Table S1 Studies documenting sex ratio variation in mosquitofish (*Gambusia affinis* and *G. holbrooki*).

Reference	Species	Country	Approx Coordinates	Proportion Males
[1]	*holbrooki*	France	43.533333, 4.5	0.974424552
[1]	*holbrooki*	France	43.533333, 4.5	0.39941691
[2]	*holbrooki*	Spain	40.671905, 0.594952	0.082725061
[2]	*holbrooki*	Spain	40.671905, 0.594957	0.71
[3]	*holbrooki*	Spain	37.000567, -6.415229	0.317291736
[4]	*holbrooki*	Hungary	46.7833333, 017.2000000	0.205882353
[4]	*holbrooki*	Hungary	46.7833333, 017.2000000	0.95
[5]	*holbrooki*	Spain	42.33, 3.1	0.111
[5]	*holbrooki*	Spain	42.33, 3.1	1
[6]	*affinis*	Dongguan,China	23.0241694°, 113.7544444°	0.786516854
[6]	*affinis*	Dongguan,China	23.0241694°, 113.7544444°	0.30952381
[7]	*holbrooki*	Spain/Portugal	38.5500000, -007.2666667	0.248447205
[7]	*holbrooki*	Spain/Portugal	38.5500000, -007.2666667	0.481848185
[8]	*affinis*	Iraq	47.75, 30.5	0.292207792
[9]	*holbrooki*	Tasmania	-41.3850000°, 147.0733333°	0.156985871
[9]	*holbrooki*	Tasmania	-41.3850000°, 147.0733333°	0.877192982
[10]	*affinis*	LA, USA	30, -090.0955556	0.066666667
[10]	*affinis*	LA, USA	30, -090.0955556	0.5
[11]	*holbrooki*	Florida, USA	30.2126950, -084.1784900	0.877192982
[11]	*holbrooki*	Florida, USA	30.2126950, -084.1784900	0.447619048
[12]	*holbrooki*	Turkey	37.0605556, 035.3255556	0.347222222
[12]	*holbrooki*	Turkey	37.0605556, 035.3255556	0.746268657
[13]	*holbrooki*	Australia	.-27.416692, 153.019472	0.518172378
[14]	*holbrooki*	Greece	39.6666667°, 020.8833333°	0.238095238
[14]	*holbrooki*	Greece	39.6666667°, 020.8833333°	0.548387097
[15]	*affinis*	NM, USA	33.4772583°, -104.3908389°	0.790322581
[15]	*affinis*	NM, USA	33.4772583°, -104.3908389°	0.098360656
[16]	*affinis*	Japan	34.0702694°, 134.5548444°	0.073490814
[16]	*affinis*	Japan	34.0702694°, 134.5548444°	0.396907216
[17]	*holbrooki*	India	29.4000000°, 079.4666667°	0.163934426
[17]	*holbrooki*	India	29.4000000°, 079.4666667°	0.62962963
[18]	*holbrooki*	Iran	37.9666667°, 053.1166667°	0.317204301
[19]	*holbrooki*	Bulgaria	42.3862639°, 025.4324556°	0.527027027
[19]	*holbrooki*	Bulgaria	42.3862639°, 025.4324556°	0.207792208
[20]	*affinis*	LA, USA	30.2265944°, -093.2173750°	0.489361702
[20]	*affinis*	LA, USA	30.2265944°, -093.2173750°	0.224299065
[21]	*affinis*	Kansas, USA	37.5581028°, -097.2489583°	0.13253012
[21]	*affinis*	Kansas, USA	37.5581028°, -097.2489583°	0.428571429
[22]	*holbrooki*	Italy	45.4428917°, 011.7072472°	0.91
[22]	*holbrooki*	Italy	45.4428917°, 011.7072472°	0.39

[23]	*affinis*	Illinois, USA	41.7870417°, -087.8104972°	0.978021978
[23]	*affinis*	Illinois, USA	41.7870417°, -087.8104972°	0.156985871
(Fryxell, D.F. 2014) *	*affinis*	California, USA	37.3074556°, -118.3448167°	0.05
(Fryxell, D.F. 2014) *	*affinis*	California, USA	37.3074556°, -118.3448167°	0.45
(Fryxell, D.F. 2014) *	*affinis*	North Island, NZ	-37.2006639°, 175.3402194°	0.79
(Fryxell, D.F. 2014) *	*affinis*	North Island, NZ	-38.4519806°, 176.1580472°	0.19

* Unpublished data

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Table S2 See additional supplementary xlsx file.

Table S3 We determined whether fish biomass explained a significant amount of variation in a subset of responses within the grouped sex ratio treatments “male-biased” and “female biased” using linear regression. Responses chosen were those showing the strongest sex ratio effects.

Group	Response	Week	R^2	Slope	p
Female-biased	Phytoplankton Abundance	2	0.074	1.540	0.393
Female-biased	NPP	2	0.008	0.066	0.772
Female-biased	pH	2	0.036	0.104	0.555
Female-biased	Temperature	2	-0.080	0.281	0.679
Female-biased	Crustacean Zoop Abundance	4	0.112	-0.860	0.287
Male-biased	Phytoplankton Abundance	2	0.193	1.921	0.153
Male-biased	NPP	2	0.001	-0.021	0.909
Male-biased	pH	2	0.003	0.028	0.861
Male-biased	Temperature	2	0.014	0.248	0.714
Male-biased	Crustacean Zoop Abundance	4	0.008	0.443	0.782

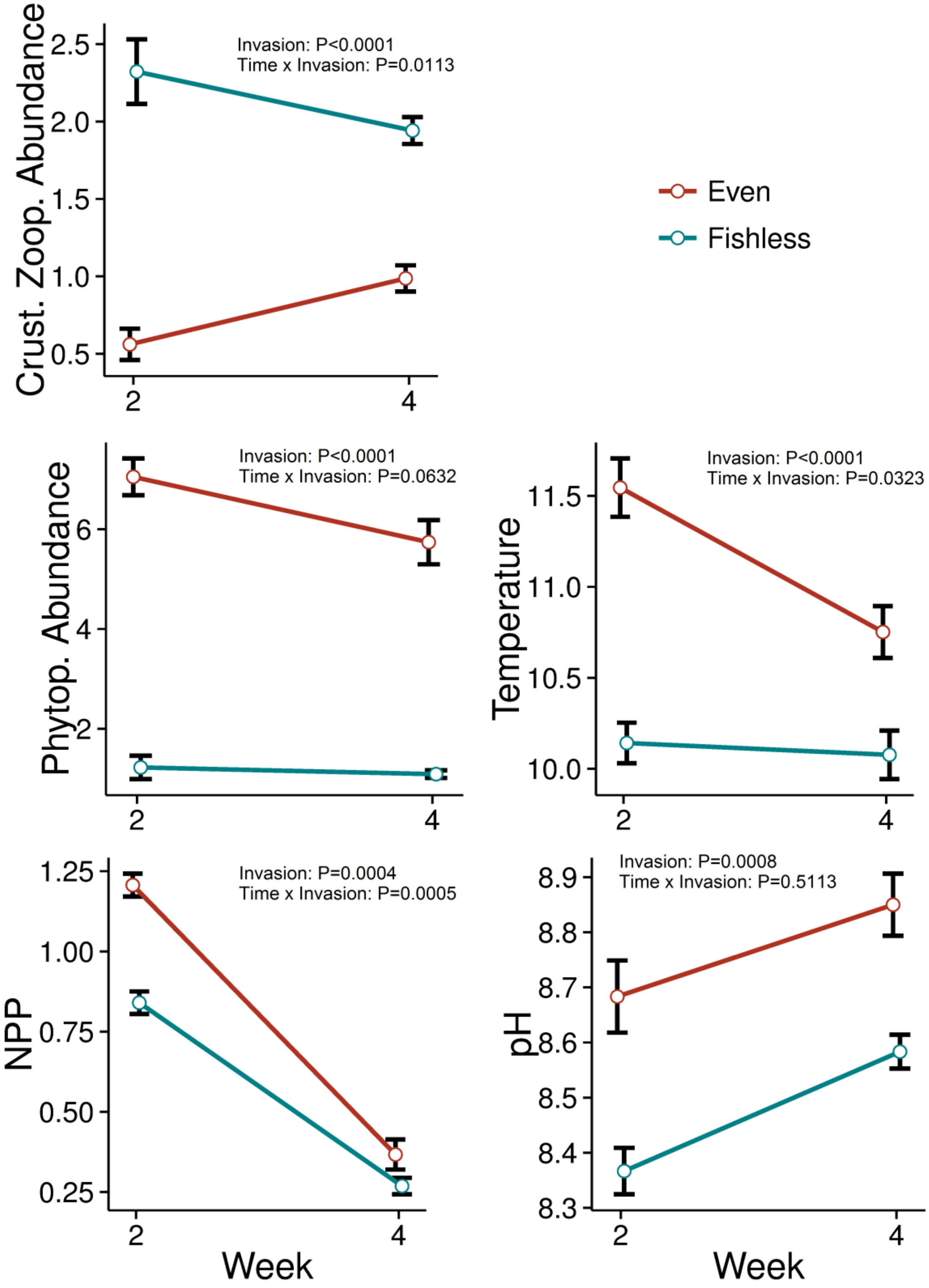

Figure S1 Pelagic responses (mean ± SE) for the fishless treatment (blue) and even sex ratio treatment (red). Significance is reported for Invasion and Time*Invasion effects from the MANOVA of repeated measures.

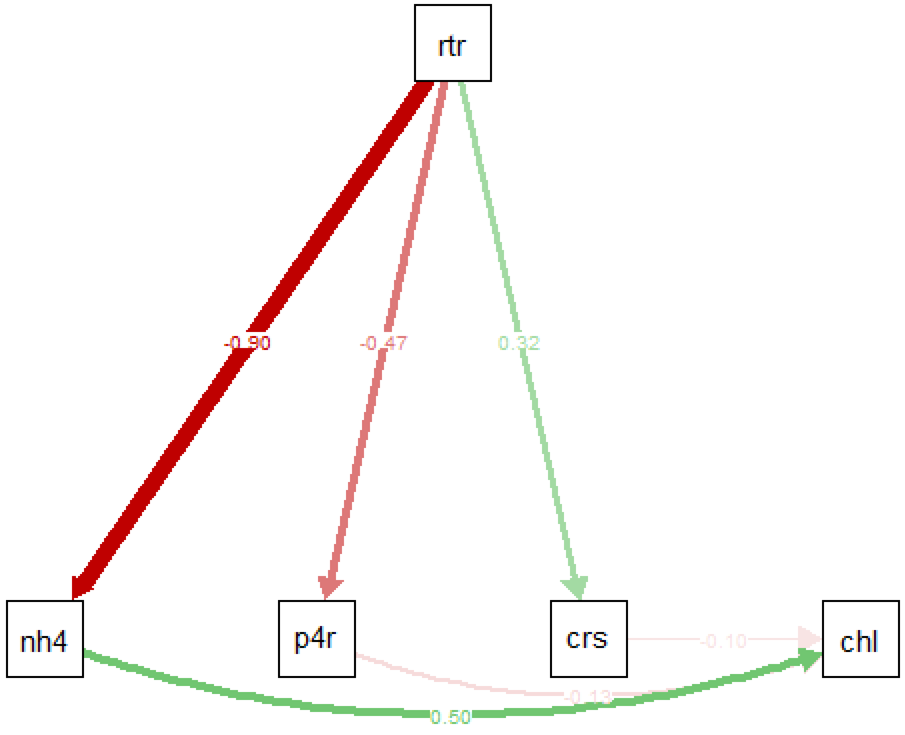

Figure S2 A directed graph showing results from a path analysis where phytoplankton abundance (chla) at week 2 is affected by the number of crustacean zooplankton (crs), the amount of fish N excretion (nh4), and the amount of fish P excretion (p4r). Inclusion of realized sex ratio treatment (rtr, measured as proportion males) shows that sex ratio had a large effect on N excretion, a significant but smaller effect on P excretion, and only a slight effect on zooplankton (at week 2). This analysis was performed to investigate the potential cause of the week 2 sex ratio effect on the trophic cascade given the lack of a sex ratio effect on crustacean zooplankton abundance at week 2. This analysis was based on standardized data from all treatments with fish, performed in R using the package lavaan, and plotted using the package SEMplot. The only significant effect on phytoplankton abundance (α=0.10) was that of N excretion (p=0.014). We do not think this analysis implies that N was the limiting nutrient for phytoplankton. However, we think N excretion was a better proxy for overall nutrient excretion than was P excretion because estimates of P excretion in our experiment were highly variable.

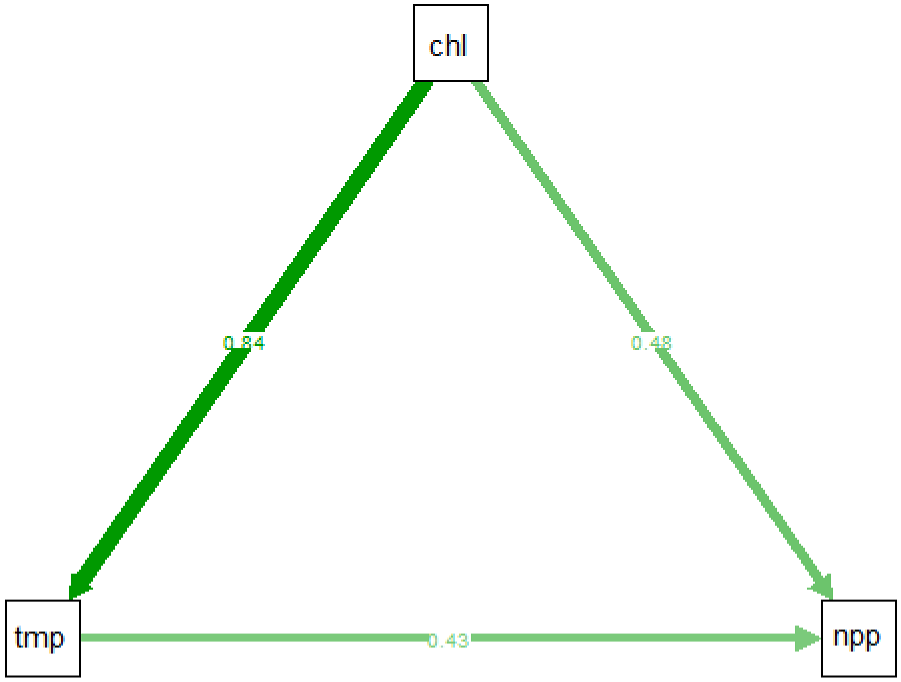

Figure S3 A directed graph showing the results from a path analysis where phytoplankton abundance (chla) affects NPP directly and indirectly through its effect on temperature (tmp). This analysis was based on standardized data from all treatments at week 2, performed in R using the package lavaan, and plotted using the package SEMplot. P-values for each arrow (i.e. parameter estimates for predictors in the underlying regressions) were significant at an α=0.005 level.

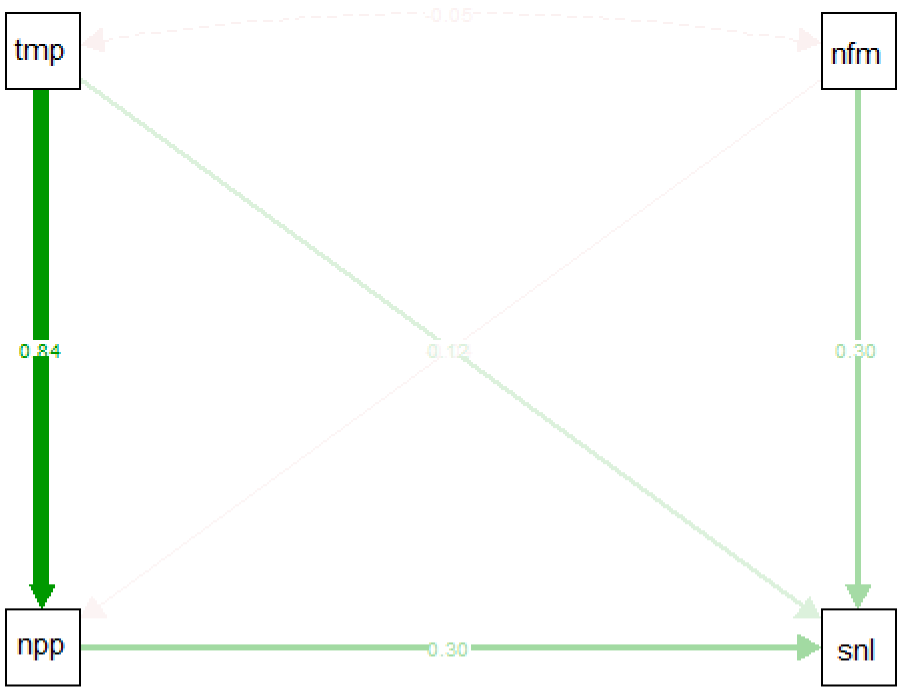

Figure S4 A directed graph showing hypothesized relationships among responses potentially affecting snail size (snl). Snail size differences were likely the result of faster growth rates, and faster growth rates could be related to the number of perceived predators (number of females (nfm) since only female mosquitofish consume snails), the pond temperature (tmp), or the amount of primary production (npp; i.e. food resources). This analysis was based on standardized data from all treatments, performed in R using the package lavaan, and plotted using the package SEMplot. Temperature and NPP values were taken from week 2 while snail size wasn't measured until week 4; this was due to the fact that snail size was determined by growth in previous ecological conditions. P-values for each direct effect on snails size (i.e. parameter estimates for predictors in the underlying regressions) were not significant (α=.10) except for the effect of number of females on snail size (p=0.050).

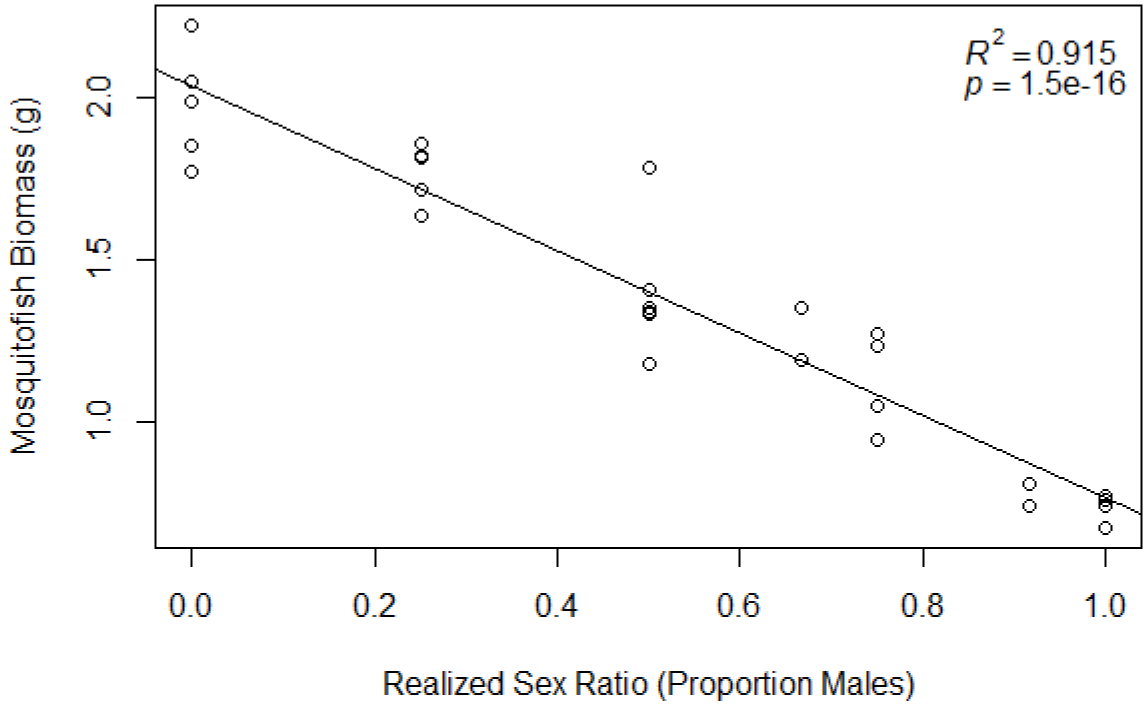

Figure S5 Realized experimental sex ratio and total fish biomass were strongly linearly related. This strong collinearity (VIF>10) precluded multiple linear regression and ANCOVA.