# 1 **Context-dependent interactions and the regulation of species richness in freshwater fish** 2 MacDougall, A.S. et al.

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 Supplementary Table 1. Species list of (A) Ontario fish species included in this study, based on species captured by BSM surveys in at least 40 of the 721 lakes, and (B) species grouped into the category of 'small prey': these species are assumed to be prey for most other fish species, and mostly inhabit the littoral zone of lakes. Further, their small size means they tend to be under-sampled by the BSM net-based fish sampling. The remaining species (C) occurred in fewer than 40 lakes, and were excluded from the analysis due to their infrequency. The 721 lakes occur over an 11-degree latitudinal gradient (43.06 – 54.52 degrees), range in area from 21-90,484 ha, have maximum depths of 1-214 m, range in phosphorus levels from 1.7-52.5 units, and have a range of total degree days from 1059-2246. 12 Average length for each species are from Ontario lakes, and taken from Holm et al  $(2010)^1$ . Thermal 13 guilds (water temperature preferences) are taken from Hasnain et al.  $2013<sup>2</sup>$ .













 Supplementary Table 2. Species association values, measured as null-adjusted Pearson correlation coefficients for abundances of 16 species identified as potential 'interaction' species by the SEM analysis (Figs. 1, Suppl. Fig. 3). The association analysis tests the direction and statistical significance of these 'interactions', correcting for bias in zero-inflated data arising because the correlation 33 coefficient is not double-zero symmetrical<sup>3</sup>. Significantly negative  $(32/120)$  pairings are grey or black highlighted cells. These results cannot test whether the significant associations derive from species interactions, or if negative associations derive from divergent habitat affinity (e.g., species x is absent when species y is present, because they prefer different lake conditions). However, by organizing 37 these results by temperature affinity  $(BLUE = cold$  water species,  $RED = cool$ , or  $GREEN = warm$ ), most negative interactions occur among temperature groups (grey cells) – for example, warm and cold water species tending not to co-occur. Only five negative species pairings were within- group (black cells). There were 37/120 positive pairings and 51/120 non-significant pairings – we assumed these associations to not reflect antagonistic species interactions, and thus could not generate restricted co-occurrence between the species pairs.



 Supplementary Table 3. Boosted regression tree (BRT) model results, for species with one or more significantly negative species associations corrected for null expectation (Supplementary Table 2). The results from the association analysis cannot distinguish between species interactions versus habitat affinity – the BRT analysis tests the relative influence (%) of 22 possible drivers of variation affecting the presence of each species, including species composition (the presence of each of the other species) and abiotic factors including degree days, pH, phosphorus, minimum depth, maximum depth, lake area, Secchi depth, and small prey species richness. Correlation refers to how much a predictor model estimates the variation within the raw data (%). The seven grey-shaded cells are the only species pairings where the explanatory species (top row) was one of the ten strongest factors negatively associated (see Suppl. Fig. 5) with the presence of the response species (vertical column on the left 55 side of the table). The species are organized by temperature affinity: blue = cold, green = cool, red = warm.



 Supplementary Table 4. Environmental range comparisons of the negatively associated species pairs identified in Supplementary Table 2. Species AB refers to lakes where both species co-occur. Ten pairs had range overlap in environmental factors yet rarely co-occur. Nine pairs had range compression in lakes of co-occurrence, where the range of environmental conditions in lakes where a species occurs in the absence of the other is wider than when the two species co-occur. BRT refers to pairs where the presence of one species significantly and directly predicts the absence of another (see Suppl. Table 3, Suppl. Fig. 5). Cold water species, cool water species, warm water species - most rarely co-occurring pairs or those with compressed co-occurrence involve species of different temperature groupings.





 \* Overlap refers to the degree of overlap in the multivariate environmental conditions of lakes where Species A and Species B occur without each other - R values closer to 0 indicate greater similarity in multivariate environmental conditions. Numbers in brackets are the percentage of 5000 randomizations for which an R value as high or higher occurred (i.e., a R value equal or greater to the calculated value less than 5% of the time is considered significant). 72 †Compression refers to a significant change in the multivariate spread or range of environmental conditions, with 'compressed' meaning that the range is NARROWER when the species is in the presence of the other species compared to when alone; 'expanded' means the range is WIDER for lakes with both species present compared to when the species is

76 alone



influence of each explanatory factor on fish.



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 Supplementary Figure 2: Bivariate relationships between abiotic factors in 721 lakes. All relationships were significant with linear fits shown, except latitude despite the concentration of human populations in the southern regions of Ontario. Again, these graphs reveal how any given combination of bivariate factors can significantly interact in lakes, suggesting that integrative analyses testing all simultaneously should better capture how they affect each other, and by extension, the fish communities found within.



# **Supplementary Note 1 – Structural Equation Modeling**

 Our model construction for the SEM analysis began with the entire presence/absence dataset, for 22 fish species in 721 lakes with ten associated environmental measures relating to climate (degree days), lake morphometry (area [ha], maximum depth [m], mean depth [m]), and lake water quality (total P concentration, ph, conductivity, dissolved solids, Secchi depth). To minimize strong co- linearity in our model and maximize parsimony, we screened the correlation matrix among the environmental variables (e.g., see some correlations displayed in Suppl. Fig. 2). This allowed us to reduce the number of environmental variables in the SEM from 10 to 5, with these 5 factors corresponding to our three hypothesized abiotic-based models of influence: climate (degree days), lake morphometry (lake area, maximum lake depth), and water quality (P, pH). All SEM analyses were 109 conducted with R 3.1.2 (R Core Team 2016), using the lavaan package version  $0.5{\text -}20^4$ . For the 'biotic' components of the SEM, we broadly classified the 22 large-sized species (average length > 10 cm) into three functional groups based on habitat affinity and foraging strategy as 112 described in Holm et al.<sup>1</sup> – major predators, littoral species, and pelagic species (Suppl. Table 1). We also grouped the 28 small-sized species (mean length < 10 cm) into a single functional group "small prey species", as described above in the Methods. We tested for influences on total numbers of species per functional group ('functional diversity') and also shifts in species composition (see Methods). For the latter (composition shifts), we identified which functional groups in the SEM were associated with significant species composition changes among lakes using PCA analyses on Hellinger transformed fish data (see Suppl. Fig. 3 and Methods for details). That is to say, which species were significantly associated with the presence of other species. Finally, to satisfy the SEM assumption of multi-normality we log-transformed variables with skewed distributions.

 Our SEM analysis was based on the recognized combined importance of abiotic and biotic 122 factors in shaping fish diversity in freshwater lakes<sup>5</sup>. Starting from our initial model that incorporated all levels of interaction among degree days, measures of lake morphometry and water quality, and 124 numbers of species per major functional group, we used modification indices<sup>4</sup> to guide decisions about 125 adding missing paths to the model. We used the "modindices" function from the lavaan package<sup>4</sup>, which provides a list of all missing linkages in the model and the expected effect of additions on 127 model fit (Maximum likelihood estimator [ML<sub>EST</sub>]). We used the modification indices incrementally, adding one path at a time, until no modification indices were higher than 2. After this process, we scanned path regressions and incrementally pruned the less significant ones until there was no more 130 gain in model fit (decreasing  $ML_{EST}^6$ ). Our final model converged after 171 iterations (n = 648, 131 ML<sub>EST</sub> = 4.91, Degree of freedom = 13,  $P=0.977$ ).

 Our SEM analysis focused on the hypothesis that an integrated model capturing abiotic and biotic interactions within and among functional groups would best capture the regulation of species 134 richness<sup>5</sup>. Yet, there is also evidence in the fish literature of more singular models shaping the regulation of richness, specifically (I) top-down regulation by major predators, (II) bottom-up models where richness in lower levels of the food chain influences diversity levels above, and (III) abiotic- based models where climate and resources primarily affect species richness at all trophic levels. To further disentangle the relative contribution of abiotic and biotic associations, we decomposed our 'Integrative' model into its different components each representing a contrasting hypothesis on the regulation of fish diversity (i.e., no biotic associations between functional groups, top-down associations only, bottom-up associations only).

 To make our models comparable we used the same variable structure for all candidate models but altered the direction of effects among functional groups. The models of 'Top-down' effects of

 consumers on prey (Suppl. Fig. 4b) and the 'Bottom-up' effects of prey on consumers (Suppl Fig. 4c) were started from an initial model including all possible top-down or bottom-up associations among fish functional groups. The abiotic factors were then added using modification indices and the less significant regression paths were pruned (using a similar approach as described above). The model without biotic associations (thereafter 'Abiotic only' model) included no effects (arrows) between functional groups so that only direct effects of environmental factors were possible (Suppl Fig. 4a). We compared the four models using Akaike Information Criterion (see Suppl. Table 5 and Suppl. Fig. 4). By examining our main 'Integrative' model against its reduced components, we were interested in contrasting the magnitude of each factor in the SEM (effect size) versus its contribution to the overall fit of the model (e.g., some factors might have large effect size, but not contribute much to our overall understanding of the system).

 Results from our model comparison indicate that the model including only abiotic factors performed the weakest, suggesting that despite being important drivers in terms of effect size, abiotic factors are insufficient to explain the system (i.e., lowest model fit, Suppl. Table 5). That is to say, ignoring biotic associations among fish functional groups led to a significant decline in analytical power (Suppl. Table 5). Alternatively, top-down factors had smaller effect sizes compared to some abiotic factors yet strongly contributed to model fit. This contribution centered largely on two top- down associations: from 'predator PCA1' to 'small prey richness' and from 'predator richness' to 'pelagic richness'. Removing either of those associations from the 'Top-Down' model lead to a jump in the Maximum Likelihood estimator of 40 and 30 points respectively, sending the model at the bottom of the ranking in the AICc comparisons. Overall, the 'Integrative' model balanced the factors with the greatest effect sizes while maximizing model fit, including the described top down

associations, the strong effects of degree days and lake morphometry, and the bottom-up effect of

- littoral richness on predator richness (Fig. 1).
- The R code for the SEM analysis is provided in full, in Supplementary Note 4.
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- Supplementary Table 5. Comparison of our best model with more simple alternative hypotheses.



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 Supplementary Figure 3. PCA analysis of Hellinger transformed species associated with significant shifts in species composition in the SEM model (Fig. 1), divided into three functional groups based on broad lake habitat associations. The mechanisms underlying the association shifts cannot be determined by the SEM, and may possibly reflect species interactions, habitat affinity not relating to species interactions, or some combination of the two. All subsequent analyses tested the likelihood of interactions versus habitat in explaining these association shifts, targeting these 16 species with the greatest vector-length separation out of the initial 22 species used in the study.



 Supplementary Figure 4. Visual representation of all the alternative hypotheses considered in this study to explain fish functional group richness and compositional shifts. Each model was built 203 iteratively with modifications indices<sup>4</sup> starting from an initial model which structure was constrained by specific rules as a function of the hypothesis tested (see Supplementary Notes 1). The a) Abiotic only model represents the effects of abiotic factors of fish functional group richness and composition 206 in the absence of any biotic associations ( $n = 648$ ,  $ML_{EST} = 3.03$ , Degree of freedom = 6, *P*=0.805), b) Top-down model represents the effects of abiotic factors interacting with top-down associations on 208 richness and composition (n = 648, ML<sub>EST</sub> = 8.66, Degree of freedom = 15,  $P=0.895$ ), c) Bottom-up model represents the effects of abiotic factors interacting with bottom-up associations on richness and 210 composition ( $n = 648$ , ML<sub>EST</sub> = 10.04, Degree of freedom = 11, *P*=0.527), and d) Integrative model represents the combined effects of abiotic factors, bottom-up and top-down associations on richness 212 and composition (n = 648, ML<sub>EST</sub> = 4.91, Degree of freedom = 13,  $P=0.977$ ).



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# **Supplementary Note 2 - Individual fish species boosted regression tree (BRT) models**

224 We developed BRT models<sup>8</sup> to assess the relative importance of 22 biotic and abiotic predictor variables for explaining the variation in fish species presence in lakes for 14 of the fish species, i.e. brown bullhead, pumpkin seed, walleye, lake whitefish, pike, cisco, yellow perch, rock bass, white sucker, large-mouth bass, lake trout, small-mouth bass, burbot, and brook trout. In the model developed for each species, we included presence data for the other 13 species to look for possible species relationships, as well as a biotic predictor of the species richness of small prey and seven abiotic characteristics of the lakes, i.e. degree days, pH, phosphorous levels, minimum depth of water, maximum depth of water, area of the lake, and Secchi depth.

 All models were fitted in R statistical computing program V. 3.2.1 using the gbm package 233 version  $2.1.1<sup>9</sup>$ . BRT models are simple classification or rule-based models that partition observations into groups based on similar values in the response variables using binary splits that are based on 235 predictor variables<sup>10</sup>. The boosting algorthim then iteratively develops a final model using a forward stage-wise approach that progressively adds trees to the model. BRT models are advantageous for analyzing our data because they can be used to assess different types of predictor variables in the same 238 model, which fits our questions well<sup>11</sup>. We used a tree complexity rate of two (which allows for possible two way interactions) and a learning rate of 0.05 to allow for a suitable predictive 240 performance<sup>10</sup>. Because response variables were based on presence and absence data from surveys conducted within each of the lakes we used a Bernoulli distribution. We show the relative influence of each of the 22 possible predictors of variation in the presence of the 14 fish species in Suppl. Table 3. We also show the number of trees included in the final model and estimates of the correlation between the observed and predicted response variables. Variation in the presence of the different fish species predicted by each BRT model is shown in Suppl. Figure 5, where values are predicted for each

- variable by keeping all other variables at mean values.
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 Supplementary Figure 5: The BRT tests if the occurrence of a species was associated with significant negative changes in the occurrence of the target species, versus all abiotic factors used in our analyses. Only two target species – brook trout (A) and lake trout (B) showed negative relationships versus the occurrence of other species, indicated by the red boxes. These data are the same as shown

 in Suppl. Fig. 3. Negative relationships are indicated by declining slopes, with the x-axis ranging from 0 (absent) the 1 (present). For the remaining 14 species tested with the BRT (Suppl. Fig. 3), all major drivers of occurrence were abiotic.

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- (A)
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(B)





# **Supplementary Note 3 - Multivariate analyses of environmental conditions for species pair**

# **combinations**

 For all species pairs, we calculated the Euclidian distance between three lake combinations (Species A only, Species B only, both) based on seven of the measured abiotic/biogeographic factors that were minimally inter-correlated. These factors are lake area, maximum depth of the lake, summer Secchi depth, conductivity, pH, total phosphorus, and degree days. We eliminated lakes for which one or more of these factors were not measured, leaving 649 of 721 lakes. We log transformed and normalized all variables before the analysis.

 To determine the overall degree of overlap in environmental space between lakes with species A only versus lakes with species B only, we used an ANOSIM analysis<sup>12</sup>. This analysis tests the null hypothesis that there are no differences in terms of multivariate environmental conditions, and is 303 equivalent to a non-parametric MANOVA, with permutation to test for significance<sup>12</sup>. The test computes the R statistic, which ranges from 0 (complete overlap in environmental space) to 1 305 (complete separation in environmental space). As recommended by Clarke et al.  $(2014)^{12}$ , we considered the pairwise test between lakes with species A only versus lakes with species B only after confirming that the global ANOSIM test (considering all lake types: species A only, species B only, both A and B, and neither) was significant. This was true in all cases. In all cases we used 5,000 random permutations to test for significant differences.

 If a difference in multivariate environmental conditions was found via the ANOSIM analysis above, it could be due to a shift in location (multivariate average of environmental conditions) and/or a difference in dispersion (multivariate variation or range of environmental conditions). To test for the latter possibility, we determined for each species pair whether lakes with both species co-occurring had a reduced range of environmental conditions compared to lakes with species A only or species B

 only. This could happen, for example, if a subordinate competitor or prey species can only co-occur with a dominant competitor or predator in deep and large lakes, with those lake conditions allowing the subordinate more possibilities to persist. Relatedly, we would predict that the subordinate could occur in smaller and shallower lakes, if the dominant species is absent from those lakes. To do this, we 319 used a test of difference in multivariate dispersions (PERMDISP<sup>13</sup>). This is a multivariate extension of Levene's test, measuring the average distance from each lake to the centroid of the group of lakes in the same category, and comparing this average distance between different categories<sup>14</sup>. If coexistence of species A and B is limited to a smaller range of environmental conditions than lakes inhabited by species A without species B, then we would expect to find a significant decline in multivariate dispersion of lakes with both species compared to lakes with species A only. This test also uses permutation to determine significance of the test. We again set the number of permutations to 5,000. We note that for species pairs where the number of lakes containing both species was very small, our power to detect differences in multivariate dispersion was likely limited. For both the ANOSIM and 328 PERMDISP analyses we used the program PRIMER<sup>14</sup>. These tests are also available in the 'vegan' package in R, but R does not allow pairwise tests in the ANOSIM function.

 To visualize the similarity among lakes in terms of their environmental conditions, we used PCA to ordinate all lakes in environmental space. We used the 'vegan' package in R to create ordination plots, with vectors to visualize the correlation of each environmental factor with the first two PCA axes.

### **Supplementary Note 4 – R code for SEM analysis**

 #..# #..........BSM project (SEM)...............# #..#

 #.. 341<br>342 #... Collaborators: Andrew MacDougall et al# # #... Author of the script: Eric Harvey (http://ericharvey.weebly.com/) # #... Creation date: January 05 2016 # #... Latest edits: November 07 2016 # #.. # #... Clear variables rm(list=ls()) #... Directories paths datapath = "~/Documents/Research/1.Projects/3.BSM\_project/3.Analysis/" #...Load needed packages library(vegan) library(ade4) library(lavaan) library(corrplot) #... Load the data setwd(datapath) presence.dat = read.delim("BSM\_presence.txt") presence.dat = na.omit(presence.dat) #... Identify species of interest for each functional group predator.of.interest = c("walleye","lake.trout","pike","smallmouth.bass","largemouth.bass","burbot") bentho.pelagic.species = c("cisco","brook.trout","round.whitefish","lake.whitefish") bentho.litoral.species = c("shorthead.redhorse","silver.redhorse","goldeye","longnose.sucker","white.sucker","br own.bullhead","channel.catfish","yellow.bullhead","black.crappie","bluegill","pumpkinsee d","rock.bass","yellow.perch","mooneye","fallfish") small.prey.species = c("blackchin.shiner","blacknose.shiner","bluntnose.minnow", "brook.stickleback","central.mudminnow","common.shiner", "creek.chub","deepwater.sculpin","eastern.blacknose.dace", "emerald.shiner","fathead.minnow","finescale.dace", "golden.shiner","Iowa.darter","johnny.darter","lake.chub", "logperch","longnose.dace","mimic.shiner","Mottled.Sculpin","ninespine.stickleback", "northern.redbelly.dace","Pearl.dace","sculpin.spp","Slimy.Sculpin", "spoonhead.sculpin","spottail.shiner","trout.perch") #... Generate presence/absence matrix with species of interest pred.comp.mat = presence.dat[,predator.of.interest] pelagic.comp.mat = presence.dat[,bentho.pelagic.species] litoral.comp.mat = presence.dat[,bentho.litoral.species] small.prey.comp.mat = presence.dat[,small.prey.species] ## ###################################

- #...Extract variables for SEM
- 389 #.....Ordinations analyses for compositional shifts<br>390 #.......Change in species composition with PCA
- #.......Change in species composition with PCA
- pred.PCA = rda(decostand(pred.comp.mat,"hell"))
- 392 pelagic.PCA = rda(decostand(pelagic.comp.mat,"hell"))<br>393 litoral.PCA = rda(decostand(litoral.comp.mat."hell"))
- 393 litoral.PCA = rda(decostand(litoral.comp.mat,"hell"))<br>394  $\#$ ..........PCA results
- #..........PCA results
- pred.PCA
- cumsum(pred.PCA\$CA\$eig/sum(pred.PCA\$CA\$eig)\*100)
- pelagic.PCA
- cumsum(pelagic.PCA\$CA\$eig/sum(pelagic.PCA\$CA\$eig)\*100)
- litoral.PCA
- cumsum(litoral.PCA\$CA\$eig/sum(litoral.PCA\$CA\$eig)\*100)
- #..........PCA diagnosis plots
- source ('http://www.davidzeleny.net/anadat-r/doku.php/en:numecolr:cleanplot.pca?
- do=export\_code&codeblock=0')
- cleanplot.pca(pred.PCA)
- cleanplot.pca(pelagic.PCA)
- cleanplot.pca(litoral.PCA)
- dev.off()
- #define "evplot" function first:
- source ("http://www.davidzeleny.net/anadat-r/doku.php/en:numecolr:evplot?
- do=export\_code&codeblock=0")
- # select the data frame with eigenvalues of particular axes:
- ev.pred <- pred.PCA\$CA\$eig
- ev.pelagic <- pelagic.PCA\$CA\$eig
- ev.litoral <- litoral.PCA\$CA\$eig
- # calculate axis-importance and draw the barplots:
- evplot (ev.pred)
- evplot (ev.pelagic)
- evplot (ev.litoral)
- dev.off()
- #.....Create variables for SEM
- #.......Axes representing changes in species composition
- pred.PCA1 = scores(pred.PCA)\$sites[,1]
- pred.PCA2 = scores(pred.PCA)\$sites[,2]
- pelagic.PCA1 = scores(pelagic.PCA)\$sites[,1]
- pelagic.PCA2 = scores(pelagic.PCA)\$sites[,2]
- litoral.PCA1 = scores(litoral.PCA,choices=c(1:4))\$sites[,1]
- litoral.PCA2 = scores(litoral.PCA,choices=c(1:4))\$sites[,2]
- #.......Species richness per functional group
- pred.richness = specnumber(pred.comp.mat)
- small.prey.richness = specnumber(small.prey.comp.mat)
- pelagic.richness = specnumber(pelagic.comp.mat)
- litoral.richness = specnumber(litoral.comp.mat)
- #.......Environmental variables
- #..........Geo
- Latitude = presence.dat\$Latitude\_DD
- Longitude = presence.dat\$Longitude\_DD 437 DD = presence.dat\$DegreeDays<br>438 #..........Morphology #..........Morphology Area = presence.dat\$Area ha Perimeter = presence.dat\$Perimeter\_km Depth = presence.dat\$Depmn\_m Depth.max = presence.dat\$Depmax m #..........Productivity 444 Secchi = presence.dat\$Secchi\_Summer\_m<br>445 Conductivity = presence.dat\$Conductivity..u Conductivity = presence.dat\$Conductivity..uS.cm.s. TDSolids = presence.dat\$TDSolids\_mgL TotPhosphorus = presence.dat\$TotPhosphorus PH = presence.dat\$pH ## ################################### #...Correlations among variables #Among biotic factors biotic.cor = cor(cbind(litoral.PCA1,litoral.PCA2,pelagic.PCA1,pelagic.PCA2,pred.PCA1,pred.PCA2, pred.richness,litoral.richness,pelagic.richness,small.prey.richness)) corrplot(biotic.cor,method="number",type="full") 457 #Among environmental variables<br>458 env.cor =  $env.cor =$  cor(na.omit(cbind(Latitude,Longitude,DD,Area,Perimeter,Depth,Secchi,Conductivity,TD Solids,TotPhosphorus,PH))) corrplot(env.cor,method="number",type="full") ## ################################### #...Data frame for SEM ananalysis #........variables are log transformed if necessary #........variables need to be all at the same variance scale - to achieve this some are divided/multiplied by a factor of 10 or 100 data.mod = data.frame(DD/100, Latitude, Area/1000,Perimeter/100,Depth, Secchi,TotPhosphorus,Conductivity/10,PH,TDSolids/10, as.numeric(pred.richness),as.numeric(small.prey.richness),pelagic.richness,litoral.richne ss, pred.PCA1\*100,pred.PCA2\*100,pelagic.PCA1\*100,pelagic.PCA2\*100,litoral.PCA1\*100, litoral.PCA2\*100) 475 colnames(data.mod) =  $c("DD", "Latitude",$  "Area","Perimeter","Depth", "Secchi","TotPhosphorus","Conductivity","PH","TDSolids", "pred.richness","small.prey.richness","pelagic.richness","litoral.richness", "pred.PCA1","pred.PCA2","pelagic.PCA1","pelagic.PCA2","litoral.PCA1","litoral.PCA2") #........Correlation matrix of the data
	- round(cov2cor(cov(data.mod)),3)
	- ##
	- ###################################
- #...Structural equation models
- 485 #Each model is built by starting from a simplified model from which paths are<br>486 sequentially added based on the modification indices until no modification ind
- sequentially added based on the modification indices until no modification indices are >2.
- #The initial simplified model for each alternative model is built as follow:
- #Model 1 Abiotic factors only
- #Model 2 Top-down regulation: Initial model was only top-down interactions and then
- abiotic factors are added sequentially
- 492 #Model 3 Bottom-up regulation: Initial model was only bottom-up interactions and then<br>493 abiotic factors are added sequentially abiotic factors are added sequentially
- #Model 4 Integrative model (main model from the paper): Abiotic + Top-down +
- Bottom-up (based on our intial meta-model see Supp.Mat)
- 496 #If the modification indice proposed to add a path that is forbidden according to the
- 497 model (e.g., between trophic groups in the abiotic model), the proposition is ignored and 498 the next best proposition is implemented. the next best proposition is implemented.
- 499 #If the modification indice value of adding a path between an abiotic factor and a
- functional group is the same as the value of adding a path between said trophic group
- and another trophic group, then it was interpreted as a signal that the effect of said
- 502 abiotic factor might be mediated by a trophic interaction<br>503 library(semTools)
- library(semTools)
- #.......Model 1 --> Model with only abiotic factors
- 505 BSM.mod1  $\lt$  "<br>506 # Regression
- # Regression
- small.prey.richness ~ DD + PH + Area + TotPhosphorus + Depth
- 508 pelagic.richness  $\sim$  DD + PH + Depth + Area + TotPhosphorus
- pelagic.PCA2 ~ DD + TotPhosphorus
- 510 litoral.richness  $\sim$  DD + Area + TotPhosphorus + PH + Depth
- 511 litoral.PCA2 ~ DD + Area + PH + TotPhosphorus
- 512 pred.richness  $\sim$  PH + Depth + Area + DD
- 513 pred.PCA1  $\sim$  DD + PH + TotPhosphorus + Depth
- 
- BSM.mod1.fit = sem(BSM.mod1,data=data.mod) #run the SEM
- BSM.mod1.fit #visualize fitting results
- summary(BSM.mod1.fit,stand=T,rsq=T) #visualize path coefficients
- modindices(BSM.mod1.fit) #modification indices
- # To inspect model estimates and parameters
- varTable(BSM.mod1.fit) #Variance table
- inspect(BSM.mod1.fit,"cov.ov") #co-variance matrix
- inspect(BSM.mod1.fit,"est") #regression estimates
- #
- 524 #.......Model 2 Top-down regulation --> paths only from higher to lower trophic groups + abiotic factors
- #Notice that Depth a factor that was very important in Model 1 is now mainly important
- for top-predators it suggests that the effect of depth on other groups is mediated by
- top-predators
- BSM.mod2 <- "
- # Regression
- 531 small.prey.richness  $\sim$  pred.PCA1 + pred.richness + pelagic.richness + pelagic.PCA2 +
- litoral.richness + PH + Area + TotPhosphorus
- 533 pelagic.richness  $\sim$  pred.richness + DD + Depth + PH + Area + TotPhosphorus + pred.PCA1
- pred.PCA1
- pelagic.PCA2 ~ pred.richness + litoral.richness + DD
- 536 litoral.richness  $\sim$  pred.richness + pred.PCA1 + DD + TotPhosphorus + Area + PH
- litoral.PCA2 ~ pred.richness + pred.PCA1 + DD + Area + pelagic.PCA2
- 538 pred.richness  $\sim$  PH + Depth + Area + DD
- pred.PCA1 ~ DD + PH + TotPhosphorus + Depth
- 540 # Residual correlations<br>541 pred.richness  $\sim$  pred.
- pred.richness ~~ pred.PCA1
- pelagic.richness ~~ pelagic.PCA2
- 543 litoral.richness ~~ litoral.PCA2
- 
- 545 BSM.mod2.fit = sem(BSM.mod2,data=data.mod)<br>546 BSM.mod2.fit
- BSM.mod2.fit
- summary(BSM.mod2.fit,stand=T,rsq=T)
- modindices(BSM.mod2.fit)
- # To inspect model estimates and parameters
- varTable(BSM.mod2.fit) #Variance table
- inspect(BSM.mod2.fit,"cov.ov") #co-variance matrix
- inspect(BSM.mod2.fit,"est") #regression estimates
- #<br> $554$  #
- #.......Model 3 Bottom-up regulation --> Ppaths only from lower to higher trohpic groups+
- abiotic factors
- BSM.mod3 <- "
- # Regression
- small.prey.richness ~ PH + Depth + Area + TotPhosphorus
- pelagic.richness ~ small.prey.richness + DD + Depth + PH + Area + litoral.PCA2
- pelagic.PCA2 ~ small.prey.richness + litoral.richness + Area + DD + TotPhosphorus
- litoral.richness ~ small.prey.richness + DD + TotPhosphorus + Area + PH
- 562 litoral.PCA2  $\sim$  small.prey.richness + DD + PH
- pred.richness ~ small.prey.richness + pelagic.richness + pelagic.PCA2 + litoral.richness
- + litoral.PCA2 + Depth + DD + Area + TotPhosphorus
- pred.PCA1 ~ small.prey.richness + pelagic.richness + pelagic.PCA2 + litoral.richness +
- litoral.PCA2 + Depth + DD + TotPhosphorus + PH
- # Residual correlations
- pred.richness ~~ pred.PCA1
- pelagic.richness ~~ pelagic.PCA2
- 570 litoral.richness ~~ litoral.PCA2
- pelagic.richness ~~ litoral.richness
- 
- BSM.mod3.fit = sem(BSM.mod3,data=data.mod)
- BSM.mod3.fit
- summary(BSM.mod3.fit,stand=T,rsq=T)
- modindices(BSM.mod3.fit)
- # To inspect model estimates and parameters
- varTable(BSM.mod3.fit) #Variance table
- inspect(BSM.mod3.fit,"cov.ov") #co-variance matrix
- inspect(BSM.mod3.fit,"est") #regression estimates
- 
- #<br> $582$  # #.......Model 4 Integrative model (main model from our meta-model) --> Abiotic factors +
- top-down + bottom-up
- BSM.mod4 <- "
- # Regression
- small.prey.richness ~ PH + Area + TotPhosphorus + pred.PCA1 + pelagic.richness +
- pelagic.PCA2
- 588 pelagic.richness ~ DD + PH + Depth + Area + TotPhosphorus + pred.richness<br>589 pelagic.PCA2 ~ DD + TotPhosphorus + pred.richness
- pelagic.PCA2 ~ DD + TotPhosphorus + pred.richness
- 590 litoral.richness  $\sim$  DD + Area + TotPhosphorus + PH + pred.richness
- 591 litoral.PCA2 ~ DD + Area + PH + TotPhosphorus
- 592 pred.richness  $\sim$  PH + Depth + litoral.richness + litoral.PCA2 + pelagic.richness +
- 593 pelagic.PCA2 + TotPhosphorus + small.prey.richness + Area<br>594 pred.PCA1 ~ DD + PH + TotPhosphorus + Depth + litoral.rich
- pred.PCA1 ~ DD + PH + TotPhosphorus + Depth + litoral.richness + litoral.PCA2
- # Residual correlations
- litoral.richness ~~ litoral.PCA2
- pelagic.richness ~~ pelagic.PCA2
- 598 pred.richness ~~ pred.PCA1<br>599 litoral.richness ~~ small.prev
- $litoral.richness \sim small.prev.richness$
- 
- BSM.mod4.fit = sem(BSM.mod4.data=data.mod)
- BSM.mod4.fit
- summary(BSM.mod4.fit,stand=T,rsq=T)
- modindices(BSM.mod4.fit) #modification indices
- resid(BSM.mod4.fit,type="raw")
- # To inspect model estimates and parameters
- varTable(BSM.mod4.fit) #Variance table
- inspect(BSM.mod4.fit,"cov.ov") #co-variance matrix
- inspect(BSM.mod4.fit,"est") #regression estimates
- #
- #######################
- #Model goodness of fit and comparaison
- #######
- #AIC comparaisons
- library(AICcmodavg)
- aictab(list(BSM.mod1.fit,BSM.mod2.fit,BSM.mod3.fit,BSM.mod4.fit),c("Abiotic","TD","BU
- ","Integrative"))
- # Model selection based on AICc:
- #
- # K AICc Delta\_AICc AICcWt Cum.Wt LL
- # TD 48 40119.91 0.00 0.61 0.61 -20008.03
- # Integrative 50 40120.85 0.94 0.38 1.00 -20006.15
- # BU 52 40130.71 10.80 0.00 1.00 -20008.72
- # Abiotic 57 40135.64 15.73 0.00 1.00 -20005.22
- #After removing 'Predator PCA1 --> 'Small prey richness' from the 'Top-down' model
- # Model selection based on AICc:
- #
- # K AICc Delta\_AICc AICcWt Cum.Wt LL
- 629 # Integrative 50 40120.85 0.00 0.99 0.99 -20006.15<br>630 # BU 52 40130.71 9.86 0.01 1.00 -20008.72
- # BU 52 40130.71 9.86 0.01 1.00 -20008.72
- # Abiotic 57 40135.64 14.79 0.00 1.00 -20005.22
- # TD 47 40157.95 37.10 0.00 1.00 -20028.22
- #After removing 'Predator richness' --> 'Pelagic richness' from the 'Top-down model'
- # K AICc Delta\_AICc AICcWt Cum.Wt LL
- # Integrative 50 40120.85 0.00 0.99 0.99 -20006.15
- # BU 52 40130.71 9.86 0.01 1.00 -20008.72
- # Abiotic 57 40135.64 14.79 0.00 1.00 -20005.22
- # TD 47 40150.85 30.00 0.00 1.00 -20024.66
- #Observations on AIC comparisons
- # 1. Abiotic only is last
- 641  $# 2$ . The difference between TD and ALL is not substantial 642  $# 3$ . The difference between TD/ALL and BU/Abiotic is sub-
- # 3. The difference between TD/ALL and BU/Abiotic is substantial
- # 4. It is important to note that no interactions in the TD model does not indicate
- of actual top-down interactions (none of them are negative!) except for one strong topdown
- interaction between top-predators and small prey richness
- 646  $\#$  5. In the context of observation  $#4$  It seems that the only reason why BU does not
- perform well is because it is lacking the very important top-down effect of top-predators
- on small prey richness
- # 6. Observation #5 can be verified easily by removing the top-down path from toppredators
- to small prey richness in the top-down model ( 'Predator PCA1' --> 'Small
- 651 prey richnes': ML increases by 40 points and the P-value  $= 0.0000$ . 'Predator richness'  $-$
- > 'Pelagic richness': ML increase 33 points) therefore the good fit of the dop-down
- model is driven by this keystone interaction in the model
- #Conclusion:
- # 1. A mixture of all variables is essential to understand the system. Both bottom-up
- and top-down effects are very important, and in many case they seem to mediate the effect of abiotic factors (e.g., Lake depth).
- # 2. Thus, Model 4 is confidently the best model to understand the system because it
- integrates all information and the mediated effects in the most parsimonious way
- # 3. Model 4 could be analyzed further to gain better insights about the system.
- #END
- #####
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# **Supplementary References**

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- 1. Holm, E., Mandrak, N. E., & Burridge, M. E. (2010). Freshwater fishes of Ontario. *Royal*
- *Ontario Museum, Toronto*.



