

### **Methods-Mixed-species infections**

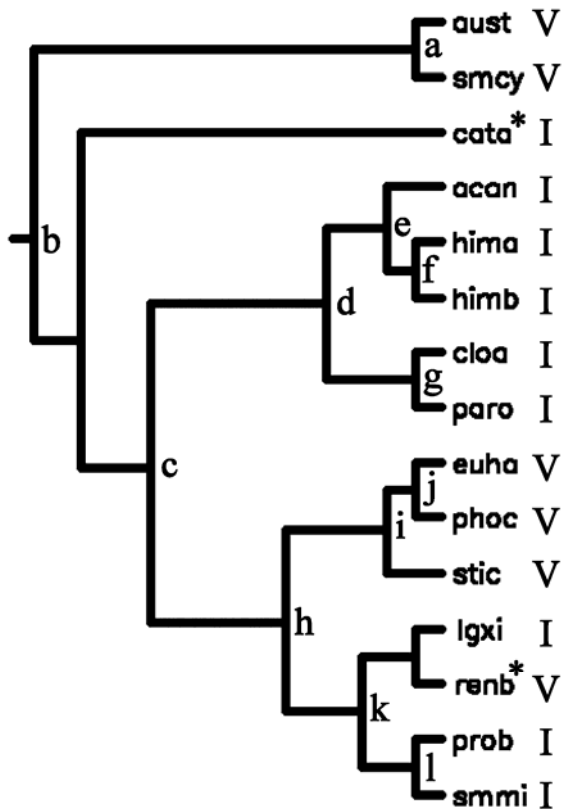
We excluded mixed-species infections from this study except for five mantle dwellers (one CATA and four RENB) found in snails with a visceral mass dweller (three of which were mature). Because the trematodes in these mixed-species infections use different host tissues, we were able to separately calculate their relative masses. We included these measurements in our analysis to increase sample sizes, allowing better estimates of the masses of these species that are frequently found in mixed-species infections. Elsewhere, we present the effects of mixed-species infections on the aggregate mass of trematode tissue (Hechinger *et al.* submitted).

### **Methods & Results-Assessment of statistical interactions**

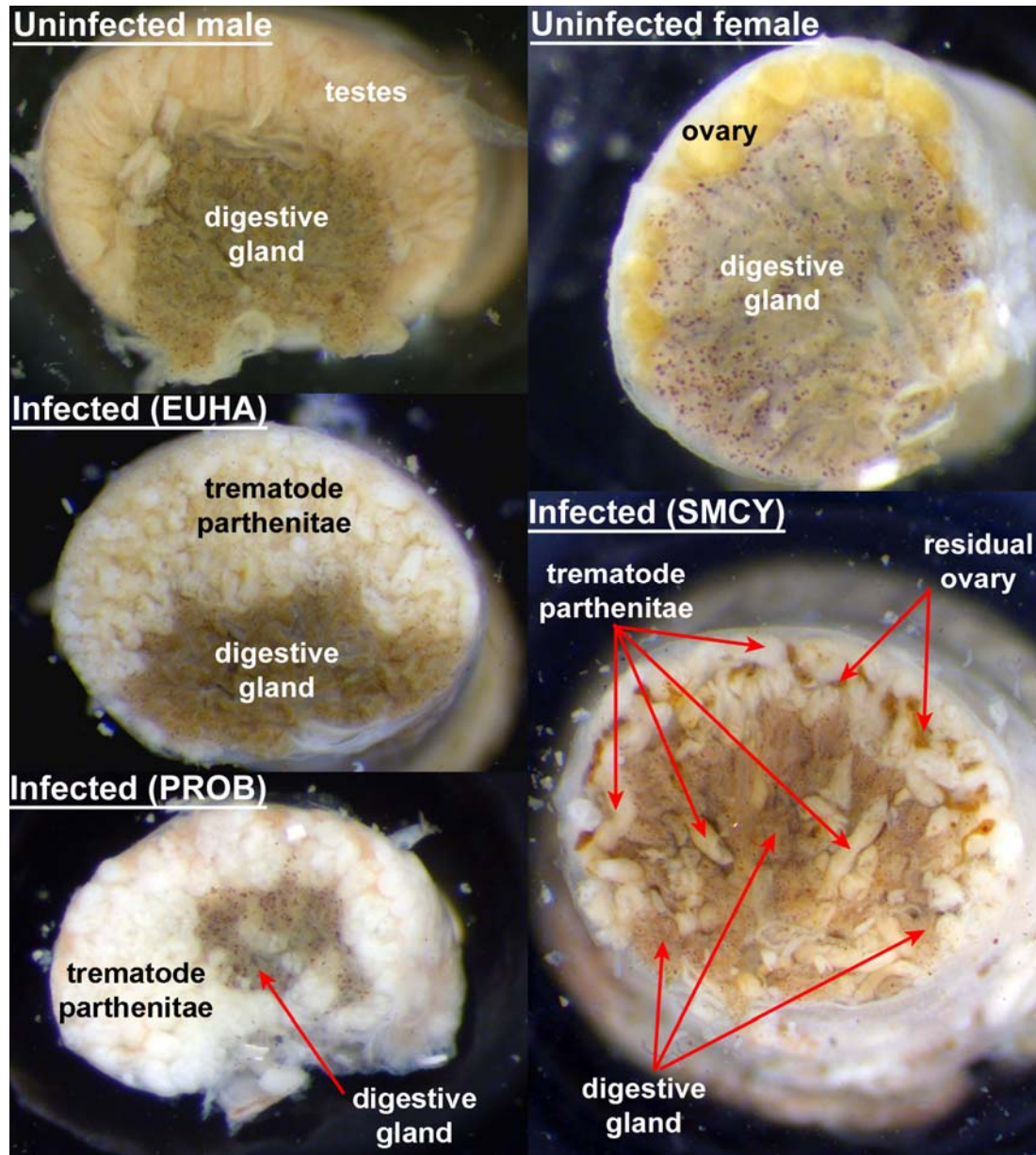
We sought to examine all higher-order interactions (i.e., whether or not the predictor variables affected trematode mass differently depending on the value of the other predictor variables). Although we had a large overall sample size (167 infections), we did not have all possible combinations of the predictor variables for each trematode species. Thus, we could not include all three- and four-way interactions into GLMs containing trematode species as a factor. However, we determined whether interactions were important in two ways. We analyzed GLMs including all interactions up to fourth-order terms (between habitat, season, snail size, and snail sex), for all trematode infections pooled (i.e., ignoring taxon), and for the one superfamily for which we had the largest sample size (the Echinostomoidea,  $n = 100$ ). To the extent that trematode taxon affects trematode mass, pooling trematodes would tend to contribute noise to the analysis when examining the effects of the other predictor variables on both trematode mass and on each other (i.e., their interactions). Given our large sample size, this would only be a problem for detection of very weak effects. We found no significant interactions in either the model with trematodes pooled (all  $P \geq 0.21$ ,  $n = 154$ ) or in the model limited to the Echinostomoidea (all  $P > 0.37$ ,  $n = 93$ ). Thus, we removed these non-significant interactions, simplifying subsequent analyses. However, trematode species could differ in how their relative mass varied across seasons, habitats, or with host size. While we could not examine such interactions for individual trematode species, we could assess two-way interactions between family, habitat, season, and host-size, for the four families that we most extensively sampled (namely, the Echinostomatidae, Philophthalmidae, Heterophyidae, and Rencolidae). This assessment of interactions should be robust because we found that most of the variation between species was due to family affiliation (see results). Here, also, all interactions were non-significant (all  $P \geq 0.11$ ,  $n = 147$ ) and we dropped them from subsequent analyses.

### **Methods & Results-lack of snail gender effect**

We dropped snail sex from analyses, because it did not affect relative trematode mass in either the model pooling single trematode infections ( $F_{1,138} = 0.74$ ,  $P = 0.39$ ) nor in the model incorporating species identity ( $F_{1,135} = 1.25$ ,  $P = 0.27$ ). This allowed us to include 16 additional infections for which we had not recorded snail sex. There was no detectable bias, with these additional infections, regarding their influence on relative trematode mass for all pooled infections ( $F_{1,191} = 0.43$ ,  $P = 0.51$ ).



**Figure esm1.** The phylogenetic hypothesis used in comparative assessments (with equal branch lengths of one) to control for confounds due to common ancestry. Species codes are as in table 1. Symbols next to species codes indicate whether offspring (cercariae) infect vertebrates (V) or invertebrates (I). The letters below the nodes indicate the position of the independent contrasts used to assess the influence of dominance rank on castrator relative mass. We imposed vertebrate use at nodes a and i for Hansen’s test examining adaptation of relative mass under the “selective regime” of offspring using vertebrates or invertebrates. The asterisks mark the two species that dwell in the mantle and were not used in comparative analyses (all other species reside in the visceral mass (full tissue-use indicated in table 1)).



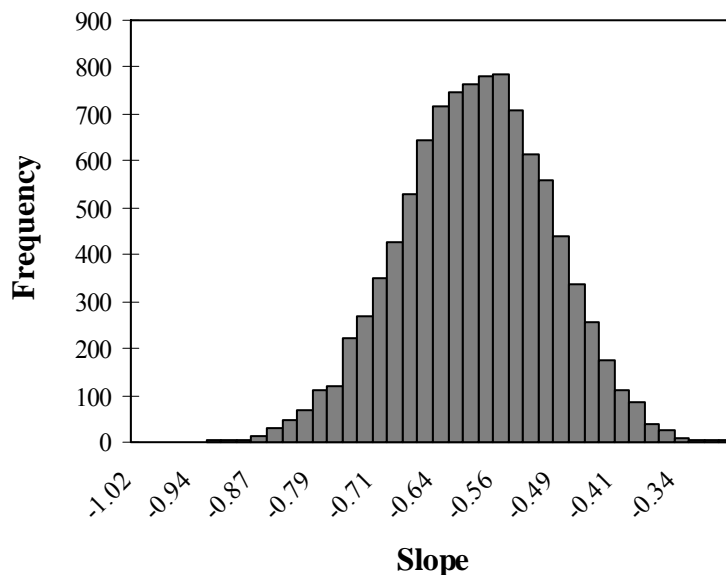
**Figure esm2.** Representative cross-sections of fixed gonadal-digestive gland regions of the visceral masses of uninfected and infected snails (*Cerithidea californica*). Red arrows indicate the major organs and trematode parthenitae. Note the difference in gonad size between uninfected males and females. EUHA (*Euhaplorchis californiensis*) represents one of the trematodes that take up the space in the visceral mass normally occupied by gonad. PROB (*Probolocoryphe uca*) and SMCY (Small cyathocotylid) represent species that also infiltrate the digestive gland. PROB achieves far greater mass by infiltrating additional tissues, whereas SMCY does not because its parthenitae are less densely packed. The residual (and non-functioning) ovary tissue is typical of SMCY infections.

### Methods & Results-Trematode relative mass not spuriously self-correlated with infected snail mass

Measures of reproductive effort ( $Y/X$ ) can spuriously self-correlate with total mass ( $X$ ) (e.g., see Kenney 1982; Jackson and Somers 1991). Therefore, an appropriate test of the relationship between such variables should be based on a null distribution of the test statistic (e.g., slope) that corrects for any spurious self-correlation (Kenney 1982; Jackson and Somers 1991). The null distribution for the value of the slope may not be centered on 0, nor normally distributed.

Following (Jackson and Somers 1991), we performed a randomization test (Edgington 1995) by generating the null distribution for the slope of the relationship between trematode relative mass and infected snail mass. We did this by randomly permuting (10,000 iterations) values of absolute trematode mass and infected snail mass (first using a GLM to control for the effects of season, habitat, and trematode species), calculating relative mass, and scoring the slope of the relationship between relative mass and infected snail mass.

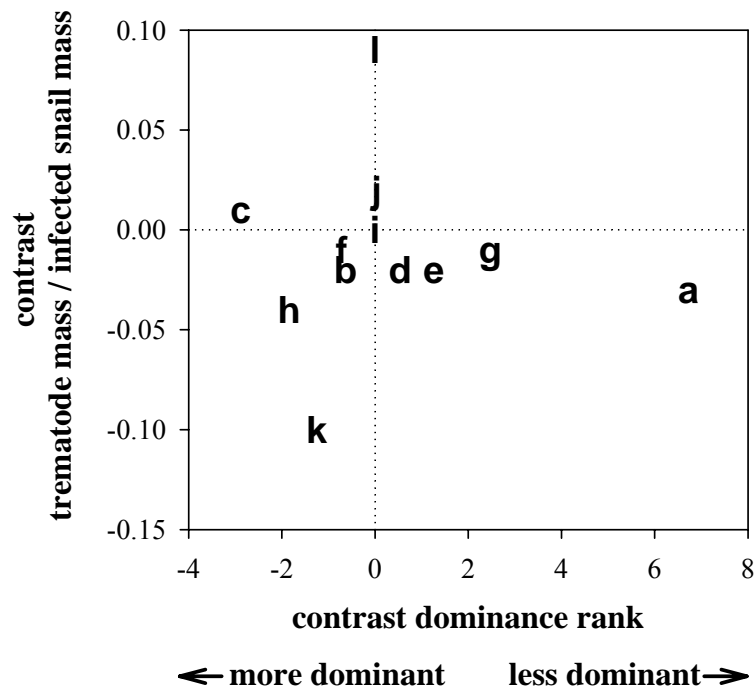
The results of this analysis supported our finding that trematode castrators were proportionally larger in larger snails. First, the observed relationship between trematode relative mass and infected snail weight, when we calculated relative mass on GLM-adjusted absolute masses, was statistically indistinguishable from the relationship observed when we incorporated relative mass directly into the GLM ( $y = 0.20x + 0.14$ , versus  $y = 0.16x + 0.13$ ). Secondly, the null distribution of the slope was centered on -0.45 and was never more positive than -0.25 (thus,  $P < 0.0000$ ) (Figure esm3). This validates that the trematodes were truly proportionally larger in larger snails.



**Figure esm3.** Null distribution of the slope between relative trematode mass and infected snail weight, based on 10,000 randomizations.

**Methods & Results-The scaling of absolute trematode mass with infected snail mass**

We bolstered the non-linear regression in two general ways. First, we used a range of starting values (0, 1, 2) for the slope and the exponent, in addition to using the values obtained from the log-transformed variable analysis (described below). For all starting values, the parameter estimation converged within six iterations on the same slope and exponent (two iterations when starting with the values obtained by back-transforming from the log-log analysis). Second, we also performed a regression analysis on  $\log_{10}$ -transformed variables. The first analysis on log-transformed variables indicated an even larger scaling exponent than did the non-linear analysis. Here, trematode mass scaled with snail mass to the 1.5 ( $P < 0.0000$ ,  $n = 167$ ). However, when logged, the datum with the smallest relative mass value had undue influence on the slope (Cook's  $D > 1$ ). We therefore performed the analysis without this outlier. The analysis on log-transformed masses then converged on the non-linear analysis. Here, trematode mass scaled with snail mass to the 1.34 (1.10-1.57 95% ci,  $P < 0.0000$ ,  $n = 165$ ) and with a coefficient of 0.29 (0.23-0.36 95% ci,  $P < 0.0000$ ).



**Figure esm4.** Phylogenetically independent contrasts for trematode relative mass versus contrasts for rank in the competitive dominance hierarchy. Symbol letters match nodes in figure esm1.

**Table esm1.** Number of trematodes for which we quantified trematode mass, by habitat and season.

	Winter	Summer	Total
Channel	23	34	57
Flat	75	35	110
Total	98	69	167

**Table esm2.** Results of general linear model assessing the role of family on the relative mass of trematodes in infected snails.

predictor	df	SS	<i>F</i> -ratio	<i>P</i>
species [family] <sup>a</sup>	7	0.044	2.05	0.0526
family	7	0.183	8.47	0.0000
season	1	0.039	12.67	0.0005
habitat	1	0.013	4.23	0.0414
tissue mass	1	0.018	5.92	0.0162
full model	17	0.665	12.69	<0.0001
residual	149	0.459		

<sup>a</sup> Species is nested within family.

**Table esm3.** Results of general linear model incorporating host-tissue site use of trematode species as an effect on their relative mass in infected snails.

predictor	df	SS	<i>F</i> -ratio	<i>P</i>
species [tiss-site] <sup>a</sup>	10	0.243	7.88	0.0000
tissue-site	4	0.062	5.01	0.0008
season	1	0.039	12.67	0.0005
habitat	1	0.013	4.23	0.0414
tissue mass	1	0.018	5.92	0.0162
full model	17	0.665	12.69	0.0000
residual	149	0.459		

<sup>a</sup> Species are nested within their tissue-site use.

**Table esm4.** Results of general linear model assessing the role of dominance rank on the relative mass of trematodes in infected snails.

predictor	df	SS	<i>F</i> -ratio	<i>P</i>
species [dominance rank] <sup>a</sup>	5	0.038	2.39	0.041
dominance rank	7	0.160	7.16	<0.0001
season	1	0.044	13.66	0.0003
habitat	1	0.012	3.61	0.0595
tissue mass	1	0.018	5.66	0.0188
full model	15	0.601	12.58	<0.0001
residual	138	0.440		

<sup>a</sup> Species is nested within dominance rank.



**Table esm5.** Results of general linear model assessing the role of the type of host infected by offspring (cercariae) on the relative mass of trematodes in infected snails.

predictor	df	SS	<i>F</i> -ratio	<i>P</i>
species [offspring host] <sup>a</sup>	11	0.252	7.20	<0.0001
offspring host	1	0.050	15.68	0.0001
season	1	0.044	13.66	0.0003
habitat	1	0.012	3.61	0.0595
tissue mass	1	0.018	5.66	0.0188
full model	15	0.601	12.58	<0.0001
residual	138	0.440		

<sup>a</sup> Species is nested within type of host infected by offspring (vertebrate or invertebrate).

**Table esm6.** Results of Hansen's test for adaptation of relative mass for trematodes with offspring infecting vertebrates versus those using invertebrates.

evolutionary constraint ( $\alpha$ )	invertebrate-user optimal relative mass ( $\theta_2$ ) $\pm$ s.e.	vertebrate vs. invertebrate-user optimal relative mass ( $\theta_1 - \theta_2$ ) $\pm$ s.e.	variation explained ( $R^2$ )	support (ln likelihood)	$t^a$	$P$
0.1	0.25 $\pm$ 0.083	-0.43 $\pm$ 0.386	10.12	19.31	1.11	0.29
0.2	0.24 $\pm$ 0.052	-0.25 $\pm$ 0.193	13.3	20.07	1.30	0.22
0.3	0.24 $\pm$ 0.039	-0.19 $\pm$ 0.130	16.27	20.61	1.46	0.17
0.4	0.24 $\pm$ 0.032	-0.16 $\pm$ 0.098	18.97	21.00	1.63	0.13
0.5	0.23 $\pm$ 0.028	-0.14 $\pm$ 0.080	21.37	21.28	1.75	0.11
0.6	0.23 $\pm$ 0.026	-0.12 $\pm$ 0.068	23.46	21.49	1.76	0.10
0.7	0.23 $\pm$ 0.024	-0.12 $\pm$ 0.060	25.25	21.64	2.00	0.07
0.8	0.23 $\pm$ 0.023	-0.11 $\pm$ 0.054	26.76	21.74	2.04	0.06
0.9	0.23 $\pm$ 0.022	-0.1 $\pm$ 0.050	28.01	21.81	2.00	0.07
1	0.23 $\pm$ 0.021	-0.1 $\pm$ 0.047	29.05	21.86	2.13	0.05
<b>1.6</b>	<b>0.24<math>\pm</math>0.02</b>	<b>-0.09<math>\pm</math>0.037</b>	<b>32.2</b>	<b>21.94</b>	<b>2.43</b>	<b>0.03</b>
2	0.24 $\pm$ 0.020	-0.08 $\pm$ 0.035	32.83	21.94	2.29	0.04
3	0.24 $\pm$ 0.020	-0.08 $\pm$ 0.033	33.09	21.91	2.42	0.03
4	0.24 $\pm$ 0.020	-0.08 $\pm$ 0.032	33.04	21.90	2.50	0.03
5	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	33.01	21.89	2.19	0.05
6	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.99	21.89	2.19	0.05
7	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.99	21.89	2.19	0.05
8	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.98	21.89	2.19	0.05
9	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.98	21.89	2.19	0.05
10	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.98	21.89	2.19	0.05
100	0.24 $\pm$ 0.020	-0.07 $\pm$ 0.032	32.98	21.89	2.19	0.05

<sup>a</sup> $t$  calculated with 12 d.f. (no. species – 1).

note: bold-font row indicates results for the value of evolutionary constraint ( $\alpha$ ) with maximum-likelihood support (using one digit in the grid search).

**Table esm7.** The family of tests for which we controlled the 'false discovery rate' (FDR).

effect <sup>a</sup>	observed <i>P</i> -value	FDR threshold <sup>a</sup>
taxon (family + species)	<0.0001	0.006
testes vs ovary	<0.0001	0.011
rank in dominance hierarchy	<0.0001	0.017
type of host used by offspring	0.0001	0.022
season	0.0005	0.028
host tissue use	0.0008	0.033
size	0.016	0.039
habitat	0.041	0.044
host sex	0.25	0.050

<sup>a</sup> This is the value below which a *P*-value must be to be deemed significant, holding the false discovery rate to 0.05.

### Esm references

- Edgington ES (1995) Randomization tests, 3rd edn. Marcel Dekker, Inc., New York
- Hechinger RF, Lafferty KD, Kuris AM (submitted) Diversity increases biomass production for trematode parasites in snails.
- Jackson DA, Somers KM (1991) The spectre of 'spurious' correlations. *Oecologia* 86:147-151
- Kenney BC (1982) Beware of spurious self-correlations! *Water Resources Research* 18:1041-1048