SUPPLEMENTARY INFORMATION

1 Schematic overview

We present a figure summarizing the trophic relationships of generalist, benthic, and limnetic sticklebacks (Figure S1). Each form has a distinct diet, with limnetics specialized on pelagic zooplankton, benthics on littoral invertebrates, and generalists feeding on both. The figure also summarizes our two main hypotheses: H1 that sticklebacks would cause the strongest cascades in their preferred foraging habitats, leading to higher levels of primary productivity with increasing predator specialization (G < B,L < BL), and H2 that distinct types of sticklebacks might differ in their engineering effects on ecosystems.

2 Experimental Design

2.1 Mesocosm construction

We constructed artificial pond mesocosms in 1136 L Rubbermaid stock tanks 2 m in diameter with a depth of 1.0 m. We added $\sim$ 40 L of a mixture of sand and gravel to the bottom of each tank to a depth of $\sim$ 5 cm. When filling tanks, we sprayed water into the tanks from a hose so to maximize chlorine water loss to the air. We filled the tanks to approximately 20 cm below the rim and let the water sit for one week. We fertilized the tanks to boost initial primary productivity with 2.46 g $NaNO_3$ and 0.18 g NaH_2PO_4 per tank. Finally, we collected and mixed benthic substrate from both Cranby and Paxton lakes on Texada island, and added equal amounts ($\sim$ 5 L) to each tank. These sediments included propagules for a wide range of benthic and pelagic organisms. After adding the substrate, we waited $\sim$ 1 week before adding the fish to the tanks.

2.2 Addition of fish to mesocosms

We collected fish from natural populations and moved them into the lab, where they were kept for at least 1 week. We added fish appropriate to each treatment so that the total mass of fish in each tank was between 5 and 6 g; total mass of fish did not differ among treatments (ANOVA, $F_{3,36} = 0.2$, P = 0.9). We also measured the standard length of each fish before adding it to the mesocosms. We transported fish to mesocosms in plastic bags, and acclimated them to the new environment by slowly mixing water from the mesocosm with water from the lab tanks. We then surveyed tanks $\sim$ 4 times per week for dead fish. When dead fish were found, we measured their standard length, and were able to identify which of the original fish had died; we then selected new fish to maintain the proper mass for that tank. New fish were collected from the natural ponds at two other times during the experiment to maintain lab populations for restocking.

2.3 Statistical analyses

Biomass estimates were unavailable for benthic invertebrates. To convert length measurements into an index of biomass, we measured the length of each individual, and cubed this measure. We then summed these indices for all individuals of a given category for each tank. This index should be approximately proportional to the total biomass of that category in that tank.

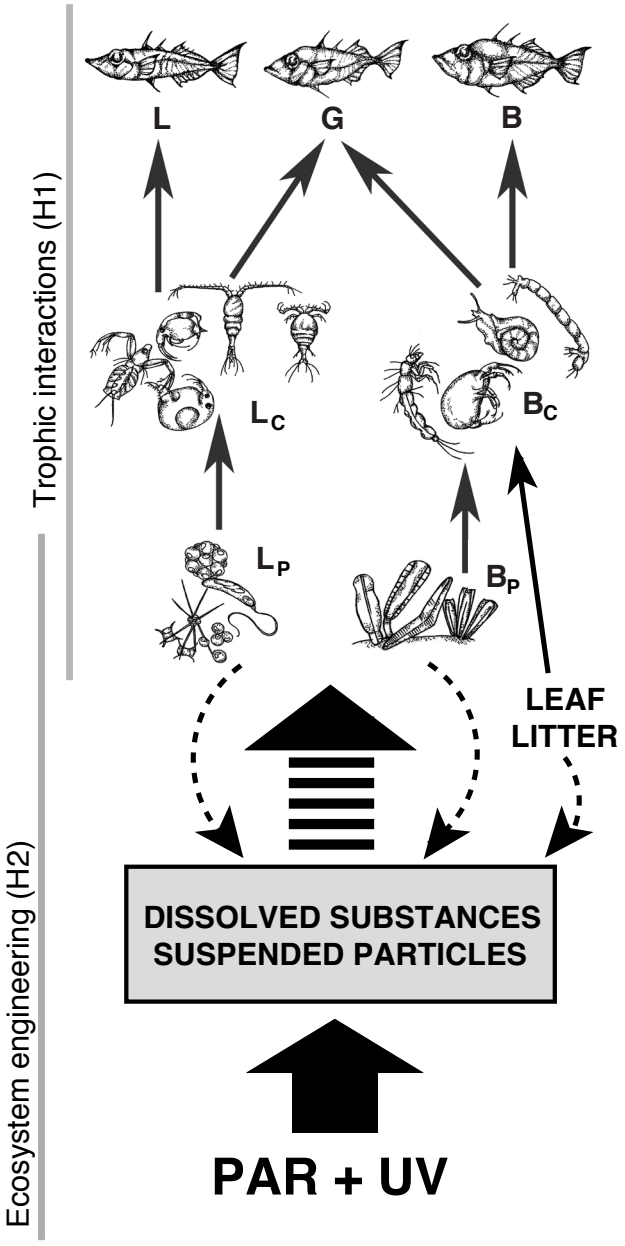

Figure S1: Hypothesized trophic interactions between fish, consumers, and producers in experimental mesocosms. L = limnetic stickleback, G = generalist stickleback, B = benthic stickleback, Lc = limnetic consumers, Bc = benthic consumers, Lp = limnetic producers, Bp = benthic producers, PAR = photosynthetically available radiation, UV = ultraviolet light. H1 and H2 denote the two main hypotheses that we tested for the effects of stickleback diversification on ecosystem function. Solid arrows represent trophic links, large dashed arrows indicate the attenuation of light by dissolved substances in the water, and small dashed arrows indicate sources of these dissolved substances.

To analyze data collected across multiple time points in the course of the experiment, we used profile analysis (PA). PA is an alternative to repeated-measures ANOVA that is particularly well-suited to time series data (Tabachnik and Fidell, 2006). In PA, the response variables are considered as profiles, here made up of a particular time series for one dependent variable. PA then involves three tests, each equivalent to a standard test from ANOVA: flatness, which tests the null hypothesis that all profiles show no change through time (described as the "time" effect in the main text); levels, which tests the null hypothesis that profiles do not differ in the average of the dependent variable among treatments (described as the "treatment" effect in the main text); and parallelism, which tests the null hypothesis that profiles of each treatment are parallel to each other (described as the "time*treatment" effect in the main text). We evaluated the statistical significance of each effect using F-distributions or MANOVA, following Tabachnik and Fidell (2006).

3 Ecosystem properties and functions

The functioning of our tank ecosystems was estimated by making weekly measurements of gross primary productivity (GPP), net primary productivity (NPP), and respiration (R), and, at the end of the experiment, by estimating the net rate of macrophyte production (MP), and the rate of leaf decomposition (DEC).

The biological properties of our tank ecosystems were estimated by measuring the zooplankton (ZP) and zoobenthos (ZB) community structure at the end of the experiment, and by making weekly measurements of pelagic algae (PA) and benthic algae (BA) concentrations (using Chlorophyll-a extractions).

We assessed the chemical properties of our tank ecosystems bi-weekly, by measuring total dissolved phosphorus (TDP), dissolved inorganic nitrogen (DIN), and ammonium (NH_4^+). In the last week, we measured the following physical properties, including: the extinction coefficient (k) of photosynthetically active radiation (PAR), absorption coefficients (a_λ) of colored dissolved organic matter (CDOM), and used scanning florescence spectroscopy to estimate the molecular size distribution (MSD) and fulvic acid concentration (FA) of DOC.

3.1 Ecosystem functions: GPP, NPP, R, MP, DEC

3.1.1 GPP, NPP, and Respiration

Gross and net primary productivity (GPP and NPP) and respiration (R) were estimated using diurnal changes in oxygen levels (Wetzel and Likens, 1991; Downing, 2005). Dissolved oxygen (DO) measurements were taken with an oxygen probe (YSI, Model 58) three times per day: at sunrise (t_o), sunset (t_1), and the following sunrise (t_2). This scheme has been shown sufficient to capture variation in productivity among treatments in other similar mesocosm experiments (Wetzel and Likens, 1991; Downing, 2005). NPP was measured as the $DO_{t_1\text{-}t_0}$, R as $DO_{t_1\text{-}t_2}$, and GPP as NPP+R. These measurements were made weekly (N=10 weeks).

3.1.2 Macrophyte Production (MP)

In lakes, dense macrophyte beds can be a significant source of DOC, reduce levels of phytoplankton growth, and influence the composition of zooplankton. To investigate the

growth of macrophytes in our tanks we harvested all the above sediment biomass of rooted macrophytes at the end of the experiment. The total production of macrophyte dry weight varied from 0-0.037 g/day, but did not differ among treatments ($F_{3,36}$= 0.16, P= 0.92).

3.1.3 Decomposition (DEC)

We measured the rate of decomposition of dried dandelions leaves over 19 days, from the middle to end of the experiment. We put 0.50 g (SD=0.003, N=40) of dried (60°C for 24 hrs) leaves in coarse mesh bags (4 mm hole diameter) and measured the change in the dry weight of the leaves. The leaves decomposed at an average rate of 0.013 g/day (SD=0.003), but this rate did not differ among treatments ($F_{3,36}$=0.66, P= 0.58).

3.2 Biological ecosystem properties: ZP, ZB, BA, and PA

3.2.1 Zooplanktonic (ZP) and benthic (ZB) community structure

To examine the effects of sticklebacks phenotypes on the pelagic zooplankton and macrobenthos community, we collected samples from the tanks at the end of the experiment. We collected pelagic samples using a 10L Schindler Patalas trap, and benthic samples by collecting 1 L of sediment from four locations in the tank. We quantified the community structure of pelagic and benthic invertebrates by counting and measuring over 8000 individuals (to the nearest 0.05 mm) using a digitizing system and a Wild M5 dissecting scope equipped with a drawing tube.We calculated biomass of pelagic individuals using published length weight regression for zooplankton (Culver et al., 1985). Species, counts per treatment, and average sizes are listed in Table 1 in the main text.

3.2.2 Pelagic (PA) and benthic algae (BA)

We analyzed Chlorophyll-*a* (Chl-*a*) of PA by filtering water through GF/F filters (Whatman), extracting the filters with 95% ethanol at 4°C overnight, and analyzing the extracts on a Trilogy fluorometer (Turner Designs). We collected samples weekly throughout the experiment and averaged measurements over two week periods to compare with parameters that were measured bi-weekly (e.g. nutrients). Overall, the average concentration of Chl-*a* growth was 4.9 μg Chl-a/L (SD=3.6, N=400). Despite clear effects of sticklebacks on the composition of pelagic zooplankton community, we saw no evidence of a classic trophic cascade, whereby we would expect that a reduction in zooplankton biomass and size would lead to an increase in pelagic phytoplankton. Instead, we found that the highest algal concentration in the generalist treatment also had the highest abundance of large *Diaptomus* copepods (Table 1), and differences among treatments in the biomass of zooplankton did not correspond to differences in PA.

We measured the growth of periphyton weekly by analyzing Chl-*a* on glass microscope slides placed in the bottom of the tanks at the beginning of the experiment. Algae were removed from the slides by rinsing with distilled water and scrubbing the slide with a wire brush. We then filtered the solution onto GF/F filters (Whatman) and analyzed Chl-*a* as above. Over the course of the experiment the average algal growth was 0.032 μg Chl-a/cm_2 (SD=0.026, N=400). The lack of significant differences in BA among treatments is consistent with minor shifts in the composition of the benthic invertebrate community among treatments (Figure 2B)

3.3 Chemical and physical ecosystem properties: TDP, DIN, NH_4^+, k, DOC, a_λ, MSD, FA

3.3.1 Nutrients concentrations: TDP, DIN, NH_4^+

Total dissolved phosphorus (TDP) and NO_3^-/NO_2^- (DIN) were analyzed on a Lachat autoanalyzer (Zellweger Analytics, QuickChem ®8000), and ammonium (NH_4^+) was analyzed on a Trilogy fluorometer (Turner Designs) following (Holmes et al., 1999). Average dissolved nutrient concentrations over the entire experiment were not significantly different among treatments (MANOVA, Wilks $\lambda = 0.71$, P= 0.20; Means: TDP =1.84 μg / L, DIN= 12.5 μg / L, NH_4^+= 8.6 μg / L), and did not explain a significant amount of variation in Chl-a among tanks (Multiple regressions of the form Chl-$a \sim$ TDP+DIN+NH_4^+ resulted in all the single factors and interactions terms being nonsignificant, P > 0.05). Overall, our nutrient analyses suggest the differences in Chl-a among treatments were not due to differing levels of nutrients.

3.3.2 Attenuation of photosynthetically available radiation (PAR): k

We measured photosynthetically available radiation (PAR: 400-700 nm, μmols / s m^2) using a 4π quantum sensor (LI-COR LI-193). A light extinction coefficient (k) was calculated for each tank as the slope of the relationship between depth (x) and $\ln(\mathrm{PAR}_{x=0.1m}/\mathrm{PAR}_{x=0.6m})$, such that high k values are associated with low light penetration through the water column.

3.3.3 Concentration and composition of dissolved organic matter: DOC, a_λ, MSD, FA

Dissolved organic matter (DOM) is a complex of pool of molecules that can profoundly affect the biology and physics of aquatic ecosystems. DOM is produced in situ by primary production and also from the decomposition of allochthonous organic matter. DOM originating from different sources of organic matter, such as algae or leaves, can have a different molecular size distribution and composition, and hence, different optical properties (McKnight et al., 2001). For example, DOM produced from algae has a smaller size distribution and is more transparent to UV than DOM produced from terrestrial plants (Retamal et al., 2007). In our experiment, we used various spectroscopy techniques to determine whether treatments differed in the concentration, origin, and composition of dissolved substances. We used ultraviolet scanning spectroscopy (UV-SS) to measure the absorption of colored dissolved organic (CDOM), which has been shown to regulate the underwater spectral light regime supporting primary production (Pienitz and Vincent, 2000). We also used synchronous florescence spectroscopy (SFS) to investigate the chemical structure, complexity, and molecular size distribution (MSD) of CDOM (Chen et al., 2004; Retamal et al., 2007), as well as florescence excitation-emission matrices (FEEM) to assess the concentration and origin of fulvic acids (FA), which are an important component of DOM in freshwaters (McKnight et al., 2001). Both SFS and FEEM provide a guide to the composition of the dissolved substances in water.

Dissolved organic carbon concentration: DOC - All water samples for dissolved organic matter analysis were filtered through 0.45 μm membrane filters (Nucleopore). The concentration of dissolved organic carbon (DOC) was determined by analyzing filtered samples on a Shimadzu 5000 TOC analyzer. Water remaining from the same samples was subsequently analyzed for its optical and fluorescence properties.

Absorption coefficients of colored dissolved organic matter: a_λ - We measured absorption coefficients of our water samples in 1-cm quartz cells using a Cary 50 (Varian) UV-scanning spectrophotomer (UV-SS). Samples were scanned at 1 nm increments, and absorption coefficients for were calculated as: $a_\lambda = 2.303 * A_\lambda / L$, where A_λ is the optical density for wavelength λ and L is the cell path length in meters. We chose the absorption coefficient at 320 nm ($\alpha_{320} m^{-1}$) to compare the light environment among tanks because it is at the boundary of UV-B (280-320) and UV-A (320-400) and is a standard method to characterize the light environment of lakes (Williamson et al., 1999). Although we emphasize results at α_{320}, the absorption coefficient of across the entire UV band (280-400 nm) was significantly lower in the generalist treatment than in all other treatments (as reported in the main text; $P < 0.05$ for $\alpha_{320} - \alpha_{400}$).

Florescence estimates of the molecular size distribution of CDOM: MSD - In situ biological processes, such as primary production or zooplankton grazing, produce low molecular weight molecules (Lλ < 5000 Da) that fluoresce at low excitation wavelengths (Lλ: 220 - 323 nm). Medium weight molecules (M$\lambda \sim$ 15,000 - 30,000 Da), such as fulvic acids, originate primarily from allochthonous sources and fluoresce at higher excitation wavelengths (Mλ: 324 - 432 nm). Humic acids, which result from the decomposition of allochthonous organic mater, have a higher molecular weight (Hλ) and fluoresce at even higher excitation wavelengths (Hλ: 433 - 593 nm). We scanned our water samples in a 1-cm quartz cell on a Cary Eclipse 3000 over an excitation and emission range of 200 - 600 nm (in synchronous mode) with a 3 nm slit width on both sides following Retamal et al. (2007). The spectra were corrected for the inner filter effect using absorption and DOC data for each sample (Mobed et al., 1996; McKnight et al., 2001). Instrument accuracy was routinely checked for wavelength accuracy using the Raman peak for water. Florescence spectra were integrated over three wavelength groupings (Lλ, Mλ, Hλ) and analyzed using ANOVA.

Total fluorescence, integrated from 220-600 nm, did not differ among treatments (ANOVA $F_{3,33} = 1.94$, $P = 0.14$), which is consistent with the similar DOC concentrations among treatments. However, the composition of the DOM differed strongly among treatments. The proportion of total florescence resulting from low molecular weight molecules ($L\lambda/(L\lambda + M\lambda + H\lambda)$) varied from 22-49% among tanks, was highest in the Cranby treatment (42%), and was significantly higher in the Cranby treatment than in the Benthic treatment (33%: Tukeys HSD, $P < 0.05$; Fig 3D). The higher florescence at lower wavelengths in the Cranby treatment was also evident from an analysis of Lλ/Mλ ratios (as in Retamal et al. 2007) (ANOVA of Lλ/Mλ: $F_{3,33} = 5.14$, $P = 0.005$), suggesting that proportionally more of the DOM in the Cranby tanks originated from in situ primary production rather than from the decomposition of leaf litter and sediment organic matter. Again, this supports our hypothesis that higher algal production in the Cranby tanks resulted in a pool of DOM with a higher proportion of low molecular weight molecules, but the same total amount of carbon. Taken together, this led to the water in the Cranby tanks being more transparent to light over a wide range of wavelengths.

The SFS analysis (Retamal et al., 2007), which is essentially a cross sectional survey of the full three dimensional excitation and emission analysis (Stedmon and Markager, 2005), also revealed significant differences among treatments within the range of excitation and emission wavelengths where fulvic acids fluoresce (ANOVA of Mλ: $F_{3,33} = 3.1$, $P = 0.04$).

In particular the Benthic treatment had a higher fluorescence than the Cranby treatment (Tukeys HSD, P = 0.05), suggesting a larger size distribution of DOM in the Benthic treatment, possibly originating from more leaf litter decomposition. To investigate this further we analyzed this wavelength region in more detail using florescence excitation and emission matrices (FEEMs).

Florescence characterization of fulvic acid concentrations: FA - The origin and relative concentration of fulvic acids, which are an important fraction of total humic substances, was assessed using florescence spectroscopy (McKnight et al., 2001; Stedmon and Markager, 2005; Klapper et al., 2002). Florescence intensity (F_λ) of each sample was measured over a range of emission wavelengths (λ range: 450-500 nm) at an excitation wavelength of 370 nm (within the Mλ range). A florescence index (FI) was calculated as the ratio of $F_{\lambda:450}$/ $F_{\lambda:500}$ following McKnight et al. (2001).

Overall, the average florescence index (FI) was 1.95, and FI was similar among treatments (ANOVA, FI: $F_{3,32} = 0.36$, P = 0.78), indicating that the organic matter in all the tanks was derived mainly from microbial degradation of autochthonous sources (McKnight et al., 2001). However, the florescence intensity at two wavelengths that are associated with fulvic acid concentration both differed strongly among treatments (ANOVA, $F_{\lambda:450}$: $F_{3,32} = 5.34$, P = 0.004; $F_{\lambda:500}$: $F_{3,32} = 6.12$, P= 0.002). The fluorescence intensity of DOM (Excitation= 370 nm, Emission= 450 nm) was lowest in the Cranby treatment, and was significantly lower in the Cranby than in the species pair treatment (Tukeys HSD, P = 0.002). This indicates that larger molecular weight molecules, such as fulvic acids that absorb proportionally more UV per unit carbon, were at a lower concentration in the Cranby treatment, leading to greater light penetration through the water column. This is also consistent with our hypothesis that the DOM pool in the Cranby tanks had the highest proportion of dissolved organic carbon originating from algal production.

3.3.4 Comparison of experimental effect sizes to variation in natural lakes

The differences among treatments in light transmission of PAR (k) are large relative to the natural variation in lakes. In a recent survey (by BM and JBS) of 40 lakes in British Columbia over a very broad range of productivity (Chl-a 0.1 to 83 ug/L), k varied from 0.1 to 1.0 (mean=0.3). Our observed experimental values are well within this range (0.3 to 0.7, mean=0.5), and the differences between G and BL are 0.1, which represents about 10% of the natural variation across lakes. Hence, the speciation of stickleback could have led to a 10% decrease in the amount light that impinges on organisms in lakes. Pienitz and Vincent (2000) found that algal communities (diatoms in particular) are extremely sensitive to even smaller changes in the concentration of dissolved substances; hence we believe that our effect size is relevant to the natural lakes. Future paleolimnological work will help reveal how the long-term changes (1000s of years) in the light environment might impact the evolution of flora and fauna in these lakes.

4 Relationships among response variables

At the end of the experiment, the total amount of dissolved organic carbon (DOC) did not differ amount treatments ($F_{3,36} = 0.38$, P = 0.77; Fig 3B), but it was positively correlated with the extinction of PAR ($k = 0.25 + 0.029 * DOC$, P = 0.001, R^2=0.24). In contrast,

Chl-*a* was uncorrelated with k (linear regression, $P > 0.05$). This supports the idea that the light used by photosynthetic organisms is primarily attenuated by the dissolved substances in the water, and not by the organisms themselves. Furthermore, α_{320} was negatively correlated with Chl-*a* ($\alpha_{320} = 8.71$ 0.14 * Chl-*a*, $P = 0.02$, $R^2 = 0.14$), suggesting the light penetration was highest in tanks with more algae. This is consistent with our hypothesis that high rates of phytoplankton production produced a pool of dissolved substances that were more transparent to both PAR (400-700 nm: Fig 3A) and UV (280-400 nm: Fig 3C) radiation.

Differences in the pelagic light environment also influenced periphyton growth in the benthic environment. At the end of the experiment, periphyton was negatively correlated with light extinction (k), and tanks with benthics (B and BL) had more periphyton per unit amount of light extinction through the water, compared with either the Limnetic or Cranby tanks (ANCOVA model: Periphtyton = Treatment + k + Treatment*k; Treatment effect: $F_{3,32}$=3.40, $P = 0.03$; Interaction: $F_{3,32} = 2.39$, $P = 0.09$). This result could either be explained by higher rates of benthic grazing on periphyton in B and BL treatments, or by higher UV inhibition of periphtyon growth in C and L treatments, however the former hypothesis is less likely given slight compositional shifts in the community structure of benthic invertebrates among treatments.

Overall, our analyses revealed that the composition of dissolved substances differed strongly among treatments. Though the total amount of DOC and concentration of fluorescently active molecules was similar among treatments, the absorbance and molecular composition of those molecules differed among treatments. Cranby tanks had higher algal biomass, but the dissolved substances extinguished less phytosyntically available radiation, were less absorbent to UV radiation, had a smaller size distribution (relatively high Lλ), and likely had a lower fulvic acid concentration (low $F_{\lambda:450}$ and $F_{\lambda:500}$). These multiple lines of evidence suggest that changing the phenotype of the top predator can influence the physical light environment of aquatic ecosystems by altering the proportion of phytoplankton derived carbon in the total pool of dissolved organic matter. Based on differences in the amount of periphyton per unit of light availability among treatments, we propose that the composition of DOC can alter levels of benthic primary production by influencing the spectral composition of light that impinges on sediments.

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