> (Jones et al. 2003), meaning that they are already sources of CO<sub>2</sub>. Further CO<sub>2</sub> production through respiration or biotic calcification may increase the amount of CO<sub>2</sub> released into the atmosphere. **Biotic** calcification may be an important aspect of stream, lake, and river outgassing of CO<sub>2</sub> that should be considered when calculating contributions to the global carbon budget. Increases in CO<sub>2</sub> concentrations, linked with heavy consumption of primary producers by consumers, can potentially lower the productivity status and may also raise local carbon emission budgets for invaded ecosystems (Cole et al. 2000, Duarte and Prairie 2005).

The freshwater snail. Melanoides tuberculata (Müller, 1774), has invaded warm aquatic habitats with unknown consequences. Melanoides has achieved high abundance and biomass in Kelly Warm Springs, Grand Teton National Park. This high biomass suggests that Melanoides has the potential to drastically change the elemental cycling, productivity, and community structure of invaded habitats (Chauvaud et al. 2003, Hall et al. 2003, Hall et al. 2006). While there is evidence that Melanoides can decrease the abundance of native macroinvertebrates (Giovanelli et al. 2005), the ecosystem-level consequences of Melanoides on carbon cycling is unknown. Introduced populations of Melanoides (as well as other exotic mollusks) may dramatically alter the CO<sub>2</sub> production and emissions of invaded habitats through respiration and shell synthesis.

This paper links the calcification rates of an exotic snail population with ecosystem level fluxes and processes. In order to evaluate the impacts of *Melanoides* on carbon cycling in Kelly Warm Springs, we asked: 1) How much  $CO_2$  is released into Kelly Warm Springs through  $CaCO_3$ shell synthesis by *Melanoides*?, and 2) To what degree do snails alter inorganic carbon cycling relative to gross primary production and community respiration? To answer these questions, we measured growth rates, biomass and shell mass of *Melanoides* in Kelly Warm Springs to calculate rates of organic and inorganic carbon production, and translated shell growth rates into rates of  $CO_2$  production via calcification. We also compared these snail-driven rates with estimates of gross primary productivity, community respiration, concentrations of carbonate species, and net  $CO_2$ flux into the atmosphere. We predicted that including *Melanoides* calcification rates in the carbon budget would increase the total  $CO_2$ production in Kelly Warm Springs due to their high abundance and biomass.

# MATERIALS AND METHODS

## Study site and Melanoides life history

Kelly Warm Springs is located in Grand Teton National Park (Wyoming, USA) at 43° 38' 21.8" N, 110° 37' 01.9" W (Figure 1) and originates from where the Gros Ventre River crosses a concealed Holocene or Pleistocene fault (Love and Love 1988). Acid neutralizing capacity and pH averaged 3.2 meq  $L^{-1}$  and 8.1 from June – September 2006, including measurements taken every three hours during 24-hour sampling periods. The average width and depth of Kelly Warm Springs measured 9.9 m and 0.3 m, respectively. Stream velocity was 7.4 m min<sup>-1</sup> (standard deviation = 1.3) and discharge was 5.7  $\text{m}^3 \text{min}^{-1}$ (standard deviation = 0.05). Temperatures in Kelly Warm Springs ranged from 22.6 – 31.3 °C (mean = 27.3 °C) along a 500 m reach.

Because of the comfortable water temperature, Kelly Warm Springs is used heavily as a swimming and kayak training area for Jackson Hole residents and park visitors. The popularity of Kelly Warm Springs has resulted in numerous introductions and the successful establishment of exotic tropical fish and snails; likely through aquarium dumping. Five species of introduced fish have been documented in the USGS Nonindigenous Species Aquatic database: Xiphophorus helleri (green swordtail), Puntius tetrazona (tiger barb), Tilapia sp. (tilapia), Cichlasoma nigrofasciatum (convict cichlid) and Poecilia reticulata (guppy). All of the species except P. tetrazona are categorized as locally established populations (USGS 2005). In summer 2003, we observed Melanoides in Kelly Warm Springs, and while we do not know the exact date of introduction, Melanoides were absent from invertebrate samples collected in 2001 (R.O. Hall and E.R. Hotchkiss, unpublished data).

*Melanoides tuberculata* (Gastropoda, Thiaridae) are likely native to eastern Asia but have established populations around the world through at least six different introductions since 1950 (Robinson 1999, Facon *et al.* 2003). Invasive populations of *Melanoides* are closely linked with the aquarium trade. They are limited to warm fresh and brackish waters with temperatures ranging from 14 to 31 °C (Dudgeon 1986, Duggan 2002). *Melanoides* burrow in substrate during the day and become more active at night (Figure 1).



Figure 1. Kelly Warm Springs, Grand Teton National Park, Wyoming in top photograph (July 2006). Bottom photograph shows a close-up of *Melanoides tuberculata* while active at night (July 2007).

Individuals can grow up to 35 mm in length (although extremes of 80 mm have been reported), which is likely equivalent to 3 to 5 years in age (Dudgeon 1986, Duggan 2002, Rader *et al.* 2003). *Melanoides* are parthenogenetic and viviparous; embryos develop in the mother and young range from 1.0 - 4.0 mm in length when they are released from the brood pouch (Berry and Kadri, 1974, Subba Rao and Mitra 1982). A majority of populations sampled worldwide consist entirely of females (Jacob 1957, Dudgeon 1986), but Livshits and Fishelson (1983) reported up to 33% males in small, isolated populations in Israel. *Melanoides* establish in new environments quickly and can out-compete other invertebrates through facilitation (specifically *Biomphalaria* spp., the intermediate host of *Schistosoma mansoni*). Because of this, *Melanoides* have been intentionally introduced in some areas of Latin America and the Caribbean as biological control against schistosomiasis (Pointier and Giboda, 1999, Giovanelli *et al.* 2003).

## **Melanoides** production

During June - September 2006, we sampled benthic macroinvertebrates along a 500 m reach of Kelly Warm Springs. We collected monthly samples from six random sites along the 500 m reach using a stovepipe sampler 15.2 cm in diameter and rinsed samples in the field with a 250-µm sieve (Hall et al. 2006). We sorted, counted and measured Melanoides and other macroinvertebrates using 1-mm and 250-µm sieves to separate all benthic invertebrates and digital calipers to measure shell lengths the nearest tenth of a millimeter. Each stovepipe sample was subsampled following Hall et al. (2006). All macroinvertebrates were preserved in 95% ethanol. We also measured and counted Melanoides collected in summer 2004 for comparison with summer 2006 population estimates.

We developed a length/mass regression for both biomass and shell mass using dried, unpreserved Melanoides collected in June 2006 to calculate biomass estimates for the population in Kelly Warm Springs. We dried, weighed, ashed (at 500 °C for four hours) and re-weighed 157 individuals from a range of size classes. Predictive equations for 27 size classes were derived using SAS PROC REG (SAS Institute 2002-2003). We calculated size-specific biomass and shell mass for Melanoides collected in 2004 (4 stovepipe samples) and monthly during summer 2006 using the relationship between shell length, shell mass, and biomass. In order to understand the severity of this invasion in terms of organic carbon cycling, we also quantified the percent of macroinvertebrate biomass that consisted of Melanoides in comparison to native species.

We measured *in situ* growth rates of *Melanoides* during July and August 2006. We placed 4 - 8 individuals from similar size classes (controlled for biomass to avoid over-crowding)

with a small rock and attached algae in 56 x 43 mm growth cages with 244- $\mu$ m nylon mesh (Toby TeaBoy Ltd., Hall *et al.* 2006). We removed any other macroinvertebrates that were embedded in the algae. Size classes were determined by shell length and binned in 0.5 mm increments. We secured the growth cages in Kelly Warm Springs for three week incubations (Hall *et al.* 2006). We immediately preserved individuals from each growth cage after collection at the end of the three week period. We re-measured shell lengths to calculate mass, from which we estimated growth rates as

Growth rate  $(day^{-1}) = \frac{ln(mass_t) - ln(mass_0)}{t}$ ,

where  $mass_0$  is the initial mass of the individuals in the growth cage,  $mass_t$  is the mass of the individuals at the end of the growth period, and *t* is the length of the growth period (in days). This equation was used for both shell mass and biomass calculations.

Because growth rates highly depend on temperature, we also measured the range of temperatures throughout the entire growth period using temperature dataloggers secured to the bottom of Kelly Warm Springs at our 0 m and 500 m sites (HOBO Water Temp Pro v2, Onset Computer Corporation). We calculated sizespecific growth rates for the Kelly Warm Springs population using SAS PROC REG to find the best predictive regression (SAS Institute 2002-2003).

After measuring size-specific growth rates and biomass for *Melanoides* in Kelly Warm Springs, we calculated secondary production for the summer season as

Somatic Secondary Production = 
$$\sum_{i=1}^{n} g_i B_i$$
,

where  $g_i$  is instantaneous growth (day<sup>-1</sup>) and  $B_i$  is biomass (g m<sup>-2</sup>) for the *i*th size class of snails (Benke 1984).

We also incorporated fecundity for *Melanoides* to estimate the relative contribution of reproduction versus growth to total biomass production in Kelly Warm Springs. All of our fecundity calculations were based on the assumption that juvenile snails were 1.5 mm in length at the time of their emergence from the brood pouch. We measured the dry and ash-free dry mass of the smallest *Melanoides* collected from Kelly Warm Springs for calculations of biomass

and shell mass production. Reproduction in native and invasive regions occurs continuously and often peaks in response to environmental variables (Berry and Kadri 1974, Dudgeon 1986, Pointer *et al.* 1992). We chose a conservative reproduction rate of 182 year<sup>-1</sup> for all individuals between 12.0 and 25.0 mm in length (Berry and Kadri 1974, Subba Rao and Mitra 1982).

## **Carbon cycling in Kelly Warm Springs**

We measured community respiration and gross primary production (g  $O_2 \text{ m}^{-2} \text{day}^{-1}$ ) using the two station open channel method for oxygen (Odum 1956). We placed two Hydrolab MiniSondes in Kelly Warm Springs at each end of a 500 m reach for three day cycles during July and August 2006 to measure changes in dissolved oxygen (DO) concentrations. Using measurements of width, depth, travel time, and  $k_{O_2}$ , we calculated instant metabolism throughout seven different 24-hour cycles (Hall *et al.* 2007) as

Instant Metabolism 
$$\left(g O_2 m^{-2} min^{-1}\right) = z \left[\frac{(C_t - C_0)}{t} - kDO_{Def}\right]$$

where  $C_t$  and  $C_0$  are the dissolved oxygen concentrations at upstream and downstream sites (g  $O_2 \text{ m}^{-2}$ ), t is water travel time between sondes (min), k is the reaeration coefficient for  $O_2$  (min<sup>-1</sup>),  $DO_{Def}$  is the average of the dissolved oxygen deficit measured upstream and downstream (g  $O_2$ m<sup>-3</sup>), and z is stream depth (m).

We used these instant metabolism measurements to calculate GPP (gross primary production) as

$$GPP\left(gO_2 \text{ m}^{-2} \text{ day}^{-1}\right) = \left[I * \sum \left(M - M_{PM}\right)\right]$$

where I is the measurement interval (min), M is instant metabolism, and  $M_{PM}$  is average night metabolism (the mean of instant metabolism rates during the nighttime). We calculated CR (community respiration) as

$$CR(gO_2 m^{-2} day^{-1}) = [M_{PM} * 1440],$$

where 1440 is total minutes day<sup>-1</sup>. We used the common assumption that CR during the daytime was equal to CR measured at night. We did not adjust for groundwater inputs because conservative tracer (NaCl) concentrations did not decline along our study reach. The one station open channel method was used during two periods in July. We converted  $O_2$  to  $CO_2$  using a photosynthetic quotient of 1.2 (Raine 1983).

4

In order to accurately measure the amount of air-water gas exchange with respect to O2 and CO<sub>2</sub> fluxes, we used tracer additions of sulfur hexafluoride  $(SF_6)$ , a biologically inert gas. We also added a conservative tracer, NaCl, to calculate travel time and any dilution from potential groundwater inputs along the reach (Wanninkhof et al. 1990). SF<sub>6</sub> is not naturally present in aquatic ecosystems and evades at a rate that can be used to calculate O<sub>2</sub> and CO<sub>2</sub> evasion (Wanninkhof et al. 1990, Cole and Caraco 1998). We collected triplicate dissolved gas samples at 5 stations along an 800 m reach downstream from the release site and measured the decline in SF<sub>6</sub> concentrations using a gas chromatograph with an electron capture detector (Shimadzu Gas Chromatograph 14A).  $k_{\text{SF6}}$ , the piston velocity (m min<sup>-1</sup>), was calculated from the three separate  $SF_6$  releases and the decline in SF<sub>6</sub>. We used our average  $k_{\text{SF6}}$  to calculate  $k_{\text{O2}}$ and  $k_{CO2}$  using the ratios of gas exchange coefficients and Schmidt numbers for the gas of interest (Wanninkhof 1992, Cole and Caraco 1998).

$$\frac{k_{gas1}}{k_{gas2}} = \left[\frac{Sc_{gas1}}{Sc_{gas2}}\right]^n,$$

where *k* is the gas exchange coefficient, gas<sub>1</sub> is SF<sub>6</sub>, gas<sub>2</sub> is CO<sub>2</sub> or O<sub>2</sub>, *Sc* is the Schmidt number, and *n* depends on processes dominating diffusion (Wanninkhof 1992, Cole and Caraco 1998). We assumed n = 1 (Portielje and Lijklema 1995, Wanninkhof and Knox 1996).

We collected data on several water chemistry and physical parameters on a weekly basis and during diel sampling throughout summer 2006 at upstream (0 m) and downstream (500 m) sites along our reach. These data inlcuded temperature, acid neutralizing capacity (ANC) calculated by titration (Wetzel and Likens 2000), pH (Orion 3-star portable pH meter with a ROSS Ultra® pH Electrode, Thermo Scientific), conductivity and dissolved oxygen (Hydrolab MiniSondes, Hach Environmental). We collected duplicate 60 mL water samples from our upstream and downstream sites that were filtered, frozen, and later analyzed for concentrations of common cations and anions (Dionex ICS-2000 Ion Chromatography System with AS40 Automated Sampler and Perkin Elmer model 372 Atomic Absorption Spectrophotometer). We measured stream depth (z) and width (w) several times throughout the summer. We also measured stream velocity (V) and discharge (Q).

Using pH, temperature and acid neutralizing capacity, we calculated dissociation constants for carbonic acid, concentrations of carbonate species, and total dissolved inorganic carbon. Carbonic acid dissociation constants (pK1 and pK2) were calculated using temperature adjustments from Cai and Wang (1998). We calculated the concentrations of different carbonate species using measurements of carbonate acid neutralizing capacity, pH, and carbonic acid dissociation constants, following Millero (1979).

$$\begin{bmatrix} H_{2}CO_{3}^{*} \end{bmatrix} = H_{2}CO_{3} + CO_{2} = \frac{\left(\frac{A_{C}a_{H}}{K_{1}}\right)}{\left(1 + \frac{2K2}{a_{H}}\right)},$$
$$\begin{bmatrix} HCO_{3}^{-} \end{bmatrix} = \frac{A_{C}}{1 + \frac{2K2}{a_{H}}},$$
$$\begin{bmatrix} CO_{3}^{2-} \end{bmatrix} = \frac{A_{C}K2}{a_{H} + 2K2},$$

where [] represents the effective concentration in mmol m<sup>-3</sup>, A<sub>C</sub> is carbonate acid neutralizing capacity (mmol m<sup>-3</sup>) and  $a_{\rm H}$  is the activity of H<sup>+</sup>. Total carbonate is the sum of [H<sub>2</sub>CO<sub>3</sub><sup>\*</sup>], [HCO<sub>3</sub><sup>-</sup>] and [CO<sub>3</sub><sup>2-</sup>]. We calculated partial pressure of CO<sub>2</sub> (in atm),

$$pCO_2 = \frac{\left[H_2CO_3^*\right]}{K_H}$$

using our indirect measurements of  $[H_2CO_3^*]$  and Henry's constant for CO<sub>2</sub> (K<sub>H</sub>, mol m<sup>-3</sup> atm<sup>-1</sup>) corrected for temperature and elevation (Langmuir 1997).

Our measurements of  $CO_2$  fluxes from Kelly Warm Springs were calculated by multiplying the  $CO_2$  deficit by the site-specific piston velocity for  $CO_2$ .

CO<sub>2</sub> Flux (mmol m<sup>-2</sup> min<sup>-1</sup>) =  $\alpha k ((pCO_2K_H) - [CO_2]_{sat})$ , where  $\alpha$  is the chemical enhancement factor, k is the piston velocity for CO<sub>2</sub> (m min<sup>-1</sup>),  $pCO_2$  is the partial pressure of CO<sub>2</sub> (mmol CO<sub>2</sub> m<sup>-3</sup>),  $K_H$  is the Henry's constant for CO<sub>2</sub> (mol m<sup>-3</sup> atm<sup>-1</sup>), and  $[CO_2]_{sat}$  is the concentration of CO<sub>2</sub> (mmol CO<sub>2</sub> m<sup>-3</sup>) at saturation (Cole and Caraco 1998). We adjusted  $K_H$  for changes in temperature and elevation when we calculated CO<sub>2</sub> saturation (Langmuir 1997). We used an average of current atmospheric levels of CO<sub>2</sub> (380 µatm) for measurements of saturation (Tans 2007).

## Contributions of Melanoides to carbon cycling

After calculating  $pCO_2$ , CR, and the rate of air-water gas exchange in Kelly Warm Springs, we measured the rate of  $CO_2$  flux into the atmosphere and the extent to which *Melanoides* were responsible for making Kelly Warm Springs a local source of  $CO_2$ . Using growth rates in combination with the CaCO<sub>3</sub> content of varying shell sizes, we calculated the amount of CaCO<sub>3</sub> produced by *Melanoides* and, consequently, the  $CO_2$  emitted through shell synthesis into Kelly Warm Springs during the summer months (Chauvaud *et al.* 2003).

We calculated the ratio ( $\Psi$ ) of released CO<sub>2</sub> to fixed CaCO<sub>3</sub> using adjustments for temperature and salinity by Frankignoulle *et al.* (1994). Approximately 0.6 moles of CO<sub>2</sub> are released for every mole of CaCO<sub>3</sub> precipitated in sea water and the ratio in freshwater is nearly 1.0, but  $\Psi$  lowers with increasing temperature (Ware *et al.* 1991). Using a temperature- and salinity-adjusted  $\Psi$  (0.85) for Kelly Warm Springs, we converted calcification rates to CO<sub>2</sub> production by *Melanoides*.

#### ✦ RESULTS

#### **Melanoides** production

The density of Melanoides in Kelly Warm Springs was 24,000 individuals m<sup>-2</sup> (standard deviation = 13,000) in summer 2006. The biomass of Melanoides in 2004 was 17.8 g AFDM m<sup>-2</sup> (standard deviation = 12.9), with a density of 17,000  $\text{m}^{-2}$  (standard deviation = 7,000). The relationship between shell length and biomass can be described using the equation: [Biomass (g AFDM  $m^{-2}$ ] = 0.0021[Shell Length]<sup>3.1153</sup> (n = 27,  $r^2 = 0.96$ , p < 0.0001). *Melanoides* biomass was 34.2 g AFDM  $m^{-2}$  (standard deviation = 18.2) in 2006. The relationship between shell length and shell mass can be described using the equation: [Shell mass (g CaCO<sub>3</sub>  $m^{-2}$ )] = 0.0223[Shell Length]<sup>2.9664</sup> (n = 27,  $r^2 = 0.99$ , p < 0.0001). Shell lengths ranged from 1.4 to 33.9 mm (Figure 2).

*Melanoides* persisted at high densities 1.5 km downstream of the spring pool and at low densities along 0.5 km of a lower reach before Kelly Warm Springs merged with Ditch Creek. Total macroinvertebrate biomass in Kelly Warm Springs was 39.1 g AFDM m<sup>-2</sup>, including 4.9 g

AFDM m<sup>-2</sup> of native mollusks, arthropods, and annelids (standard deviation = 2.65). *Melanoides* made up 87% of the total invertebrate biomass during summer 2006.



Figure 2. Frequency of biomass represented by each *Melanoides* size class in Kelly Warm Springs during summer 2004 and summer 2006. Error bars represent standard deviations.



Figure 3. Size-specific growth rates for *Melanoides* in Kelly Warm Springs measured *in situ* from June – September 2006. Growth rates are best predicted using the following equation: [growth rate (day<sup>-1</sup>)] =  $0.0142e^{-0.1454[\text{shell length}]} + 0.1686e^{-0.7165[\text{shell length}]}$  (n = 46, r<sup>2</sup> = 0.9507, p < 0.0001).

Growth rates were measured for Kelly Warm Springs *Melanoides* ranging from 1.5 to 13 mm in length. Growth rates were best predicted using the following equation: [growth rate  $(day^{-1})$ ] = 0.0142e <sup>-0.1454[shell length]</sup> + 0.1686e <sup>-0.7165[shell length]</sup> (n = 46, r<sup>2</sup> = 0.95, p < 0.0001). Because we did not measure individuals with an initial length less than 1.5 mm in our growth chambers, we assumed that they have the same growth rates as snails in the 1.5 mm size class, even though the growth rate of smaller snails is likely higher. By weighting size-

6

specific growth rates with the relative abundance of each size class, we calculated an average growth rate of 0.02 (day<sup>-1</sup>). We also assumed that individuals with an initial length greater than 13 mm had a growth rate of zero (day<sup>-1</sup>). These large snails have a low abundance and we were unable to measure significant growth during our three week incubations (Figure 3).

Secondary production of Melanoides biomass in Kelly Warm Springs was 0.31 g AFDM  $m^{-2} day^{-1}$  in 2006, with a P:B (production: biomass) of 3.3 year<sup>-1</sup> (without accounting for fecundity or potential seasonal changes in growth). The density of fecund individuals was 370 m<sup>-2</sup> during summer 2006, yielding an estimated annual production of 67,000 young m<sup>-2</sup> year<sup>-1</sup> and a daily production rate of 0.07 g AFDM (of young)  $m^{-2}$ day<sup>-1</sup>. Combining production from the growth of the current population and the predicted fecundity of individuals 12.0 to 25.0 mm in length, Melanoides produced 0.4 g AFDM m<sup>-2</sup> day<sup>-1</sup> and had a P:B of 4.1 year<sup>-1</sup> during summer 2006. Melanoides in Kelly Warm Springs produced 12.2 moles CaCO<sub>3</sub> m<sup>-2</sup> day<sup>-1</sup> through biotic calcification.

## **Carbon cycling in Kelly Warm Springs**

The differences in CR and GPP between one and two station calculations were similar to the ranges measured between various dates during summer 2006. After comparing GPP and CR calculated using both the two station and the one station approach for the dates when we deployed two MiniSondes, we included our one station calculations from the end of July in our overall estimates of metabolism for Kelly Warm Springs. One and two station CR measurements differed by an average of 6.4% (n = 3, standard deviation = 6.8), while the average difference between one and two station GPP measurements was 9.6% (n = 5, standard deviation = 5.5).

Our releases of SF<sub>6</sub> yielded a  $k_{SF6}$  value of 0.00155 m<sup>-1</sup> (95% confidence interval for all three releases = 5.66 x 10<sup>-5</sup>) and a  $k_{600}$  value of 0.00136. Gross primary productivity (GPP) and community respiration (CR) measurements over 14 days in July and August averaged -379 mmol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (standard deviation = 131) and 445 mmol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (standard deviation = 37.5). Net ecosystem production (NEP) was 65.9 mmol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (standard deviation = 128.9). Despite a much higher GPP from 21-22 July in comparison to our other measurements (the measurements

responsible for the high standard deviation), we do not believe these calculations are due to measurement error and included these data in our metabolism calculations for Kelly Warm Springs.

Kelly Warm Springs was consistently super-saturated with CO<sub>2</sub> (with respect to current atmospheric levels of 380 ppm). CO<sub>2</sub> partial pressure values ranged from 476.0 to 5421.5 µatm, with a mean of 2725.8 µatm over 24-hour cycles. CO<sub>2</sub> evasion rates ranged from 0.0015 to 0.0556 mmol CO<sub>2</sub> m<sup>-2</sup> min<sup>-1</sup>, with higher fluxes during late evening and early morning and lower fluxes in the afternoon (Figure 4). Kelly Warm Springs contributed to an average flux of 37.2 mmol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (standard deviation = 5.6).



Figure 4. Net  $CO_2$  evasion over 3 different 24-hour periods in July and August 2006. The average  $CO_2$  flux from Kelly Warm Springs was 37.2 mmol  $CO_2$  m<sup>-2</sup> day<sup>-1</sup>.

## Contributions of Melanoides to carbon cycling

Using our direct measurements of *Melanoides* population density, growth rates and size frequency distributions, shell mass in Kelly Warm Springs was 1.9 moles CaCO<sub>3</sub> m<sup>-2</sup> and calcification rates averaged 12.2 mmol CaCO<sub>3</sub> m<sup>-2</sup> day<sup>-1</sup>. During summer months, we estimated that biotic calcification by *Melanoides* contributed 10.4 mmol CO<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> to the water column inorganic carbon pool. We assumed that CO<sub>2</sub> produced as a byproduct of calcification was constant throughout day and night.

Compared to the daily swings of  $CO_2$ production and consumption in Kelly Warm Springs, calcification produced a relatively small amount of  $CO_2$  (Figure 5). However, this  $CO_2$ released from biotic calcification increased total  $CO_2$  production estimates for Kelly Warm Springs. Including calcification in the inorganic carbon budget for Kelly Warm Springs would increase net ecosystem production of CO<sub>2</sub> from 65.9 mmol CO<sub>2</sub>  $m^{-2}$  day<sup>-1</sup> to 76.3 mmol CO<sub>2</sub>  $m^{-2}$  day<sup>-1</sup>. Because the bicarbonate system in streams is dominated by HCO<sub>3</sub>, a large proportion of the CO<sub>2</sub> produced via biological processes was transformed to HCO<sub>3</sub>. Relatively little CO<sub>2</sub> was lost due to diffusion and reaeration (only 37.2 mmol CO<sub>2</sub>  $m^{-2}$  day<sup>-1</sup>, including losses from high-CO<sub>2</sub> groundwater inputs upstream), despite Kelly Warm Springs being a net source of CO<sub>2</sub> to the atmosphere (Figures 4 & 6).



Figure 5. Comparison of  $CO_2$  production by stream metabolism versus calcification.



Figure 6. Carbonate pools and fluxes within and from Kelly Warm Springs, Wyoming. Arrows represent fluxes (mmol of  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$ ) and represent the relative contributions of each process to  $CO_2$  production. Boxes are standing stock. Note that primary production by biota in the organic carbon pool can take up  $CO_2$  or  $HCO_3^-$ , depending on the species. We did not measure dissolution of shells or carbonate minerals on the benthos for this specific study and assumed they were at steady state.

# + DISCUSSION

## **Melanoides** production

Warm temperatures and high primary production in Kelly Warm Springs have likely facilitated one of the highest densities reported for *Melanoides* in the literature to date. Biomass production and shell calcification rates for *Melanoides* individuals were slower than rates published for other invasive snail species, but were important in terms of total invertebrate biomass due to the high density of *Melanoides* established in Kelly Warm Springs.

*Melanoides* tend to grow slowly and have lower fecundity compared to other successful invasive freshwater snails (Facon *et al.* 2006) Therefore, it is no surprise that the *Melanoides* annual P:B of 4.1 was lower than 9 and 12 year<sup>-1</sup> calculated for the invasive New Zealand mud snails in the greater Yellowstone area (Hall *et al.* 2006). Our calculations of annual P:B for the measured ranges of *Melanoides* fecundity (4.1 to 4.9 year<sup>-1</sup>) are on either end of the reported values of 4.4 in Lake Kariba, Zimbabwe (Kiibus and Kautsky 1996) and 4.81 in Ping Long, Hong Kong (Dudgeon 1986).

While we calculated the density of *Melanoides* from only four stovepipe samples collected in 2004 and measurements in 2006 were not statistically higher (t = -0.9976, df = 6, p = 0.3570), overall trends suggest the population has continued to increase over the past few years. Giovanelli *et al.* (2005) found *Melanoides* populations capable of exponential growth to over 1,000 individuals m<sup>-2</sup> just a few months after establishment, so it is reasonable to assume that the introduction of *Melanoides* did take place around 2001.

## **Carbon cycling in Kelly Warm Springs**

Kelly Warm Springs was a highly productive yet net heterotrophic stream during summer 2006. The 500 m reach was supersaturated with  $CO_2$  with respect to atmospheric concentrations during the entire study period; and Kelly Warm Springs acted as a net source of  $CO_2$ throughout diel cycles and the summer months in general. Rates of primary production and respiration in Kelly Warm Springs were higher than most streams (Mulholland *et al.* 2001) as well as Polecat Creek, another highly productive stream in the greater Yellowstone ecosystem (Hall *et al.* 2003). Despite extremely high rates of productivity, Kelly Warm Springs was net heterotrophic during six of the seven 24-hour cycles we recorded changes in dissolved oxygen.

As with many freshwater ecosystems, Kelly Warm Springs was consistently supersaturated with respect to atmospheric  $pCO_2$ . While some of this  $CO_2$  saturation can be attributed to a P:R (GPP:CR) of -0.85, much of this  $CO_2$  likely comes from the upwelling of  $CO_2$ -rich spring water above our study reach. This constant source of inorganic carbon, along with warm temperatures, may be important factors behind the high productivity in Kelly Warm Springs.

While methods have been developed to quantify inorganic carbon dynamics within aquatic ecosystems as well as evasion rates of  $CO_2$  into the atmosphere, it is still uncertain how much of this  $CO_2$  evasion is driven by biological versus physical and geological processes. We show here that photosynthesis and respiration, which alter pH in aquatic systems, can drive 24-hour cycles in  $CO_2$  evasion.

## Contributions of Melanoides to carbon cycling

*Melanoides* calcification and  $CO_2$ production rates in Kelly Warm Springs were similar to rates calculated for other aquatic invertebrates, but were small in comparison to daily inorganic carbon fluxes driven by photosynthesis and respiration. However, the rate of  $CO_2$  production via shell synthesis was significant in comparison to daily net ecosystem production (NEP) of  $CO_2$ . When considering the role of invasive snails in freshwater inorganic carbon cycling, the introduction and establishment of *Melanoides* in Kelly Warm Springs has increased net biological  $CO_2$  production and, consequently,  $CO_2$  evasion from the water into the atmosphere.

Beyond dominating the invertebrate organic carbon pool, the shell mass of *Melanoides* may be an important inorganic carbon reserve that could alter biogeochemical cycling. *Melanoides* have thick shells (and grow to a much larger size than any of the other mollusks present in Kelly Warm Springs) that will take several years to dissociate after death, especially in Kelly Warm Springs with high calcium (Ca<sup>2+</sup>) concentrations

and pH, which impede  $CO_2$  dissolution (Strayer and Malcom 2007). Decreases in  $CaCO_3$ precipitation can also reverse this trend of  $CO_2$ production and act as a  $CO_2$  sink, especially during exoskeleton dissolution (Orr *et al.* 2005).

Melanoides CO<sub>2</sub> production via biotic calcification was similar to other mollusks. including the invasive freshwater bivalves Dreissena polymorpha and Corbicula fluminea (Chauvaud et al. 2003). We calculated additional annual CO<sub>2</sub> production estimates for several different marine calcifying organisms for further comparisons, most of which were in a similar range to Melanoides CO<sub>2</sub> production after accounting for the difference in  $\Psi$  between salt and freshwater systems (Table 1). If Melanoides consumed a larger proportion of primary producers in Kelly Warm Springs, it is possible that they would have more of an impact on ecosystem inorganic carbon cycling and CO<sub>2</sub> fluxes (Schindler et al. 1997). On the other hand, it is unlikely that ecosystems could support higher densities and/or higher production rates of snails without a preceding increase in GPP.

CO<sub>2</sub> production by *Melanoides*, while only a fraction of daily inorganic carbon cycling in relation to GPP and CR, was 13.6% of the net biological CO<sub>2</sub> production in Kelly Warm Springs (Figure 6). Despite the large proportion of  $CO_2$ that was quickly transformed to HCO; within the carbonate pool, we estimated the potential impacts of losing calcifying Melanoides from Kelly Warm Springs. Compared to the total net CO<sub>2</sub> production from biological processes (NEP + calcification), diffusion and reaeration were responsible for the evasion of 48.7% of the net biological CO<sub>2</sub> production. Holding all other processes equal, the loss of calcifying Melanoides would decrease Kelly Warm Springs CO<sub>2</sub> evasion rates from 37.2 to 32.1 mmol  $CO_2 \text{ m}^{-2} \text{ day}^{-1}$ .

This study demonstrates the potential role of invasive species as a source of  $CO_2$  to stream inorganic carbon budgets as well as the ecosystemlevel impacts of an exotic snail. *Melanoides* can contribute substantially to total biological  $CO_2$ production through calcification, which must be calculated independently from stream metabolism. Biotic calcification did increase calculations of total  $CO_2$  production and evasion in Kelly Warm Springs. In freshwater systems that are already super-saturated with  $CO_2$ , successful invasions by calcifying organisms may contribute to higher  $CO_2$  evasion rates. The escalating introduction and spread of invasive mollusks worldwide will have important consequences when considering the biological processes responsible for  $CO_2$  evasion from aquatic systems.

Table 1. Approximate  $CO_2$  production via calcification by several different freshwater and marine organisms. Freshwater organisms are noted with a "\*" and calcification values calculated from other sources by Chauvaud *et al.* (2003) are noted with a "\*".

Organism	CO <sub>2</sub>	Citation
0	Production	
	(moles CO <sub>2</sub>	
	m <sup>-2</sup> year <sup>-1</sup> )	
Dreissena	0-18.0	Chauvaud et
polymorpha*		al. 2003 <sup>+</sup>
(zebra mussels)		
Heteropod	$4.7 \times 10^{-4} -$	Fabry 1990
mollusks	16.6 x 10 <sup>-4</sup>	
Pteropod	$3.3 \times 10^{-3} -$	Fabry 1990
mollusks	15.6 x 10 <sup>-3</sup>	
Bryozoans	0.4	Smith &
		Nelson 1994
Potamocorbula	1.8	Chauvaud et
amurensis		al. 2003
(Asian clam)		
Corbicula	1.8 - 10.8	Chauvaud et
fluminea*		al. 2003 <sup>+</sup>
(freshwater		
Asian clam)		
Bryozoans,	2.4	Smith 1972
coralline algae,		
echinoderms and		
mollusks		
Seagrass	3.2	Chauvaud et
epiphytes		al. 2003 <sup>+</sup>
Crepidula	3.6	Martin et al.
fornicata		2006
(slipper limpet)		
Melanoides	3.8	This study
tuberculata*		
(red-rim		
melania)		
Ophiothrix	4.8	Migné et al.
fragilis (brittle		1998
star)		
Coral reef systems	6.0	Gattuso et al.
		1998
Crustose coralline	9.7 - 66.9	Chisholm
algae		2000
Foraminifera	12.0	Chauvaud et
		al. 2003 <sup>+</sup>

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