Vol. 115: 121–128, 2015
doi: 10.3354/dao02893

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 23

Consistent isotopic differences between *Schistocephalus* spp. parasites and their stickleback hosts

Antti P. Eloranta[1,2,*], Rune Knudsen[3], Per-Arne Amundsen[3], Juha Merilä[4]

[1]Norwegian Institute for Nature Research, PO Box 5685 Sluppen, 7485 Trondheim, Norway
[2]University of Jyväskylä, Department of Biological and Environmental Sciences, PO Box 35, 40014 Jyväskylä, Finland
[3]UiT The Arctic University of Norway, Faculty of Biosciences, Fisheries and Economics, Department of Arctic and Marine Biology, 9037 Tromsø, Norway
[4]University of Helsinki, Department of Biosciences, Ecological Genetic Research Unit, PO Box 65, 00014 University of Helsinki, Finland

ABSTRACT: Parasite–host systems show markedly variable patterns in isotopic fractionation: parasites can be either depleted or enriched in ^{15}N and ^{13}C as compared to their hosts. However, it remains unknown whether isotopic fractionation patterns are similar in comparable parasite–host systems from markedly different ecosystems. Results of this study show that large-sized *Schistocephalus* spp. endoparasites are consistently depleted in ^{15}N (by on average −2.13 to −2.20‰) as compared to their nine-spined stickleback *Pungitius pungitius* and three-spined stickleback *Gasterosteus aculeatus* hosts. The differences between parasites and host for both $\delta^{15}N$ and $\delta^{13}C$ were consistent in both study systems despite marked biogeographical differences between the study localities. Although the stable isotope values in general were strongly correlated between the hosts and their parasites, *Schistocephalus* specimens occupying the same nine-spined stickleback host showed sometimes substantial individual variation in $\delta^{13}C$. This might be due to selective use of different carbon sources, or different metabolic or feeding rates. Further studies on selective feeding, physiology and metabolism of parasites are needed to better understand the role of parasites in the structure and functioning of aquatic food webs.

KEY WORDS: Endoparasite · *Pungitius pungitius* · *Gasterosteus aculeatus* · Tapeworm · Platyhelminth · Stable isotope analysis · Nutrient assimilation

INTRODUCTION

Recent ecological studies have highlighted the central role of parasites in aquatic food webs (Lafferty et al. 2008), both as consumers (Amundsen et al. 2009) and as prey (Thieltges et al. 2013). For instance, parasites have been shown to increase food-chain length and the degree of omnivory, thereby affecting the structure and function of aquatic food webs (Lafferty et al. 2008, Amundsen et al. 2009, Thieltges et al. 2013). During the past decade, stable isotopes have been widely used in studies of food webs (Boecklen et al. 2011, Layman et al. 2012, and references therein), host–parasite interactions (e.g. Pinnegar et al. 2001, Deudero et al. 2002, Navarro et al. 2014), as well as starvation and nutrient stress in e.g. fish and humans (Reitsema 2013, Bowes et al. 2014). However, it has remained unclear why parasites, which are expected to be assimilating energy and nutrients at a higher apparent trophic level than their host, can be either depleted or enriched in ^{15}N (Pinnegar et al. 2001, Deudero et al. 2002), and hence show inconsistent trophic enrichment as compared to most diet–consumer relationships (cf. McCutchan et al. 2003).

One example of a widely studied parasite–host system that shows an unexpected isotopic fractionation

*Corresponding author: antti.eloranta@nina.no

pattern (i.e. depletion in ^{15}N) is the large-sized *Schistocephalus solidus* (Cestoda) tapeworm and its three-spined stickleback *Gasterosteus aculeatus* host. *S. solidus* has 3 consecutive hosts during its lifecycle: a cyclopoid copepod, the three-spined stickleback and a fish-eating bird (Barber et al. 2008). As the total mass of *S. solidus* tissue can exceed that of the three-spined stickleback, it can reduce the growth and fecundity of the host (Barber 2007). In addition, in order to increase transmission success to its final bird host, *S. solidus* can also manipulate the behavior of its fish host (Barber 2007 and references therein).

S. solidus lives in the body cavity of the three-spined stickleback, where it is fuelled by nutrients from assimilated food ingested by the host (Barber et al. 2008). In spite of this, Pinnegar et al. (2001) observed that *S. solidus* occupied a lower apparent trophic level than its three-spined stickleback host as inferred from stable nitrogen isotopes ($\delta^{15}N$). For most predator–prey isotopic relationships, it is usually observed that predators are enriched in ^{15}N by 2 to 4‰ as compared to their prey (cf. Post 2002, McCutchan et al. 2003). As such, it has been argued that the unexpected isotopic differences between parasites and their fish hosts (i.e. parasites being depleted rather than enriched in ^{15}N) could be due to a range of physiological or behavioural processes including prey selectivity, diet quality and feeding rate (Pinnegar et al. 2001, Power & Klein 2004, and references therein). However, Pinnegar et al. (2001) analysed only a small number (n = 5) of parasites and hosts collected from a single lake, and it is possible that isotopic differences between *S. solidus* parasites and their fish hosts differs between populations. Moreover, it is unknown how nitrogen and carbon ($\delta^{13}C$) stable isotopes fractionate in ostensibly similar parasite–host systems, or whether parasites occupying the same individual host can show individual differences in isotope ratios and/or niche use, similar to that observed among numerous consumer taxa (Bolnick et al. 2003).

The aim of this study was to explore the differences in stable nitrogen and carbon isotopes between *Schistocephalus* spp. endoparasitic tapeworms and their stickleback hosts. The differences between parasite and host $\delta^{15}N$ and $\delta^{13}C$ values were studied in 2 similar parasite–host systems inhabiting biogeographically different ecosystems: (1) in a lake inhabited by three-spined sticklebacks and *S. solidus* parasites, and (2) in a pond inhabited by nine-spined sticklebacks *Pungitius pungitius* and *S. pungitii* parasites. In both study systems, the $\delta^{15}N$ and $\delta^{13}C$ values of parasites and their stickleback hosts were hypothesized to show significant correlation, with parasites having consistently lower $\delta^{15}N$ values than their fish hosts, as observed for several endoparasite–fish relationships (e.g. Pinnegar et al. 2001, Deudero et al. 2002). Secondly, differences between parasite and host $\delta^{15}N$ and $\delta^{13}C$ were hypothesized to be similar in both studied parasite–host systems, despite biogeographical differences between the study locations. Thirdly, if the fish host was infected by several *Schistocephalus* individuals, the parasites were hypothesized to have equal isotope values, and hence niche use, inside the body cavity of the stickleback host. Fourthly, parasitized sticklebacks were hypothesized to be enriched in ^{15}N and have higher C:N ratios as compared to unparasitized individuals due to nutrient stress and catabolism leading to higher $\delta^{15}N$ (Reitsema 2013, Bowes et al. 2014).

MATERIALS AND METHODS

Parasitized (n = 20) and unparasitized (n = 40) nine-spined sticklebacks of similar size were collected from a small (surface area ca. 0.05 km^2, maximum depth ca. 5 m), isolated pond in northeastern Finland (Rytilampi; 66° 23′ N, 29° 18′ E) during 7 to 10 August 2012. Apart from introduced whitefish *Coregonus lavaretus*, which may already be extinct, the nine-spined stickleback is the only fish species present in this locality (Herczeg et al. 2009). The fish were caught with metallic minnow traps set overnight (see Merilä et al. 2013 for details).

Infected three-spined sticklebacks (n = 6) were collected from Lake Sagelvvatn in northwestern subarctic Norway (69° 11′ N, 19° 06′ E) during 9 to 11 August 2010. Sagelvvatn is an oligotrophic and relatively deep lake (maximum depth ca. 80 m, surface area ca. 5 km^2) harbouring 2 sympatric salmonid species, Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta*. The three-spined sticklebacks were caught with gillnets from the shallow littoral zone (see Eloranta et al. 2013 for details). Their parasite community is described by Kuhn et al. (2015). The three-spined stickleback and *Schistocephalus* spp. parasite samples from Sagelvvatn were originally collected for another study (Eloranta et al. 2013) and were thus not measured or weighed, but otherwise the preparation procedure followed that in Rytilampi.

Fish from both localities were stored at –20°C until examination in the laboratory. Each nine-spined stickleback from Rytilampi was measured (total length to nearest 1 mm) and weighed (wet mass to nearest 0.1 g), and a piece of dorsal white muscle

tissue was dissected for stable isotope analysis. This tissue was used for isotope analyses because it is usually the major contributor to fish body mass (Plimmer 1921). *Schistocephalus* spp. parasites were isolated from the fish body cavity, weighed, identified and rinsed with distilled water. The frozen fish muscle tissue and *Schistocephalus* spp. parasite samples were freeze-dried (Alpha 1-4 LD Plus, Martin Christin Gefriertrocknungsanlagen) for 48 h, homogenized with a metallic pestle and weighed (0.500–0.600 mg) into tin cups. The samples were analysed with a FlashEA 1112 elemental analyser connected to a Thermo Finnigan DELTAplus Advantage mass spectrometer at the University of Jyväskylä, Finland. Analytical precision (i.e. SD of an internal standard made from pike *Esox lucius* white muscle tissue) was <0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$ in each run.

Pearson correlation was used to test for relationships between host and parasite $\delta^{15}N$ and $\delta^{13}C$ values. As the normality and homoscedasticity assumptions for parametric tests were met, pairwise comparisons of $\delta^{15}N$ and $\delta^{13}C$ values were made between each fish host and its respective parasite using a paired *t*-test. The 'Anova' function in the 'car' package (Fox & Weisberg 2011) was used to perform analysis of covariance (ANCOVA) for testing differences in parasite–host isotopic differences between Rytilampi and Sagelvvatn parasite–host systems, with parasite isotope values being treated as response variables, host isotope values as covariates and lake as a fixed factor. Finally, the $\delta^{15}N$ and $\delta^{13}C$ values and C:N ratios of parasitized and unparasitized nine-spined sticklebacks from Rytilampi were compared using *t*-tests. All statistical analyses were performed in R 3.1.1 (R Core Team 2014).

RESULTS

The parasitized nine-spined sticklebacks caught from Rytilampi ranged from 45 to 88 mm (mean ± SD = 56 ± 1 mm) in total length and from 0.7 to 3.4 g (mean 1.4 ± 0.8 g) in wet mass. Most of these fish (65%) had only 1 *Schistocephalus pungitii* parasite in the body cavity, but some fish had more: 3 fish had 2 parasites, 2 fish had 3, 1 fish had 4, and 1 fish had 5 parasites, the latter making up to 34% of the host's total wet mass. The wet mass of individual *S. pungitii* ranged from 0.03 to 0.32 g (mean = 0.12 ± 0.08 g).

The $\delta^{15}N$ and $\delta^{13}C$ values of the *Schistocephalus* spp. parasites and their hosts were strongly correlated, both in Rytilampi and in Sagelvvatn (Table 1, Fig. 1). *Schistocephalus* spp. parasites had significantly lower $\delta^{15}N$ values than their *Pungitius pungitius* and *Gasterosteus aculeatus* hosts (Table 1). In contrast, there were no significant differences in $\delta^{13}C$ values between *Schistocephalus* spp. parasites and their *P. pungitius* and *G. aculeatus* hosts (Table 1, Fig. 1).

The results from ANCOVA indicated no significant differences in parasite–host isotopic differences between the 2 parasite–host systems ($\delta^{15}N$: $F_{2,37}$ = 0.84, p = 0.12; $\delta^{13}C$: $F_{2,37}$ = 0.03, p = 0.97). The mean difference of $\delta^{15}N$ between the parasite and the fish host was −2.13 ± 0.45‰ in Rytilampi and −2.20 ± 0.18‰ in Sagelvvatn, whereas the respective mean differences of $\delta^{13}C$ were −0.18 ± 0.90‰ and −0.45 ± 1.12‰.

The differences between individual parasite and host $\delta^{13}C$ values ranged from −2.23 to 1.51‰ in Rytilampi and from −1.64 to 1.40‰ in Sagelvvatn. In Rytilampi, individual parasites occupying the same host showed a maximum of 2.18‰ difference in $\delta^{13}C$ values (Fig. 2). Contrary to $\delta^{13}C$, the differences between parasites and individual host $\delta^{15}N$ values were much more consistent, ranging from 0.68 to 2.78‰ in Rytilampi and from 1.92 to 2.34‰ in Sagelvvatn. Individual parasites occupying the same host in Rytilampi showed a maximum difference of 0.54‰ in $\delta^{15}N$ values (Fig. 2). In most cases, larger parasites were more enriched in ^{13}C and ^{15}N than their smaller conspecifics (see Fig. A1 in the Appendix).

The parasitized nine-spined sticklebacks from Rytilampi were significantly enriched in ^{15}N (by an average of 0.33‰) but not in ^{13}C as compared to unparasitized conspecifics of the same size ($\delta^{15}N$: $t_{35.98}$ = −2.17, p = 0.04; $\delta^{13}C$: $t_{39.68}$ = −1.09, p = 0.28). The parasitized nine-spined sticklebacks had also significantly higher C:N ratios than the unparasitized individuals ($t_{40.56}$ = −2.36, p = 0.02).

DISCUSSION

Both *Schistocephalus* spp. species had consistently lower $\delta^{15}N$ values than their stickleback hosts as also observed in some other endoparasite–fish host systems (e.g. Pinnegar et al. 2001, Deudero et al. 2002, Power & Klein 2004). The host and parasite isotope values were strongly correlated, although parasites occupying the same individual host showed marked isotopic variation, particularly in $\delta^{13}C$. As hypothesized, the differences between parasite and host for $\delta^{15}N$ (by ca. −2.1‰) and $\delta^{13}C$ (by ca. 0.2‰) were similar in both study locations, indicating similar isotopic routing in parasite–host systems consisting of ecolog-

Table 1. Mean ± SD and range of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes analysed from stickleback hosts and their *Schistocephalus* spp. parasites. Results from Pearson correlation (r) and paired *t*-test comparisons of hosts and their parasites are also reported (statistically significant differences shown in **bold**)

Host	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Parasite	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Pearson $\delta^{15}N$	Pearson $\delta^{13}C$	*t*-test $\delta^{15}N$	*t*-test $\delta^{13}C$
Pungitius pungitius	20	6.0 ± 0.5 (4.9 to 6.9)	−31.7 ± 1.2 (−33.9 to −29.2)	*Schistocephalus pungitii*	34	4.0 ± 0.7 (2.2 to 5.1)	−31.4 ± 1.3 (−34.3 to −28.5)	r = 0.74 **p < 0.001**	r = 0.72 **p < 0.001**	*t* = −27.33 **p < 0.001**	*t* = −1.13 p = 0.27
Gasterosteus aculeatus	6	11..5 ± 1.2 (9.5 to −12.6)	−26.5 ± 2.3 (−29.1 to −23.8)	*Schistocephalus solidus*	6	9.3 ± 1.3 (7.1 to 10.7)	−26.9 ± 2.6 (−30.4 to −24.1)	r = 0.99 **p < 0.001**	r = 0.90 **p < 0.001**	*t* = −30.52 **p < 0.001**	*t* = −0.97 p = 0.38

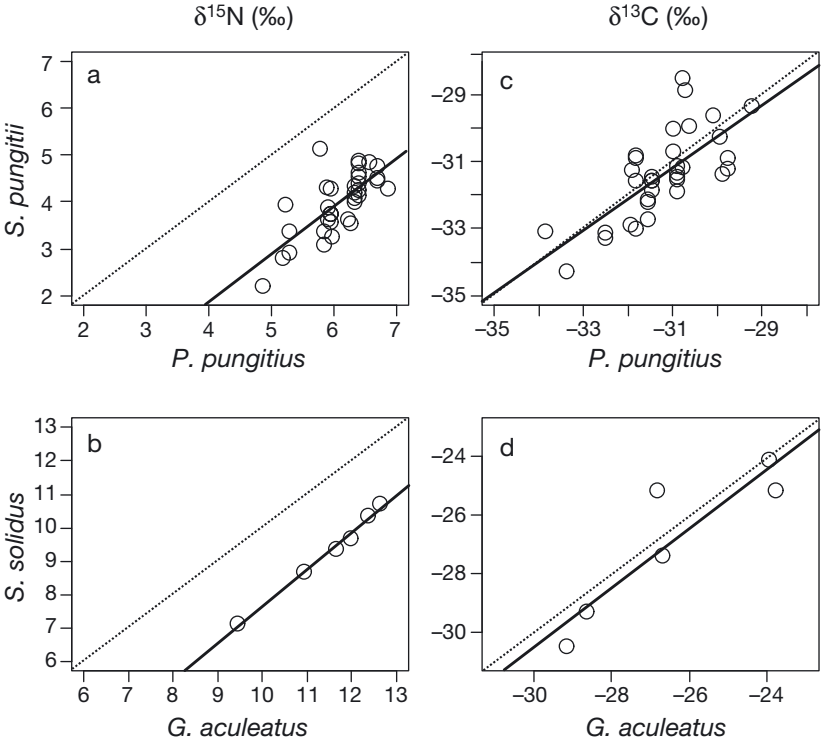

Fig. 1. Relationships between (a, b) nitrogen and (c, d) carbon isotopes in *Schistocephalus pungitii* and *S. solidus* parasites and their respective nine-spined stickleback *Pungitius pungitius* and three-spined stickleback *Gasterosteus aculeatus* hosts. Dashed lines indicate 1:1 diagonals between host (*x*-axis) and parasite (*y*-axis) isotope values

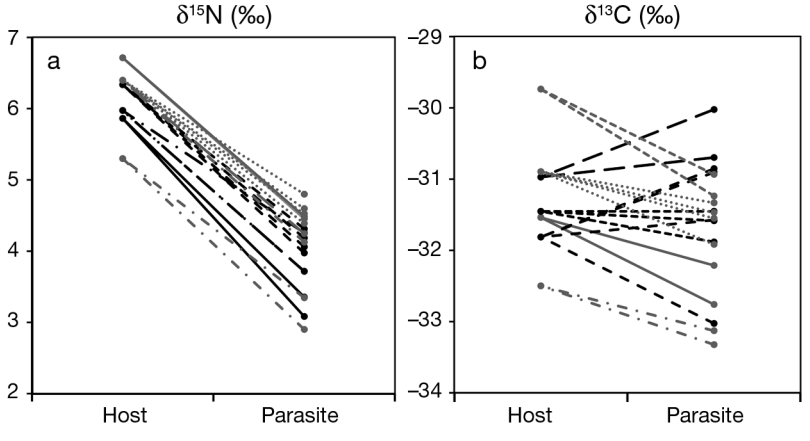

Fig. 2. Relationships between (a) nitrogen and (b) carbon isotopes from individual nine-spined stickleback *Pungitius pungitius* hosts and their respective *Schistocephalus pungitii* parasites in Rytilampi pond, northeastern Finland. Only nine-spined sticklebacks (n = 7) with multiple parasites (n = 2–5) are shown to illustrate isotopic variation among parasites occupying the same individual host

ically similar species living in markedly different ecosystems. As hypothesized, the parasitized nine-spined sticklebacks from Rytilampi were enriched in ^{15}N and had higher C:N ratios as compared to unparasitized conspecifics, indicating nutrient stress and catabolism within the host muscle tissue (Reitsema 2013, Bowes et al. 2014).

Stable isotope analyses have been widely used in studies of food webs (Boecklen et al. 2011, Layman et al. 2012, and references therein) and trophic interactions between hosts and their parasites (e.g. Gómez-Díaz & Gonzáles-Solís 2010, Sánchez et al. 2013, Navarro et al. 2014). Based on stable isotope data, Doucett et al. (1999) proposed 3 criteria to judge whether an organism is truly parasitic. Firstly, the parasite should be more enriched in ^{15}N and ^{13}C than its host. Secondly, isotopic differences between the host and the parasite should fall within the expected values for diet–consumer fractionation measured in laboratory studies. Thirdly, the isotope ratios should be correlated across individual hosts and their parasites. Several studies have now tested these predictions in fish parasite–host systems, and found that these expectations are frequently not met (e.g. Pinnegar et al. 2001, Deudero et al. 2002, Power & Klein 2004, Navarro et al. 2014). Our findings are in agreement with previous stable isotope studies showing that *S. solidus* parasites are consistently depleted in ^{15}N as compared to their stickleback hosts (Pinnegar et al. 2001, Power & Klein 2004). This phenomenon has also been observed in roach *Rutilus rutilus* hosts and their *Ligula intestinalis* parasites, which have a similar life-history strategy and life cycle as the 2 *Schistocephalus* species studied here. It has also been observed in many other parasite–host relationships, including both teleost and elasmobranch fish as well as invertebrate hosts, and cestode, nematode and trematode parasites (Iken et al. 2001, Persson et al. 2007, Dubois et al. 2009, Navarro et al. 2014). This suggests that depleted $\delta^{15}N$ values of parasites, particularly of endoparasites, may represent a general pattern in many parasite–host relationships.

Several factors may contribute to the observed ^{15}N-depletion between endoparasites and their hosts: (1) the parasites may utilize ^{15}N-depleted nitrogen (e.g. ammonia) that they have excreted themselves (Barrett 1981, Olive et al. 2003); (2) they may take up ^{15}N-depleted amino acids or ammonia from the host (Barrett 1981, Hare et al. 1991); and/or (3) they may be unable to synthesize amino acids and show a low rate of excretion, tegument diffusion and respiration (Dubois et al. 2009). In general, the critical difference in the metabolic processes between endoparasites and typical consumers, i.e. that the parasites often are relying on anaerobic and simplified metabolic systems to increase the efficiency of energy utilization (Barrett 1981), is probably the main factor explaining why commonly observed isotopic fractionation patterns do not directly apply to parasite–host relationships (Power & Klein 2004). In fact, similar isotopic fractionation patterns have also been observed in non-parasitic, fluid-feeding insects, which are ^{15}N-depleted as compared to their diet plants (McCutchan et al. 2003).

Bowes et al. (2014) demonstrated from experimental and field observations that starved guppies *Poecilia reticulata* had significantly higher $\delta^{15}N$ values than guppies that were satiated. Parasitized hosts may starve due to the presence and energy drainage of the parasite, a phenomenon that is particularly well-known from *Schistocephalus* spp. and their stickleback hosts (Milinski 1990, Barber et al. 2008, Heins & Baker 2014). The starved host may accordingly become enriched in ^{15}N relative to its parasites, which may explain the higher $\delta^{15}N$ values in the stickleback hosts observed here. Our results show that parasitized nine-spined sticklebacks indeed have higher $\delta^{15}N$ values and C:N ratios as compared to unparasitized conspecifics, likely resulting from the parasites' energy and nutrient stealing, leading to host starvation, catabolism and thus ^{15}N-enrichment (Reitsema 2013, Bowes et al. 2014). However, further studies are needed to confirm whether the higher $\delta^{15}N$ values of parasitized nine-spined sticklebacks is due to the parasite infection per se or due to e.g. specialized foraging on carnivorous copepod zooplankton, which in Rytilampi had much higher $\delta^{15}N$ values than most zoobenthos taxa (mean ± SD $\delta^{15}N$: 3.10 ± 0.23 versus 1.10 ± 1.54; A. Eloranta & J. Merilä unpubl. data).

As hypothesized, our results demonstrate that, despite the marked difference in lake size, climate and community structure in the studied ecosystems, the differences between parasite and host for $\delta^{15}N$ and $\delta^{13}C$ are similar in the 2 similar parasite–host systems. This observation is probably due to the ecological similarity of the three- and nine-spined stickleback hosts (Wootton 1976), but possibly also due to the specialized feeding and metabolism of *Schistocephalus* spp. endoparasites. Although previous stable isotope studies on three-spined sticklebacks and *S. solidus* parasites largely correspond to the present results, the reported mean differences between parasite and host $\delta^{15}N$ values have been variable, ranging from ca. −2.4‰ (Pinnegar et al. 2001) to ca. −1.4‰ (Power & Klein 2004). Whether this is due to differ-

ences in, for example, sampling period (e.g. pre- or post-spawning) or physiological status of the host or the parasite is unknown, and thus represents an avenue for future research. Nevertheless, the mean differences in $\delta^{13}C$ reported for three-spined sticklebacks and *S. solidus* parasites have been more consistent, ranging from ca. −0.45‰ (this study) to 0.14‰ (Pinnegar et al. 2001, Power & Klein 2004).

The consistently higher $\delta^{15}N$ and $\delta^{13}C$ values of the three-spined sticklebacks and their parasites in Sagelvvatn compared to the nine-spined sticklebacks and their parasites in Rytilampi can be due to differences in the fish diets and/or in isotopic baselines between the study locations (e.g. France 1995, Cabana & Rasmussen 1996). Based on unpublished and published data (Eloranta et al. 2013), sticklebacks in both study locations mainly feed on littoral benthic macroinvertebrates and thus isotopic differences at the bottom of the food webs (i.e. in $\delta^{15}N$ and $\delta^{13}C$ values of the pelagic and littoral primary producers and consumers) is a more likely explanation. Unlike Rytilampi with a pristine catchment area, Sagelvvatn is surrounded by small patches of farmland and thus the lake is subjected to higher nutrient loading, which may have led to ^{15}N-enrichment of the entire food web (Cabana & Rasmussen 1996). In contrast, the small and shallow Rytilampi is surrounded by bog and forest areas and thus subjected to higher load of terrestrial carbon (i.e. allochthonous dissolved organic carbon), which may explain the generally lower $\delta^{13}C$ values of the organisms. In any case, our study focuses on isotopic fractionation within the systems and not on differences in $\delta^{15}N$ and $\delta^{13}C$ values of fish and their parasites between the study systems, making it unnecessary to consider any differences in isotopic end members and baselines between the 2 study locations (Layman et al. 2012).

There were marked individual differences in the $\delta^{13}C$ values among *S. pungitii* parasites occupying the same nine-spined stickleback host. For example, some *S. pungitii* individuals were depleted and some were enriched in ^{13}C relative to the host. Larger parasites were generally more enriched in ^{13}C and ^{15}N than their smaller conspecifics inhabiting the same fish host (Fig. A1). This may imply selective use of different carbon sources, or different metabolic or feeding rates of individuals of different sizes or occupying different locations in the host body cavity. Parasites having direct access to the host muscle tissue are probably more efficient in their energy utilization than those that are blocked by other, possibly larger and older individuals. Such individual differences in supply:demand ratios for carbon (energy) may further explain some of the individual differences in $\delta^{13}C$ observed among parasites infecting the same host (cf. Fry 2006). Additionally, sticklebacks — including the Sagelvvatn three-spined sticklebacks (Kuhn et al. 2015) — are also infected by a range of other parasite species in variable densities, which may also explain variation in isotopic fractionation among the individual hosts. Furthermore, some of the observed minor (<0.2‰) individual differences may, of course, simply arise from analytical measurement error. Although we cannot currently differentiate in between these alternative explanations, the differences between parasite and host for both $\delta^{13}C$ and $\delta^{15}N$ showed negative, albeit statistically non-significant, correlations ($\delta^{13}C$: $r = -0.49$, $p = 0.06$; $\delta^{15}N$: $r = -0.45$, $p = 0.10$), with increasing size of parasites (measured as parasite:host mass ratio) infecting the same host. In other words, large parasites seemed to isotopically resemble their host more than small parasites, possibly due to a more neutral nitrogen balance of large, non-growing parasites (Martínez del Rio et al. 2009).

In conclusion, the present study gives further support that endoparasites can be consistently depleted in ^{15}N as compared to their host. Despite living in markedly different ecosystems, the 2 studied parasite–host pairs (i.e. *S. pungitii*–*P. pungitius* and *S. solidus*–*G. aculeatus*) revealed similar isotope routing for both $\delta^{15}N$ and $\delta^{13}C$. The results confirm that individual parasites infecting the same host can show marked differences in $\delta^{13}C$, possibly due to specialized feeding on different carbon sources or due to differential age, size and physiological status of the parasites. Moreover, our results demonstrate that parasites can cause nutrient stress and thereby lead to ^{15}N-enrichment of the host tissues, as observed among starving and sick humans (Reitsema 2013). Future studies applying e.g. compound specific stable isotope methods and/or fatty acid analyses (see Boecklen et al. 2011 and references therein) could increase our understanding of the trophic relationships between different host and parasite taxa, thereby enabling better evaluation of the role of parasites in the structure and functioning of aquatic food webs.

Acknowledgements. We thank Hanna-Kaisa Lakka for help with the field work and Oulanka Research Station (University of Oulu) for logistic support. Jacquelin DeFaveri kindly provided comments on this manuscript. Our research was supported by the Ellen and Artturi Nyyssönen Foundation (personal grant to A.P.E.), the Research Council of Norway (project number NFR 213610/F20 to P.A.A.) and the Acad-

emy of Finland (grant numbers 200940, 108601 and 118673 to J.M.). The nine-spined sticklebacks were collected with permission from Metsähallitus (permission number 3087/41/2011).

LITERATURE CITED

Amundsen PA, Lafferty KD, Knudsen R, Primiciero R, Klemetsen A, Kuris AM (2009) Food web topology and parasites in the pelagic zone of a subarctic lake. J Anim Ecol 78:563–572

Barber I (2007) Host-parasite interactions of the three-spined stickleback. In: Östlund-Nilsson S, Mayer I, Huntongford FA (eds) Biology of the three-spined stickleback. CRC Press, Boca Raton, FL, p 271–318

Barber I, Wright HA, Arnott SA, Wootton RJ (2008) Growth and energetics in the stickleback–*Schistocephalus* host–parasite system: a review of experimental infection studies. Behaviour 145:647–668

Barrett J (1981) Biochemistry of parasitic helminths. MacMillan, London

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Syst 42:411–440

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161:1–28

Bowes RE, Lafferty MH, Thorp JH (2014) Less means more: nutrient stress leads to higher $\delta^{15}N$ ratios in fish. Freshw Biol 59:1926–1931

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci USA 93:10844–10847

Deudero S, Pinnegar JK, Polunin NVC (2002) Insights into fish host–parasite trophic relationships revealed by stable isotope analysis. Dis Aquat Org 52:77–86

Doucett RR, Giberson DJ, Power G (1999) Parasitic association of *Nanocladius* (Diptera: Chironomidae) and *Pteronarcys biloba* (Plecoptera: Pteronarcyidae): insights from stable-isotope analysis. J N Am Benthol Soc 18: 514–523

Dubois SY, Savoye N, Sauriau PG, Billy I, Martinez P, de Montaudouin X (2009) Digenean trematodes–marine mollusc relationships: a stable isotope study. Dis Aquat Org 84:65–77

Eloranta AP, Knudsen R, Amundsen PA (2013) Niche segregation of coexisting Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) constrains food web coupling in subarctic lakes. Freshw Biol 58:207–221

Fox J, Weisberg S (2011) An R companion to applied regression. Sage Publications, Thousand Oaks, CA

France RL (1995) Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Can J Fish Aquat Sci 52:651–656

Fry B (2006) Stable isotope ecology. Springer Science+Business Media, New York, NY

Gómez-Díaz E, Gonzáles-Solís J (2010) Trophic structure in a seabird host-parasite food web: insights from stable isotope analyses. PLoS ONE 5:e10454

Hare PE, Fogel ML, Stafford TW Jr, Mitchell AD, Hoering TC (1991) The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. J Archaeol Sci 18:277–292

Heins DC, Baker JA (2014) Fecundity compensation and fecundity reduction among populations of the three-spined stickleback infected by *Schistocephalus solidus* in Alaska. Parasitology 141:1088–1096

Herczeg G, Gonda A, Merilä J (2009) Evolution of gigantism in nine-spined sticklebacks. Evolution 63:3190–3200

Iken K, Brey T, Wand U, Voigt J, Junghans P (2001) Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog Oceanogr 50:383–405

Kuhn JA, Jakobsen J, Kristoffersen R, Marcogliese MJ and others (2015) Parasite communities of two three-spined stickleback populations in subarctic Norway — effects of a small spatial-scale host introduction. Parasitol Res 114: 1327–1339

Lafferty KD, Allesina S, Arim M, Briggs CJ and others (2008) Parasites in food webs: the ultimate missing links. Ecol Lett 11:533–546

Layman CA, Araújo MS, Boucek R, Hammerschlag-Peyer CM and others (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev Camb Philos Soc 87:545–562

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev Camb Philos Soc 84:91–111

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390

Merilä J, Lakka HK, Eloranta A (2013) Large differences in catch per unit of effort between two minnow trap models. BMC Res Notes 6:151

Milinski M (1990) Parasites and host decision-making. In: Barnard CJ, Behnke JM (eds) Parasitism and host behavior. Taylor & Francis, London, p 95–116

Navarro J, Albo-Puigserver M, Coll M, Saez R, Forero MG, Kutcha R (2014) Isotopic discrimination of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in a host-specific holocephalan tapeworm. J Helminthol 88:371–375

Olive PJ, Pinnegar JK, Polunin NV, Richards G, Welch R (2003) Isotope trophic-step fractionation: a dynamic equilibrium model. J Anim Ecol 72:608–617

Persson ME, Larsson P, Stenroth P (2007) Fractionation of $\delta^{15}N$ and $\delta^{13}C$ for Atlantic salmon and its intestinal cestode *Eubothrium crassum*. J Fish Biol 71:441–452

Pinnegar JK, Cambell N, Polunin NVC (2001) Unusual stable isotope fractionation patterns observed for fish host-parasite trophic relationships. J Fish Biol 59: 494–503

Plimmer RHA (1921) Analysis and energy values of foods. HM Stationery Office, London

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718

Power M, Klein GM (2004) Fish host–cestode parasite stable isotope enrichment patterns in marine, estuarine and freshwater fishes from northern Canada. Isotopes Environ Health Stud 40:257–266

R Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.R-project.org/

Reitsema LJ (2013) Beyond diet reconstruction: stable isotope applications to human physiology, health, and nutrition. Am J Hum Biol 25:445–456

Sánchez MI, Varo N, Matesanz C, Ramo C, Amat JA, Green AJ (2013) Cestodes change the isotopic signature of

brine shrimp, *Artemia*, hosts: implications for aquatic food webs. Int J Parasitol 43:73–80

Thieltges DW, Amundsen PA, Hechinger RF, Johnson PTJ and others (2013) Parasites as prey in aquatic food webs: implications for predator infection and parasite transmission. Oikos 122:1473–1482

Wootton RJ (1976) The biology of the sticklebacks. Academic Press, London

Appendix

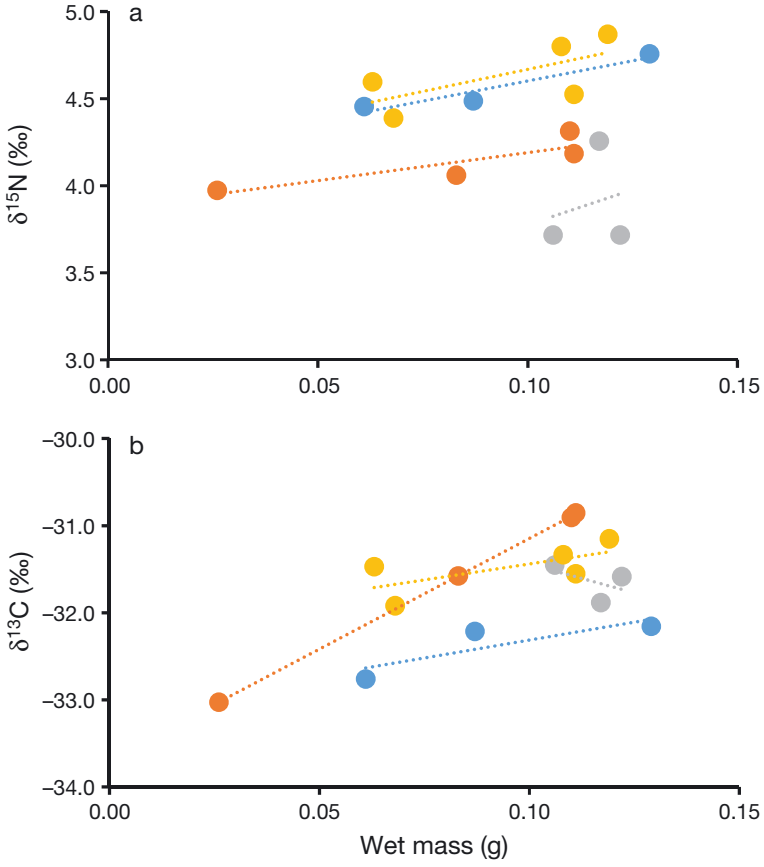

Fig. A1. Relationships between parasite wet mass and stable (a) nitrogen and (b) carbon isotopes from *Schistocephalus pungitii* occupying the same individual host (i.e. nine-spined stickleback *Pungitius pungitius* from Rytilampi pond, northeastern Finland), which are marked with different colours

Editorial responsibility: Sven Klimpel, Frankfurt, Germany

Submitted: October 28, 2014; Accepted: June 12, 2015
Proofs received from author(s): July 14, 2015