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Stoichiometry and parasitism: changes in nutrient concentrations in the three-spined Stickleback-Schistocephalus solidus system

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Stoichiometry and parasitism: changes in nutrient concentrations in the three-spined Stickleback-*Schistocephalus solidus* system

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Abstract:

Ecological stoichiometry is a rapidly growing field of study that assesses the elemental composition of organisms in ecosystems and the impact that changes in these elemental compositions have on populations. Parasitism is widely studied for its role in altering the physiology and behavior of hosts within a population. However, the interaction between parasitism and the stoichiometry of the host-parasite system has not been well-established. To determine the impact of parasitism on stoichiometry of host, we measured the elemental composition (%C, %N, and %P) and ratios (C:N, C:P, and N:P) of a population of parasitized and unparasitized *Gasterosteus aculeatus* (three-spined stickleback) and their parasite, *Schistocephalus solidus*. Infection presence resulted in an overall decrease in some stoichiometric ratios, specifically C:N, with larger infection intensities resulting in a greater decrease of C:N ratios. Body mass was also an important predictor of infection status and stoichiometry of cestode, as larger hosts had a greater chance of infection and contained larger cestodes with lower carbon, nitrogen, and phosphorus concentrations. These results demonstrate a relationship between stoichiometry and parasitism, but whether variation in elemental composition of host is a cause or a result of parasitism has not yet been determined and requires experimental infections.

Key words: Ecological stoichiometry, parasitism, elemental composition, *Gasterosteus aculeatus*

Introduction

Ecological stoichiometry (ES) is the study of relative mass quantities of the chemical elements of different types of matter in organisms, communities, or ecosystems (Bernot & Poulin, 2018). Redfield et al. (1963) discovered the Redfield ratio, which is the consistent ratio of elements found throughout seawater (Hill, 2005). Since this ratio consisted of carbon (C), nitrogen (N), and phosphorus (P), these are the elements most commonly studied ecological stoichiometry as potential limiting nutrients (Bernot & Poulin, 2018). Mass balance is used to estimate elemental ratios and fluxes in organisms and ecosystems (Chodkowski & Bernot, 2017), as organisms need to maintain certain homeostatic levels of these elements for sufficient growth and reproduction. Overall fitness of the organism may be reduced if they fail to maintain homeostasis with a particular ratio of carbon, nitrogen, and phosphorus (Persson et al., 2010). However, this ratio can vary among species, or among populations or genotypes. Fish species show significant variation in nutrient concentrations. In a study of 20 species of freshwater fish (Hendrixson et al., 2007), phosphorus concentrations ranged from <2% to >4% of the individual's total body mass. Nitrogen ranged from 9% to 12%, and carbon ranged from <40% to >50% of total mass. Members of Salmonidae, Cyprinidae and Catostomidae families were found to have lower average phosphorus percentages, while members of Centrarchidae, Esocidae and Ictaluridae families averaged higher phosphorus percentages. Individuals with high phosphorus levels also typically had low N:P and C:P ratios (Hendrixson et al., 2007). The degree of a stoichiometric shift that impairs fitness in fish species is species-dependent, with some species tightly regulating their nutrient concentrations, and others, like *Oncorhynchus mykiss* and *Synechogobius hasta*, only weakly regulating nutrient homeostasis with more variation among individuals (Persson et al., 2010). When a nutrient imbalance leads to impaired

fitness, an organism may conserve the limiting nutrient, excrete excess nonlimiting nutrients, limit their growth, or change their feeding behavior (Bernot & Poulin, 2018).

Parasites exploit their hosts for resources to fuel growth, ensure successful reproduction, and continue their life cycles. Depending on the species pairing, parasites can act as competitors or consumers in a parasite-host relationship. Gut-dwelling parasites, like tapeworms, compete with their host for absorption of nutrients like carbon, nitrogen, and phosphorus and use these resources to reach reproductive maturity. When infection intensity increases, some species of parasite will compete with each other for host resources. This competition puts further strain on the host (Michaud et al., 2006). Trematodes, another species of parasite, act as consumers, as they consume host tissue to meet their nutrient requirements (Bernot & Poulin, 2018).

Parasites might plausibly alter host stoichiometry in several ways. They can change host foraging decisions, shifting the ratio of consumed nutrients. They can preferentially absorb certain elements from the host, altering the ratio of elements remaining. When parasites have different stoichiometric requirements than their hosts, imbalance occurs in the host's homeostasis, which may increase parasite virulence (Bernot & Poulin, 2018). Since maintaining elemental homeostasis requires energy expenditure, parasitic infection that disrupts host homeostasis increases energy expenditure and diverts energy and resources from other important physiological functions of the host (Sanders & Taylor, 2018). Alternatively, parasites can induce physiological changes in the host (growth rate, immune responses) that might alter host stoichiometry.

To understand these alternative stoichiometric effects, we need to answer three key questions. First, do parasites' C:N:P ratios match that of their hosts, or do they have a different stoichiometric ratio? Second, are parasites' stoichiometric ratios correlated with their hosts'

ratios (increasing or decreasing together, even if they are not equal)? Lastly, do parasites alter their hosts' stoichiometric ratios? Some studies have found that shifts in stoichiometry of a host have been met with corresponding shifts in stoichiometry of their respective parasites (Frenken et al., 2017). However, we remain largely uncertain how host and parasite stoichiometry influence each other.

Elemental phenotype of an organism can potentially be indicative of an organisms' growth patterns. Organisms grow using protein synthesis, a process that occurs on subcellular structures called ribosomes. These structures, which are required in higher quantities for growth, are particularly rich in phosphorus, so organisms with higher growth rates tend to have a greater demand for phosphorus and lower C:P and N:P ratios. One study of diverse species, including *Daphnia*, zooplankton, *Drosphila melanogaster*, found a strong correlation between total phosphorus content of organisms and their growth rate, with r^2 values ranging from 0.46 to 0.98 and an average r^2 value of 0.75 (Elser et al., 2003). As a result, growth rate can be used to estimate phosphorus levels, and phosphorus levels can be used to estimate growth rate (Elser et al., 1996). Parasites with complex lifestyles are likely to have stoichiometric ratios that are different from their hosts, as the host and its parasite will have innately different physiological requirements and growth rates (Michaud et al., 2006). As a result, these organisms will have different nutrient requirements and will use and excrete these nutrients at different rates. This leads to a fundamental difference in elemental ratios between host and parasite (Michaud et al., 2006).

Parasites often exhibit tissue specificity within their host organism, and stoichiometry of host tissues can provide an explanation for this specificity, as different cells and tissues within the organism could potentially have different stoichiometric levels (Bernot & Poulin, 2018).

Some parasites can selectively extract certain nutrients from their host and alter host ratios (Sanders & Taylor, 2018). Parasites that are capable of selective exploitation of host will likely not experience changes in stoichiometry that are correlated with their host. This is likely species specific, however, as some studies have demonstrated correlated shifts in host and parasite elemental ratios. A Frenken et al. (2017) study found that increasing N:P ratios in phytoplankton corresponded with increasing N:P ratios in their respective parasites, chytrids ($r = 0.96$, $P < 0.001$). Evidence of a correlation in shifts of elemental concentrations and ratios between a host and its parasite would provide information on parasitic exploitation of the host in a host-parasite relationship.

Parasitic infection can have drastic, diverse, and detrimental impacts on the host, as it has been shown to impact host behavior (Ezenwa, 2004), fitness, mating tendencies (Campbell et al., 2017), and survival (Fredensborg et al., 2005). Parasites can change the physiology of the host, by means of an immune response, which can, in turn, impact nutrient retention and allocation of specific nutrients. Alternatively, parasites with drastically different stoichiometric traits from their host can have different rates of metabolic activity and can, therefore, impact nutrient excretion (Sanders & Taylor, 2018). Varying dietary phosphorus levels have been shown to also impact immunity, with high levels supporting cell-mediated immunity and low levels diminishing the gut microbiome and encouraging infection (Heyer et al., 2015). This suggests that hosts with high C:N and C:P ratios could put the host at a greater risk of infection (Sanders & Taylor, 2018). Conversely, if infection alters host growth rate, phosphorous levels might change accordingly, altering stoichiometric ratios. Behavioral modification by parasites on their hosts can also change their choices in prey and susceptibility to predation, which could, in turn, affect the bioavailability of different nutrients throughout the ecosystem (Cézilly et al., 2010).

Overall, parasites have been shown to impact host stoichiometry in a variety of ways, but knowledge of host-parasite stoichiometry is still limited. The degree to which nutrient ratios of host change upon parasitic infection and the impact that hosts have on their parasite's stoichiometry is still largely unknown.

The Impact of *Schistocephalus solidus* infection in Three-spined Stickleback

The three-spined stickleback (*Gasterosteus aculeatus*), which exists in both freshwater and marine ecosystems, is a model organism for studying the host-parasite interaction. The species has an expansive geographical distribution, is highly suitable for laboratory experimentation, and has a well-documented and understood evolutionary history (Barber, 2013). *Schistocephalus solidus* is a freshwater cestode and parasite to the three-spined stickleback. *Schistocephalus solidus* begins its life cycle through ingestion by a copepod, which serves as its first host. Infected copepods experience changes in growth, reproduction (Wedekind, 1997), and behavior. These behavioral changes initially decrease copepod activity and susceptibility to predation, as to promote parasite maturation and prevent premature predation (Weinreich et al., 2013). Once *S. solidus* maturation is complete, however, behavioral modulation shifts to promote activity and encourage predation of the copepod by the three-spined stickleback (Hammerschmidt et al., 2009).

The three-spined stickleback, the second host of *S. solidus*, experiences changes in physiology (Barber & Scharsack, 2010), immunity (Scharsack et al., 2004), morphology (Dingemanse et al., 2009) (Ness & Foster, 1999), and behavior (Talarico et al., 2017) as a result of infection. *Schistocephalus solidus* enters the last stage of its life cycle when it is ingested by a bird, its final host. *Schistocephalus solidus* lives within the three-spined stickleback for a period

of about 7 to 16 weeks. While inside the body cavity of its second intermediate host, *S. solidus* goes through two distinct phases that impact both parasite and host. The first phase, the non-infective phase, does produce an immune response in the stickleback, with a fibrotic response occurring within 24 hours of infection (Hund et al., 2020). Major genome-wide reprogramming events within the cestode instigate the shift to the second phase, the infective phase (Hébert et al., 2017). This phase is accompanied by significant changes to the host's morphology and behavior. Three-spined sticklebacks in the second phase of infection become more exploratory, bolder, and less-anxious, leading to an increased risk of predation by the final avian host (Barber et al., 2004).

Schistocephalus Solidus mechanisms for manipulation of host

Behavioral manipulation by the parasite on its host should be mediated by the production and release of manipulation factors that disrupt the central nervous system and key physiological systems of the host. Parasites release molecules into their external environment called a secretome that consist of lipids, nucleic acids, and proteins. These molecules are sometimes released within extracellular vesicles. The secretome of *S. solidus* contains many proteins that are not present in the proteome (Berger et al., 2020). These proteins have neural, immune, and cell communication functions that could modulate stickleback behavior upon infection. The secretome also contained proteins that have GTP binding and GTPase activity. These proteins could have numerous roles from hormone signaling to secretion of virulence factors (Berger et al., 2020). While the secretome of late-infection worms has not been found to have an effect on stickleback behavior, the secretome for early infection worms has resulted in more cautious behavior from allopatric hosts. This indicates that the secretome of *S. solidus* does have the

potential to modulate behavior in its host, but the behavioral changes exhibited by the three-spined stickleback as a result of infection are multifactorial in nature (Berger & Aubin-Horth, 2019). In order to produce the molecules excreted in the secretome of the parasite, the parasite must acquire the necessary nutrients. The acquisition of these nutrients may also have implications on the host. In addition, these behavioral, physical, and immune alterations that the secretome generates may alter the stickleback's elemental concentrations.

The stickleback immune response to *S. solidus* infection is multifaceted, as effective immunity requires a variety of components and pathways (Fuess et al., 2020). When *S. solidus* infects a stickleback's peritoneum, genes responsible for reactive oxygen species (ROS) production are expressed. The ROS-recycling system involves several enzymes (Lohman et al., 2017), and increased production of these enzymes could have unique nutritional requirements that alter host stoichiometry. *Schistocephalus solidus* also induces different immune responses between different populations of stickleback due to divergent evolution of host resistance (Lohman et al., 2017). For example, upon infection, stickleback from Roberts Lake had an increase in granulocyte proportions, while stickleback from nearby Gosling Lake showed no increase in granulocyte production (Weber et al., 2017). This increase in granulocyte production could alter elemental content and ratios of the stickleback from Roberts Lake, as nutritional requirements may be altered in a disease state, and therefore, infection could have a differing impact on the stoichiometry of different populations. The proteome of *S. solidus* could be a mechanism for alteration of host immunity. An enzyme in the proteome of *S. solidus*, SsFAAH-like enzyme, breaks down the signaling molecules of the endocannabinoid class, which are important for immune activation in host. This may decrease host immune response to *S. solidus* infection (Berger et al., 2020).

Research Objectives and Hypotheses

For this study, we collected samples of both infected and uninfected three-spined stickleback from a wild population and determined their overall carbon, nitrogen, and phosphorus content and their respective ratios. We also measured the ratios of the *S. solidus* parasite. The aim of this study is (i) to test whether *S. solidus* elemental concentrations correspond with its host or deviate from it (ii) to evaluate if shifts in elemental ratios of host correspond to shifts in elemental ratios of parasite, and (iii) to determine if parasitic infection by *Schistocephalus solidus* impacts the host elemental concentrations and ratios of the *Gasterosteus aculeatus*, the three-spined stickleback. We also assess the role of mass of both the cestodes and three-spined stickleback on the total nutrient concentrations of the cestode. We predicted that mass of both cestode and host will impact *S. solidus* stoichiometry, as larger hosts and cestodes may indicate a more advanced stage of infection, and a cestode that is more developed may have a larger change in stoichiometry.

Methods:

Stickleback Collection

We collected samples from a population of three-spined stickleback specimens from Merrill Lake on northern Vancouver Island in British Columbia, Canada on October 5, 2020. A total of 95 individuals were collected from the lake using a dipnet and were stored in a freezer prior to experimentation. We chose specimens from this sample collected until we reached a total of 30 infected individuals and 30 uninfected individuals. In total, 70 of the 95 individuals collected

were used in this experiment. Stickleback were sampled with approval from the University of Connecticut Institutional Animal Care and Use Committee (IACUC; protocol A18-008), and with a scientific fish collection permit from the British Columbia Ministry of the Environment (permit NA20-600806).

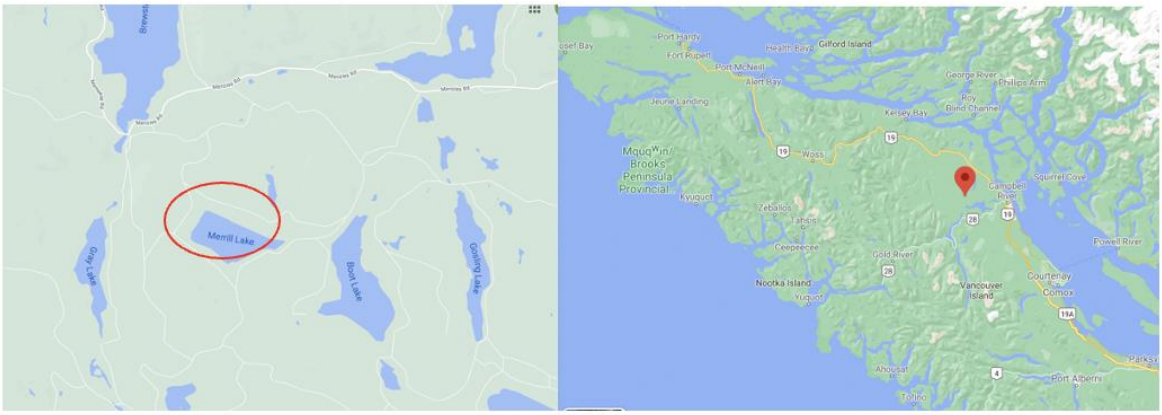

Figure 1: Map images of Merrill Lake: Infected and Uninfected Gasterosteus aculeatus were collected from Merrill Lake in northern Vancouver Island, B.C., Canada. (Google Maps, n.d.)

Dissection, Infection Status, and Ecomorphology

Individuals were thawed, and standard length and mass were measured for each specimen. Mass was measured on a microbalance, and standard length was measured from snout to the distal end of the caudal peduncle. Specimen were subsequently dissected and sexed through visual inspection of gonads. A fibrosis score was determined through fibrosis score criteria found in previous studies (Hund et al., 2020). A score of 0 indicated no fibrosis. A score of 1 means that there is some fibrosis present, and the organs of the specimen do not move freely. Presence of fibrosis that adheres the organs together is a score of 2. A score of 3 indicates that the organs are adhered to both each other and the peritoneal wall. A score of 4 is the most severe form of fibrosis and presents with difficulty opening the peritoneal cavity (Hund et al.,

2020). Video documentation of this ordinal scoring system is available at https://www.youtube.com/watch?v=yKvcRVCSpWI . *S. solidus* presence and *S. solidus* intensity (number of cestodes present) were also determined through dissection. *S. solidus* collected from infected individuals were also measured for mass on a microbalance.

CNP Analysis Sample Preparation

After dissection, each individual stickleback specimen was placed in an aluminum foil packet of predetermined mass. *S. solidus* from infected individuals were placed in separate aluminum foil packets that corresponded to their host. All samples were placed into a drying oven at 60 °C for 7 days. *G. aculeatus* and *S. solidus* specimens were removed from the drying oven after 7 days and placed on a microbalance to assess dry mass of each individual. Each specimen was homogenized into a fine powder with a mortar and pestle and placed into a microcentrifuge tube. The mortar and pestle were cleaned with 95% EtOH and were wiped dry after each specimen was homogenized. Homogenized samples in microcentrifuge tubes were stored in a desiccator until CNP analysis. 1-2 mg of each homogenized sample of *G. aculeatus* and *S. solidus* were placed in encapsulating tins using a spatula and a microbalance. Overall mass of tin packet and samples were recorded, and completed tin packets were placed in a labeled 96-well microtitre plate.

Samples were sent to the University of Georgia Center for Applied Isotope Studies Stable Isotope Ecology Laboratory for total nitrogen and total carbon analysis. Micro-Dumas combustion analysis and an isotope ratio mass spectrometer (IRMS) were used to determine the total percentages of carbon and nitrogen within each sample. Micro-Dumas combustion analysis uses rapid and complete flash combustion to transform solid homogenized samples into a

gaseous phase. Samples are placed into a quartz combustion tube by a rotating multiplace sample dropper. The quartz combustion tube, which contains granulated chromium III oxide combustion catalyst, is kept at a temperature of 1200 °C. After placement in the combustion tube, each sample receives a pulse of pure O_2. The resulting combustion of the sample generates thermal energy and produces a temperature as high as 1700 °C, causing all sample material to enter a gaseous phase. A nonreactive helium carrier gas moves the sample material gas from the furnace.

When the samples are converted into gas, all carbon within the solid sample is converted to gaseous CO_2, and all nitrogen is converted into N_2 and NOx. All nitrogenous combustion products pass through a reduction column, which contains Cu wire heated to 600 °C. Through this process, nitrogen oxides transfer their oxygen onto the copper and become N_2. A gas tap, consisting of magnesium perchlorate, removes water vapor from the gaseous samples. Once water vapor is removed and the samples are purely CO_2 and N_2, the gasses are separated from each other as they are passed through a gas chromatograph column. A detector determines differences in thermal conductivity between the N_2 and CO_2 sample gas pulses as they pass through the device. These differences are recorded as numerically integrated areas and displayed as visible peaks. Through linear regression of combustion of a known standard material, ultra-high purity acetanilide, a regression line is generated. Using the regression line and peak areas from the unknown samples, total elemental values are calculated. Spinach leaves were used for calibration of the Autoanalyzer after every twelfth sample (*SIEL Animal Analysis « Center for Applied Isotope Studies (CAIS)*, n.d.).

The University of Georgia Center for Applied Isotope Studies Stable Isotope Ecology Laboratory also conducted total phosphorus analysis. 0.5 mg of each sample were measured and placed into scintillation vials. 0.2 mL of 0.17M Magnesium Sulfate ($MgSO_4$) was added to each

sample. Samples were placed into a drying oven set at 95 °C for 2.5 hours to evaporate, and the lids to the scintillation vials were removed for this process. Once removed from the drying oven, lids were loosely replaced, and samples were placed into a muffle oven set at 500 °C and baked 2 hours. Samples were removed from the muffle oven and cooled at room temperature. Once cooled, 3 mL of 0.75M Hydrochloric Acid (HCL) was added to the samples to begin hydrolysis. The lids to the samples were screwed on tightly, and the samples were placed back into the oven at 80 °C for 20 minutes. After 20 minutes, 7 mL of distilled water were added to the samples, and they were kept in the oven for an additional 10 minutes. Samples were cooled again at room temperature and transferred into test tubes. 1 mL of standard mixed reagent was added to each test tube of sample material. 10 minutes after adding the reagent, absorbance was measured at 885 nm in a 1 cm cuvette, and percentage of total phosphorus was determined from absorbance (Solórzano & Sharp, 1980).

Statistical Analysis

Statistical analysis was performed in R studio ver. 1.4.1106 (R Core Team, 2020). From the total percentages of carbon, nitrogen, and phosphorus, moles of carbon, nitrogen, and phosphorus were generated. To calculate the moles of each element, the following equation was used: $moles\ of\ element = total\ percentage\ of\ element \times \frac{(atomic\ mass\ of\ element)^{-1}}{100}$. These values were used to calculate molar ratios. To determine whether stickleback and *S. solidus* differ in elemental ratios, we used t-tests to compare the species' means for C:N, C:P, or N:P (in separate tests). A significant difference would imply that stickleback have different elemental ratios than their respective parasites. We also performed correlation tests to determine if changes in elemental ratios of host correspond to shifts in elemental ratios of parasite. A significant

correlation (at $\alpha = 0.05$) would indicate that changes in host stoichiometry coincide with changes in their respective parasite's stoichiometry. To test whether cestode infection alters host stoichiometry, we estimated a linear model in which host C:N (or, C:P or N:P) depends on host mass and the presence or absence of the cestode. A significant infection effect (at alpha = 0.05) would imply that infection presence induces significant changes in stoichiometry of the host. We tested the impact of infection intensity on stoichiometry of host by estimating a linear model in which host C:N (or, C:P or N:P) depends on host mass and the quantity of cestodes. A significant change in host stoichiometry upon greater infection intensities (at alpha = 0.05) would indicate that infection intensity amplifies the changes that infection presence makes on stoichiometry of host. To test the role of cestode mass on stoichiometry of cestode, a linear model was estimated in which cestode %C, %N, or %P depends on cestode mass. A significant mass effect (at alpha = 0.05) would indicate that cestode nutrient concentrations changes as size of cestode changes. We also tested the impact of host mass on cestode nutrient concentrations by estimating a linear model in which cestode %C, %N, or %P depends on host mass. A significant mass effect (at alpha = 0.05) would indicate that increasing host mass across a sample impacts stoichiometry of their respective parasite. The data collected on host sex and fibrosis score was inconclusive, as gonads were underdeveloped (the fish were subadults) and fibrosis was not present in our sample, so these measures were not used as covariates in our sample.

Results

Infection Status

Of the 70 stickleback samples used in this experiment, 30 individuals were infected with *S. solidus* and 40 of the individuals were uninfected. Note that this is not indicative of the infection prevalence in the population as a whole, because we retained all infected individuals (to achieve our target minimum sample size of 30), but randomly excluded some uninfected individuals (which were more abundant). The mean wet mass of the *G. aculeatus* used in this experiment is 678.8 mg, with a mean standard length of 38.1 mm. The mean wet mass of the *S. solidus* found within the infected specimens was 200.7 mg, with a mean intensity of 1.9 cestodes per infected stickleback.

Does *G. aculeatus* and *S. solidus* have different elemental ratios, and are they correlated?

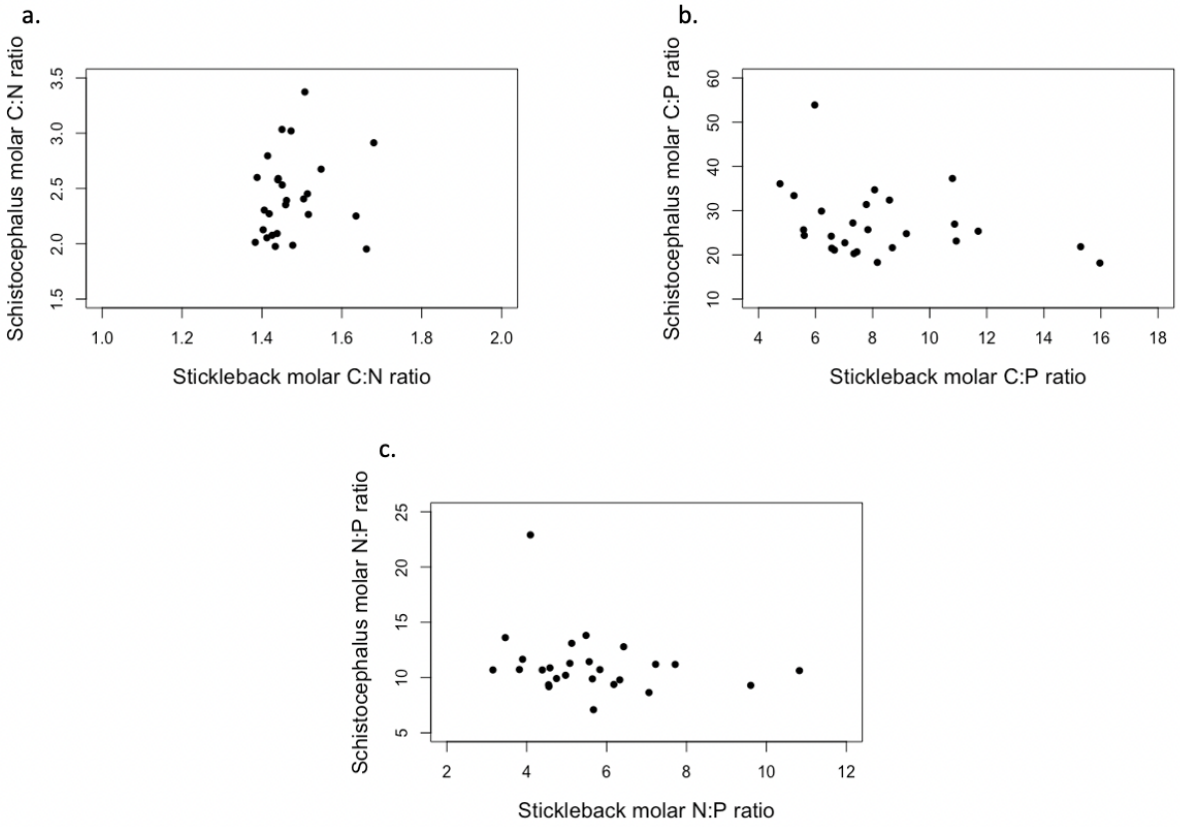

Figure 2: Stoichiometric Ratios of S. solidus vs. G. aculeatus. (a) Carbon-Nitrogen ratios of total stickleback and total S. solidus (b) Carbon-Phosphorus ratios of total stickleback and total S. solidus (c) Nitrogen-Phosphorus ratios of total stickleback and total S. solidus

Stoichiometric ratios differed between *S. solidus* and their stickleback host, both on average and among individuals. In general, sampled cestodes had a higher C:N ratio than the stickleback (mean C:N ratio of 2.426 and 1.492 respectively, t-value = 12.6, $p = 1.3 \times 10^{-12}$). Cestodes had a higher mean C:P ratio than the stickleback (mean C:P ratio for cestodes = 27.02; mean C:P ratio for stickleback = 8.398, t-value = 12.0, $p = 1.6 \times 10^{-12}$). Mean N:P ratio of the cestodes was also higher than mean N:P ratio of the stickleback (mean N:P ratio for cestodes = 11.156; mean N:P ratio for stickleback = 5.626, t-value = 9.1, $p = 1.0 \times 10^{-12}$).

There was no significant correlation between nutrient ratios of the *S. solidus* and the *G. aculeatus* in our sample population. Stickleback with higher Carbon-Nitrogen (C:N) ratios were not necessarily infected with cestodes that had high C:N ratios ($r = 0.14$, $p = 0.48$; figure 2a.). Stickleback with high Carbon-Phosphorus (C:P) levels also did not have cestodes with higher C:P ratios ($r = -0.31$, $p = 0.13$, figure 2b.). Nitrogen-Phosphorus levels followed the same trend with no correlation between *G. aculeatus* N:P ratios and *S. solidus* N:P ratios ($r = -0.25$, $p = 0.22$; figure 2c.).

Does infection presence impact *G. aculeatus* stoichiometry?

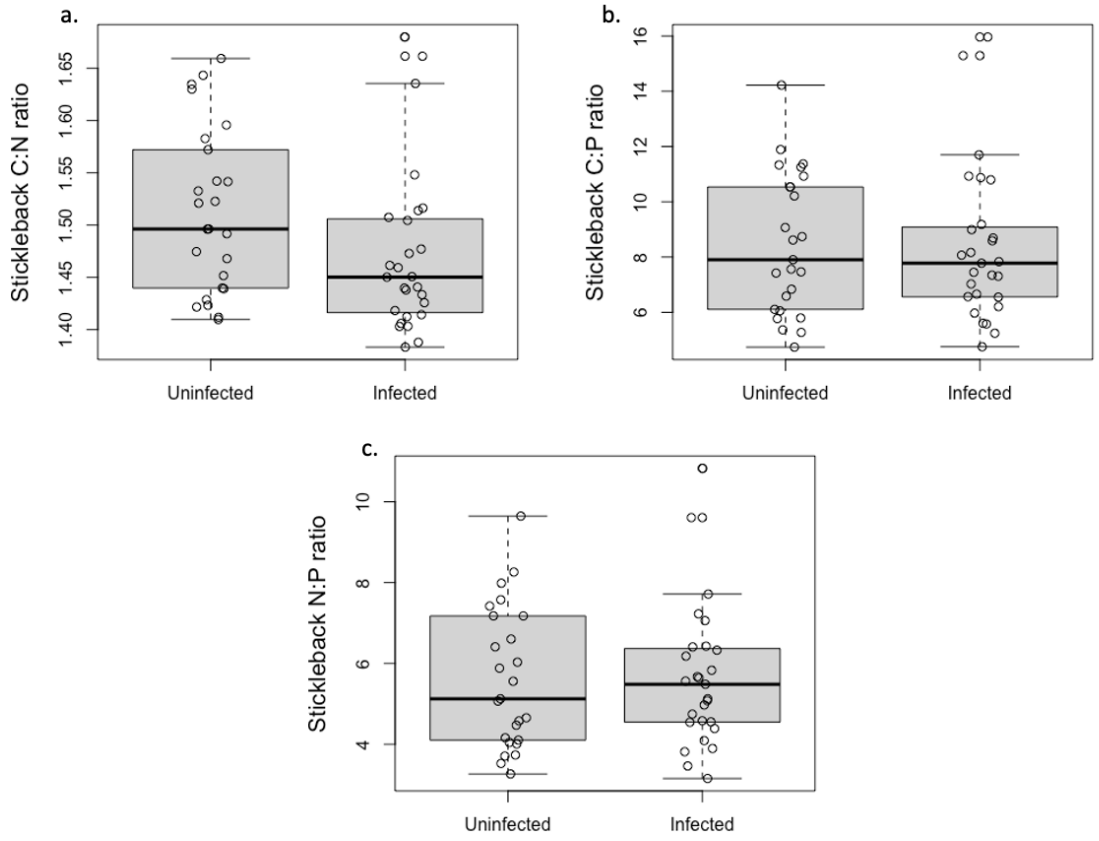

Figure 3: Nutrient Ratio changes as a result of S. solidus infection. (a) C:N ratios of infected and uninfected stickleback (b) C:P ratios of infected and uninfected stickleback (c) N:P ratios of infected and uninfected stickleback

We compared the stoichiometric ratios of the infected and uninfected stickleback within our sample population. The C:N ratios of the stickleback significantly decreased in the presence of *S. solidus* infection (slope = – 0.055, p = 0.02), when accounting for (non-significant) effect of the wet mass of the stickleback samples (p = 0.16; figure 3a). C:P ratios were not significantly impacted by infection presence (p = 0.59), when accounting for wet mass of the stickleback samples (p = 0.363; figure 3b). Similarly, N:P ratios were not significantly different between infected and uninfected samples (p = 0.81; figure 3c), when accounting for wet mass of the stickleback samples (p = 0.48).

How does infection intensity (quantity of *S. solidus*) impact host stoichiometry?

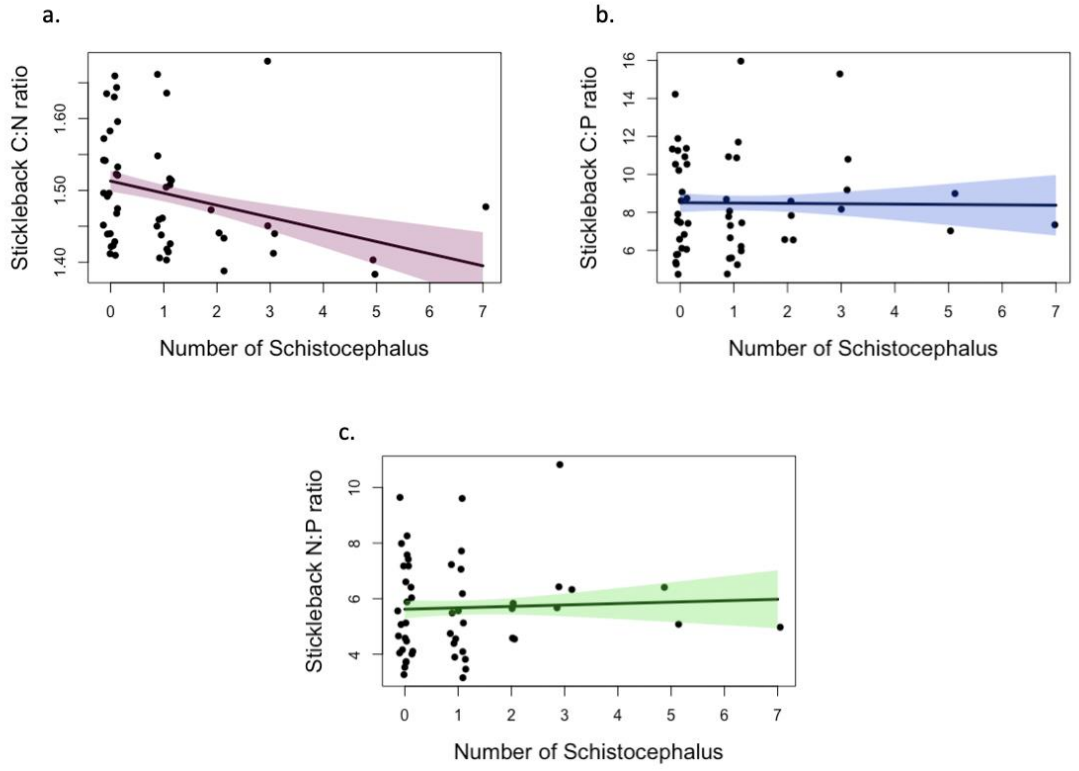

Figure 4: Stoichiometric Ratios of Stickleback of Varying Infection Loads. (a) C:N ratios of stickleback at different S. solidus infection intensities (b) C:P ratios of stickleback at different S. solidus infection intensities (c) N:P ratios of stickleback at different S. solidus infection intensities

We assessed the impact of infection intensity on stoichiometric ratios of the stickleback using three linear models (figure 4a, b, and c), while accounting for wet mass of the stickleback. The results of these linear models followed a similar pattern to the results of infection presence. Stickleback with higher infection intensities had lower C:N ratios (slope = – 0.17, p = 0.035; figure 4a), and mass values were not significant (p = 0.28). C:P and N:P ratios were not impacted by infection intensity, with nutrient ratio p-values of 0.94 and 0.77 and wet mass p-values of 0.46 and 0.58, respectively (figure 4b and c).

Do the masses of the cestode or the host impact cestode stoichiometry?

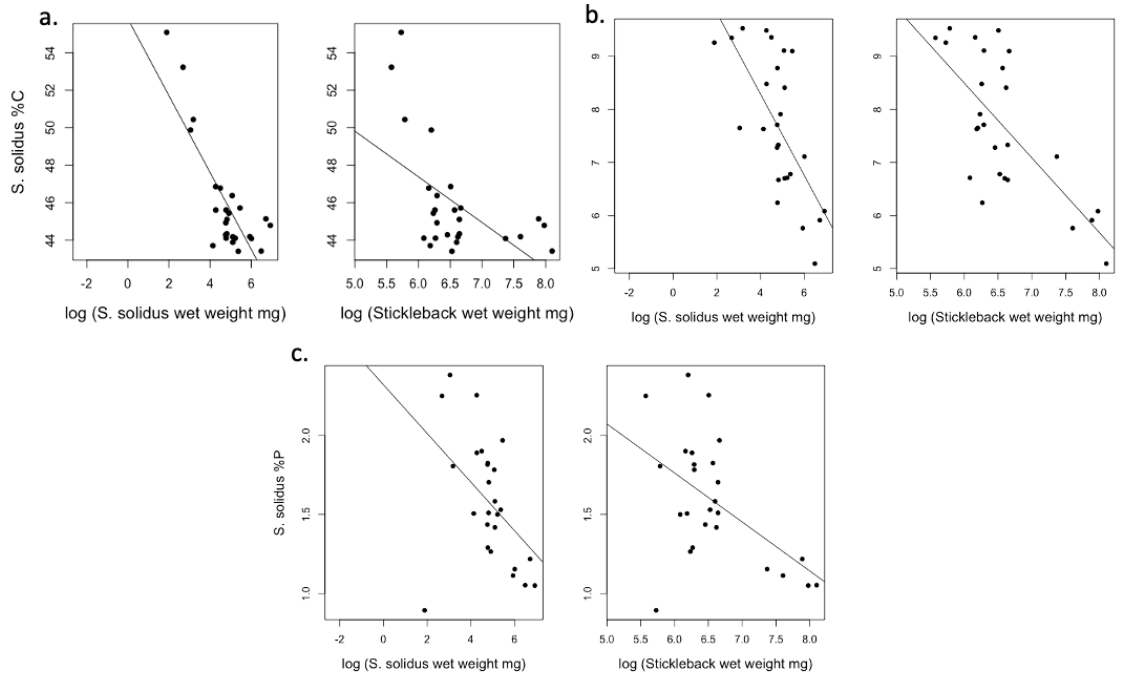

Figure 5: Impact of wet mass of S. solidus and G. aculeatus on total nutrient percentages of S. solidus (a) Total Carbon percentages of S. solidus of varying wet mass values of stickleback and cestode with regression line (b) Total Nitrogen percentages of S. solidus of varying wet mass values of stickleback and cestode with regression line (c) Total Phosphorus percentages of S. solidus of varying wet mass values of stickleback and cestode with regression line

Total carbon percentages of mass exhibited the largest decrease in cestodes of larger mass values. As cestode mass increased, total carbon percentages of the cestode decreased (slope = – 2.05, $p = 5.33 \times 10^{-7}$; figure 5a). Total nitrogen values decreased as cestode mass increased, as well (slope = – 0.77, $p = 1.23 \times 10^{-4}$; figure 5b). Total phosphorus percentages also decreased as mass increased (slope = – 0.15, p = 0.019; figure 5c). Note that carbon, nitrogen, and phosphorus percentages of total mass did not add up to 100% in our data.

The mass of the *S. solidus* host, *G. aculeatus*, also impacted the total nutrient values of the *S. solidus*. Total carbon values of the *S. solidus* decreased as its host size increased (slope = – 2.44, p = 0.004; figure 5a). *S. solidus* of larger hosts also had lower total nitrogen values (slope = – 1.41, $p = 3.93 \times 10^{-5}$; figure 5b). Host size also impacted phosphorus levels of *S. solidus*, with larger hosts containing less phosphorus than smaller hosts (slope = – 0.31, p = 0.0059; figure 5c). Hosts of larger mass values were also found to have cestodes with larger mass values (slope = 0.24, $p = 2.19 \times 10^{-4}$). Hosts with larger masses were also found to have a greater chance of infection (slope = 0.39, p = 0.006).

Discussion:

Parasites have the potential to shift host ecological stoichiometry, which might have substantial effects on host fitness and ultimately on their ecosystem function. This is especially likely if parasites exhibit different C:N:P ratios than their hosts. However, few studies have tested for stoichiometric differences between, or correlations among, host and parasites, or how infection changes host stoichiometry. In this study, we provide evidence that parasitic infection is related to host stoichiometry in the *G. aculeatus* – *S. solidus* system. While *S. solidus* had a significantly different stoichiometric composition than its host, and parasites with complex life

cycles and different stoichiometric ratios than their host often exploit their hosts for resources (Michaud et al., 2006), evidence of this relation between parasitic infection and changes in host stoichiometry is novel. Stoichiometry of both host and parasite varied as a factor of host mass, cestode mass, cestode intensity, and infection presence. The changes in stoichiometry of both host and parasite could provide insight about the specifics of this host-parasite interaction and could explain the impact of parasitism on ecological systems, overall.

Nutrient Ratios of *G. aculeatus* and *S. solidus*

While C:N, C:P, and N:P ratios of *S. solidus* did differ from those of their host, shifts in these ratios did not correlate with shifts in the ratios of *G. aculeatus*. Essentially, hosts with higher concentration of an element, for example carbon, do not necessarily have parasites with higher concentrations of that element. This suggests that *S. solidus* selectively exploits its host for nutrients, rather than tracking availability within the host. Conversely, it means that parasites, which absorb more carbon (for instance), do not lead to corresponding carbon-depleted hosts.

Elemental Variation in Parasitized and Unparasitized *G. aculeatus*

C:N ratios were impacted by infection presence in our stickleback population, with infected individuals having lower C:N ratios than uninfected. This could be the result of a host's coping strategy to infection. Hosts could be consuming more of their excess protein to compensate for the behavioral, morphological, and physiological changes implemented onto the host by the parasite. Alternatively, *S. solidus* could be directly outcompeting *G. aculeatus* for its resources, specifically carbon. This nutrient deficit within the host could impact host immunity and the degree to which the parasite changes its hosts physiology.

Infection intensity also impacted C:N ratios with C:N ratios of the stickleback decreasing as quantity of parasites infecting the stickleback increased. High C:N ratios put a host at a greater risk of infection, but in our sample, host C:N decreased as infection intensity increased. Since *S. solidus* have higher C:N ratios than their host, they are likely depleting their host of carbon for nutritional purposes. As a result, host C:N ratios are likely higher prior to infection, and upon infection, *S. solidus* depletes *G. aculeatus* of its carbon, so infected individuals have lower C:N ratios than their healthy counterparts. These effects increase as intensity increases, because exploitation likely increases, as well. Past research indicates that C:N ratios decrease as parasites reach maturity (Paseka & Grunberg, 2019). C:N ratios of *S. solidus* in our sample decreased as mass of *S. solidus* increased. This could indicate that larger cestodes were closer to reaching their reproductive stage and preparing to be transferred to their final avian host.

Body Size of Host and Parasite and Parasitic Elemental Composition

Mass of both cestode and host increased as total nutrient composition of cestode decreased. Hosts with larger masses had cestodes with larger masses, so this negative correlation could be indicative of resource competition between host and parasite. Since the host is larger, it may be more capable of outcompeting its parasite for carbon, nitrogen, and phosphorus. As a result, *S. solidus* in larger hosts would have lower total nutrient levels, even if the parasites, themselves, are large. Since, carbon, nitrogen, and phosphorus levels of cestodes collectively decreased with increasing mass values, the relative percentages of elements not analyzed in this study may increase within larger individuals to account for the loss of carbon, nitrogen, and phosphorus.

Caveats

While the focus of this paper was to determine if parasitic infection altered host stoichiometry, the variations in stoichiometry among our sample population may be a causation of parasitism rather than an effect of it. Since these stickleback samples were analyzed post-infection, there is no way to determine if their innate stoichiometry prior to infection was different from the uninfected samples. Innate stoichiometry variation could indicate susceptibility to parasitic infection via impaired immune response, physiological stress, or some other mechanism. Age of specimens could also implicate the role of mass on infection status and stoichiometry. Larger hosts may have been older, and this could have led to more opportunities for infection. Similarly, older hosts could have been infected for a longer period of time, which could implicate stoichiometric changes to *S. solidus* as a result of mass of host. In addition, we derived elemental concentrations from the whole organism. Large differences in stoichiometry between the tissues within the stickleback where *S. solidus* lives and the whole organism could implicate our results. Finally, all samples studied within our population had successfully survived up until the time of capture, which indicates a potential for survival bias. Individuals who are more tolerant of infection are more likely to be sampled in wild collections. If stoichiometric ratios covary with tolerance, we might observe differences between surviving infected fish, compared to a typical uninfected fish. Individuals who died as a result of parasitic infection were not examined in this study and could have had different C:N:P ratios than the surviving individuals.

Expanding Knowledge of Ecological Stoichiometry

Controlled infection experiments with live specimen are needed to overcome these implications and determine if stoichiometry is the result of parasitic infection or a leading factor causing it. Since this is a relatively novel area of study, expansion on species-parasite pairing models is necessary to determine if these results are accurate across taxa. This also would allow for effect strength to be determined throughout different biological scenarios. Finally, future research should address the reason why infection changes stoichiometry and how this could impact ecosystems. When an organism deviates from elemental homeostasis, they will likely excrete elements as different ratios from which they ingest them. This leads to changes in stoichiometry of the broader ecosystem, which could, in turn, lead to nutrient imbalances of other organisms within the ecosystem. These imbalances could be carried through trophic levels, and nutrient consumption could be impacted, as well. Mass balance can be used to predict the limits of nutrient availability within an ecosystem (Bernot & Poulin, 2018).

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