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Appendix 1

Table A1. Patterns of parasite infection from paired host structures reported from the literature, with patterns of ‘random’ included. References given by numbered footnotes at the end of the table. The proposed mechanism, if offered by the authors, is listed in the final column. See text for description of patterns.

Parasite type	Parasite species or genus	Host type/species	Paired host structure	Pattern	Proposed mechanism
Trematode	*Echinostoma* sp. (Rudolphi 1809)	larval amphibians (*Rana clamitans*, *Lithobates sylvaticus*)	kidneys	right side[1,2,3]	discrepancy in kidney size[1], increased host survival[1]
Trematode	*Diplostomum* sp. (Rudolphi 1819)	fish	eyes	inconsistent[4, 5, 6,7,8] random[9]	invading parasites alter eye blood supply[4], first parasites create 'pathway'[4,5], limit cataract

					formation and effects on feeding, predation[6], increase cryptic coloration response time[7]
Trematode	*Stictodora hancocki* (Looss 1899)	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	eyes	random[10]	
Trematode	unknown cyathocotylid sp.	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	eyes	random[10]	
Trematode	*Phocitremoides* sp. (Martin 1950)	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	eyes	random[10]	
Trematode	*Stictodora hancocki*	fish (*Clevelandia*	fins	random[10]	

		ios and *Fundulus parvipinnis*)			
Trematode	unknown cyathocotylid sp.	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	fins	random[10]	
Trematode	*Phocitremoides* sp.	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	fins	random[10]	
Trematode	*Stictodora hancocki*	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	gills	random[10]	
Trematode	unknown cyathocotylid sp.	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	gills	random[10]	
Trematode	*Phocitremoides* sp.	fish (*Clevelandia ios* and *Fundulus parvipinnis*)	gills	random[10]	

Trematode	*Haematoloechus* sp. (Looss 1899)	frogs	lungs	random[11]	
Nematode	*Dioctophyma renale* (Goeze 1782)	mink	kidneys	right side[12,13,14]	ingested larvae migrate through liver into nearby right kidney[15]
Dipteran (blow fly)	*Protocalliphora* sp. (Hough 1899)	hawk *Buteo lineatus*	ears	inconsistent[16]	
Mite	*Unionicola* sp. (Haldeman 1842)	midge (*Paratrichocladius* sp.)	wings	uniform[17]	asymmetric loads interfere with flying[17]
Mite	*Dicrocheles phalaenodectes* (Treat 1954)	noctuid moths (mostly *Leucania* sp.)	ears	inconsistent[18,19]	first mites create 'path'[18] decreased host predation by bats[18,19]

Mite	*Arrenurus* sp. (Dugès 1834)	dragonfly	wings	random[20]	
Mite	*Ensliniella trisetosa* (Cooreman 1942)	Wasp (*Ancistrocerus antilope*)	Acarinaria ('mite chambers/pockets')	uniform[21]	
Isopod	suborder Epicaridea	fossil decapods (*Munida* sp.)	Branchial region	right side[22,23]	greater parasite mortality in left side[24]
Monogenea	*Dactylogyrus* sp. (Diesing 1850)	eels (*Barbus martorelli*)	gills	random[25]	
Monogenea	*Bothrithrema bothi* (Hendrix 1990)	eels (*Scophtalmus aquosus)*	external surface	inconsistent	dissymmetrical host body[27]
Monogenea	*Pseudodactylogyrus anguillae* (Yin & Sproston 1948)	eels (*Anguilla anguilla*)	gills	left side[26]	niche partitioning[28]
Monogenea	*Pseudodactylogyrus bini* (Kikuchi 1949)	eels (*Anguilla anguilla*)	gills	right side[28]	niche partitioning[28]

Monogenea	*Entobdella soleae* (van Beneden & Hesse 1863)	flatfish (*Solea solea)*	body	nadiral (bottom) side (biological right)[29]	reduced predation on parasites[29], easier egg attachment to substrate[29]
Myxosporidia	*Myxobolus* sp. (Walliker 1968)	eels (*Barbus martorelli*)	gills	random[25]	
Copepod	*Leposphilus labrei* (Hesse 1866)	fish (*Crenilabrus melops*)	lateral line	left side[30]	asymmetric fish locomotion ('handedness')[30]
Copepod	*Lepeophtheirus thompsoni* (von Nordmann 1832)	flatfish (*Psetta maxima*)	gill cavity	nadiral (bottom) side (biological right)[31]	host physiology (mucus, respiratory

					flow)[31]
Copepod	*Lepeophtheirus europaensis* (Zeddam *et al* 1988)	flatfish (*Scophthalmus rhombus*)	gill cavity	nadiral (bottom) side (biological right) [31]	host physiology (mucus, respiratory flow)[31]
Copepod	*Lepeophtheirus europaensis*	flatfish (*Platichthys flesus*)	gill cavity	zenithal (top) side (biological right) [31]	host physiology (mucus, respiratory flow)[31]

[1]Thiemann and Wassersug 2000), [2]Holland 2009, [3]Holland et al. 2007, [4]Rau et al. 1979, [5]Graczyk 1991, [6]Karvonen and Seppala 2008, [7]Rintamaki-Kinnunen et al. 2004, [8]Dwyer and Smith 1989, [9]Machado et al. 2005, [10]Shaw et al. 2005,[11]Whitehouse 2002,[12]Mace 1976, [13]Mech and Tracy 2001, [14]Loukmas et al. 2010, [15]Measures 2001, [16]King et al. 2010, [17]McLachlan et al. 2008, [18]Treat 1957, [19]Payne et al. 1966), [20]Mitchell 1959, [21]Cooper 1954, [22]Rayner 1935, [23]Rasmussen et al. 2008, [24]Housa 1963, [25]Jeannette et al. 2010, [26]Hendrix 1990, [27]Bilong-Bilong 1995, [28]Buchmann 1988, [29]Kearn 1984, [30]Donnelly and Reynolds 1994, [31]de Meeus et al. 1995.

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Table A2. Amphibian species sampled as part of the field survey. Presented are the number of individuals with detectable echinostome infections (‘N’) and the mean percentage of metacercariae recovered from the right kidney. Species codes are also used in Fig. 1 of the text.

Species name	Species code	n	Right kidney (%)
Acris crepitans (Baird 1854)	ACCR	11	71.2
Ambystoma gracile (Baird 1859)	AMGR	8	60.7
Ambystoma macrodactylum (Baird 1849)	AMMA	18	54.5
Ambystoma tigrinum (Green 1825)	AMTI	29	51.7
Anaxyrus americanus (Holbrook 1836)	ANAM	118	58.9
Anaxyrus boreas (Baird & Girard 1852)	ANBO	575	57.8
Anaxyrus cognatus (Say 1823)	ANCO	5	52.5
Anaxyrus woodhousii (Girard 1854)	ANWO	8	62.6
Hyla versicolor (LeConte 1825)	HYVE	133	71.3
Pseudacris crucifer (Wied-Neuwied 1838)	PSCR	10	79.5
Pseudacris maculata (Agassiz 1850)	PSMA	16	78.2
Pseudacris regilla (Baird & Girard 1852)	PSRE	3462	63.2
Pseudacris triseriata (Wied-Neuwied 1838)	PSTR	50	60.3
Rana aurora (Baird & Girard 1852)	RAAU	159	60.9
Lithobates blairi (Mecham, Littlejohn, Oldham, Brown, & Brown 1973)	RABL	21	35.7
Lithobates catesbeianus (Shaw 1802)	LICA	344	59.4
Lithobates chiricahuensis (Platz & Mecham 1979)	LICH	12	50.9
Lithobates clamitans (Latreille 1801)	LICL	397	60.8

Rana luteiventris (Thompson 1913)	RALU	30	58.9
Lithobates palustris (LeConte 1825)	LIPA	14	56.8
Lithobates pipiens (Schreber 1782)	LIPI	323	54.6
Lithobates septentrionalis (Baird 1854)	LISEP	41	65.4
Lithobates sphenocephalus (Cope 1886)	LISP	134	56.8
Lithobates sylvaticus (LeConte 1825)	LISY	41	62.2
Taricha granulosa (Skilton 1849)	TAGR	1	100
Taricha torosa (Rathke 1833)	TATO	40	64.9

Appendix 2

Additional details on methodological approaches, analyses and results

Overview of approach and alternative hypotheses

We utilized a combination of field and experimental data to test hypotheses about the patterns, potential mechanisms, and implications of echinostome parasite distributions within amphibian hosts. Building from past studies that have reported a right-kidney bias in amphibian echinostome infections (Thiemann et al. 2000, Holland et al. 2007, Holland 2009), we began by using the field data to test whether the distribution of cysts between kidneys differed from a random binomial expectation (0.5) and then explored competing mechanistic hypotheses derived from the literature, including variation in kidney volume or position (host asymmetry), avoidance or attraction to previously colonized parasites ('follow the leader'), congenital handedness by parasites, and differential mortality by hosts or parasites (Treat 1957, Payne et al. 1966, Rau et al. 1979, Thiemann et al. 2000,Holland 2009).

For the first, we incorporated measures of kidney volume or the disparity in volume between the left and right kidneys into statistical models of parasite distribution, first with the field data and subsequently with the experimental data. Based on potential hypotheses, we tested whether right-biased infections were greatest when the right kidney was larger than the left and/or when total infections were high. Using an additional set of larval bullfrogs with highly detailed anatomical measurements on the size and position of the kidneys, we further evaluated whether kidney position in the antero–posterior axis affected parasite distributions between the kidneys. For 'follow the leader', we expected that the degree of right-kidney bias would increase with infection intensity (a quadratic, exponential or power relationship) and that the distribution of early infection events would positively influence colonization by subsequent parasites in experiments with fluorescently labeled cercariae. We used an experimental approach involving cercarial choice assays infused with different cues (water or one of two concentrations of kidney extract) to assess whether parasites had a congenital directional preference or responded to host cues. To examine whether differential mortality could explain non-random distributions in echinostome infection, we compared the right-side bias in our experimental studies (where there was little to no host mortality) with that observed in our field data. If hosts in nature with a relatively balanced (i.e. random) distribution of echinostomes are dying due to renal failure or secondary stressors, we would expect that the lab-observed bias in favor of right-side infections would be much less pronounced or even absent. We also evaluated whether total parasite recovery differed as a function of parasite distribution, which would provide evidence of differential mortality by the parasites. Finally, we assessed the relationship between the degree of right-side bias in experimental hosts and measures of host fitness, including survival and growth, to determine whether asymmetric infections led to a reduction of parasite impacts on hosts.

Experimental exposures

For the experimental exposures of larval amphibians to varying numbers of echinostome cercariae, hosts were maintained individually in 1.0 lL of de-chlorinated tap water and were fed a 1:1 mixture of ground Tetramin fish flakes and *Spirulina* (*Pseudacris regilla*) or rabbit food pellets (*Lithobates sylvaticus*) with water and containers changed twice weekly. *Echinostoma trivolvis* cercariae were obtained from naturally infected snails (*Helisoma trivolvis*) within 2 to 4 hours of release and pooled among snails to account for variation among genotypes. Cercariae were identified as *E. trivolvis* lineage A based on the number of collar spines and verified using molecular markers (ITS1, ITS2, CO1 and ND1) with sequences from our samples incorporated into a Bayesian phylogenetic analysis (Schell 1985, Jadin et al. unpubl.).

Fluorescent dye experiment

In the experiment involving fluorescently labeled parasites, *Lithobates sphenocephalus* larvae were exposed individually to labeled echinostome cercariae within 1.0 l of treated tap water. In addition to the main experimental treatments (see main text), we maintained an additional five larvae that were not exposed to any cercariae were maintained as a control. This experiment allowed to us examine whether earlier infections affected the distribution of subsequently colonizing parasites, either positively (follow the leader) or negatively (competitive avoidance). To do this, we used a red (BODIPY® 558/568 C_{12}, Invitrogen) and a green (BODIPY® FL C_{12}, Invitrogen) fluorescent dye to label the cercariae. Cercariae were labeled with one fluorescent dye for the first exposure (green) and the other for the second exposure (red). Two days after the second exposure event, we necropsied each host and removed the kidneys for imaging. For each host, we captured three images of the kidneys (i.e. bright-field, GFP filtered, TX Red filtered) and channel-combined these images using ImageJ software to create a composite image. The number and location of the parasites from each exposure event was determined using these composite images.

Cercarial choice assay

We created Y-shaped mazes out of high-density plastic measuring 4 × 3.5 × 0.5 (L × W × H) cm with a dark brown coloration for background contrast with the pale cercariae. A Y-shape was carved into each block with mean measurements (+ SD) in cm as follows: main arm = 1.33 + 0.05, left arm = 1.23 + 0.15, right arm = 1.3 + 0.16, width and depth = 0.31 + 0.02. The water choice consisted of de-chlorinated tap water while kidney solutions were made by extracting both kidneys from 10 bullfrog tadpoles (Gosner (1960) stages 30–35), placing them into 1.5 ml microcentrifuge tubes (five kidney pairs/tube) with 0.5 ml frog Ringer's solution (1 l distilled water, 6.5 g NaCl, 0.42 g KCl and 0.25 g $CaCl_2$), and then crushing them with a tissue macerator to achieve a viscous state. We then added enough frog Ringer's solution to achieve a total volume of 1.5 ml. Tubes were centrifuged for 1 min and the supernatant transferred to new tubes. For each experimental run, eight mazes were used, with four in an 'upright' Y-position and the other four rotated by 180° to avoid any unintended environmental cues. For trials that offered a choice between two different solutions, four mazes received one cue (e.g. water) in the left arm while the other four received the alternate cue (e.g. kidney) in the right arm. Mazes were rinsed with hot water, followed by 70% ethanol and a second water rinse, and then fully dried before re-use. The treatment water/kidney extract combinations were as follows (L = left arm, R = right arm, W = water, K = kidney extract): LW-RW, LK-RK, LK-RW and LW-RK. For the assay involving kidney extract at different concentrations, the choices were LlowK-RhighK and LhighK-RlowK, where the low concentration represented a 50% dilution of the kidney extract with frog Ringer's solution. Echinostome cercariae were collected from naturally infected snails from a wetland in Ontario, Canada, known to support both *E. trivolvis* lineage A and *Echinoparyphium* lineage 3 based on molecular markers (ITS1, ITS2, CO1 and ND1) (Jadin et al. unpubl.); within 2 to 6 hours of release from snails, we pooled cercariae among snails to account for variation among species or genotypes. After 20 min, the number of parasites in each arm was counted under a dissecting microscope.

Field analysis

We used generalized linear mixed models to evaluate how kidney identity (left vs right), kidney volume (calculated using the volumetric formula for an ellipsoid: $4 / 3 \times \pi \times L / 2 \times W / 2 \times 1$, where L and W are the length and width and the depth is held constant) affected the number of detected metacercariae in a given kidney (modeled following a negative binomial distribution). Random effects included host (with kidneys nested within host), wetland, and host species. Analyses were performed using the R package and function glmmADMB, which generates AIC values that can be used to compare among full and reduced models. If the right-side bias was explained by kidney volume, we expected there to be no effect of kidney identity (right vs left) after accounting for volume and the hierarchically nested random effects. To estimate the overall probability of right kidney infection while accounting for any effects of kidney volume disparity or total infection, we used generalized linear models with a binomial distribution and both fixed effects (total number of echinostome cysts and logit transformation of the ratio of right kidney volume to left kidney volume) and random effects (host species and site-visit) using the lmer function in the R package lme4. This analysis allowed us to then use the anti-logit function to back-calculate the probability of right side infection.

Experimental analysis

Among larval amphibians exposed to echinostome cercariae, we analyzed how both the number of detected metacercariae (either in the kidneys alone or summed between the kidneys and pronephros) as well as the proportion of the metacercariae noted in the right kidney influenced metrics associated with host fitness. For *P. regilla*, this included host survival, Gosner (1960) stage, snout-vent length (mm), and wet mass (blotted dry, mg). For *L. sylvaticus*, only one individual died and thus mortality was not examined as a response. We instead tested the effects of the number of cysts and proportion of metacercariae in the right kidney on the log-transformed proportional change in body size (final mass minus initial mass divided by initial mass). Analyses involved least squares regression with appropriate transformations to response variables, when necessary.

Field results

In the mixed model analysis of infection within each kidney, variance associated with the random effects was greatest for site-visit (1.52), followed by host individual (0.82), and least for host species (0.59). Based on the subset of larval bullfrogs for which we more intensively measured kidney dimensions, kidney volume based on mass correlated with our indirect measure of volume ($r = 0.69$ for left and 0.77 for right kidney; both $p < 0.005$), helping to validate our approach. Among an additional set of field-collected animals without detectable echinostome infection ($n = 1382$), the right kidney was still ~11% larger (in estimated volume) than the left kidney (paired-t = 11.123, $p > 0.0001$, DF = 1381). This suggests that echinostome infection, on its own, was not responsible for the observed asymmetry in amphibian kidneys. After accounting for host species, the proportional volume occupied by the right kidney (right kidney volume divided by the volumetric sum of both kidneys) was unrelated to host size (snout–vent length) (full model of size, species and size × species interaction $p = 0.22$). For the most well represented species (*P. regilla*, $n = 1110$), host size explained <<0.1% of the variation in kidney asymmetry ($p = 0.89$).

Experimental results

On average (± 1 SE), 5.54 ± 1.05 metacercariae were recorded in the right kidney of Pacific chorus frogs (range: 0 to 18) relative to 2.77 ± 0.814 in the left kidney (range: 0 to 18), whereas 18.73 ± 1.603 and 13.423 ± 1.255 parasites were detected in the right and left pronephroi, respectively. Approximately 11.1 ± 0.58 of the 25 parasites administered to each wood frog were detected upon necropsy. On average (± 1 SE), 5.04 ± 0.45 metacercariae were recorded in the right kidney (range: 0 to 18) relative to 2.30 ± 0.32 in the left kidney (range: 0 to 12) of wood frogs, whereas 1.59 ± 0.21 and 1.91 ± 0.22 parasites were detected in the right and left pronephroi, respectively. Among *P. regilla*, for which we had measurements of the host kidneys, the volume of the right kidney averaged 19% larger than the left (paired t-test: two-tailed $t = -3.07$, $DF = 25$, $p < 0.006$); however, this asymmetry was insensitive to variation in host size or stage (both $p > 0.45$).

Fluorescent dye results

We also saw no relationship between the degree of right-side bias and the length, volume, or proportional volume occupied by the right kidney, despite the fact that the right kidney was generally larger than the left kidney (paired t-test of kidney volumes, $t = 12.60$, $p < 0.0001$). Total parasite detection averaged 50.2 ± 1.98% of those administered, of which 65.3 ± 2.69% were found in the kidneys (mesonephroi). These fractions were unrelated to the color of dye used or the number of exposure events, helping to validate the efficacy of the approach.

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