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Supplemental Information

Ecological Impacts of Reverse Speciation in Threespine Stickleback

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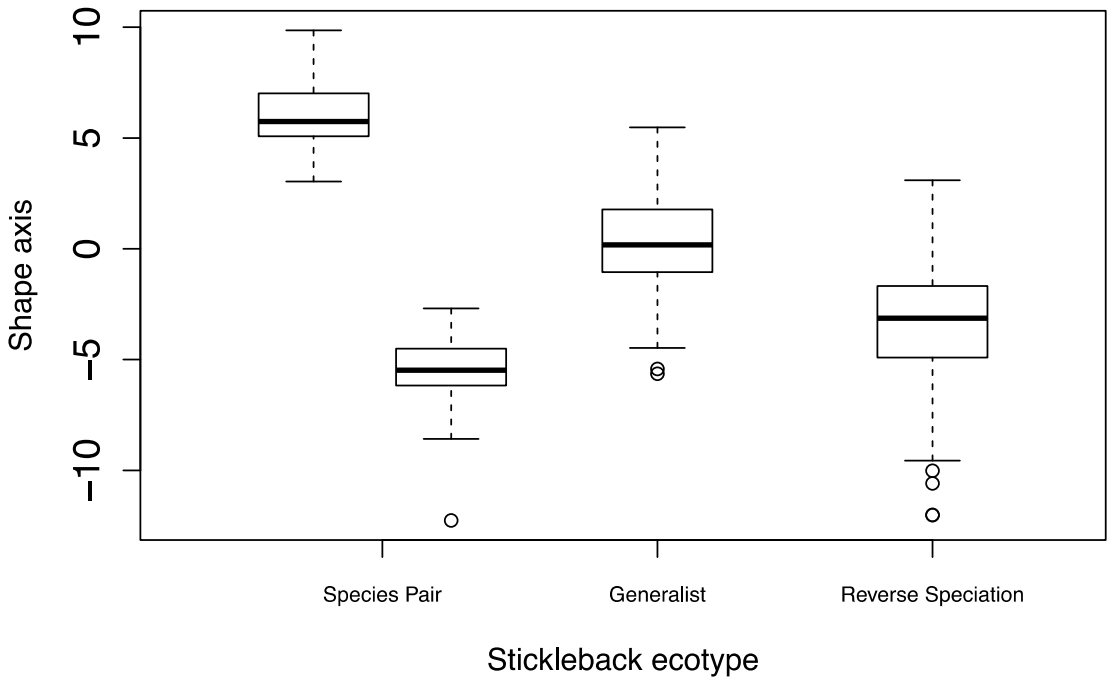


FIG. S1 (*related to Fig. 1*): Body shape scores of stickleback in experimental treatments. Scores are derived from a discriminant function maximally separating benthic and limnetic ecotypes.

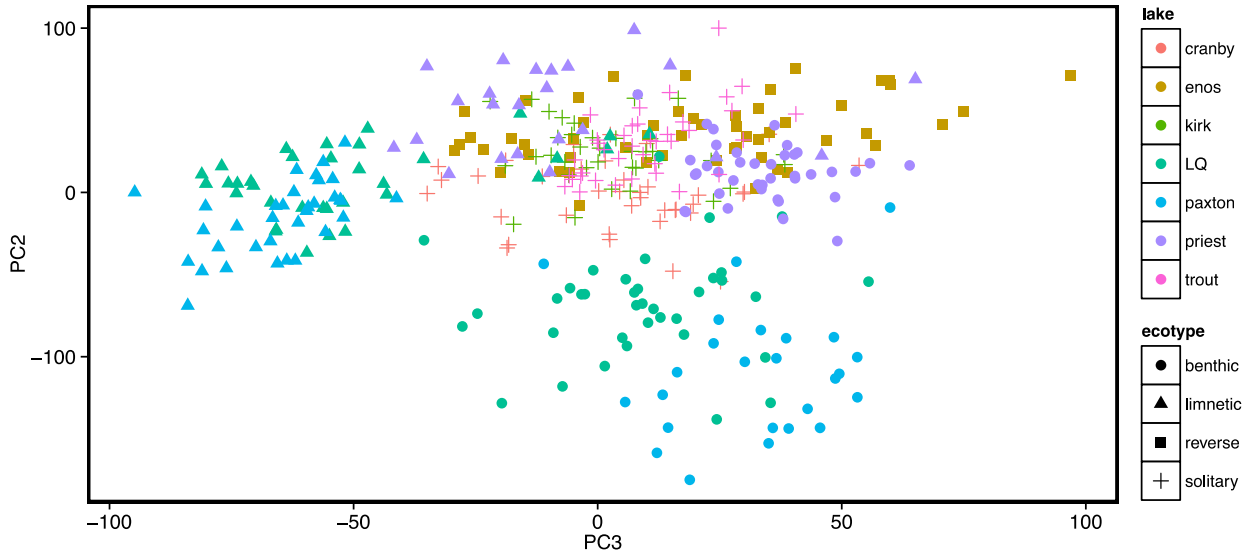
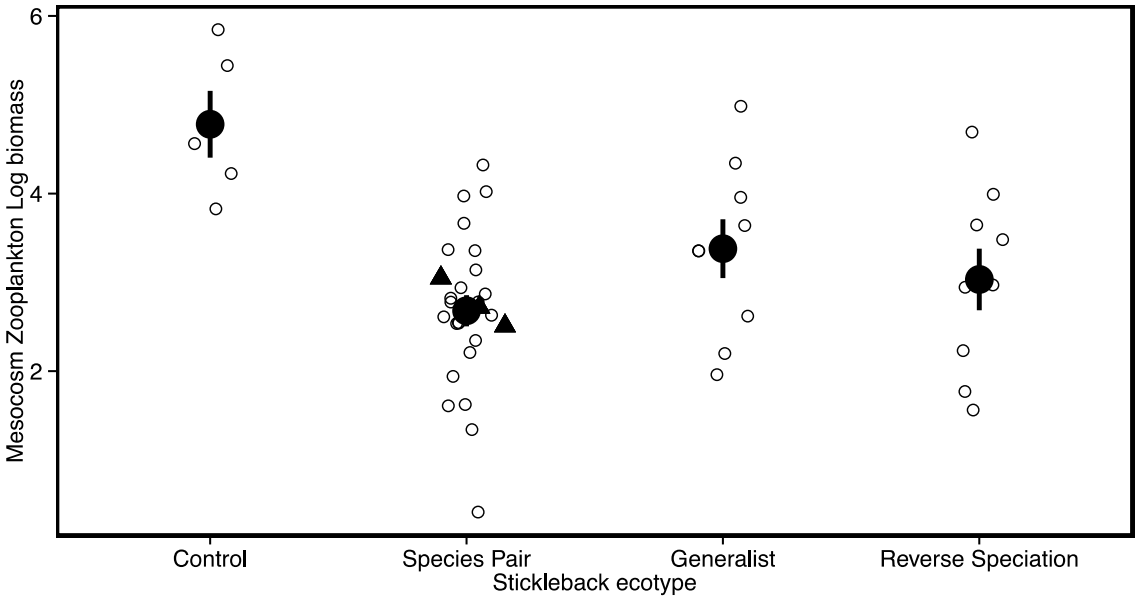


Fig. S2 (*related to Fig. 1*): The two principal components that best accounted for variation in shape (after accounting for specimen bending). Fish from all populations used in the mesocosm experiment and the lake sampling are represented here.

A)



B)

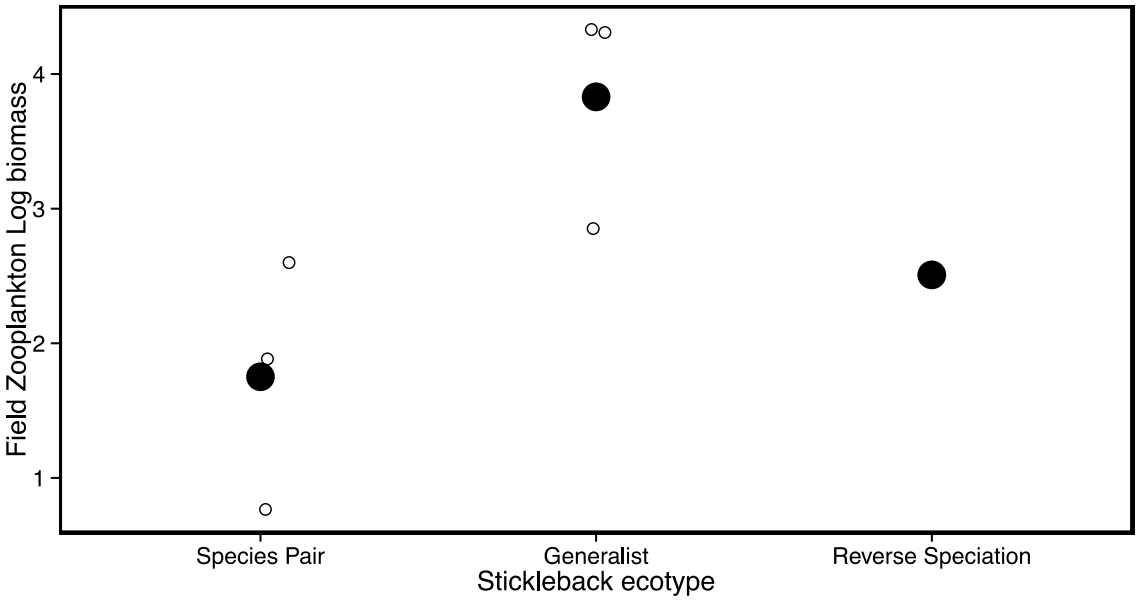


Fig. S3 (*related to Fig. 4*): The biomass of zooplankton in: A) experimental mesocosms at the end of the study and B) May field zooplankton samples demonstrating a trend towards zooplankton reduction when species pairs of stickleback are present. Biomass is corrected to the same volume of water per sample (11L) for both field and mesocosm samples.

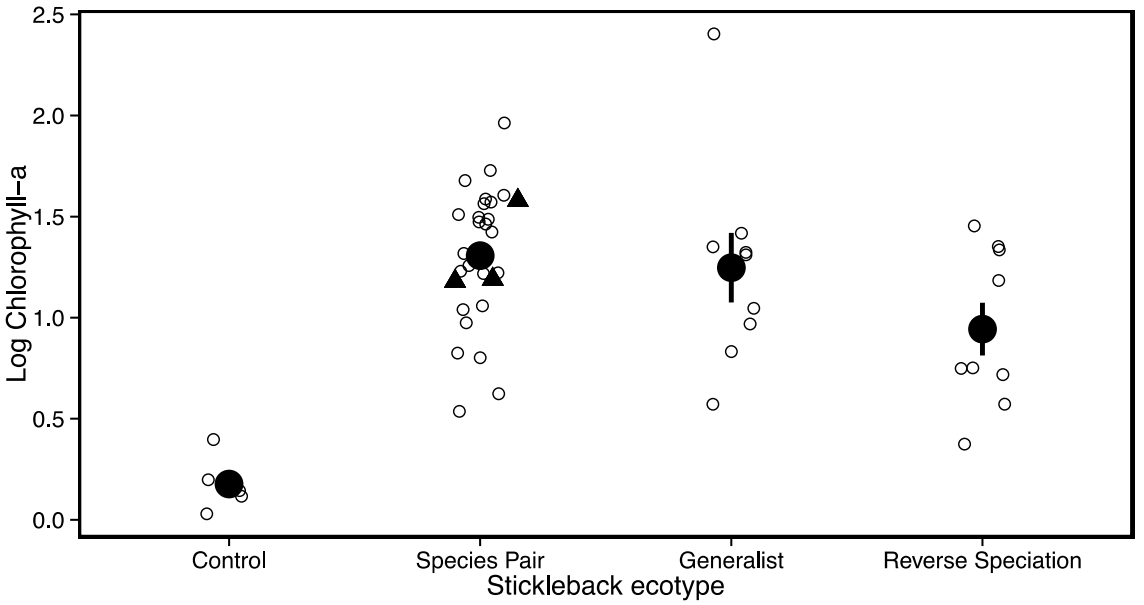


Fig. S4 (*related to Fig. 4*): The effects of stickleback ecotype on the phytoplankton concentration as measured by chlorophyll-a concentration at the end of the mesocosm experiment. Open circles are measurements of individual tanks. Filled circles are means of each treatment (shown with ± 1 standard error). Triangles are the mean for each of the three extant species pairs.

Table S1 (*related to Fig. 2*): The abundances of the nine most common zooplankton taxa in each of the seven lakes sampled in May and August 2012. Each sample contains 1/16th of the total from two 5 m and one 10 m vertical zooplankton tows.

Zooplankton species abundances in May 2012

Taxa	Species Pair			Generalist			Collapse
	Little Quarry	Paxton	Priest	Cranby	Kirk	Trout	Enos
Bosmina	43	0	12	12	33	9	913
Calanoid copepod	54	12	132	87	104	233	67
Calanoid copepodid	7	0	0	107	114	81	62
Chydorus	0	4	0	0	0	0	20
Copepod nauplii	555	335	463	891	714	175	440
Cyclopoid copepod	19	662	126	87	1088	384	190
Daphnia	2	11	164	4	28	74	8
Diaphanosoma	2	5	4	0	21	18	0
Holopedium	0	0	3	0	432	373	0

Zooplankton species abundances in August 2012

Taxa	Species Pair			Generalist			Collapse
	Little Quarry	Paxton	Priest	Cranby	Kirk	Trout	Enos
Bosmina	15	21	1	1	8	18	1911
Calanoid copepod	85	157	496	1489	176	387	239
Calanoid copepodid	55	38	53	97	9	58	7
Ceriodaphnia	18	293	39	45	17	6	784
Copepod nauplii	667	938	427	2506	763	2624	266
Cyclopoid copepod	79	645	33	45	470	255	165
Daphnia	1	19	12	23	78	303	0
Diaphanosoma	78	163	1495	97	704	352	187
Holopedium	0	0	0	0	168	71	0

Table S2 (*related to Fig. 2*): Abundances of zooplankton species found in the stomachs of 40 benthic and 40 limnetic stickleback collected from Enos lake in 1988. Stickleback were collected using seines and minnow traps which were checked hourly to minimize prey digestion. For additional details on the sampling see [S4].

Taxa	Stickleback ecotype	
	Benthic	Limnetic
Bosmina	0	39
Calanoid copepod	1	80
Chaoborus	0	1
Chydorus	34	6
Unknown cladoceran	6	3
Calanoid copepod	1	18
Cyclopoid copepod	28	36
Daphnia	8	9
Harpacticoid copepod	14	9

Supplemental Experimental Procedures

Morphometric methods

Fish from all populations were collected at a single time point using minnow traps. We collected fish from each independently evolved species pair, the reverse speciation population of Enos Lake, and three generalist populations. Generalist populations were chosen based on four criteria: 1) the fish community had to match that of species pairs lakes which contain only stickleback and cutthroat trout 2) they had to roughly similar in size 3) they had to be at a similar elevation to the species pair lakes and Enos lake (between 30m and 100m) 4) they had to be within a 30km radius of the species pair lakes and Enos Lake. Once caught, fish were euthanized with a lethal dose of MS-222 and placed in 95% ethanol. Specimens were transferred from ethanol to formalin using a "step-down" dilution procedure (i.e. 75% ethanol, 50% ethanol, 25% ethanol, water, 10% formalin) and then stained with alizarin red to facilitate the identification of body landmarks.

To determine the position of each fish on a benthic to limnetic continuum, we used a discriminant function analysis to identify an axis that best differentiated between benthic and limnetic individuals from extent species pair populations. We randomly sub-sampled half of the species pair fish from each population to create this axis, and then we tested the ability of this axis to differentiate between benthic and limnetic ecotypes using the other half of the species pair fish. The axis correctly identified all benthic and limnetic individuals from the second subset. We then used this axis to score each fish from each population on this benthic to limnetic continuum (shown in Fig. S1). Upon visual inspection of these data, we ran

planned contrasts to determine whether Enos Lake fish differed in their mean position on this axis compared to species pairs and generalist fish. We produced a principal components analysis to visualize the morphometric differences. We ran a generalized procrustes analysis in the 'shapes' package [S1] that uses rotation, translation, and scaling to optimize landmark configuration. We obtained the first 5 principal components and visualized the shape change associated with each. PC1 represented specimen bending, so here we plot PC2 and PC3 [Fig. S2].

Field zooplankton collections

Zooplankton samples were collected with a 30cm mouth diameter, 120cm long simple plankton net composed of 80μm mesh. Samples were stored in 70% ethanol. Before counting, samples were sub-sampled to $1/16^{th}$ using a Folsom plankton splitter (Aquatic Research Instruments, Hope, ID, USA) and stained with rose bengal. Zooplankton were identified to the lowest readily recognizable taxonomic unit and the length of at least the first 20 individuals of each group was measured.

Mesocosm experiment

Fish collection and stocking

Fish were collected in the spring and held in the lab for at least one week prior to the start of the study. We selected adult stickleback that were not in obvious breeding condition (i.e. not gravid or showing male nesting coloration). We did not sex fish present in the mesocosms. Sex could have introduced experimental noise if sex ratios differed between replicates.

Community responses

To examine the zooplankton community within the mesocosms, we collected vertical columns of water using a PVC pipe (10 cm diameter × 110 cm length) with care taken to sample water from the full depth of each mesocosm. A total of 11 liters of water was taken from each tank and sieved through 66 μm mesh. Samples were stored in Lugol's iodine solution to preserve and stain the specimens before counting. Each individual in each sample was counted, measured, and identified to the lowest readily identifiable taxonomic unit. We used published length-weight regressions to estimate dry biomass from each sample (i.e. μg/11L), which is shown in figure S3 [S2]. To estimate total phytoplankton abundance, we measured the amount of Chlorophyl-a (CHLA) *in vivo* using a fluorometer (Turner Designs, Sunnyvale, CA, USA) in each mesocosm. 50ml samples were taken at 5cm depth, placed in a cooler, and analyzed immediately (Fig. S4). To sample the benthic invertebrate community, we used a small dip net to collect two 120 cm^2 scoops from different areas of the benthic substrate within each mesocosm [S3]. These samples were then combined and searched for live organisms by two people working simultaneously for ten minutes (i.e. a total of 20 search minutes). Collected specimens were placed into 95% ethanol and were then counted and identified to the lowest feasible taxonomic unit. We analyzed chironomids separately from all other benthic invertebrates. Emerged insects were collected using floating traps built from no-see-um netting on top of mesocosms. Traps had an opening of 40cm and were set out mid-day. The following day, traps were emptied with a modified hand vacuum (BioQuip Products, Rancho Dominguez, CA, USA) to remove all emerged insects. All insects were transferred to vials containing 95% ethanol and were later identified to the lowest taxonomic unit and measured. Mesocosms were surveyed daily for dead or dying stickleback, which were removed and

replaced within 12 hours. Stickleback mortality was 16%; ANOVA revealed no significant differences between stickleback treatments in mortality ($f=0.26$, $df=2$, $p=0.77$).

Ecosystem responses

Tank-level gross primary productivity (GPP) was estimated at 3-4 week intervals throughout the experiment using diurnal changes in oxygen levels [S2, S3]. Dissolved oxygen measurements were taken with an oxygen probe (YSI, Pro ODO2030, Yellow Springs, OH, USA) three times per sample period: at sunrise (T0), sunset (T1), and the following sunrise (T2). Net primary productivity was calculated as $NPP= DO_{T1}-DO_{T0}$, respiration as $R= DO_{T1}-DO_{T2}$, and finally GPP as NPP+R. Decomposition was measured by placing two 25mm filter papers and 0.75 grams of alder leaves (collected during the fall and dried) into a mesh bag. At the end of the study the bag was removed and the remaining paper and leaves were dried and weighed to calculate decomposition. 200ml water samples were collected at a depth of 10 cm at the end of the study to measure dissolved organic carbon (DOC). Water was filtered through 0.45 μm membrane filters and analyzed with a TOC analyzer (Shimadzu Corp., Kyoto, Japan). We measured photosynthetically active radiation (PAR) using a LI-193 (LI-COR Biosciences, Lincoln, Nebraska, USA). We took measurements at 5cm and 28cm on a day with full sun. We calculated the amount of light extinguished per centimeter as $PAR_{28cm}-PAR_{5cm}/23cm$.

Analysis of data

All community and ecosystem response variables from the mesocosm study were first visualized and tested for normality (Shapiro test). Any data that deviated significantly from normal distribution were log-transformed before analysis. We treated contrasts between both extant species pairs and generalist populations with the Enos Lake population as planned contrasts, with the lake of origin treated as a fixed effect due to limitations in the number of independent origins of species pairs.

Supplemental references

S1. Dryden, I.L (2014). shapes: Statistical shape analysis. R package version 1.1-10. http://CRAN.R-project.org/package=shapes.

S2. Watkins, J.M., L. G. Rudstam and K. T. Holeck. (2011). Length-weight regression for zooplankton biomass calculations- a review and a suggestion for standard equations. Available at eCommons Cornell (http://ecommons.library.cornell.edu/handle/1813/24566). Accessed May 2013.

S3. Ingram, T., R. Svanbäck, N. J. B. Kraft, P. Kratina, L. Southcott, and D. Schluter. 2012. Intraguild predation drives evolutionary niche shift in threespine stickleback. Evolution 66: 1819–1832.

S4. Wetzel, R. G. and Likens, G. E. (1991). Limnological analyses. Saunder College Publishing, New York, NY, second edition.

S5. Schluter, D. & McPhail, J.D. Ecological character displacement and speciation in sticklebacks. *Am Nat* **140**, 85-108 (1992).