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Parasite-Induced Changes in Nitrogen Isotope Signatures of Host Tissues

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ABSTRACT: To estimate isotopic changes caused by trematode parasites within a host, we investigated changes in the carbon and nitrogen isotope ratios of the freshwater snail *Lymnaea stagnalis* infected by trematode larvae. We measured carbon and nitrogen stable isotopes within the foot, gonad, and hepatopancreas of both infected and uninfected snails. There was no significant difference in the $\delta^{13}C$ and $\delta^{15}N$ values of foot and gonad between infected and uninfected snails; thus, trematode parasite infections may not cause changes in snail diets. However, in the hepatopancreas, $\delta^{15}N$ values were significantly higher in infected than in uninfected snails. The ^{15}N enrichment in the hepatopancreas of infected snails is caused by the higher ^{15}N ratio in parasite tissues. Using an isotope-mixing model, we roughly estimated that the parasites in the hepatopancreas represented from 0.8 to 3.4% of the total snail biomass, including the shell.

Parasitic infections introduce additional demands on host resources, either through direct competition for energy (Coop and Holmes, 1996; Sorensen and Minchella, 1998) or by stimulating the host's immune system (Moret and Schmidt-Hempel, 2000). Recently, parasites have been shown to modify the feeding patterns of their intermediate hosts (e.g., Thompson, 1990; Miura et al., 2006). Hosts may attempt to compensate for the increased nutritional demands caused by parasites by increasing their foraging effort (Thompson, 1990).

Stable isotope ratios of carbon and nitrogen are increasingly being used to analyze the food sources of macroinvertebrates in various ecosystems (e.g., Vander Zanden and Vadeboncoeur, 2002; Doi et al., 2004, 2006). Stable isotope techniques can provide continuous measures of trophic positions that integrate the assimilation of energy or mass flow through all the various trophic pathways leading to an organism (McCutchan et al., 2003).

The freshwater snail *Lymnaea stagnalis* is an intermediate host for many species of trematode parasites (Yurlova, 2003). *Lymnaea stagnalis* is the first intermediate host for at least 15 trematode species that parasitize the hepatopancreas and the second intermediate host for 18 trem-

TABLE I. The dry weight of the total body, foot, gonad, and hepatopancreas from infected and uninfected *Lymnaea stagnalis* and the percentage of each tissue in the total dry weight of the snails (mean ± 1 SD, n = 10 infected and 10 uninfected snails). The *t*- and *P*-values are for *t*-tests comparing infected and uninfected snails.

	Dry weight (μg)	*t*	*P*	% of Total weight	*t*	*P*
Total body of snail with shell						
Uninfected	794 ± 116					
Infected	838 ± 118	−0.8	0.41			
Foot						
Uninfected	104 ± 26			10.0 ± 1.9		
Infected	88 ± 27	−1.3	0.20	9.0 ± 2.0	−1.1	0.28
Gonad						
Uninfected	59 ± 17			5.6 ± 1.5		
Infected	33 ± 10	−4.2	<0.001	3.3 ± 0.8	−4.2	<0.001
Hepatopancreas						
Uninfected	41 ± 11			3.9 ± 0.8		
Infected	58 ± 15	2.9	0.01	6.0 ± 1.6	4.0	<0.001

atode species that occur in other snail tissues, such as the mantle and foot (Sudarikov et al., 2002; Yurlova, 2003; Yurlova and Serbina, 2004; Yurlova et al., 2006).

We investigated changes in the isotope ratios of infected and uninfected *L. stagnalis* to determine whether parasites can affect the nature and concentration of isotopes. Changes in food sources of hosts because of infection have been reported for marine gastropods (Miura et al., 2006); the phenomenon was observed using stable isotopes. We hypothesized that (1) an infected snail will change food sources because of changes in feeding behavior and habitat, and (2) the isotope ratios in the tissues of infected snails will differ from those of uninfected snails. The confirmation of these hypotheses will provide key information for understanding host–parasite interactions because changes in host food sources and feeding habits because of parasites could affect survival and reproductive rates within a host population.

We studied the common freshwater snail *L. stagnalis* inhabiting the riverine portion of Lake Chany, Siberia, Russia. *Lymnaea stagnalis* is a dominant gastropod snail in western Siberia (Yurlova and Vodyanitskaya, 2005). Lake Chany is located in the Barabinskaya lowland of West Siberia, Russia (54°30′–55°09′N, 76°48′–78°12′E). The lake is located in a forest–steppe region at an altitude of 106 m above sea level. It is a shallow, inland, saline system (average depth, 2.2 m; maximum depth, 8.5 m; Aladin and Plotnikov, 1993; Doi et al., 2004). The study was conducted in the inflow part of the Kargat River (54°37.76′N, 78°13.07′E) of Lake Chany. The substrata at the site varied from detritus to sediment. Information regarding larval trematodes and their effects on the behavior, growth, fecundity, and population dynamics of their snail hosts in West Siberia has been reported previously (Yurlova, 1987, 2003, 2006; Yurlova et al., 2000, 2006).

Specimens of *L. stagnalis* and their parasites were sampled in August 2004. Snails were collected by hand at a depth of 0.1–0.5 m. We collected 20 *L. stagnalis* (10 infected and 10 uninfected). Under laboratory conditions, the snails were measured using a slide caliper (length of shell from apex to aperture), then dissected, and examined for the presence of trematode parasites using a microscope. We saved the foot, gonad, and hepatopancreas for isotope ratio analyses (n = 20). The tissues were first dried at 60 C and then stored at −20 C. Before analysis, the dry weight of each tissue was measured. We calculated the relative contribution of each tissue to the dry weight of the body. We used *t*-tests to examine the effect of infection on tissue dry weight.

Before isotope measurement, the lipids in all tissues were removed using a chloroform:methanol mixture (2:1 by weight) because of the high lipid content in muscle tissue (Kling, 1992). The isotopic ratios of carbon and nitrogen in the samples were measured with a mass spectrometer (Integra CN, SerCon Co., Cheshire, U.K.). The results are presented using common delta notation, calculated as: $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1{,}000$ (‰), where R is the $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$ ratio for $\delta^{13}C$ or $\delta^{15}N$, respectively. Pee Dee Belemnite and atmospheric nitrogen were used as international standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. The errors during the overall analyses were within ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$. The effects of infection on the carbon and nitrogen isotope values in the different tissues were determined using *t*-tests.

Four of 10 infected *L. stagnalis* had sporocysts of *Plagiorchis mutationis*; 6 of 10 snails were infected by sporocysts of *Plagiorchis* sp. larvae. We determined the total dry weight of each snail by calculating the dry weight and the percentage of total weight represented by each tissue (foot, gonad, and hepatopancreas) from infected and uninfected *L. stagnalis* (Table I). The shell lengths of uninfected and infected snails were 41.9 ± 0.43 and 42.5 ± 0.42 mm, respectively, and were not significantly different ($P = 0.90$). The dry weight and percentage of total weight of infected hepatopancreases were significantly higher than those of uninfected hepatopancreases ($P < 0.01$; Table I). However, the dry weight and percentage of the total weight of infected gonads were significantly lower than those of uninfected snails' gonads ($P < 0.001$; Table 1). The dry weight and percentage of total weight of foot tissue did not differ between infected and uninfected snails (Table I).

The $\delta^{15}N$ values from infected hepatopancreases were significantly higher than those of uninfected hepatopancreases ($P = 0.03$, Table II). However, there were no significant differences in $\delta^{15}N$ between infected and uninfected foot or gonad tissues, or in $\delta^{13}C$ values for foot, gonad, or hepatopancreas tissues ($P > 0.09$; Table II). Except for the $\delta^{15}N$ of the hepatopancreas, there were no significant effects of infection on the $\delta^{13}C$ and $\delta^{15}N$ values of the different tissues (Fig. 1). There were no significant differences in the $\delta^{13}C$ and $\delta^{15}N$ values of tissues between snails infected with *Plagiorchis* sp. and *P. mutationis* ($P = 0.3$). It is possible that our small sample sizes limited our ability to detect differences between snails infected by different trematode species and between infected and uninfected snails. We tested 2 hypotheses: (1) the food sources of host snails are altered by trematode infection, and (2) the isotope ratios of infected tissues differ from uninfected tissues because of parasites. We did not observe the migration of cercariae through the tissues. Parasites can become significantly ^{15}N-enriched from the host because parasites feed mainly on host tissues (Boag et al., 1998; Doucett et al., 1999; Power and Klen, 2004; O'Grady and Dearing, 2006). Changes in host food sources because of infection have been reported for marine gastropods (Miura et al., 2006). Thus, parasites can modify the feeding patterns of snails that serve as their intermediate hosts (Levri, 1999; Levri and Fisher, 2000). However, the $\delta^{13}C$ and $\delta^{15}N$ values of foot and gonad tissues did not differ significantly between the infected and uninfected snails that we examined. Moreover, we did not find differences in the habitat use or feeding behavior of infected or uninfected snails in August. Also, we collected all snails (infected and uninfected) at the same sites and observed that they were moving in many directions. Thus, trematode infection may not have a strong effect on snail diet and may not affect the $\delta^{13}C$ and $\delta^{15}N$ values in snail tissues, expect within the hepatopancreas. An additional explanation for the observed foot and gonad isotope values is that significant isotope changes were not detected because the snails fed on various food sources

TABLE II. The $\delta^{13}C$ and $\delta^{15}N$ values of the foot, gonad, and hepatopancreas of infected and uninfected *Lymnaea stagnalis* (mean ± 1 SD, n = 10 infected and 10 uninfected snails). The *t*- and *P*-values are for *t*-tests comparing infected and uninfected snails.

	$\delta^{13}C$ (‰)	*t*	*P*	$\delta^{15}N$ (‰)	*t*	*P*
Foot						
Uninfected	−32.3 ± 1.6			3.4 ± 1.5		
Infected	−31.1 ± 0.9	−1.77	0.10	4.3 ± 1.7	−1.24	0.23
Gonad						
Uninfected	−32.2 ± 1.2			3.9 ± 1.5		
Infected	−31.5 ± 1.2	−1.36	0.19	3.9 ± 2.0	0.11	0.91
Hepatopancreas						
Uninfected	−31.6 ± 1.5			3.1 ± 1.5		
Infected	−32.3 ± 1.0	0.87	0.39	4.5 ± 1.1	−2.23	0.03

with various isotope ratios, such as sediment organic matter, benthic microalgae, and macroalgae.

In addressing our second hypothesis, the $\delta^{15}N$ values of hepatopancreases from infected snails were significantly higher than those of uninfected hepatopancreases. Moreover, the dry weight of infected hepatopancreases was significantly greater than that of uninfected hepatopancreases. Many generations of trematode asexual larval stages, i.e., sporocysts and/or rediae, develop and reproduce in the hepatopancreas of an infected snail. In most snail–trematode systems, the growth and reproduction of trematode larval sporocysts and rediae occur in resource-rich host tissues, such as the digestive gland and gonads (Kube et al., 2006). Thus, a possible explanation for the greater weight of infected hepatopancreases is the altered composition of tissues such as the increased storage of calcium granules. Also, the dry weight of infected gonads was significantly lower than that of uninfected gonads. Some trematode species possess larval stages that castrate their snail hosts, either chemically or directly (mechanical castration), when the larvae are located in the gonads of the host and cause complete destruction of the gonad (Wilson and Denison, 1980; Sluiters et al., 1984; De Jong-Brink, 1990; Probst and Kube, 1999). In infected snails, the hepatopancreas contains trematode parasite tissues.

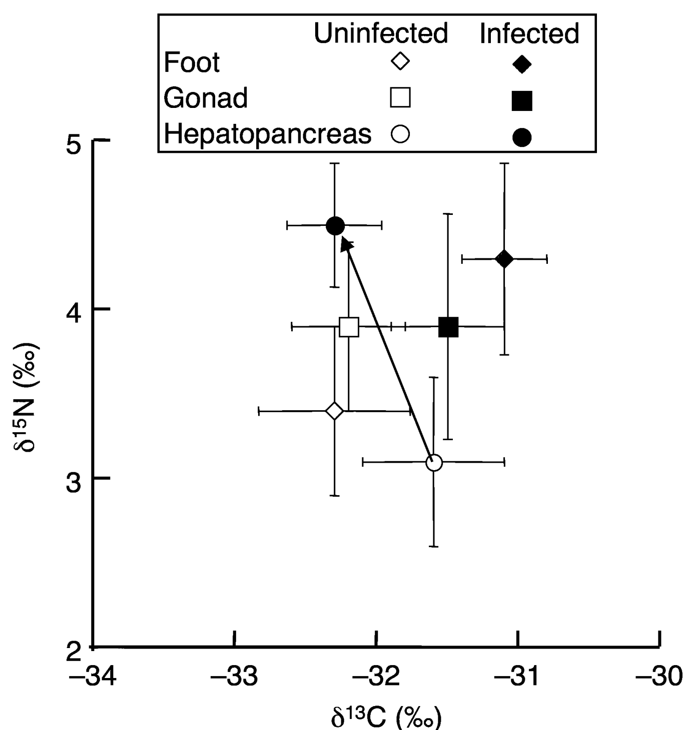

FIGURE 1. $\delta^{13}C$ and $\delta^{15}N$ values of the foot, gonad, and hepatopancreas of infected and uninfected *Lymnaea stagnalis* (mean ± 1 SE). Arrow indicates the significant changes in the $\delta^{13}C$ and $\delta^{15}N$ values of uninfected and infected tissues (*t*-test, $P = 0.03$).

We conclude that the trematode parasites caused the ^{15}N enrichment and increased the weight of infected hepatopancreases, even though Cheng et al. (1983) indicated that the soft tissues of infected and uninfected snails do not differ after trematode parasites are removed. Parasites such as cestodes and nematodes become significantly ^{15}N-enriched from their hosts because the parasites feed mainly on host tissues (Boag et al., 1998; Doucett et al., 1999; Power and Klen, 2004; O'Grady and Dearing 2006). Parasite ^{15}N-enrichment likely causes the $\delta^{15}N$ values of infected and uninfected hepatopancreases to differ, although we did not directly measure the $\delta^{15}N$ values of the trematode parasites.

We calculated the contribution of parasites to the isotope values of infected hepatopancreases using the mass–balance isotope-mixing model of Phillips (2001) with various isotope fractionations of parasites as follows:

$$\delta^{15}N_{\text{infected hepatopancreas}} = a(\delta^{15}N_{\text{uninfected hepatopancreas}} + \Delta^{15}N) + (1 - a)\delta^{15}N_{\text{uninfected hepatopancreas}},$$

where $\Delta^{15}N$ is the isotope fractionation of the cestode and nematode parasites, ranging from +1.0 to +4.0‰ (Boag et al., 1998; Doucett et al., 1999; Power and Klen, 2004; O'Grady and Dearing, 2006). Based on the isotope-mixing model with various isotope fractionations, the contribution of parasites to the isotope values of infected hepatopancreases ranged from 12.5 to 50.0%. Thus, the dry weight of the parasites was calculated using the assumed isotopic contribution from the parasites and the dry weight of the hepatopancreas. In this way, we roughly estimated that the dry weight of parasites in an infected hepatopancreas ranged from 7.3 to 29.0 μg. Parasites in the hepatopancreas thus accounted for between 0.8 and 3.4% of the total snail biomass. The high proportion of parasite biomass within the host probably causes remarkable changes in host reproduction and tissue weight. However, we acknowledge several caveats with this approach that affect its scope. Thus, the species composition of trematode parasites varies among individual snails, and the isotope estimation includes the contributions of many trematode species. In future studies, we plan to estimate and compare parasite biomass in various hosts and parasite species using stable isotopes.

We tested 2 hypotheses using stable isotopes of carbon and nitrogen in snail tissues. We showed that the food sources of snails are not changed by trematode infection, but the nitrogen isotope ratio differs between infected and uninfected hepatopancreases, probably because of the direct effect of parasite biomass. Using an isotope mixing model, we estimated the biomass of parasites in the snails. Our results provide an initial step for estimating the presence and biomass of parasites in host tissues using stable isotope techniques.

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