

ARTICLE

Freshwater Ecology

Benthic–limnetic morphological variation in fishes: Dissolved organic carbon concentration produces unexpected patterns

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Abstract

Variation in traits related to foraging and locomotion in benthic and limnetic habitats has been observed in many fishes. Benthic and limnetic food chain productivity in lakes is strongly influenced by the concentration of dissolved organic carbon (DOC) in the water, suggesting that DOC might indirectly impose selection on these traits and lead to classic benthic forms at low DOC concentrations and limnetic forms at high DOC concentrations. We tested this hypothesis via geometric morphometric and meristic analyses of bluegill sunfish (*Lepomis macrochirus*, Centrarchidae) from 14 lakes with DOC concentrations ranging from 4 to 24 mg/L. These lakes, located in close proximity to each other, straddle the drainage divide between the Mississippi River and Laurentian Great Lakes basins in northern Wisconsin, USA. Bluegill morphology was consistently related to lake DOC concentration in both drainage basins, despite differences in morphology between basins. Fish from higher DOC lakes had deeper bodies and smaller heads, among other differences, though the proportion of shape variation described by DOC was low. Gill raker length and inter-raker spacing were positively related to DOC concentration. Although some traits were thus related to DOC concentration, the directions of these relationships did not match the predicted benthic–limnetic patterns. Further, no relationships were evident between DOC and gill raker number, eye width, pectoral fin dimensions, or pectoral fin insertion angle in univariate analyses. These variable outcomes suggest that selection linked to DOC does not map neatly onto the classic benthic–limnetic axis, that high DOC favors a benthic–limnetic generalist rather than a limnetic specialist, or that the benthic–limnetic morphological dichotomy is less clear and universal than is often suggested.

KEYWORDS

benthic, dissolved organic carbon, fish, food web, geometric morphometrics, lake, limnetic, littoral, pelagic, phenotype

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INTRODUCTION

Inputs of terrestrially derived dissolved organic matter play an important role in structuring lake food webs (Creed et al., 2018; Jones, 1992; Solomon et al., 2015). These inputs support heterotrophic respiration and microbial production and bring nutrients that stimulate autotrophic production. Terrestrially derived dissolved organic matter (measured as dissolved organic carbon [DOC]) is often darkly colored, imparting a tea-stained color that reduces light penetration and changes the vertical distribution of heat. Collectively, these effects of DOC have substantial implications for benthic (bottom) and limnetic (open water) food chains, including primary production of algae and secondary production of benthic and planktonic invertebrates. Benthic primary and secondary productions are strongly negatively related to DOC concentration (Ask et al., 2009; Craig et al., 2015; Godwin et al., 2014; Karlsson et al., 2009). In the limnetic or pelagic habitat, the situation is more complex: Pelagic primary production can be stimulated by intermediate DOC concentrations, though it falls off at high concentrations, and pelagic secondary production seems to follow the same pattern (Batt et al., 2015; Bergström & Karlsson, 2019; Carpenter et al., 2016; Kelly et al., 2014, 2016, 2018; St-Gelais et al., 2017). Thus, benthic production is strongly negatively related, and pelagic production somewhat less strongly negatively related, to DOC concentration. These effects of DOC at the base of benthic and limnetic food chains can propagate up to influence the productivity of fishes, which often link those food chains via their mobile foraging behaviors (Benoit et al., 2016; Craig et al., 2015; Finstad et al., 2014; Karlsson et al., 2009; Koizumi et al., 2018; Vander Zanden & Vadeboncoeur, 2002).

DOC concentrations can differ substantially among lakes, even those in close proximity to one another. Differences in watershed area, terrestrial vegetation, hydrologic flowpaths, lake volume, and other factors create heterogeneity in the delivery of terrestrial organic matter to lakes and its processing within lakes, and thus in the concentrations of DOC (Canham et al., 2004; Jones et al., 2018; Mulholland, 2003). Although the DOC concentration of a given lake can vary at multiple time scales and is increasing through time in some regions, substantial differences between lakes are fairly stable over long time periods in the absence of major anthropogenic disturbances (Jane et al., 2017; Meyer-Jacob et al., 2019; Monteith et al., 2007).

This spatial variation in an important environmental parameter sets the stage for natural selection or phenotypic plasticity to generate adaptive variation among populations (Hendry, 2017; Richardson et al., 2014). Yet,

we are aware of only a few studies that have considered whether fish phenotypes are related to DOC concentrations. Bartels et al. (2016), examining Eurasian Perch in lakes with DOC concentrations between 4 and 34 mg/L, found that that increasing DOC concentration was associated with longer heads, large eyes, and narrow caudal peduncles. Additionally, several studies considered the relationship between DOC concentration and fish growth rates or other life history traits (Benoit et al., 2016; Craig et al., 2017; van Dorst et al., 2020). For instance, Craig et al. (2017), examining bluegill sunfish in lakes with DOC concentrations between 3 and 24 mg/L, found that populations in lakes with high DOC had low initial growth rate, late maturity, and low lifetime fecundity, whereas these traits showed more variation among low-DOC lakes.

Given that DOC concentrations influence the productivity of benthic and limnetic food chains and differ substantially and stably between lakes, we expected that DOC differences might shape divergence in the traits of fishes that can forage on both benthic and limnetic prey. Trait polymorphisms linked to the different demands of benthic and limnetic foraging have been observed in populations of a number of fish species, including sticklebacks *Gasterosteus aculeatus*, whitefishes *Coregonus* spp., and sunfishes *Lepomis* spp., among others (Robinson & Wilson, 1994; Schluter & McPhail, 1993; Skulason & Smith, 1995). Relative to benthic specialists, limnetic specialists tend to have fusiform bodies; longer, more numerous, and more closely spaced gill rakers; and other traits suited to sustained open water swimming and capturing small zooplankton rather than maneuvering in complex littoral habitats to capture benthic invertebrates. These environment–trait associations typically reflect a mixture of genetic differences (evolution) and environmental influences (plasticity) (Bentzen & McPhail, 1984; Ehlinger & Wilson, 1988; Skulason & Smith, 1995).

We investigated the relationship between lake DOC concentrations and traits related to foraging in bluegill (*Lepomis macrochirus*, Centrarchidae). Bluegill are among the most common and widespread freshwater fishes in North America and are generalists found in a wide range of conditions. They exhibit morphological and behavioral polymorphisms linked to trade-offs between benthic and limnetic foraging, as well as diverse life history strategies (Aday et al., 2003; Belk, 1995; Dominey, 1980; Ehlinger & Wilson, 1988; Gross, 1991; Gross & Charnov, 1980; Mittelbach, 1981). We sampled bluegill from a set of lakes across a wide range of DOC concentrations (4–24 mg/L), hypothesizing that foraging traits of bluegill would be consistent with lower reliance on benthivory, and greater reliance on zooplanktivory, in high-DOC lakes because of the effects of DOC on benthic and limnetic food web productivity. Specifically, we

expected that DOC concentration would be positively associated with fusiform body shape, shorter and narrower pectoral fins inserted more anteriorly and horizontally, longer and more numerous gill rakers with smaller inter-raker spacing, and larger eyes.

METHODS

Study design and sampling

We conducted morphometric analyses on bluegill sampled from 14 lakes with DOC concentrations between 4 and 24 mg/L (Craig et al., 2017, Solomon et al., 2018, Carpenter et al., 2021; Table 1, Appendix S1: Figure S1). DOC concentrations in lakes commonly range between 1 and 20 or 30 mg/L globally and in our study region, and the effects of DOC on benthic and limnetic productivity are apparent at concentrations above 5–10 mg/L (Godwin et al., 2014; Hanson et al., 2007; Kelly et al., 2018; Sobek et al., 2007; Toming et al., 2020). All of the lakes were within a radius of ~30 km, roughly 100 km south of Lake Superior, near the Wisconsin–Michigan border, USA. This is a low-relief, lake-rich region that straddles the divide between the Great Lakes (Lake Superior) and Mississippi River (Wisconsin River) basins. Different lineages of bluegill could be present on either side of the drainage divide (Kawamura et al., 2009); to minimize the risk of confounding DOC and

lineage effects, we chose to study lakes such that our distribution of DOC concentrations and our sampling effort would be approximately equal in the two basins.

We collected ~30 bluegill specimens from each lake between June and August 2018, using fyke nets, boat electrofishing, and angling (Table 1). All of these sampling methods targeted the littoral habitats that we observe bluegill using in these lakes. Fyke nets (12-m lead, 1.3-cm bar mesh, 1-m³ car, four hoops, and two throats) were set from afternoon to morning, with the lead set perpendicularly from shore. Electrofishing was conducted at night using alternating current. Angling was conducted near sunrise or sunset using standard tackle. Captured fish between approximately 75 and 150 mm in total length, or longer when we could not obtain a sufficient number of fish within that size range, were euthanized by overdose of buffered MS-222. We suspended euthanized fish from a rack by the tail before freezing them, a procedure that reduced bending and thus facilitated later geometric morphometric analysis. Animal care procedures were approved by the Animal Care and Use Committee at the Cary Institute of Ecosystem Studies under protocol #2016-06.

Morphometrics and meristics

We quantified body shape of each specimen from a photograph by digitizing 19 two-dimensional (2D) true type II landmarks using tpsDig2 v.2.31 (Rohlf, 2006)

TABLE 1 Summary of lake characteristics and sampling for survey lakes

Lake	Lat.	Long.	Area (ha)	Max depth (m)	DOC (mg/L)	Basin	Fish collected	Fish length (mm)	Capture method
Bay (BA)	46.24418	–89.49733	67	12.2	7.4	Great Lakes	29	64–165	FN, A
Birch (BH)	46.21767	–89.83838	205	13.7	10.9	Mississippi	27	61–149	FN, E
Crampton (CR)	46.20965	–89.47368	26	18.5	5.0	Great Lakes	30	56–135	FN
Found (FD)	45.95051	–89.45320	136	6.4	6.2	Mississippi	30	56–118	E
Hummingbird (HB)	46.24368	–89.50587	1	7.6	24.5	Great Lakes	30	123–180	FN, A
Little Crooked (LC)	46.15085	–89.69511	62	9.1	4.1	Mississippi	30	60–121	E
Lost (LT)	45.96554	–89.48312	218	6.1	4.0	Mississippi	30	70–149	FN, E
McCullough (MC)	46.19831	–89.57043	89	8.2	14.3	Great Lakes	30	65–150	FN
Muskellunge (MS)	45.95180	–89.37989	109	5.8	6.7	Mississippi	30	62–120	E
Oxbow (OB)	46.19967	–89.49964	212	13.4	11.1	Great Lakes	30	81–166	FN, E
Papoose (PS)	46.18476	–89.80266	171	19.8	6.5	Mississippi	30	77–128	FN
Red Bass (RS)	46.19825	–89.66405	11	6.7	18.9	Great Lakes	30	115–158	FN
Squaw (SQ)	45.88447	–89.99458	298	6.4	21.6	Mississippi	28	76–180	FN
Towanda (TO)	45.93854	–89.70771	56	8.2	5.3	Mississippi	30	69–167	FN, E

Note: Lake gives the name of each lake and the abbreviation used to identify the lake in the figures. *Lat.* and *Long.* are the latitude (positive values are decimal degrees north) and longitude (negative values are decimal degrees west). *Fish collected* gives the number of fish collected and *Fish length* gives the range of standard lengths for these fish.

Abbreviations: A, angling; DOC, dissolved organic carbon; E, electrofishing; FN, fyke net.

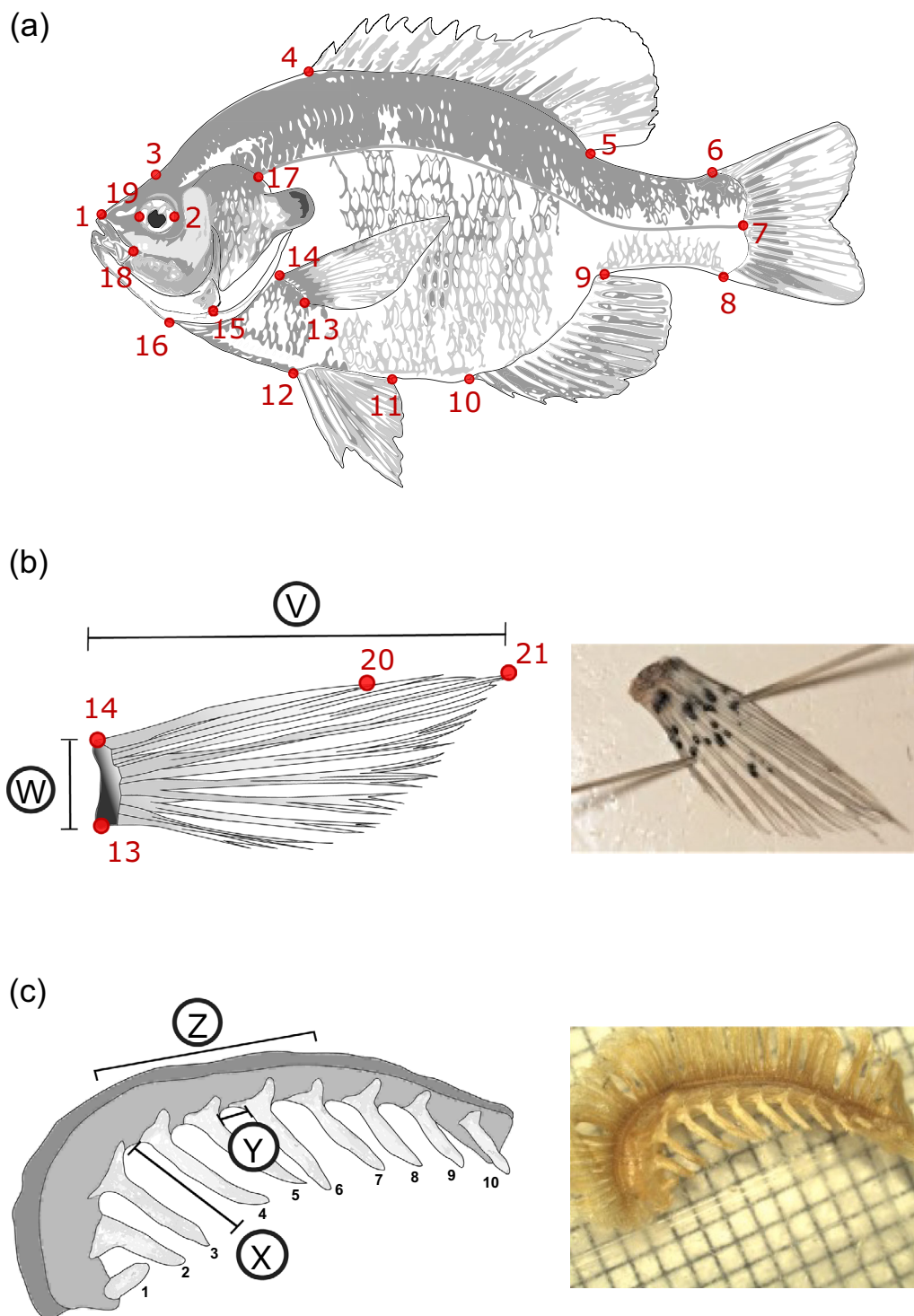


FIGURE 1 Landmark positions and meristic measurements for bluegill sunfish (*Lepomis macrochirus*). (a) Full body landmark positions: 1. Dorsal tip of premaxillary. 2. Right-centered edge of eye. 3. Forehead above center of eye. 4. Dorsal fin anterior insertion point. 5. Dorsal fin posterior insertion point. 6. Caudal fin dorsal insertion point. 7. Caudal fin mid insertion point intersecting with lateral line end point. 8. Caudal fin ventral insertion point. 9. Anal fin posterior insertion point. 10. Anal fin anterior insertion point. 11. Pelvic fin posterior insertion point. 12. Pelvic fin anterior insertion point. 13. Pectoral fin ventral insertion point. 14. Pectoral fin dorsal insertion point. 15. Intersection point between suboperculum and interoperculum. 16. Anterior intersection point between isthmus and gill structures. 17. Intersection point between lateral line and operculum. 18. Dorsal, posterior tip of maxillary. 19. Left-centered edge of eye. (b) Pectoral fin landmarks and linear measurements: 20. Tip of first dorsal ray. 21. Tip of third/longest ray. V. Fin length measurement. W. Fin base width measurement. (c) Gill arch with raker linear measurements: X. Raker length. Y. Raker spacing. Z. Rakers 4-7 and spaces between them were those used in analyses. Image of bluegill is adapted from illustration by Duane Raver, US Fish and Wildlife Service website

(Figure 1a). Digital photographs were taken under consistent lighting with a Nikon D300 camera on a tripod, with the F-stop and ISO set to 15 and 200, respectively. We photographed the left side of the body after thawing the specimen, pinning its fins out (so that fin insertions were clear), ensuring that its mouth was closed, and including a scale reference. We calculated landmark measurement error based on quadruplicate landmarking of a subset of 60 fish, following Zelditch et al. (2012). Landmark placement was 94.7% repeatable (interindividual variation $F = 187.2$, $p = 0.001$, which was much larger than intra-individual variation $F = 2.6$, $p = 0.001$). Due to this high repeatability, we measured the remaining specimens only once. We calculated eye width as the distance between landmarks 2 and 19, and the angle of pectoral fin insertion from landmarks 13 and 14 (Wainwright et al., 2002).

After photographing each specimen, we removed the right pectoral fin and preserved it in a vial in 95% ethanol. In some cases, the preserved fins dried out and became unusable due to evaporation of ethanol from poorly sealed vials, so we were able to analyze fins from only a subset of the captured fish. We spread and pinned each fin and photographed it using the procedure described above for body photos. Four 2D true type II landmarks were digitized onto the images using tpsDig2 (Figure 1b). Due to high variability in how far the fin could be spread apart, we could not place a replicable landmark along the ventral side. This limitation prevented us from obtaining a pectoral fin width measurement, and so we instead measured fin base widths as the distance between landmarks 13 and 14 and fin length as the distance between landmarks 14 and 21. We divided the pectoral fin length by its base width to get the fin length : width ratio.

We examined the gill rakers from the left anterior-most gill arch, which we dissected from the buccal cavity. We counted the rakers under a dissecting microscope

and then took a photo of the gill arch to measure raker length and spacing. We took photos using a Leica microscope with DMC camera attachment connected via USB to a computer with Leica software. A scale reference was included in each photograph. Following Robinson et al. (1993), we measured the length of the four rakers that follow the apex raker located at the crown of the arch (Figure 1c). For each raker, we measured length as a straight line from the insertion point in the arch to the distal point of the raker and raker spacing as a straight line along the base of arch from the posterior edge of one raker to the anterior edge of the next (Figure 1c). These measurements were made using the measurement tool included in tpsDig2.

Statistical analyses

We conducted statistical analyses in R v.4.0.3 (R Core Team, 2016). We assumed that our sample of fish from each lake provided an unbiased estimate of mean phenotype, aside from any effects of sampling method, which were accounted for in our models (see below). We also assumed a monomorphic distribution of phenotypes in each lake, based on preliminary examination of the data.

We conducted geometric morphometric analyses using the geomorph v.3.1.2 package (Adams et al., 2019). We subjected raw coordinates to a generalized Procrustes analysis to remove isometric size effects and achieve uniform orientation and position (Rohlf & Slice, 1990). We then conducted a principal component (PC) analysis to identify the major axes of shape variation among all bluegill specimens. To visualize occupied morphospace along the first two PCs, we created a backtransform morphospace using the StereoMorph package (Olsen, 2017). We evaluated the relationship between DOC and body shape along all axes by performing a Procrustes ANOVA using the random

TABLE 2 Summary of Procrustes ANOVA describing the relationship between body shape of bluegill (*Lepomis macrochirus*) and predictors, including centroid size (a proxy for fish size calculated from the two-dimensional shape data), dissolved organic carbon concentration (DOC; mg/L), drainage basin (0 for Great Lakes basin, 1 for Mississippi River basin), capture method, and a random lake effect

Term	df	SS	MS	R^2	F	Z	p
Log(centroid size)	1	0.042	0.042	0.068	51.4	9.29	0.0001
Log(DOC)	1	0.010	0.010	0.016	12.2	6.05	0.0001
Basin	1	0.026	0.026	0.043	32.2	7.31	0.0001
Capture method	2	0.008	0.004	0.012	4.60	5.14	0.0001
Lake	11	0.092	0.008	0.148	1.0	0	0.5
Residuals	402	0.329	0.001	0.530			
Total	416	0.620					

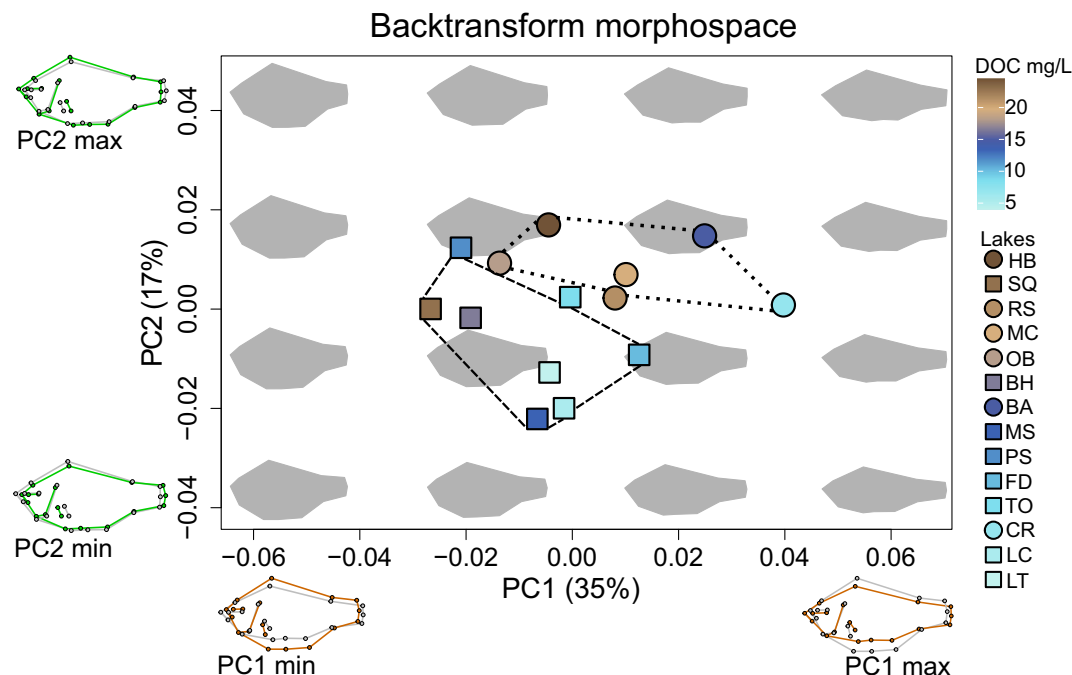


FIGURE 2 Variation in bluegill (*Lepomis macrochirus*) shape across lakes is related to dissolved organic carbon (DOC) concentration. Points indicate the mean fish shape from each lake, and point color indicates the DOC concentration of the lake. Circles connected with dotted lines represent lakes in the Great Lakes basin, and squares connected by dashed lines represent lakes in the Mississippi River basin. Lake abbreviations are given in Table 1. Fish shapes are plotted in the background for reference. Fish shapes outside the axes illustrate the change in shape from the minimum to maximum value for individual fish along the principal component (PC) axis; the corresponding PC scores are $(-0.061, 0.041)$, $(-0.061, -0.047)$, and $(0.066, -0.047)$ for the top left, bottom left, and bottom right extremes. PC1 is positively correlated with fusiform body shape, a more horizontal pectoral fin insertion angle, and a larger eye diameter. PC2 is positively correlated with smaller head size and a more anterior pectoral fin insertion. Axis ranges encompass all individual fish and thus are wider than the distribution of the plotted lake-level mean shapes

TABLE 3 Summary of mixed effects models describing the relationships between lake dissolved organic carbon concentration (DOC) and eye, gill raker, and pectoral fin metrics of bluegill (*Lepomis macrochirus*)

Term	Pectoral fin				Eye width	Raker length	Raker spacing	Raker count
	Length	Width	Length : width	Insertion angle				
Intercept	3.36 (3.14, 3.58)	1.77 (1.56, 2.00)	1.60 (1.37, 1.82)	4.24 (4.00, 4.46)	2.23 (2.09, 2.38)	2.58 (2.31, 2.83)	1.85 (1.66, 2.06)	2.39 (2.29, 2.48)
Log(DOC)	0.06 $(-0.02, 0.14)$	0.04 $(-0.04, 0.11)$	0.02 $(-0.06, 0.10)$	0.01 $(-0.07, 0.09)$	$-0.02 (-0.07, 0.04)$	0.20 (0.10, 0.29)	0.09 (0.02, 0.17)	$-0.00 (-0.03, 0.03)$
Basin	0.29 (0.03, 0.53)	0.21 $(-0.05, 0.44)$	0.04 $(-0.21, 0.29)$	0.05 $(-0.22, 0.31)$	0.02 $(-0.15, 0.20)$	0.27 $(-0.02, 0.57)$	0.23 $(-0.00, 0.46)$	$-0.03 (-0.14, 0.08)$
Log(DOC) : basin	$-0.12 (-0.22, -0.01)$	$-0.09 (-0.18, 0.02)$	$-0.02 (-0.12, 0.09)$	0.04 $(-0.07, 0.15)$	$-0.00 (-0.08, 0.07)$	$-0.08 (-0.21, 0.05)$	$-0.10 (-0.20, 0.00)$	$-0.01 (-0.06, 0.04)$
Capture method E	$-0.04 (-0.11, 0.06)$	$-0.07 (-0.16, 0.03)$	0.05 $(-0.03, 0.15)$	$-0.16 (-0.24, -0.06)$	$-0.02 (-0.05, 0.02)$	$-0.02 (-0.10, 0.10)$	$-0.12 (-0.20, -0.03)$	0.11 (0.07, 0.16)
Capture method FN	$-0.02 (-0.08, 0.05)$	$-0.02 (-0.10, 0.05)$	0.01 $(-0.06, 0.09)$	$-0.14 (-0.21, -0.06)$	$-0.01 (-0.04, 0.02)$	$-0.06 (-0.13, 0.04)$	$-0.08 (-0.15, -0.01)$	0.04 (0.00, 0.08)
σ_{lake}	0.05 (0.02, 0.07)	0.04 (0.00, 0.06)	0.05 (0.00, 0.07)	0.06 (0.02, 0.07)	0.04 (0.02, 0.05)	0.07 (0.03, 0.09)	0.05 (0.00, 0.06)	0.02 (0.00, 0.02)
σ_{residual}	0.10 (0.10, 0.12)	0.15 (0.14, 0.17)	0.12 (0.11, 0.14)	0.14 (0.13, 0.15)	0.05 (0.05, 0.05)	0.15 (0.14, 0.16)	0.16 (0.15, 0.17)	0.11 (0.10, 0.11)
R^2_{marginal}	0.07	0.03	0.02	0.13	0.03	0.22	0.11	0.09
$R^2_{\text{conditional}}$	0.27	0.11	0.15	0.25	0.40	0.35	0.18	0.11

Note: Each column summarizes a univariate model for the log-transformed response variable indicated in the column header; all response variables except for gill raker count were size-standardized prior to log transformation. Data in the first four rows are the restricted maximum-likelihood point estimates (and likelihood profile 95% confidence intervals) for the fixed effects, including an intercept, DOC, drainage basin in which the lake is located (0 for Great Lakes basin and 1 for Mississippi River basin), the interaction between DOC and basin, and capture method. Effects for which the confidence interval does not include zero are highlighted in bold. The σ_{lake} and σ_{residual} rows give the point estimates for the standard deviations of the random lake effect and the residuals. R^2_{marginal} is the proportion of the variance described by the fixed effects in the model, and $R^2_{\text{conditional}}$ is the proportion of the variance described by the full model including the random effects; both R^2 values were computed using the `r.squaredGLMM` function in the package MuMIn (Barton, 2020). Abbreviations: E, electrofishing; FN, fyke net.

residual permutation procedure (Anderson & ter Braak, 2003; Collyer et al., 2015). Specifically, we used a Procrustes ANOVA to describe variation in shape as a function of

DOC, basin, capture method, a random lake effect, and centroid size (a proxy for fish size calculated from the 2D shape data). This model structure assumes that the

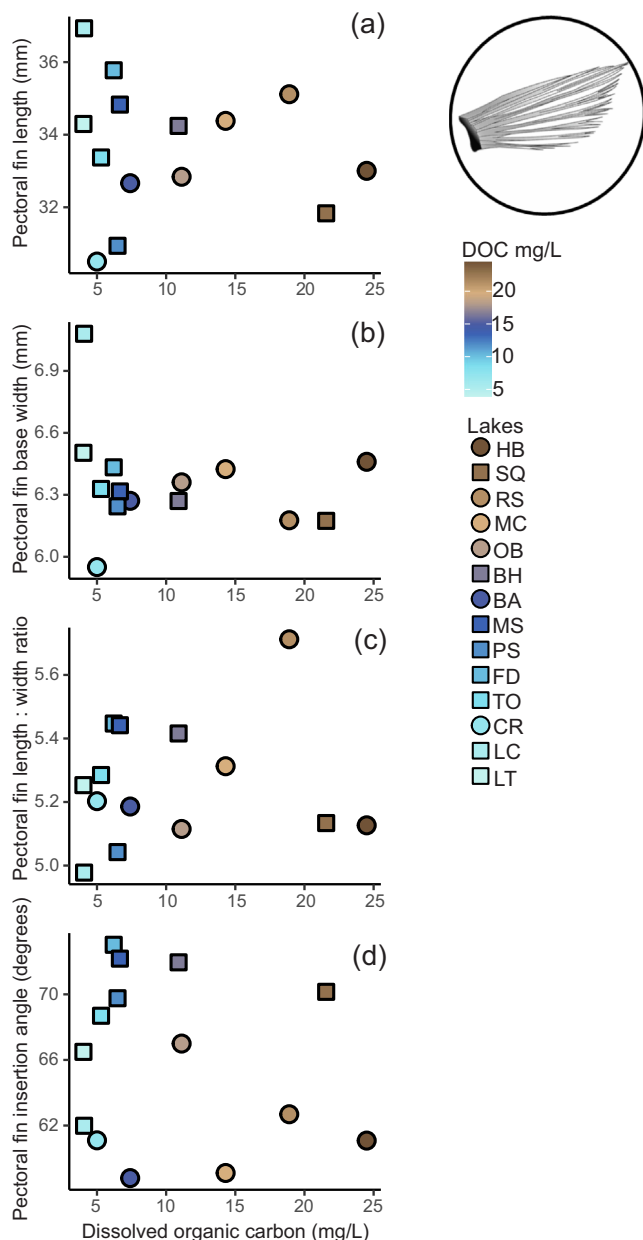


FIGURE 3 Pectoral fin length (a), width at base (b), length : width ratio (c), and insertion angle (d) of bluegill in lakes with different dissolved organic carbon (DOC) concentrations. Points indicate the fitted, size-corrected estimate for a fish captured by fyke net. Point color indicates the DOC concentration of the lake, and point shape indicates the basin, in which the lake is located (circles = Great Lakes basin, squares = Mississippi River basin). Lake abbreviations are given in Table 1. Pectoral fin length was longer for bluegill in the Mississippi basin, and there was some evidence for a negative relationship between pectoral fin length and DOC for fish in this basin; otherwise, there were no significant effects of DOC or basin on these traits (Table 3)

lake-level intercepts might depend linearly (with error) on lake DOC.

We also used univariate models to analyze phenotypic traits other than overall body shape, including pectoral

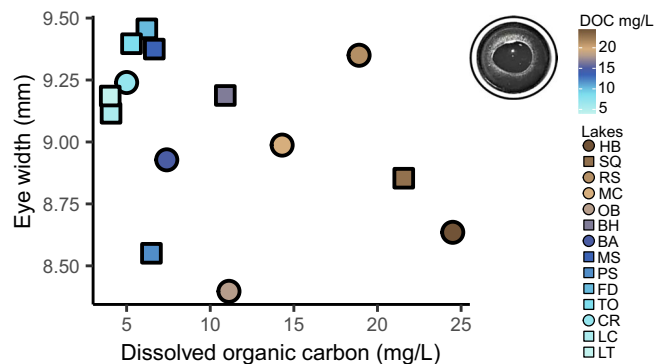


FIGURE 4 Eye width of bluegill in lakes with different dissolved organic carbon (DOC) concentrations. Points indicate the fitted, size-corrected estimate for a fish captured by fyke net. Point color indicates the DOC concentration of the lake, and point shape indicates the basin, in which the lake is located (circles = Great Lakes basin, squares = Mississippi River basin). Lake abbreviations are given in Table 1. Neither drainage basin nor DOC was a significant predictor of eye width (Table 3)

fin dimensions, gill raker characteristics, and eye width. These models had the same basic structure as the Procrustes ANOVA, describing variation in the trait measurement as a function of DOC concentration, basin, capture method, and a random lake effect. Trait measurements were size-standardized when necessary before models were fit, using allometric coefficients estimated from common-slope regressions of the log-transformed trait measurement on log-transformed fish standard length (Reist, 1986). We used the `lmer()` function to fit linear models to the log-transformed trait measurements for all traits (Bates et al., 2015). All of the data and code for our analyses is publicly available (Bishop et al., 2022; Solomon et al., 2018).

RESULTS

Body shape of bluegill was significantly related to DOC concentration, although the variation explained by DOC was low (Table 2, Figure 2). There was also a significant difference in shape between the Great Lakes and Mississippi basins and a significant allometric relationship between centroid size and shape. The first two PCs of the overall body shape data explained 52% of the individual-level variation in shape. Increasing values of PC1 described a shift from a shorter, deeper body to a longer and more fusiform body, including from a short and deep caudal peduncle to a long and narrow one, from a shorter to longer eye diameter, and from a more vertical to a more horizontal insertion of the pectoral fin (Figure 2). Increasing values of PC2 described a shift from a larger

head and more posteriorly positioned pectoral fin to a smaller head and more anteriorly positioned pectoral fin (Figure 2). Although average shapes of Great Lakes basin and Mississippi basin bluegill were different, within each basin the gradient from low to high DOC was associated with lower PC1 values and somewhat higher PC2 values—that is, bluegill in lakes with higher DOC tended to have deeper bodies, smaller heads, smaller eyes, and pectoral fin insertions that were more anteriorly and vertically positioned.

Pectoral fin length was longer for bluegill in the Mississippi basin, and there was some evidence for a negative relationship between pectoral fin length and DOC for fish in this basin (Table 3, Figure 3). The width, length : width

ratio, and insertion angle of pectoral fins were not significantly related to DOC concentration, drainage basin, or their interaction in univariate analyses. There was considerable lake-level variation in these traits; the random lake effect explained 20% of the variance in pectoral fin length and 8%–12% of the variance in width, length : width ratio, and insertion angle. Pectoral fin insertions of angled fish were more vertical than those of fish caught by electrofishing or fyke nets (Table 3).

While variation in eye width was apparent in the multivariate analysis, there was no significant relationship between eye width and DOC, basin, or their interaction in the univariate analysis (Table 3, Figure 4). There was also no relationship between eye width and capture method. The random lake effect explained 37% of the variation in eye width.

Gill rakers were longer, and the spacing between rakers wider, in lakes with higher DOC concentrations (Table 3, Figure 5). There was also some evidence that rakers were longer and more widely spaced in the Mississippi basin than in the Great Lakes basin, although the 95% confidence intervals for these effects narrowly overlapped zero. The number of rakers on the gill arch ranged from 9 to 15 and was strongly related to fish size, but not to DOC or drainage basin. Angled fish had fewer, more widely spaced rakers than those caught by electrofishing or fyke nets.

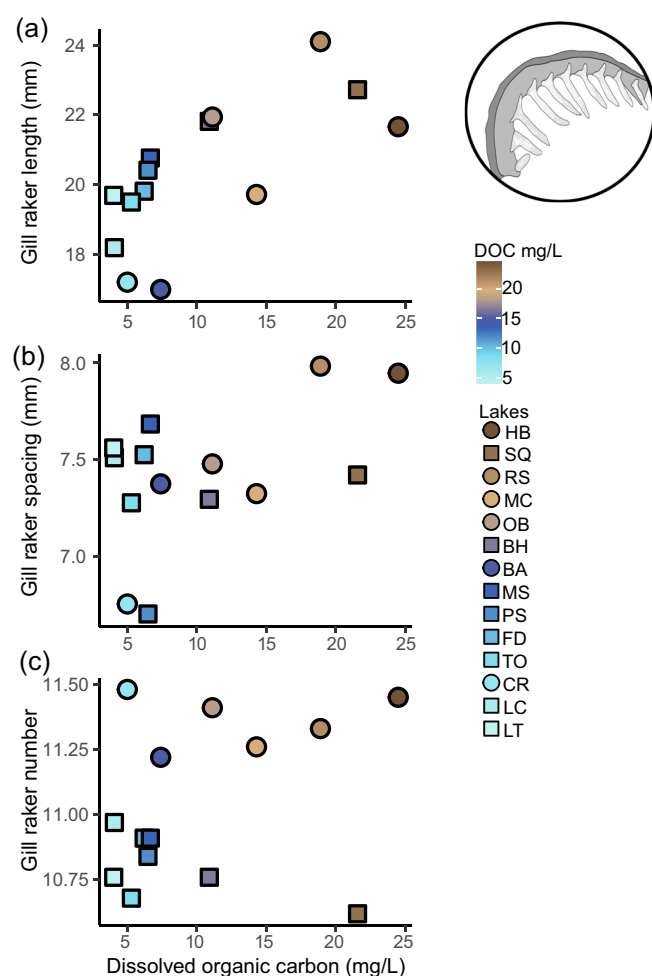


FIGURE 5 Gill raker length (a), spacing between gill rakers (b), and number of gill rakers (c) of bluegill in lakes with different dissolved organic carbon (DOC) concentrations. Points indicate the fitted, size-corrected (for length and spacing) estimate for a fish captured by fyke net. Point color indicates the DOC concentration of the lake, and point shape indicates the basin, in which the lake is located (circles = Great Lakes basin, squares = Mississippi River basin). Lake abbreviations are given in Table 1. Raker length and spacing were positively related to DOC concentration (Table 3)

DISCUSSION

We documented morphological differences among bluegill populations across the DOC gradient, and these trends were largely consistent between the Mississippi and Great Lakes drainages. Yet, these differences did not neatly match our expectation of classic “benthic” traits at low DOC versus classic “limnetic” traits at high DOC. Instead, we observed a mixture of expected, unexpected, and null relationships between DOC concentration and the examined traits (Table 4). We can see at least three plausible explanations for this outcome.

Before turning to those explanations, it is useful to recognize that the null relationships we observed (Table 4) probably indicate that DOC does not impose strong selection on those traits in bluegill. One alternative explanation is that the genetic or plastic potential to respond to selection is limited for these traits, but this does not seem likely; studies with pumpkinseed, a congener of bluegill, report that most of the traits in question can show rapid plastic and/or genetic responses when fish are found or reared in littoral versus open water habitats (Robinson & Wilson, 1996; Yavno & Fox, 2014). Another possibility is that genetic and plastic effects offset each other, as in “counter-gradient variation” (Conover et al., 2009;

TABLE 4 Summary of the expected and observed relationships between fish traits and lake dissolved organic carbon (DOC) concentration

Trait	Expectation	Opposing evidence	Observed (body shape)	Observed (univariate)
Body shape	Fusiform [1, 2, 3, 4, 5]	No relationship [6 ^a]	<i>Deep</i>	—
Caudal peduncle depth	Shallow [7, 8]	No relationship [4, 6 ^a]	<i>Deep</i>	—
Caudal peduncle length	Long [3, 4, 6]		<i>Short</i>	—
Head length	Long [8, 9]	[4, 6 ^a]	<i>Short</i>	—
Head depth	Shallow [9]		Shallow	—
Eye size	Large [4, 8, 9]		<i>Small</i>	No relationship
Pectoral fin insertion point	Anterior [1]		Anterior	—
Pectoral fin insertion angle	Horizontal [1]	No relationship [6]	Vertical	No relationship
Pectoral fin length	Short [1,3, 5]	Long [6]	—	No relationship ^b
Pectoral fin width	Narrow [4, 5]		No relationship	No relationship
Gill raker length	Long [10]		—	Long
Gill raker spacing	Narrow [2]	No relationship [6 ^a]	—	<i>Wide</i>
Gill raker number	More [2]	No relationship [6 ^a]	—	No relationship

Note: Numbers in brackets refer to reference citations. *Expectation* is the expected relationship. *Opposing evidence* highlights studies that counter the expectation. *Observed (body shape)* is the trend that we observed in the multivariate analysis of body shape (Figure 2). In this column and the next one bold indicates that the observation matched the expectation, while italics indicate that the observation showed the reverse of the expected pattern. Traits not considered in a given analysis are indicated with a dash (—). *Observed (univariate)* summarizes the result of a univariate test of significance of the relationship between the trait and DOC concentration. In all columns, table entries indicate the morphology associated with high-DOC lakes; for instance, body shape was expected to be more fusiform in high-DOC lakes (and therefore less fusiform, or more deep, in low-DOC lakes). [1] Ehlinger and Wilson (1988); [2] Robinson and Wilson (1994); [3] Svanbäck and Eklöv (2002); [4] Weese et al. (2012); [5] Robinson et al. (2000); [6] Robinson et al. (1993); [7] Webb (1982); [8] Bartels et al. (2016); [9] McPhail (1984); [10] Schluter and McPhail (1993).

^aThis study examined benthic and limnetic forms of pumpkinseed in a lake without bluegill. Limnetic fish had (nonsignificant) shallower bodies and caudal peduncles. There was no difference in number of gill rakers or gill raker length, but rakers were wider and angled differently in limnetic form, such that they overlapped more. Head length measured as predorsal length was shorter in limnetic fish; for other measures of head length, there was a significant interaction between habitat (benthic or limnetic) and fish size.

^bSome evidence for a positive relationship between pectoral fin length and DOC in the Mississippi River basin.

Conover & Schultz, 1995). Regardless, any logically consistent explanation for our results should accommodate the null relationships with DOC as well as the significant ones—and we now suggest three general possibilities.

One possibility is that selection imposed by DOC does not map neatly on to the classic benthic/limnetic axis. Certainly, strong evidence exists that DOC influences the productivity of benthic and pelagic food chains, as we described in Introduction section. Yet, these ecosystem-level metrics might not adequately describe the effects of DOC on either the benefits or costs of benthic versus limnetic foraging strategies, which depend on the ability of fish to efficiently find and ingest prey while avoiding predators. For instance, bluegill foraging in shallow waters might enjoy similar densities of zoobenthos regardless of DOC concentrations, because zoobenthos production at shallow depths can be relatively constant even as high DOC concentrations limit the range of depths at which substantial zoobenthos production occurs (Craig et al., 2015). At the same time, DOC might also influence the rate at which bluegill can locate their prey, or themselves be located by potential predators.

High DOC concentrations can reduce the ability of fish to see zooplankton and thus the rate at which they can consume them, or can interfere with the ability of zooplankton to detect chemical cues of fish presence and make a behavioral avoidance response (Estlander et al., 2010, 2012; Jönsson et al., 2012; Santonja et al., 2017; Weidel et al., 2017). High DOC might also reduce the ability of piscivorous fishes to locate their prey, which could alter the predation risks that bluegill smaller than the gape limit of the piscivore face while foraging in benthic or limnetic habitats (Ranåker et al., 2012). The unexpected trait associations that we observed—such as the trend toward deeper bodies but longer gill rakers in high-DOC lakes—might reflect the net effect that DOC or other covarying factors have on the benefits and costs of benthic and limnetic foraging strategies. Future work to more carefully quantify how DOC influences selection pressures on foraging and other traits would be a useful step forward from the results that we present here.

A second possibility is that high-DOC concentrations might favor generalist foragers, rather than limnetic specialists as we had hypothesized. Werner and Hall (1974)

developed and tested a model for the optimal diet breadth of a fish foraging on prey of different sizes available at different densities, using bluegill as a model species. Their model considers only different size classes of *Daphnia* zooplankton as prey, allowing variation in handling time to be ignored. In the model, search time varies among prey items because prey size is linked to the distance from which it is visible. When search times overall are long—because of low prey density, poor visual conditions in the water, or low fish activity, for instance—then broader diets are favored. Interestingly, all three of these contributors to long search times might occur in high-DOC lakes, because prey densities might be low and high DOC reduces light availability and mean water column temperature (Craig et al., 2015; Kelly et al., 2014; Solomon et al., 2015). Two features of our results provide at least some support for the idea that we see generalists at high DOC. First, the mix of traits seen in high-DOC lakes does not match either of the classic limnetic or benthic morphotypes (see above), but instead seems like a mixture of the two (Table 4). Second, the body shapes of fish from high-DOC lakes tended to be fairly similar across lakes, whereas much more variation is evident among the low-DOC lakes (Figure 2). Limited pilot data on bluegill diets in two of our study lakes ($n = 28$ –91 fish per lake, mean total length ~ 75 mm) also provide some support for this idea: In Crampton Lake (5.0 mg DOC/L), fish were benthic specialists, consuming 69% benthic prey and 11% limnetic prey by mass, whereas in Hummingbird Lake (24.5 mg DOC/L), fish consumed 45% benthic and 50% limnetic prey. This result contrasts with data from stable isotope analyses of Eurasian perch, which showed nearly equal benthic and limnetic reliance at low DOC but predominantly limnetic reliance at high DOC (Bartels et al., 2016).

A third possibility is that the link between benthic or limnetic foraging behavior and fish morphology is not as strong or consistent as we had assumed, at least at an intraspecies level. Certainly, there are iconic examples of benthic and limnetic morphotypes of several fish species (Robinson & Wilson, 1994; Schluter & McPhail, 1993; Skúlason & Smith, 1995). On the other hand, exceptions exist to the expected patterns (Table 4). Some studies with *Lepomis* sunfishes (bluegill or pumpkinseed) provide evidence both for and against a relationship, and in some cases for relationships in opposite directions. For instance, a study of bluegill in a single lake observed that fish collected from deep water had shorter pectoral fins than those collected from shallow vegetated habitats, while a study of pumpkinseed collected from similar habitats in another lake observed the opposite pattern (Ehlinger & Wilson, 1988; Robinson et al., 1993). In general, cases of “parallel” evolution along repeated environmental gradients may actually be quite variable and not very parallel,

suggesting the opportunity and need for richer understanding of the mechanisms at work (Oke et al., 2017).

Bluegill evolutionary history

Our observation that bluegill from the Great Lakes and Mississippi River basins were morphologically divergent provides some interesting, albeit preliminary, new insight into aspects of their phylogeography. Kawamura et al. (2009) described the phylogeography of bluegill using mitochondrial ND1 gene sequences from nearly 400 individuals sampled from the Mississippi River, Lake Michigan, and Lake Ontario basins. They concluded that the formerly glaciated portions of these basins were recolonized, after the end of the Wisconsin glaciation 11,000 years ago, by bluegill from two clades that diverged in the Ouachita–Ozark Highlands around 524,000 years ago. All 149 bluegill that Kawamura et al. (2009) sampled from five sites in the upper Mississippi River basin had one of four closely related haplotypes from one of these clades, while 30 of the 35 fish they sampled from three sites in the Lake Michigan basin had a single haplotype from the other clade. These results provide strong evidence that different lineages are present in the Mississippi River and Great Lakes basins. However, the study did not include any fish from the region that we studied around the drainage divide between the upper Wisconsin River and Lake Superior, nor from anywhere else in the Lake Superior basin. Our morphometric results suggest the possibility that, as in the regions sampled by Kawamura et al. (2009), different lineages may predominate on either side of the drainage divide in our study region. Of course, these ideas need to be confirmed via sequencing from our study area and studies assessing the association between lineage and morphology (see work by Weese et al., 2012 with pumpkinseed sunfish, a congener of bluegill).

CONCLUSIONS

Although the central role of DOC in structuring lake food webs is now widely recognized, understanding of the mechanisms by which DOC influences consumer populations remains limited. Our work here adds to a growing body of literature examining the relationships between DOC and the phenotypes of fishes and other consumers (Bartels et al., 2016; Benoit et al., 2016; Craig et al., 2017; Minguez et al., 2020; van Dorst et al., 2020). At the same time, the unexpected and variable nature of DOC–trait associations suggests that fully untangling the

effects of DOC on phenotypes will require careful and thoughtful exploration of the multiple ways, in which DOC influences the fitness landscape in lake ecosystems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data and code (Bishop et al., 2022) are available from Zenodo: <https://doi.org/10.5281/zenodo.5880086>. The code pulls additional data from the Cary Institute via Figshare: <https://doi.org/10.25390/caryinstitute.7438598>.

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