Supporting Information

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SI Materials and Methods

Mesocosm Preparation. Mesocosm tanks were 300 gallon polyethylene cattle watering tanks manufactured by Rotonics Manufacturing Inc. Tanks were 72 inches in diameter and 24 inches in height. These tanks had been used for the previous 2 years for amphibian experiments. Each tank was scrubbed, bleached and rinsed at the beginning of the season to remove dirt and debris and allowed to dry for a day. On February 23, 2007 we filled all tanks using Lake Berryessa reservoir water (unchlorinated), which was then allowed to sit for a day. On February 24, 2007 we added 35 pounds of washed Monterey beach sand to each mesocosm as a bottom substrate. On February 25 we added 25 g of rabbit chow as a nutrient base. On February 27 we began zooplankton additions, which continued through March 7. We collected zooplankton from 8 natural ponds in the region, including ponds also used to collect native *Ambystoma*. We combined collections in a single receptacle, then mixed and distributed 3 separate 0.25L aliquots into each tank on each introduction occasion. After the commencement of zooplankton additions, we added a second 25 g of rabbit chow and 3L of recently-clipped grass to each tank. Grass was collected from Jepson Prairie, a grassland site occupied by a population of *A. californiense*, on March 5. We provided time for temporary eutrophic conditions due to nutrient additions to pass—i.e., waited for water to clear entirely before introducing experimental animals.

Before introduction of *Ambystoma* and *Taricha* larvae, we began introducing *Pseudacris* tadpoles. On March 25, we introduced 8 tadpoles to each mesocosm to help reduce growth of filamentous algae, freeing basal nutrients for phytoplankton, which support zooplankton populations. *Pseudacris* also breed over an extended period in central California, so the sequential addition of *Pseudacris* tadpoles is designed to simulate ongoing breeding of *Pseudacris* in natural populations. Please see details of tadpole collection and introduction dates below.

Wild Embryo Collection. *Pseudacris* embryos were collected from natural substrates (submerged sticks and aquatic vegetation) and artificial substrates [rectangular pvc grids with nylon string (1)] and transported in natural pond water to the Haring Hall animal room facility at University of California Davis. There, groups of 3 egg masses were housed in plastic shoebox containers with 10% Holtfreters solution and monitored daily for hatching or inviability. Inviable eggs were removed daily. Upon hatching, tadpoles were grouped by pond in batches of 25 and fed spirulina tablets for up to a week before introduction to mesocosms.

We collected hybrid *Ambystoma* embryos on 8 March from 3 ponds of known hybrid index in Salinas Valley (Monterey County, CA). Hybrid index varies from 0 to 1, 0 being native *Ambystoma californiense* and 1 being introduced *A. tigrinum mavortium* across all sampled loci. Hybrid indices in 2007 from our collection ponds were 0.697, 0.830 and 0.963 (sites 49, 55 and 62 respectively in the supporting information text in ref. 2). We collected eggs from natural substrates (sticks, vegetation). Upon collection, we placed *Ambystoma* embryos in plastic bags of natural pond water for transport to the University of California, Davis animal rooms. Once in the animal rooms, eggs were transferred in batches of 10 to plastic shoeboxes containers containing 10% Holtfreter's solution and monitored daily for hatching or inviability. Embryos collected at later developmental stages were housed at 16 °C whereas less developed eggs were housed in a separate room at 19 °C to synchronize hatching times. As individuals hatched, they were removed from their shoebox and placed in individual plastic cups with 10% Holtfreter's solution and maintained at 16 °C.

We collected *Taricha* egg masses from 3 ponds in Contra Costa County (intermediate to Solano and Monterey Counties and within the region of range overlap of *Ambystoma* and *Taricha*) on March 22 and transported them as described above. In the animal rooms, we divided egg masses into groups of 10 and placed them in plastic shoeboxes filled with 10% Holtfreter's solution. These were monitored daily as above for hatching and embryo death.

Ambystoma Breeding and Generation of Line Cross Larvae. The Shaffer laboratory maintains a colony of tiger salamanders including parental *A. californiense, A. t. mavortium* and F1 hybrids. During the year, the salamanders' indoor temperatures are synchronized with real burrow temperatures measured at Jepson Prairie where a large *A. californiense* population resides. In December animals began to come into breeding condition and in January 2007 we introduced single females and up to 4 males into outdoor breeding mesocosms (separate from experimental mesocosms) according to a breeding design that would generate multiple directions of each cross for the first 2 generations (i.e., FI, F2, backcrosses to each parental species) and both parental crosses. We monitored breeding mesocosms for egg deposition and transferred embryos to the animal rooms when they were found, where they were treated identically to wild-caught embryos. Treatment animals were ultimately derived from the following total number of family crosses: NCTS-2; BN-4; F1–2; F2–2; BI-3; IBTS-2.

Hatching and Larval Development. Field-collected *Ambystoma* hybrids hatched between 13 March and 23 March. Captive-bred *Ambystoma* hatched as follows: NCTS: 13–27 February; BN: 1 February through 8 March; F1: 8–21 March; F2: 21 February through 13 March; BI: 22 February through 1 April; IBTS: 9 March through 9 April. *Taricha* larvae hatched 29 March through 4 April. *Ambystoma* larvae were housed at either 16 °C or 19 °C depending on hatch date to standardize sizes upon introduction to mesocosms (cooler temperature for earlier hatchers).

We fed all salamander larvae (*Ambystoma* and *Taricha*) a combination of lab-reared brine shrimp (*Artemia spp.*) and field-collected zooplankton (primarily microcrustaceans: various branchiopod and copepod spp.). Newly hatched *Ambystoma* larvae were housed in small plastic cups and transferred to larger plastic cups when they reached 40 mm total length. *Taricha* hatchlings were grouped in batches of 5 and housed in large plastic cups of 10% Holtfreter's solution. *Ambystoma* larvae in small plastic cups were fed one 10-mL pipetter of suspended zooplankton per day, and larvae in larger plastic cups were fed 2 pipetters and transitioned to blackworms (*Lumbriculus variegatus*) for 10 days before introduction to mesocosms. *Taricha* larvae are smaller—batches of 5 were fed 2 pipetters full of zooplankton per day.

Larval Collection. The highly unusual winter season in central California made embryo collection in native ponds extremely difficult. A range-wide drought resulted in limited breeding and embryos from the first and most significant round of breeding were largely destroyed by a severe winter freeze, and in many localities a second round of breeding did not occur. We were able

to collect hatched larvae from 4 ponds in Solano and Monterey Counties on March 16, March 20, and March 28, and used these in our experiments. Based on previous observations of early larval growth rates, we estimate that our collected larvae were ≈4–8 days old, with most measuring 20–30 mm total length. We transported larvae in bags of natural pond water to the Haring Hall animal room facility, and gradually transitioned them from natural pond water to 10% Holtfreter's solution over 2 days. We housed all larvae in individual plastic cups at either 16 °C or 19 °C depending on their size relative to wild-collected hybrid and lab-reared larvae.

Later in the season we also collected hatched *Pseudacris* tadpoles from natural ponds in Contra Costa and Alameda Counties for later introductions into mesocosms. We collected tadpoles by seine and transported them in plastic bags of natural pond water and grouped in batches of fifty per bag for transport and immediate release into mesocosms. We added tadpoles sequentially over the course of the experiment. Introduction dates and numbers were as follows: March 25 (8 tads per pond; noted above), April 27 (10 tads/pond), May 3 (15 tads per pond), May 10 (10 tads per pond), May 15 (20 tads per pond), May 25 (12 tads per pond), June 1 (25 tads per pond).

Introduction to Mesocosm Tanks. We massed and measured all *Ambystoma* larvae before introduction to mesocosms. We excluded unusually large and small larvae from our population of experimental animals, and any particularly early or late hatchlings. We randomized experimental animals within treatments. *Taricha* larvae were uniform in size (relative to our ability to measure), so were not massed and measured. We included equal numbers of larvae from each collection pond in our randomized population of experimental animals. To prevent cannibalism during introductions, each *Ambystoma* larva was transported in an individual plastic bag of Holtfreter's solution to our experimental mesocosm array on the campus of the University of California Davis on 16 April. *Taricha* larvae were likewise transported 2 days later in groups of twenty-five per bag. (Although cannibalism can occur in newt larvae, it is very infrequent in small larvae and was not observed. Each bag was checked before release into experimental mesocosms to assure that no newt larvae had perished or been injured due to cannibalism.) Once at the experimental array, we floated each bag of larvae in their mesocosm for 1 h to allow laboratory water temperatures to adjust to pond water temperature. We then exchanged half of the Holtfreter's solution in the bag with mesocosm pond water and left animals to adjust in their bags for another 6 h, after which we released them into the mesocosms. (An extra water change was conducted for mesocosm experiment 1, after which animals were allowed to adjust for an additional 4 h.) Introduction into the ponds constituted the beginning of the experiment.

Monitoring. We monitored mesocosms daily and conducted full counts of *Ambystoma* in each mesocosm biweekly. In addition to the *Pseudacris* additions mentioned above, we added California blackworms (*Lumbriculus variegatus*) on 2 occasions as an additional foodbase as zooplankton and *Pseudacris* were depleted: June 7 (60 mL) and June 15 (60 mL).

As animals approached metamorphosis, we conducted *Ambystoma* counts semiweekly until finding the first metamorphs. Once animals began metamorphosing, we monitored ponds daily for metamorphs for the remainder of the experiment. Our counts primarily focused on *Ambystoma* because *Taricha* are small, camouflaged and hard to detect in mesocosms before changes in color and skin texture associated with metamorphosis, which make them far more easily visible for the week preceding metamorphosis and afterward. So although we only estimated *Taricha* during the majority of the experiment, we did keep careful counts during the final weeks of the experiment approaching metamorphosis. We likewise estimated *Pseudacris* numbers during the majority of the experiment but monitored carefully for metamorphosing animals from May onwards.

1. Alvarez JA (2004) Use of artificial egg laying substrate to detect California tiger salamanders (*Ambystoma californiense*) *Herp Rev* 35:45–46.

2. Fitzpatrick BM, Shaffer HB (2007) Introduction history and habitat variation explain the landscape genetics of hybrid tiger salamanders. *Ecol Apps* 17:598–608.

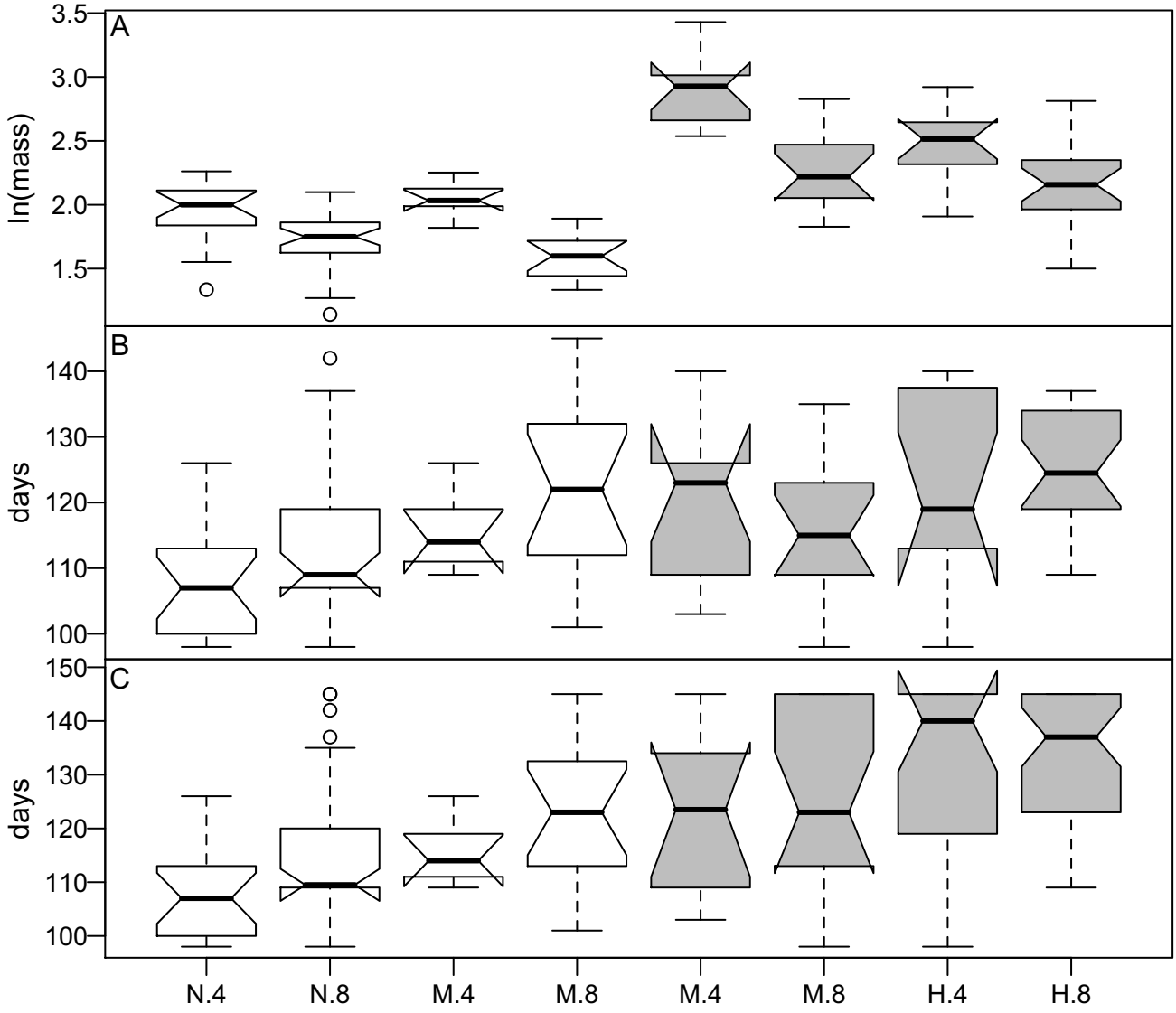

Fig. S1. Experiment 2: NCTS and hybrid responses. Plots in white are NCTS; plots in gray are hybrids. NCTS in plots A&B are the same data shown in Fig. 6. A) Gray plots: hybrid log mass was best described by the full model including the effects of density, genetic composition and their interaction (AIC = 71.17, test vs. null likelihood ratio test: $\chi^2 = 36.904$, df = 3, $P < 0.00001$). B) Gray plots, censored data (metamorphs only): hybrid time to metamorphosis was significantly influenced by genetic composition (AIC = 429.29, test vs. null likelihood ratio test: $\chi^2 = 5.1732$, df = 1, $P = 0.0229$). C) Uncensored data (metamorphs + non-transforming individuals). White plots: NCTS data are identical to those shown in Fig. 6B with the exception of 2 outliers, which were sick animals unable to metamorphose from treatment N.8. Gray plots: hybrid time to metamorphosis was best described by the model including only genetic composition ($\chi^2 = 7.50$, df = 1, $P = 0.0062$).

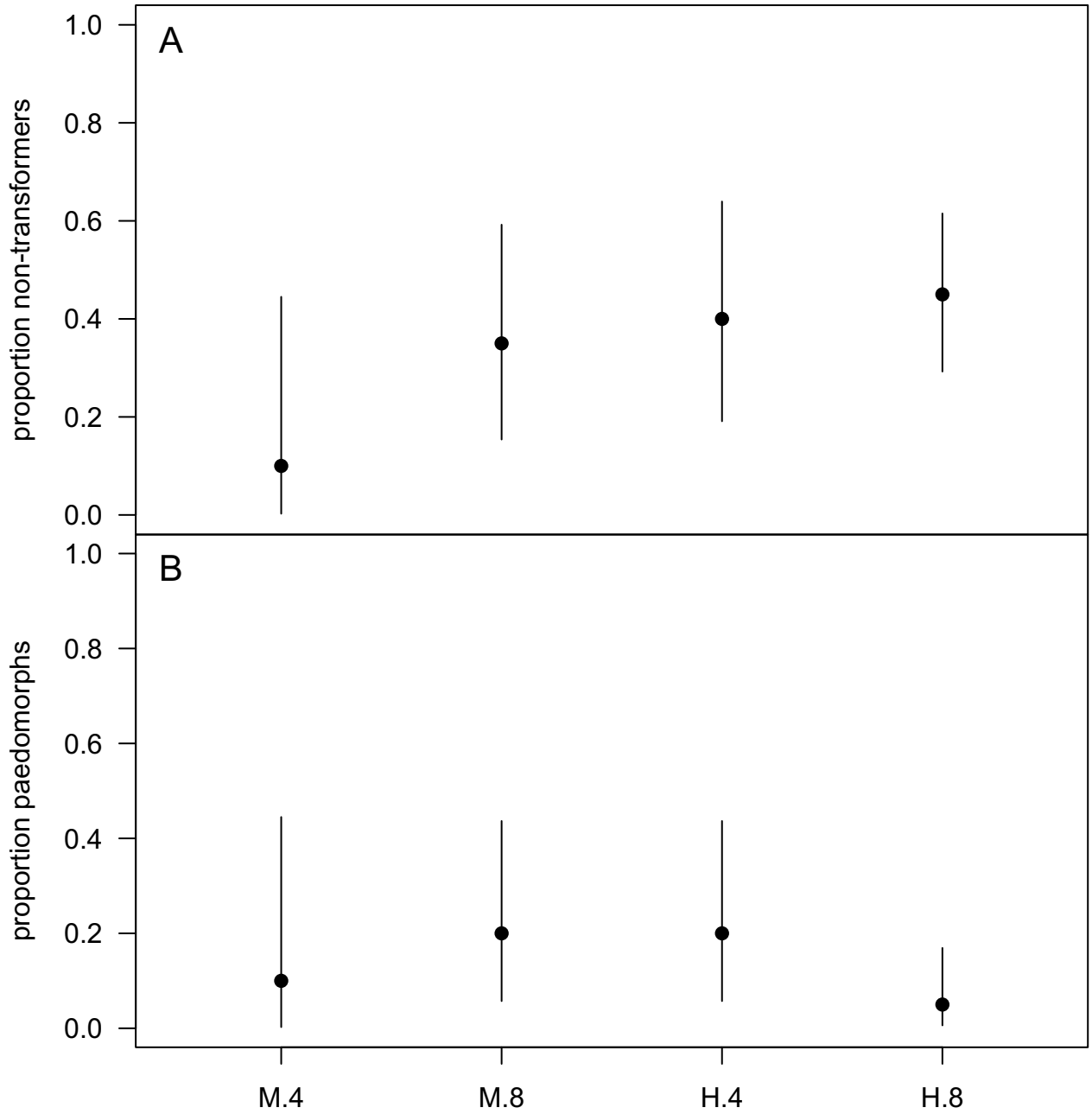

Fig. S2. Proportion of hybrid nontransforming individuals and putative paedomorphs by treatment type. M.4 and M.8 refer to low and high density mixed tanks respectively. H.4 and H.8 refer to low and high hybrid-only tanks. Nontransforming individuals include both paedomorphs and animals that were not able to metamorphose in time due to genetic, competitive or other environmental constraints. We considered larvae that exceeded the grand mean for size (12.01 g) to be putative paedomorphs for purposes of this graph, recognizing that this is a somewhat arbitrary cut-off. Data show a trend toward increasing numbers of nonmetamorphosing animals with increases in overall tiger salamander density and hybrid relative density, and humped distribution of paedomorphs. However, there was no significant overall effect of treatment on nonmetamorphosis or paedomorphosis.

Table S1. Experiment 1: Model comparison for tiger salamander cross type responses

	Cross type survival	Ln(mass)	Ln(days to metamorphosis)
Model	AIC	AIC	AIC
Null	198.66	113.777	−68.933
Treatment (cross type)	163.25	45.972	−95.116

For the best fitting model (treatment), Shapiro–Wilk test (normality) for mass was not significant ($W > 0.95$) ($W = 0.9619$, $P = 0.001534$); Levene test (homogeneity of variances) for mass was significant ($P = 0.02329$). Shapiro–Wilk and Levene tests for time to metamorphosis were both significant (Shapiro–Wilk $W = 0.9137$, $P < 0.00001$ and Levene $P = 0.008479$). Although modal time to metamorphosis was very similar among cross types, NCTS and BN had slightly negative skew, whereas F2, BI and IBTS had strongly positive skew. F1 hybrids tended to have the shortest time to metamorphosis with a roughly symmetrical distribution. Tests of normality and homogeneity of variances fail due to this heterogeneity of distributions among cross types, which could not be captured by any model assuming a consistent error function. A similar problem existed for mass.

Table S2. Experiment 1: Model comparison for native California Tiger Salamander responses

	NCTS survival	Mass	Days to metamorphosis
Model	AIC	AIC	AIC
Null	175.309	434.43	945.05
Treatment (cross type)	169.432	422.87	913.06

For the best fitting model (treatment), Shapiro–Wilk and Levene tests for NCTS mass and time to metamorphosis were not significant (mass: Shapiro–Wilk $W = 0.9551$ and $P = 0.0002516$, Levene $P = 0.2359$; time to metamorphosis: Shapiro–Wilk $W = 0.9675$ and $P = 0.002969$, Levene $P = 0.651$).

Table S3. Experiment 1: Model comparison for impacts of cross-type on third party species

	Frog survival	Newt survival
Model	AIC	AIC
Null (block only)	407.46	246.553
Null (block + cross type survival)	334.30	268.743
Treatment (cross type)	239.87	101.170
Treatment (cross type) + Cross type survival	156.78	98.067

Error is Poisson-distributed.

Table S4. Experiment 1: Model comparison for generation means analysis for predation on frogs and newts

	Frog survival	Newt survival
Model	AIC	AIC
Null (block only)	407.46	246.553
Null (block + cross type survival)	221.013	213.558
Additive	203.93	141.095
Dominance	180.68	125.335
Additive x additive epistasis	159.81	113.559
Dominance x dominance epistasis	160.55	110.216
Full	162.31	103.170

Each model includes all lower order effects. Error is Poisson-distributed.

Table S5. Experiment 2: Model comparison for native California Tiger Salamander responses

	Mass	Days to metamorphosis
Model	AIC	AIC
Null	293.13	590.96
Density	262.53	583.89
Genetic composition	295.12	588.42
Density + Genetic composition	264.50	581.40
Full	260.17	583.32

Shapiro–Wilk and Levene tests were not significant for NCTS mass or time to metamorphosis (mass: Shapiro–Wilk $W = 0.9885$ and $P = 0.7269$, Levene for density $P = 0.3284$, for genetic composition $P = 0.1231$; time to metamorphosis: Shapiro–Wilk $W = 0.9587$ $P = 0.01537$, Levene for density $P = 0.07802$, for genetic composition $P = 0.5754$). Results for time to metamorphosis in this analysis are concordant with survival analysis using time to metamorphosis as an endpoint.

Table S6. Experiment 2: Model comparison for hybrid responses

	Ln(mass)	Days to metamorphosis (censored)	Days to metamorphosis (uncensored)
Model	AIC	AIC	AIC
Null	102.079	432.46	736.37
Density	78.798	434.44	737.78
Genetic composition	98.263	429.29	730.87
Density + genetic composition	73.227	431.22	732.27
Full	71.174	432.04	734.24

For mass (ln(mass)), Shapiro–Wilk test was not significant ($W = 0.9877$, $P = 5734$); Levene test for density was not significant ($P = 0.68$), but was significant for genetic composition ($P = 0.03207$) for similar reasons as described in Table S1. Shapiro–Wilk and Levene tests were not significant for time to metamorphosis when we eliminated censored individuals, i.e., those who did not metamorphose before the end of the experiment (Shapiro–Wilk $W = 0.9837$, $P = 0.6591$; Levene for density $P = 0.3811$, for genetic composition $P = 0.6254$). Genetic composition was also the preferred model for uncensored data, but data strongly violate assumptions of normality and homogeneity of variances.

Table S7. Experiment 2: Model comparison for impacts on third party species.

	Frog survival	Newt survival
Model	AIC	AIC
Null (block only)	453.12	218.904
Null (block + tiger salamander survival)	412.34	234.303
Density	448.67	217.135
Genetic composition	344.49	88.546
Density + genetic composition	340.04	86.777
Full	282.41	78.945
Full + Tiger salamander survival	200.70	80.484

Error is Poisson-distributed.