Morphology of bluegill sunfish (*Lepomis macrochirus*) varies with lake dissolved organic carbon concentration

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ABSTRACT

Inter- and intra-population trait polymorphisms related to foraging and locomotion in benthic and pelagic habitats have been observed in many fishes. The concentration of dissolved organic carbon (DOC) in the water strongly influences the productivity of benthic and pelagic food chains in lakes , suggesting that DOC might impose selection on these traits and lead to classic benthic-foraging "littoral" forms at low DOC concentrations and pelagic-foraging "limnetic" forms at high DOC concentrations. We tested this hypothesis via geometric morphometric and meristic analyses of body, fin, eye, and gill raker morphometry of Bluegill sunfish (*Lepomis macrochirus*, Centrarchidae) from fourteen lakes with DOC concentrations ranging from 4 to 24 mg L^{-1}. These lakes, located in close proximity to one another, straddle the drainage divide between the Mississippi River and Laurentian Great Lakes watersheds. Many of the traits that we considered were significantly related to DOC concentration, and in most cases the sign (though not always the magnitude) of these relationships was consistent across the two watersheds. Fish from lakes with high DOC concentrations tended to have deeper bodies, deeper and shorter caudal peduncles, shorter and shallower heads, and larger eyes in shape space. When examined as an individual trait, there was no relationship between eye width and DOC concentration. Pectoral fins tended to be inserted more anteriorly and vertically when examined in shape space, but no significant correlation was found between the univariate insertion angle and DOC. Pectoral fin length varied with DOC, but the direction of this effect differed between the two watersheds. The gill rakers on the first branchial arch were longer and more widely spaced at high DOC, but the number of gill rakers did not vary with DOC. While many of the traits that we quantified were significantly related to DOC concentration, the directions of these relationships did not match with the predicted littoral-limnetic patterns. This may be because DOC has complex effects on the fitness landscape for foraging and locomotion traits; because high DOC favors a littoral-limnetic generalist rather than a limnetic specialist; or because the littoral-limnetic morphometric dichotomy is less clear and universal than is commonly thought.

RÉSUMÉ

Des polymorphismes de caractères inter et intra-populations liés à la recherche de nourriture et à la locomotion dans les habitats benthiques et pélagiques ont été observés chez de nombreux poissons.). La concentration de carbone organique dissous (COD) dans l'eau influence fortement la productivité des chaînes alimentaires benthiques et pélagiques dans les lacs, ce qui suggère que le COD pourrait imposer une sélection sur ces caractères et conduire à des formes classiques de «littoral» benthique fourrager à de faibles concentrations de COD et formes «limnétiques» pélagiques à des concentrations élevées de COD. Nous avons testé cette hypothèse au moyen d'analyses géométriques morphométriques et méristiques de la morphométrie du corps, des nageoires, des yeux et des branchies du crapet crapet (Lepomis macrochirus, Centrarchidae) de quatorze lacs avec des concentrations de COD allant de 4 à 24 mg L-1. Ces lacs, situés à proximité les uns des autres, chevauchent la ligne de partage des eaux entre le fleuve Mississippi et les bassins versants des Grands Lacs laurentiens. Bon nombre des traits que nous avons examinés étaient significativement liés à la concentration de COD, et dans la plupart des cas, le signe (mais pas toujours l'ampleur) de ces relations était cohérent dans les

deux bassins versants. Les poissons des lacs à fortes concentrations de COD avaient tendance à avoir des corps plus profonds, des pédoncules caudaux plus profonds et plus courts, des têtes plus courtes et moins profondes et des yeux plus grands dans l'espace de forme. Lors de l'examen en tant que trait individuel, il n'y avait pas de relation entre la largeur des yeux et la concentration de COD. Les nageoires pectorales avaient tendance à être insérées plus en avant et verticalement lorsqu'elles étaient examinées dans l'espace de forme, mais aucune corrélation significative n'a été trouvée entre l'angle d'insertion univarié et le COD. La longueur des nageoires pectorales variait avec le COD, mais la direction de cet effet différait entre les deux bassins versants. Les branchies branchiales sur le premier arc branchial étaient plus longues et plus espacées à un COD élevé, mais le nombre de branchies branchiales ne variait pas avec le DOC. Alors que de nombreux traits que nous avons quantifiés étaient significativement liés à la concentration de COD, les directions de ces relations ne correspondaient pas aux schémas littoraux-limnétiques prédits. Cela peut être dû au fait que le DOC a des effets complexes sur le paysage de la condition physique pour les traits de recherche de nourriture et de locomotion; parce qu'un DOC élevé favorise un généraliste littoral-limentique plutôt qu'un spécialiste limnétique; ou parce que la dichotomie morphométrique littoral-limnétique est moins claire et universelle qu'on ne le pense généralement.

PREFACE

Acknowledgments

I would first like to extend my sincerest thanks to my supervisors Chris Solomon and Andrew Hendry who have supported me through all the ups and downs of my master's thesis. Chris, you've been so patient throughout my ongoing battle with scientific writing and I appreciate all of your feedback and advice, that said I hope to produce a thesis that will make you proud. Andrew, you were always there to listen when I had crazy ideas to bounce off of you and I doubt I would've kept my sanity if you hadn't encouraged my involvement in various field work. You've both helped me navigate through this thesis during difficult and unprecedented times and I cannot thank you enough for believing in me when I often lost belief in myself.

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Contribution of authors

This thesis contains one chapter in manuscript-form that is intended for publication, of which I am the primary author. The original research ideas and sampling methodologies were developed by myself, my co-supervisors Christopher T. Solomon and Andrew Hendry, along with Stuart E. Jones. All authors contributed to revisions of the manuscript. Further details of contributions are as follows:

Chelsea Bishop: Conducted field work, data collection, as well as statistical and geometric morphometric analyses. Undertook writing of manuscript and other thesis-related material.

Madlen Stange: Provided guidance and helped to conduct geometric morphometric and statistical analyses. Provided feedback and suggestions throughout the project. Provided critical review of the manuscript.

Stuart E. Jones: Provided facilities, manpower, and guidance with fieldwork logistics required to conduct sample collection. Provided feedback and suggestions throughout the project. Provided critical review of the manuscript.

Andrew P. Hendry: Provided guidance with fieldwork logistics, study design, and statistical analyses. Provided critical review of the manuscript and thesis throughout the project and the writing process.

Christopher T. Solomon: Provided facilities, manpower, and guidance with fieldwork logistics. Provided guidance with study design, and statistical analyses. He also provided critical review of the manuscript and thesis throughout the project and the writing process.

INTRODUCTION AND LITERATURE REVIEW

Dissolved organic carbon (DOC) can have far-reaching consequences for freshwater ecosystems, impacting both their structure and productivity (Jones 1992; Hessen and Tranvik 1998; Prairie 2008; Solomon *et al.* 2015). Lakes located in boreal or forested areas commonly obtain heterotrophic energy in the form of terrestrially derived dissolved organic matter (tDOM), of which DOC is a major component (Vadeboncoeur *et al.* 2001; Ask *et al.* 2009b; Finstad *et al.* 2014; Solomon *et al.* 2015). Plant materials, among other forms of tDOM, accumulate in freshwater ecosystems and break down to release various compounds including humic and fulvic acids (Jones 1992; Thomas 1997; Roulet and Moore 2006; Solomon *et al.* 2015). The acids range in color from yellow to black and have high molecular weights giving lakes their distinctive brown color when DOC concentrations are high (Jones 1992; Roulet and Moore 2006). Further, the properties of DOC allow for strong light attenuation which can alter the stratification of heat and light throughout the water column (Jones 1992; Roulet and Moore 2006). Accumulation of DOC alters how energy is transferred throughout the lake and can play a significant role in the function of aquatic ecosystems (Jones 1992; Thomas 1997; Roulet and Moore 2006; Solomon *et al.* 2015).

Ranges of DOC concentrations vary widely and have been suggested to be regulated through a hierarchical system, in which climate and vegetation coverage establish regional DOC ranges while individual lakes regulate fluctuations through their own properties (Sobek *et al.* 2007). It has also been suggested that over recent decades there have been widespread increases in DOC concentrations -proposing anthropogenic atmospheric deposits and climate change as potential explanations (Freeman *et al.* 2004; Monteith *et al.* 2007). In a world where lakes appear to be ‘browning’, it is necessary to understand how increasing DOC concentrations impact aquatic ecosystems and what repercussions that might have.

DOC and productivity

The relationship between increasing DOC and freshwater ecosystems is complex and non-linear (Ask *et al.* 2009b; Karlsson *et al.* 2009; Finstad *et al.* 2014; Solomon *et al.* 2015). Previous research indicates a potential threshold for positive DOC effects such as nutrient input and UV protection above which, negative impacts including light attenuation begin to reduce production (Jones 1992; Ask *et al.* 2009a; Solomon *et al.* 2015a). There has yet to be a set concentration at which the threshold is defined making it difficult to categorize DOC concentrations as either “high” or “low”. That being said, several studies have established that there is a unimodal response of both primary and secondary production to DOC (Finstad *et al.* 2014; Seekell *et al.* 2015a, b; Solomon *et al.* 2015; Kelly *et al.* 2018; Olson *et al.* 2020) . Further, previous work on establishing what has been called the “critical DOC concentration” have found it to range between 4-15 mg/L with the most recent stating it to be 16-26 mg/L (Hanson *et al.* 2003; Solomon *et al.* 2013; Seekell *et al.* 2015a; Bergström and Karlsson 2019; Olson *et al.* 2020). Using this research, we could make the statement that our study which includes lakes ranging from 4-24 mg/L contains both “high” and “low” DOC concentrations.

Initial influxes of DOC provide a necessary substrate for bacteria and provide necessary nutrients to support phytoplankton production (Jones 1992; Ask *et al.* 2009a; Solomon *et al.* 2015). However, surges in bacterial abundance can suppress phytoplankton due to resource competition and thereby hinder primary production in the pelagic habitat (Ask *et al.* 2009b; Solomon *et al.* 2015). Reduced light intensity has been shown to have a greater impact on benthic processes due to their location at the sediment level, requiring light to be able to penetrate deeper than the water column (Vadeboncoeur *et al.* 2001; Vander-Zanden and Vadeboncoeur 2002; Ask *et al.* 2009b). In high DOC lakes the light climate is reduced at the benthic level thereby impeding production of primary benthic algae (Vadeboncoeur *et al.* 2001; Vander-Zanden and Vadeboncoeur 2002). As the 'browning' process intensifies, the water drops in temperature and becomes anoxic, extending impacts upwards through the benthic food chain to zoobenthos (Vadeboncoeur *et al.* 2001; Vander-Zanden and Vadeboncoeur 2002). Zoobenthos, which include insect larvae and other invertebrates, largely rely on warm, well-oxygenated habitat in order to thrive (Craig *et al.* 2015). Further, increasing levels of DOC have been linked with the formation of 'fluffy' substrate – also unsuitable for zoobenthos (Hessen and Tranvik 1998). As benthic pathways have been found to significantly support many freshwater fishes (Vadeboncoeur *et al.* 2001; Vander-Zanden and Vadeboncoeur 2002), one assumes high concentrations of DOC will propagate further up the food chain and impact fish as well.

DOC and fish

Previous research examining DOC effects on fish have largely focused on population abundance and growth rate across several species. Walleye (*Sander vitreus*) productivity decreased with DOC in one study while in another on largemouth bass *(Micropterus salmoides*), diet composition changed due to reduced preferred epilimnion, but productivity was unaffected (Benoît *et al.* 2016; Koizumi *et al.* 2018). In a study examining bluegill sunfish

(*Lepomis macrochirus*), those from high DOC lakes reached smaller sizes overall and grew more slowly (Craig 2016).

Freshwater fish populations have commonly shown intraspecific variation in trophic traits and morphologies depending on their resource use leading to morphological divergence along the littoral-pelagic axis. Those more towards the 'littoral end' of the axis exploit zoobenthos while those in pelagic areas predominantly exploit zooplankton (Ehlinger & Wilson, 1988; Svanbeck & Eklov, 2002; Spotte, 2007; Bartels et al., 2016; Moran et al., 2018). Few studies have been conducted investigating high DOC impacts on the trophic morphology of fish. One study examined the diets and morphology of the European perch *(Perca fluviatilis*), a top predator, and revealed a shift in diet towards predominantly pelagic resources due to the reduction in available zoobenthos, as well as a shift from two distinct trophic morphologies to one more similar (Bartels *et al.* 2016).

The literature has recently begun to investigate how changes in DOC concentrations may propagate up through aquatic food webs to fish. However, gaining a good understanding of how fish species will respond to lake browning will require further research, particularly how changes in prey availability affect the phenotypes associated with their capture. The main objective of this thesis is to examine the effects, if any, of increasing DOC concentrations on trophic polymorphisms of fish which feed directly on both zoobenthos and zooplankton. To do so, I examine the morphology of bluegill sunfish (*Lepomis macrochirus*) from various north-temperate lakes along the DOC gradient. Bluegill sunfish are a widely distributed species among northern-temperate lakes and a popular study specimen (Werner and Hall 1974; Ehlinger and Wilson 1988; Werner *et al.* 1988; Ellerby and Gerry 2011; Gerry *et al.* 2011). This species varies phenotypically based on whether they feed in the littoral zone or open-water areas (Ehlinger and Wilson 1988; Gerry *et al.* 2011). Those present in the littoral area, feeding predominantly on zoobenthos, have deeper bodies and longer fins for better navigation and swimming efficiency within vegetation (Ehlinger and Wilson 1988). The opposite is found for those in open-water habitats as a plankton-dominated diet is associated with a more streamline body shape with short fins adapted for quick-burst swimming (Ehlinger and Wilson 1988). This thesis will examine these, among other traits associated with feeding in fish, in association with lake DOC concentration. Determining how increasing DOC concentrations influence bluegill trophic traits will allow us to further understand the mechanisms behind how fish populations will respond to a browning world.

REFERENCES

Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P. and Jansson, M. 2009a. Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. *American Society of Limnology and Oceanography*, **54**, 2034–2040.

Ask, J., Karlsson, J., Persson, L., Ask, P., Pa¨, P., Byström, P., Byström, B. and Jansson, A.M. 2009b. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology*, **90**, 1923–1932.

Bartels, P., Hirsch, P.E., Svanbäck, R. and Eklöv, P. 2016. Dissolved Organic Carbon Reduces Habitat Coupling by Top Predators in Lake Ecosystems. *Ecosystems*, **19**, 955–967, https://doi.org/10.1007/s10021-016-9978-x.

Benoît, P.O., Beisner, B.E. and Solomon, C.T. 2016. Growth rate and abundance of common fishes is negatively related to dissolved organic carbon concentration in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **73**, 1230–1236, https://doi.org/10.1139/cjfas-2015-0340.

Bergström, A.K. and Karlsson, J. 2019. Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, **25**, 2021–2029, https://doi.org/10.1111/gcb.14623.

Craig, N. 2016. *Impacts of Dissolved Organic Carbon on Productivity of Fish and Benthic Macroinvertebrates in North Temperate Lakes*.

Craig, N., Jones, S.E., Weidel, B.C. and Solomon, C.T. 2015. Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, **60**, 2079–2089, https://doi.org/10.1002/ln.

Ehlinger, T.J. and Wilson, D.S. 1988. Complex foraging polymorphism in bluegill sunfish. *Proc. Nail. Acad. Sci. USA*, **85**, 1878–1882.

Ellerby, D.J. and Gerry, S.P. 2011. Sympatric Divergence and Performance Trade-Offs of Bluegill Ecomorphs. *Evolutionary Biology*, **38**, 422–433, https://doi.org/10.1007/s11692-011-9130-y.

Finstad, A.G., Helland, I.P., Ugedal, O., Hesthagen, T. and Hessen, D.O. 2014. Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**, 36–43, https://doi.org/10.1111/ele.12201.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S. and Hudson, J. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, **430**, 195–198, https://doi.org/10.1038/nature02707.

Gerry, S.P., Wang, J. and Ellerby, D.J. 2011. A new approach to quantifying morphological variation in bluegill Lepomis macrochirus. *Journal of Fish Biology*, **78**, 1023–1034, https://doi.org/10.1111/j.1095-8649.2011.02911.x.

Hanson, P.C., Bade, D.L., Carpenter, S.R. and Kratz, T.K. 2003. *Lake Metabolism: Relationships with Dissolved Organic Carbon and Phosphorus*.

Hessen, D.O. and Tranvik, L.J. 1998. Aquatic humic substances: ecology and biogeochemistry. *Ecological Studies*, **133**.

Jones, R.I. 1992. Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator. *Hydrobiologia*, **229**, 73–91.

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L. and Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, 506–509, https://doi.org/10.1038/nature08179.

Koizumi, S., Craig, N., Zwart, J.A., Kelly, P.T., Ziegler, J.P., Weidel, B.C., Jones, S.E. and Solomon, C.T. 2018. Experimental whole-lake dissolved organic carbon increase alters fish diet and density but not growth or productivity. *Canadian Journal of Fisheries and Aquatic Sciences*, **75**, 1859–1867, https://doi.org/10.1139/cjfas-2017-0283.

Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopécek, J. and Vesely, J. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540, https://doi.org/10.1038/nature06316.

Yves T Prairie. 2008. Carbocentric limnology: looking back, looking forward. *Canadian Journal of Fisheries and Aquatic Sciences*. **65**(3): 543-548. https://doi.org/10.1139/f08-011

Roulet, N. and Moore, T. 2006. Browning the waters. *Nature*, **444**, 283–284.

Seekell, D.A., Lapierre, J.F., Ask, J., Bergstreom, A.K., Deininger, A., Rodriguez, P. and Karlsson, J. 2015a. The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, **60**, 1276–1285, https://doi.org/10.1002/lno.10096.

Seekell, D.A., Lapierre, J.F. and Karlsson, J. 2015b. Trade-offs between light and nutrient availability across gradients of dissolved organic carbon concentration in swedish lakes: Implications for patterns in primary production. *Canadian Journal of Fisheries and Aquatic Sciences*, **72**, 1663–1671, https://doi.org/10.1139/cjfas-2015-0187.

Sobek, S., Tranvik, L.J., Prairie, Y.T., Kortelainen, P. and Cole, J.J. 2007. Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and Oceanography*, **52**, 1208–1219.

Solomon, C.T., Jones, S.E., Weidel, B.C., Buffam, I., Fork, M.L., Karlsson, J., Larsen, S., Lennon, J.T., Read, J.S., Sadro, S. and Saros, J.E. 2015a. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, **18**, 376–389, https://doi.org/10.1007/s10021-015-9848-y.

Thomas, J.D. 1997. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biology*, **38**, 1–36.

Vadeboncoeur, Y., Lodge, D.M. and Carpenter, S.R. 2001. *Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats*.

Vander-Zanden, M.J. and Vadeboncoeur, Y. 2002. *Fishes as integrators of benthic and pelagic food webs in lakes*.

Werner, E.E. and Hall, D.J. 1974. Optimal Foraging and the Size Selection of Prey by the Bluegill Sunfish (Lepomis macrochirus). *Source: Ecology*, **55**, 1042–1052.

Werner, E.E., Gilliam, J.F., Hall, D.J. and Mittelbach, G.G. 1983. *An Experimental Test of the Effects of Predation Risk on Habitat Use in Fish*.

Werner, E.E., Hall, D.J. and Kellogg, W.K. 1988. ONTOGENETIC HABITAT SHIFTS IN BLUEGILL: THE FORAGING RATE-PREDATION RISK TRADE-OFF’. *Ecology*, **69**, 1352–1366.

THE LITTORAL-LIMNETIC AXIS OF MORPHOMETRIC VARIATION IN FISHES: DISSOLVED ORGANIC CARBON CONCENTRATION PRODUCES UNEXPECTED PATTERNS.

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Abstract

Inter- and intra-population trait polymorphisms related to foraging and locomotion in benthic and pelagic habitats have been observed in many fishes. The concentration of dissolved organic carbon (DOC) in the water strongly influences the productivity of benthic and pelagic food chains in lakes , suggesting that DOC might impose selection on these traits and lead to classic benthic-foraging "littoral" forms at low DOC concentrations and pelagic-foraging "limnetic" forms at high DOC concentrations. We tested this hypothesis via geometric morphometric and meristic analyses of body, fin, eye, and gill raker morphometry of Bluegill sunfish (*Lepomis macrochirus*, Centrarchidae) from fourteen lakes with DOC concentrations ranging from 4 to 24.5 mg L^{-1}. These lakes, located in close proximity to one another, straddle the drainage divide between the Mississippi River and Laurentian Great Lakes watersheds.

Many of the traits that we considered were significantly related to DOC concentration, and in most cases the sign (though not always the magnitude) of these relationships was consistent across the two watersheds. Fish from lakes with high DOC concentrations tended to have deeper bodies, deeper and shorter caudal peduncles, shorter and shallower heads, and larger eyes in shape space. When examined as an individual trait, there was no relationship between eye width and DOC concentration. Pectoral fins tended to be inserted more anteriorly and vertically when examined in shape space, but no significant correlation was found between the univariate insertion angle and DOC. Pectoral fin length varied with DOC, but the direction of this effect differed between the two watersheds. The gill rakers on the first branchial arch were longer and more widely spaced at high DOC, but the number of gill rakers did not vary with DOC. While many of the traits that we quantified were significantly related to DOC concentration, the directions of these relationships did not match with the predicted littoral-limnetic patterns. This may be because DOC has complex effects on the fitness landscape for foraging and locomotion traits; because high DOC favors a littoral-limnetic generalist rather than a limnetic specialist; or because the littoral-limnetic morphometric dichotomy is less clear and universal than is commonly thought.

Introduction

Inputs of terrestrially derived dissolved organic matter play an important role in structuring lake food webs (Jones 1992; Thomas 1997; Roulet and Moore 2006; Solomon et al. 2015). Lakes located in boreal or forested areas commonly obtain heterotrophic energy in the form of terrestrially derived dissolved organic matter (measured as dissolved organic carbon, DOC)

(Vadeboncoeur et al. 2001; Ask et al. 2009; Finstad et al. 2014; Solomon et al. 2015). These inputs include plant materials which accumulate and break down to release various compounds including humic and fulvic acids, giving lakes a distinct brown color (Jones 1992; Thomas 1997; Roulet and Moore 2006; Solomon et al. 2015). The coloring properties of DOC reduces light penetration and alters the vertical distribution of heat in the water column (Jones 1992; Roulet and Moore 2006). Collectively, these effects have substantial implications for benthic (bottom) and pelagic (open water) food chains, including primary production of algae and secondary production of benthic and planktonic invertebrates (Karlsson et al. 2009, Ask et al. 2012, Godwin et al. 2014, Craig et al. 2015). The relationship between increasing DOC and freshwater ecosystems is complex and non-linear with a threshold -above which, concentrations have negative effects on production (Ask et al. 2009; Karlsson et al. 2009; Finstad et al. 2014). Initial influxes of DOC provide a necessary substrate for bacteria and provide necessary nutrients to support phytoplankton production (Jones 1992; Ask et al. 2009; Solomon et al. 2015). However, surges in bacterial abundance can suppress phytoplankton due to resource competition and thereby hinder primary production in the pelagic habitat (Ask et al. 2009; Solomon et al. 2015). Reduced light intensity has been shown to have a greater impact on benthic processes due to their location at the sediment level, requiring light to be able to penetrate deeper than the water column (Vadeboncoeur et al. 2001; Vander Zanden and Vadeboncoeur 2002; Ask et al. 2009). In high DOC lakes the light climate is reduced at the benthic level thereby impeding production of primary benthic algae (Vadeboncoeur et al. 2001; Vander Zanden and Vadeboncoeur 2002). As the ‘browning’ process intensifies, the water drops in temperature and becomes anoxic, extending impacts upwards through the benthic food chain to zoobenthos

(Vadeboncoeur et al. 2001; Vander Zanden and Vadeboncoeur 2002). Zoobenthos, which include insect larvae and other invertebrates, largely rely on warm, well-oxygenated habitat in order to thrive (Craig et al. 2015). These effects of DOC at the base of benthic and pelagic food chains can propagate up to influence to productivity of fishes, which often link those food chains via their mobile foraging behaviors (Vander Zanden and Vadeboncoeur 2002, Karlsson et al. 2009, Finstad et al. 2014, Craig et al. 2015, Benoit et al. 2016, Koizumi et al. 2018).

Previous research examining DOC effects on fish have largely focused on population abundance and growth rate across several species. Walleye (*Sander vitreus*) productivity decreased with DOC in one study while in another on Largemouth bass (*Micropterus salmoides*), diet composition changed due to reduced preferred epilimnion, but productivity was unaffected (Benoît et al. 2016; Koizumi et al. 2018). In a study examining bluegill sunfish (*Lepomis macrochirus*), those from high DOC lakes reached smaller sizes overall and grew more slowly (Craig et al. 2017). Freshwater fish populations have commonly shown intraspecific variation in trophic traits and morphologies depending on their resource use leading to morphological divergence along the littoral-pelagic axis. Those more towards the ‘littoral end’ of the axis exploit zoobenthos while those in pelagic areas predominantly exploit zooplankton (Ehlinger & Wilson, 1988; Svanbeck & Eklov, 2002; Bartels et al., 2016; Moran et al., 2018). In particular, Bluegill display longer, more stream-line body shapes and shorter pectoral fins when predominantly exploiting open-water habitat and shorter, deeper bodies with longer fins in littoral habitat (Ehlinger and Wilson 1988). Gill rakers also exhibited trophic plasticity as raker length and number increase in mainly planktivorous, open-water bluegill and gaps between rakers decrease (Mittelbach, 1984; Ehlinger & Wilson, 1998). Studies using yellow perch

(Svanbeck & Eklov, 2002) and stickleback (Bentzen & McPhail, 1984) found similar effects on body shape and trophic traits. Yet, we know of only one study that has considered the relationship between these traits and DOC concentration. Examining Eurasian Perch, Bartels et al. (2016) collected samples from lakes with DOC concentrations between 4 and 34 mg L-1. The study found that that increasing DOC concentration was associated with longer heads, large eyes, and narrow caudal peduncles; fish diet shifted towards pelagic resources (Bartels et al. 2016).

This paper aims to investigate the correlation between the trophic alterations caused by increasing DOC and the morphology of fish populations. We sampled Bluegill from a set of lakes in northern Wisconsin, USA, varying in DOC concentration from 4 to 24.5 mg L-1 and straddling the drainage divide between the Great Lakes and Mississippi River. We believe that DOC could be affecting the littoral-limnetic axis on which fish phenotypes change. As increasing levels of DOC often result in a reduction of benthic prey availability, we hypothesize that fish in high DOC lakes will tend to be morphologically suited for zooplanktivory. Fish sampled from lakes with higher concentrations of dissolved organic carbon are expected to have streamline body shapes with shorter fins. Further, we believe they will have longer and more numerous gill rakers with small inter-raker gaps and larger eyes for optimal capture of planktonic prey.

Methods

Study Design and Sampling

We conducted morphometric analyses on Bluegill sampled from 14 lakes with DOC concentrations between 4 and 24.5 mg L^{-1} (Table 1, Figure 1). Fish assemblages of the lakes were similar with all of them containing largemouth bass (*Micropterus salmoides*) and the majority of them containing northern pike (*Esox Lucius*), muskellunge (*Esox masquinongy*), and walleye (*Sander vitreus*). All lakes were within a radius of ~40 km, near the Wisconsin-Michigan border, USA. This is a low-relief, lake-rich glacial region that straddles the divide between the Great Lakes and Mississippi River drainages. Phylogeographic work using mitochondrial DNA sequences from bluegill collected to the south and east of our study site does not indicate deeply rooted phylogenetic diversity in this region (Kawamura *et al.* 2009). Nonetheless, we chose study lakes such that our sample size and distribution of DOC concentrations would be approximately equal in the two drainages, to reduce the chance of confounding DOC effects with effects of lineage/drainage.

We sampled ~30 Bluegill individuals from each lake between June and August 2018, using a variety of methods including fyke nets, boat electrofishing, and angling (Table 1). In order to maintain a balanced study design 30 individuals were collected from each lake as some lakes proved more difficult to capture enough specimens than others and required multiple capture days to acquire. Three to four fyke nets (12 m lead, 1.3 cm bar mesh, 1 m^3 car, four hoops and two throats) were set from afternoon to morning, with the lead set perpendicular to the shore. Electrofishing was conducted at night using alternating current. Angling was conducted near sunrise or sunset using standard tackle and was added as a sampling method due to the inability to perform initial capture methods in Hummingbird Lake. Fish were selected to be between 75 and 150mm standard length (or longer if necessary) to avoid ontogenetic niche shifts (Werner *et al.* 1988). Captured fish 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. The fish used for morphometric analyses came from 1 to 3 different samples (location-day combinations) per lake, covering 29 total sample days and 3 capture methods. Animal care procedures were approved by the Cary Institute of Ecosystem Studies animal care and use committee under protocol number 2016-06.

Morphometrics and Meristics

Digital photographs of each sampled fish 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 so that fin insertion points were clear, ensuring that its mouth was closed, and including a scale reference. We separately calculated eye width as the distance between landmarks #2 and #19, and angle of pectoral fin insertion from landmarks #13 and #14 (Figure 2A) After photographing each specimen, we removed the right pectoral fin and preserved it in 95% ethanol. Later, we spread and pinned each fin and photographed it using the procedure described above for body photos. 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 2C). For each, we measured raker 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 2C). These measurements were made using the measurement tool included in the tpsDig2 v.2.31 program (Rohlf, 2006).

We quantified body morphometry of each specimen from the full body photographs by digitizing 19 true type II two-dimensional landmarks using tpsDig2 v.2.31 (Rohlf & Bookstein 1990, Rohlf 2006) (Figure 2A). To assess digitizing error, a subset of 60 specimens underwent four landmarking procedure replications. We then conducted a Procrustes ANOVA of the replicates and following Zelditch et al. (2004) we calculated landmark repeatability to be 95%. As this result was sufficiently high, we measured the remaining specimens only once. Pectoral fin morphometry was also examined using the same landmarking procedures and four true type landmarks were digitized onto the images (Rohlf, 2006) (Figure 2B). 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 the fin insertion width as the distance between landmarks #13 and #14 and fin length as the distance between landmarks #14 and #21 (Figure 2B). Following Robinson et al. (1996), which also used insertion point width, we calculated pectoral fin aspect ratio as the length of the fin divided by the width of the insertion point. We separately calculated the angle of pectoral fin insertion from landmarks #13 and #14 of the full body photos and eye width as the distance between landmarks #2 and #19 (Figure 2A).

Statistical Analyses

We conducted geometric morphometrics analyses using the geomorph v.3.1.0 package in R v.3.5.3 (Adams et al., 2019; R Core Team, 2019). We subjected raw coordinates to a generalized Procrustes analysis (Zelditch *et al.* 2004) to remove isometric size effects and achieve uniform orientation. We then conducted a principal components analysis to identify the major axes of shape variance among all the Bluegill in our sample. To visualize morphospace based on shape along the first two principal components, we created a backtransform morphospace (Olsen, 2017) (Figure 3).

We evaluated the relationship between DOC and body shape by using the random residual permutation procedure with 1000 iterations within the geomorph package (Collyer & Adams, 2019). Using the procD.lm function we subjected the shape data to a four-factor multivariate analysis of covariance with (Goodall, 1991; Anderson, 2001; Collyer et al., 2015). Centroid size was included as a covariate, DOC and watershed as fixed effects, and lake as a random effect. Centroid size, a proxy for fish size calculated from the 2D shape data, is the square root of the sum of squared distances of all landmarks from their centroid, which is obtained by averaging landmark x and y coordinates (Klingenberg 2016). We want to test the impact of the co-variate (centroid size) on shape to eventually remove the variance in shape that is explained by the co-variate (Zelditch *et al.* 2004). We determined interactions between the covariate and multiple factors (DOC, lake, and watershed) thus preventing us from being able to remove the variation by regression (Zelditch *et al.* 2004). Instead, the focus of our analysis must be on the interactions between the factors and the covariate rather than the main effects of the factors (Zelditch *et al.* 2004). To analyze other phenotypic traits, including eye width and characteristics of pectoral fins and gill rakers, we used univariate models similar in structure to the model described above for the shape data, including DOC and watershed as fixed effects and lake as a random effect. Most of these traits covaried with fish size (standard length), so we size standardized using the equation given in Kaeuffer et al. (2012). We fit these models using R packages lme4 and lmerTest (Kuznetsova et al., 2017; Bates et al. 2018).

Results

Body Shape

There was a significant allometric effect in bluegill sunfish depending on lake DOC and watershed (z=2.04, p=0.02) (Table 2). Variation in shape was strongly correlated with size, resulting in a consistent allometric effect between watersheds but a significant, varying effect between DOC concentrations. Overall, the variation in allometric slopes depended on both the DOC of the sample lake and the watershed the lake is in. Principal components 1 and 2 for all individuals represented 52% of the total variance in shape. When examining mean body shape per lake, the first two PC's described 80% of the shape variation. The first principal component (55%) described a shift from a longer, streamline body shape to a shorter, deeper body; a shorter to longer eye diameter; and from a more horizontal to a more vertical insertion of the pectoral fin (Figure 3). The second principal component (25%) described a shift from a smaller head, more anteriorly positioned pectoral fin and longer caudal peduncle to a larger head, more posteriorly positioned pectoral fin and shorter tail (Figure 3). The mean fish shape for each lake and corresponding DOC concentration showed that lakes with DOC concentrations less than 10mg/L occupy almost the entire shape space of both PC's. However, lakes with higher DOC concentrations (>10mg/L) contained fish shapes with more positive PC1 values and negative PC2 values. Fish from lakes in the Great Lakes watershed occupied lower PC value shape space than those from lakes in the Mississippi watershed. There were 14 outlier fish shapes, 7 of which came from Crampton Lake.

Pectoral Fins

Pectoral fin length was significantly related to dissolved organic carbon and watershed while other characteristics of the fin were not (Figure 4). Length increased with DOC concentration for lakes within the Great Lakes watershed, but the opposite was observed for the Mississippi watershed (Figure 4A). The longest pectoral fins were from Hummingbird Lake (size standardized mean of 33.9 mm) which had the highest DOC concentration. Squaw Lake, which had a similar DOC concentration but belonged to a different watershed, was one of the lakes with shorter pectoral fins (size standardized mean of 32.7mm). The shortest pectoral fins, with a size corrected mean of 29.9mm, were from Crampton Lake. Overall, it was found that the effect of lake DOC on fin length was dependent on the watershed the lake is from (t= -2.3, p=0.05). Neither DOC concentration nor watershed influenced pectoral fin base width, aspect ratio, or insertion angle (Figure 5). While there was a visible change in pectoral fin insertion angle displayed in the shape data along PC1, it was not directly associated with DOC nor watershed. Fish size also had no correlation with fin insertion angle.

Gill Rakers

Gill raker length and inter-raker spacing were significantly related to DOC concentration and watershed while raker number was not (Figure 5). Gill raker length varied by lake DOC (t=2.8, p=0.02) and watershed (t=2.3, p=0.046). Raker length increased with DOC concentration and was longer overall in lakes from the Mississippi Watershed (Figure 5A). The steepness (but not the direction) of the relationship between raker spacing and DOC varied by watershed (t= - 2.8, p=0.017). Width of inter-raker spaces increased with DOC concentration but at a slower rate in the Mississippi Watershed (Figure 5B). Size standardized raker length means ranged from 17.5mm in Crampton Lake to 24.5mm in Red Bass Lake while spaces ranged from 5mm in Crampton Lake to 7.6mm in Squaw Lake. The number of rakers on the outer-most gill arches ranged from 9 to 15 and was strongly related to fish size but not to DOC concentration (Figure 6C).

Eyes

Eye widths ranged from 7.1mm to 11.3mm across lakes (Figure 6). Unsurprisingly, eye size was strongly positively correlated with fish size.

Discussion

Our results demonstrate changes in several morphometric traits, independent of size, correlating with increasing DOC concentrations. While we expected the bluegill in high DOC lakes to exhibit ‘pelagic-type’ traits suited for zooplanktivory and open-water swimming, we instead found that they display a combination of different traits. Several were ‘littoral-type’, associated with consuming zoobenthos and living in vegetated habitat, while others were ‘pelagic-type’ or inconclusive. This contradicts what we know about DOC effects on resource availability and production and what was thought to be a clear division of phenotypic traits based on trophic habitat.

Pelagic Traits

Previous research is consistent in showing that while higher concentrations of DOC in lakes negatively impact primary and secondary production, zoobenthos are more negatively impacted than zooplankton (Vadeboncoeur et al. 2001; Vander Zanden and Vadeboncoeur 2002; Ask et al., 2009; Craig et al., 2015). Therefore, in lakes with high DOC concentrations, the diet of bluegill should be dominated by zooplankton. Previous research shows bluegill that feed predominantly on zooplankton have a more streamline, elongated body shape with smaller, more anteriorly placed pectoral fins that are also more horizontally angled to facilitate open-water swimming (Webb, 1984; Ehlinger and Wilson, 1988; Drucker and Lauder, 2002; Svanback and Eklov, 2002; Spotte, 2007). Planktivorous diets are also associated with longer, more numerous gill rakers with smaller gaps between them for more efficient capture of smaller prey (Bentzen and McPhail, 1984; Mittelbach, 1984; Gibson, 1987; Wimberger, 1994; Berchtold et al, 2015). However, our study showed fish from high DOC lakes having only a subset of the hypothesized 'pelagic traits'. Independent of watershed, raker length increased with DOC and the multivariate analysis included an anterior shift of pectoral fin position. Further, the pectoral fins of fish from the Mississippi watershed decreased in length with increasing DOC concentrations, potentially lending the idea that this trait is more strongly impacted by some other factor as the exact opposite relationship was observed across the Great Lakes watershed. Previous studies evaluating the evolutionary history of fish from the Great Lakes and Mississippi drainage basins have yet to determine the extent of their relatedness (Kawamura *et al.* 2009). This leaves evolutionary history as a potential explanation for the morphological variations such as fin length we have observed between the two basins.

Littoral Traits

Commonly associated with enhanced maneuverability in littoral or complex vegetated habitats, bluegill from higher DOC lakes tended towards deeper body shapes with shorter caudal peduncles and heads, and long pectoral fins but only in the Great Lakes watershed. Independent of watershed or size, they had larger gaps between gill rakers which is indicative of larger prey consumption.

Deeper, shorter bodies have been linked with navigating littoral zones of several marine and freshwater fish species for decades. It could be that ontogeny or external pressures such as predation risk are also at play here and pushing bluegill to remain in complex habitat for longer periods of time resulting in the unexpected 'littoral' shape. Bluegill exhibit habitat partitioning and phenotypic variation even as juveniles, with young-of-year's being more fusiform in deeper areas of the lake and having deeper bodies in shallow, vegetated areas (Layzer and Clady, 1987). The environment in which bluegill reside throughout early development is highly influential upon determining their phenotypes and the trophic polymorphisms they exhibit as adults (Layzer and Clady, 1987). Moreover, bluegill go through several ontogenetic shifts, one of which is returning to the littoral area as fry of a certain size to feed in the vegetation for protection until they are large enough to avoid predation risks associated with open-water habitat (Werner and Hall, 1988). We know that bluegill in high DOC lakes grow slower (Craig et al, 2017), and so the ontogenetic shift from littoral to pelagic habitat may take longer. Moreover, the predator that small bluegill try to avoid is the largemouth bass, whose density increases in lakes with high DOC concentrations as the volume of their preferred habitat in the epilimnion is reduced (Koizumi et al. 2018). Further, there is an intermediate transition by bluegill to the water above the vegetation to exploit zooplankton before they make the full transition to the pelagic zone (Werner and Hall, 1988). It is possible that in high DOC lakes, bluegill exploit this resource as well as reside in the vegetation longer for safety which could explain their combination of littoral and pelagic traits.

Pectoral fin lengths, as mentioned previously, exhibit different relationships with increasing DOC based on which watershed they are from. While the Mississippi watershed showed the expected negative relationship with DOC, the Great Lakes watershed did not. This insinuates that there are other effects at play within watersheds which influence how DOC may interact with pectoral fin lengths.

Wider gill raker gaps are an interesting result coupled with longer gill rakers as their relationship is typically inverted. One possible explanation for this could be that DOC impacts on bluegill prey availability require them to increase the range of their diet and wider raker spaces could allow them to do that. Previous bluegill research revealed that they increase their breadth of prey selection and search times when faced with lower temperatures in dark waters (Werner and Hall, 1974). Longer hover and search times have also been associated with feeding in more complex environments where prey are more cryptic (Ehlinger and Wilson, 1988; Spotte, 2007).

Finally, several littoral trophic traits may be expressed by bluegill in high DOC lakes because not all high DOC lakes are created equal. Despite increases in DOC, shallow lakes have a higher threshold for the negative effects than deep lakes (Finstad et al., 2014). This is because the smaller depths of shallow lakes allow for the, albeit smaller, euphotic zone to be better exploited for photosynthesis (Finstad et al., 2014). Therefore, shallow lakes with high DOC concentrations could potentially have enough zoobenthos availability to influence bluegill trophic traits. The lakes with the highest DOC concentrations in our study are also those within the lower range of max depths exhibited, again providing possible context as to why the fish from these lakes have trophic traits associated with littoral habitats as well as pelagic (Table 1).

Inconclusive Traits

Several traits examined were not significantly influenced by increases in DOC concentrations, despite their association with trophic habitat in the literature, and include eye size, raker number, and pectoral fin insertion angle.

Eye size has been positively related to visual acuity in other fish species and this relationship was found to increase in salmonids when exposed to high turbidity and DOC resulting in lower visibility (Drinan et al., 2012; Bartels et al., 2016; Caves et al., 2017). However, eye size did not increase as a function of DOC in our study, indicating potentially insufficient visual acuity for hunting prey in darker, 'browning' waters. While our study only examined eye diameter there are other more complex traits associated with vision including opsin gene expression (Carleton et al., 2008). Opsin genes are found in the rods and cones of the eye and determine the visual spectral range or wavelengths a fish can see (Carleton et al., 2008). It could be that dissolved organic carbon is having an impact on these genes rather than eye diameter which is significantly related to size. Other research involving zooplankton revealed that their ability to detect predator chemical cues was reduced with increasing dissolved organic matter and humic substances (Santonja et al., 2016). Perhaps increased eye diameter is not selected upon by DOC because bluegill prey are already more susceptible to predation.

Raker number was also found not to be influenced by increasing DOC concentrations. Previous research describes other species including herring and whitefish having increased zooplankton foraging success when having more gill rakers (Gibson, 1988; Kahilainen et al, 2011). It should be noted however that another study investigating gill raker morphology of salmonids found that there was no relationship between raker number and successful planktivory (Langeland and Nøst, 1995). Moreover, salmonids and herring are typically 'cruisers' which filter-feed and their gill raker numbers range anywhere from 20 to 50+ (Gibson, 1988; Kahilainen et al, 2011) whereas bluegill from our study exhibited a range of 9-15. Therefore, it would make sense that species that are not visual hunters and have such a wide variation in gill raker numbers would be more reliant on their ability to sieve prey than a species such as the bluegill.

The final inconclusive trait-DOC relationship examined was the pectoral fin angle of insertion. The angle at which this fin is positioned determine the forces the fin can generate for turning and helps with maneuverability and stability (Drucker and Lauder, 2002; Spotte, 2007). In a study comparing the wake dynamics of bluegill sunfish and trout the more vertically angled pectoral fins of the bluegill allowed for a significant increase in turning power than the horizontally angled trout fins (Drucker and Lauder, 2002). While there was a visible change in pectoral fin angle along the PC1 axis of the multivariate data showing a slightly more vertically angled pectoral fin at the PC1 max, when the angle was examined as a univariate it was found to have no relationship with DOC. This suggests that pectoral fin angle could be an important contributor to an axis of variation associated with DOC and correlated with other shape changes but is not strongly selected upon by DOC individually. Pectoral fin width and aspect ratio were also unrelated to changes in DOC concentration. Following literature stating that larger fins help with turning and braking in complex littoral areas (Spotte, 2007), we predicted a decrease in fin width and aspect ratio as DOC levels increase. A lack of response in these traits suggest that they may not be as mailable to environmental changes compared to pectoral fin length and perhaps they are not as vital to maneuverability.

Evolution

The unexpected combination of results may fall under the umbrella of a larger evolutionary effect. We propose two possible evolutionary explanations for how selection of DOC may be acting upon bluegill trophic traits lending to our results. First, we propose that DOC selection maps directly on to the littoral-limnetic axis, but traits are reacting to differently. Within this theory there are two potential selective pathways the traits could follow one of which is that traits have different genetic variation and thus different evolutionary potentials to respond. For example, raker length and gap width may have similar selection (fitness x trait value) but length has more variation (frequency x trait value) and has evolved appropriately in response to increased DOC concentrations. Another possibility, under the same selective theory, is that plasticity varies between traits. As such, raker length could simply be more plastic than number or gap width and is the first to respond. A study examining the genetic and plastic contributions to a population of pumpkinseed sunfish (*Lepomis gibbosus*), a morphologically similar and closely related species to the bluegill, found that trophic traits such as body depth and pectoral fin position were plastic while body and pectoral fin lengths were genetic contributions

(Robinson and Wilson, 1996). They also found that traits such as pectoral fin base width and aspect ratio are contributed by an interaction of both genetic variation and phenotypic plasticity (Robinson and Wilson, 1996). This supports the idea that morphological trait variations can be adapted either through genetic or plastic means and some are more variable than others which lends to the idea that traits could be adapting at different rates to the presence of high DOC concentrations. In another pumpkinseed study by Yavno and Fox (2014), fish from different environments were raised in pelagic and littoral enclosures and they found that fish from certain areas that were placed in the pelagic enclosures actually had deeper bodies, longer gill rakers and wider gaps- the same polymorphisms that we observed. They suggested that a potential explanation for this could be that the environment that these fish were originally from were highly variable and experienced relatively large perturbations and then went on to say that the fish may be exhibiting reduced plasticity because of such perturbations (Yavno & Fox, 2014). However, a major issue with being able to definitively attribute our results to this selection theory is time. The road to evolutionary potential through genetic variation or plasticity is relatively short, for example the plastic and genetic variations observed in the aforementioned studies occurred over one generation or field season. However, our study lakes have been ‘high’ or ‘low’ DOC lakes for a long time with only slight changes to concentrations often to the first decimal point. This suggests that the bluegill in these lakes should have surpassed the stages of different trait ‘reaction times’ and should have reached their overall response to their respective DOC environments by the time this study was conducted.

A more likely selection theory, we believe, is that DOC selection does not map directly on to the littoral-limnetic axis and there are other indirect effects of DOC creating opposing outcomes. In other words, it may be that increases in DOC may be stronger and act on the traits differently resulting in unexpected responses. For example, increases in DOC and limnetic foraging may have successfully selected for longer rakers but DOC-induced changes in something like growth or predation risk, as mentioned earlier, could be stronger and thus selecting for traits associated with remaining in the safety of vegetated habitat such as deep body shape and wider gaps between rakers. The opposing indirect effects could also be 'battling' with the direct effects of increased limnetic foraging and leading to a lack of any response at all as with our inconclusive results. Moreover, it seems clear that there is some indirect effect of DOC impacting how pectoral fin length responds in separate watersheds as they positively correlated with DOC concentration increases in the Great Lakes watershed and negatively so in the Mississippi watershed.

The final possibility that should be considered is the potential for the development of a generalist at high concentrations of DOC. As both selection theories imply a reduction in phenotypic divergence and the fish exhibit trophic traits adapted for both habitats, it is possible that an intermediate form or generalist is more beneficial than specializing for one habitat over the other. This could be why the bluegill have not only longer rakers to optimize zooplankton capture but also wider gaps between rakers to accommodate predation of larger prey such as invertebrates if the opportunity should present itself as bluegill are known to follow the optimal foraging theory (Spotte, 2007). Additionally, in a paper by Ehlinger (1991) stated that bluegill have an intermediate generalist form which more so resembles the shape of the 'maneuverer', i.e. a deep body. Moreover, previous research on the evolution of a single generalist says it is expected to evolve as a result of beneficial mutations that improve fitness across different patches likely being fixed (Bailey et al., 2015).

There are numerous components, both external and internal, in lake ecosystems which could have influenced our results. Several biotic factors may have impacted our results including the fact that every lake sampled contained major predators of bluegill which are known to increase bluegill (particularly those below 50 mm SL) use of vegetated areas as refuge (Werner *et al.* 1983). In the absence of predators, bluegill of all sizes have been observed shifting between both open and vegetated habitats depending on foraging success (Werner *et al.* 1983). Future studies would likely get a clearer image of DOC effects on trophic morphology sampling lakes without predators. It may also be beneficial to include more lakes with larger sample sizes and perhaps a single, more consistent sampling method.

While DOC does have an effect on bluegill body shape and other select trophic traits, it is clear that the effects do not lead to a distinct pelagic phenotype. There is still a lot we do not understand about how DOC impacts freshwater ecosystems, particularly higher up the food chain at the level of fish. Future studies should examine bluegill from specifically littoral and limnetic areas of the lake, as well as examine them on a temporal scale. While this study did not allow for the examination of individual fish diets, it would be useful to know exactly what the fish are eating in high DOC lakes even though the literature provides us with an idea of the primary and secondary producer composition. Additional studies are required for us to better understand how DOC effects move throughout the food web of freshwater ecosystems.

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REFERENCES

Adams, D.C. and Collyer, M.L. 2019. Geomorph: Software for geometric morphometric analyses. R package Version 3.1. 0.

Ask, J., Karlsson, J., Persson, L., Ask, P., Byström, P. and Jansson, M. 2009a. Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. *American Society of Limnology and Oceanography*, **54**, 2034–2040.

Ask, J., Karlsson, J., Persson, L., Ask, P., Pa¨, P., Bystro¨m, P., Bystro¨m, B. and Jansson, A.M. 2009b. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. *Ecology*, **90**, 1923–1932.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32-46.

Anderson MJ. and C.J.F. terBraak. 2003. Permutation tests for multi-factorial analysis of variance. Journal of Statistical Computation and Simulation 73: 85-113.

Bailey, S. F., Rodrigue, N., & Kassen, R. 2015. The effect of selection environment on the probability of parallel evolution. *Molecular biology and evolution*, *32*(6), 1436-1448.

Bartels, P., Hirsch, P.E., Svanbäck, R. and Eklöv, P. 2016. Dissolved Organic Carbon Reduces Habitat Coupling by Top Predators in Lake Ecosystems. *Ecosystems*, **19**, 955–967, https://doi.org/10.1007/s10021-016-9978-x.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G. and Green, P. 2018. Package 'lme4'. *Version*, *1*, p.17.

Benoît, P.O., Beisner, B.E. and Solomon, C.T. 2016. Growth rate and abundance of common fishes is negatively related to dissolved organic carbon concentration in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **73**, 1230–1236, https://doi.org/10.1139/cjfas-2015-0340.

Bentzen, P., and McPhail, J. D. 1984. Ecology and evolution of sympatric sticklebacks (Gasterosteus): specialization for alternative trophic niches in the Enos Lake species pair. *Canadian Journal of Zoology*, *62*(11), 2280-2286.

Bergström, A.K. and Karlsson, J. 2019. Light and nutrient control phytoplankton biomass responses to global change in northern lakes. *Global Change Biology*, **25**, 2021–2029, https://doi.org/10.1111/gcb.14623.

Carleton, K. L., Spady, T. C., Streelman, J. T., Kidd, M. R., McFarland, W. N., & Loew, E. R. 2008. Visual sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC biology*, *6*(1), 22.

Caves, E. M., Sutton, T. T., & Johnsen, S. (2017). Visual acuity in ray-finned fishes correlates with eye size and habitat. *Journal of Experimental Biology*, *220*(9), 1586-1596.

Collyer, M.L., D.J. Sekora, and D.C. Adams. 2015. A method for analysis of phenotypic change for phenotypes described by high-dimensional data. Heredity. 115:357-365.

Craig, N. 2016. *Impacts of Dissolved Organic Carbon on Productivity of Fish and Benthic Macroinvertebrates in North Temperate Lakes*.

Craig, N., Jones, S.E., Weidel, B.C. and Solomon, C.T. 2015. Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, **60**, 2079–2089, https://doi.org/10.1002/ln.

Drucker, E. G., & Lauder, G. V. 2002. Wake dynamics and locomotor function in fishes: interpreting evolutionary patterns in pectoral fin design. *Integrative and Comparative Biology*, *42*(5), 997-1008. 42

Drinan, T. J., McGinnity, P., Coughlan, J. P., Cross, T. F., & Harrison, S. S. 2012. Morphological variability of Atlantic salmon Salmo salar and brown trout Salmo trutta in different river environments. *Ecology of Freshwater Fish*, *21*(3), 420-432.

Ehlinger, T.J. 1991. Allometry and Analysis of Morphometric Variation in the. *Source: Copeia*, **1991**, 347–357.

Ehlinger, T.J. and Wilson, D.S. 1988. Complex foraging polymorphism in bluegill sunfish. *Proc. Nail. Acad. Sci. USA*, **85**, 1878–1882.

Ellerby, D.J. and Gerry, S.P. 2011. Sympatric Divergence and Performance Trade-Offs of Bluegill Ecomorphs. *Evolutionary Biology*, **38**, 422–433, https://doi.org/10.1007/s11692-011-9130-y.

Finstad, A.G., Helland, I.P., Ugedal, O., Hesthagen, T. and Hessen, D.O. 2014. Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**, 36–43, https://doi.org/10.1111/ele.12201.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S. and Hudson, J. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature*, **430**, 195–198, https://doi.org/10.1038/nature02707.

Gerry, S.P., Wang, J. and Ellerby, D.J. 2011. A new approach to quantifying morphological variation in bluegill Lepomis macrochirus. *Journal of Fish Biology*, **78**, 1023–1034, https://doi.org/10.1111/j.1095-8649.2011.02911.x.

Goodall, C.R. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society B 53:285-339.

Hanson, P.C., Bade, D.L., Carpenter, S.R. and Kratz, T.K. 2003. *Lake Metabolism: Relationships with Dissolved Organic Carbon and Phosphorus*.

Hessen, D.O. and Tranvik, L.J. 1998. Aquatic humic substances: ecology and biogeochemistry. *Ecological Studies*, **133**.

Jones, R.I. 1992. Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator. *Hydrobiologia*, **229**, 73–91.

Kahilainen, K. K., Siwertsson, A., Gjelland, K. Ø., Knudsen, R., Bøhn, T., & Amundsen, P. A. 2011. The role of gill raker number variability in adaptive radiation of coregonid fish. *Evolutionary Ecology*, *25*(3), 573-588.

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L. and Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, 506–509, https://doi.org/10.1038/nature08179.

Kawamura, K., Yonekura, R., Katano, O., Taniguchi, Y. and Saitoh, K. 2009. Phylogeography of the bluegill sunfish, Lepomis macrochirus, in the Mississippi River Basin. *Zoological Science*, **26**, 24–34, https://doi.org/10.2108/zsj.26.24.

Kelly, P.T., Solomon, C.T., Zwart, J.A. and Jones, S.E. 2018. A Framework for Understanding Variation in Pelagic Gross Primary Production of Lake Ecosystems. *Ecosystems*, **21**, 1364–1376, https://doi.org/10.1007/s10021-018-0226-4.

Klingenberg, C.P. 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. *Development genes and evolution*, *226*(3), pp.113-137.

Koizumi, S., Craig, N., Zwart, J.A., Kelly, P.T., Ziegler, J.P., Weidel, B.C., Jones, S.E. and Solomon, C.T. 2018. Experimental whole-lake dissolved organic carbon increase alters fish diet and density but not growth or productivity. *Canadian Journal of Fisheries and Aquatic Sciences*, **75**, 1859–1867, https://doi.org/10.1139/cjfas-2017-0283.

Kuznetsova, A., Brockhoff, P.B. and Christensen, R.H., 2017. lmerTest package: tests in linear mixed effects models. *Journal of statistical software*, *82*(13), pp.1-26.

Langeland, A., & Nøst, T. 1995. Gill raker structure and selective predation on zooplankton by particulate feeding fish. *Journal of Fish Biology*, *47*(4), 719-732.

Layzer, J. B., & Clady, M. D. 1987. Phenotypic variation of young-of-year bluegills (Lepomis macrochirus) among microhabitats. *Copeia*, 702-707.

Mittelbach, G. G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). *Ecology*, *65*(2), 499-513.

Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopécek, J. and Vesely, J. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540, https://doi.org/10.1038/nature06316.

Olsen, A.M., 2017. Feeding ecology is the primary driver of beak shape diversification in waterfowl. *Functional Ecology*, *31*(10), pp.1985-1995.

Olson, C.R., Solomon, C.T. and Jones, S.E. 2020. Shifting limitation of primary production: experimental support for a new model in lake ecosystems. *Ecology Letters*, **23**, 1800–1808, https://doi.org/10.1111/ele.13606.

Robinson, B. W., & Wilson, D. S. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (Lepomis gibbosus). *Evolutionary Ecology*, *10*(6), 631-652.

Roulet, N. and Moore, T. 2006. Browning the waters. *Nature*, **444**, 283–284.

Santonja, M., Minguez, L., Gessner, M. O., & Sperfeld, E. (2017). Predator–prey interactions in a changing world: humic stress disrupts predator threat evasion in copepods. *Oecologia*, *183*(3), 887-898.

Seekell, D.A., Lapierre, J.F., Ask, J., Bergstreom, A.K., Deininger, A., Rodriguez, P. and Karlsson, J. 2015a. The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, **60**, 1276–1285, https://doi.org/10.1002/lno.10096.

Seekell, D.A., Lapierre, J.F. and Karlsson, J. 2015b. Trade-offs between light and nutrient availability across gradients of dissolved organic carbon concentration in swedish lakes: Implications for patterns in primary production. *Canadian Journal of Fisheries and Aquatic Sciences*, **72**, 1663–1671, https://doi.org/10.1139/cjfas-2015-0187.

Sobek, S., Tranvik, L.J., Prairie, Y.T., Kortelainen, P. and Cole, J.J. 2007. Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and Oceanography*, **52**, 1208–1219.

Solomon, C.T., Bruesewitz, D.A., et al. 2013. Ecosystem respiration: Drivers of daily variability and background respiration in lakes around the globe. *Limnology and Oceanography*, **58**, 849–866, https://doi.org/10.4319/lo.2013.58.3.0849.

Solomon, C.T., Jones, S.E., Weidel, B.C., Buffam, I., Fork, M.L., Karlsson, J., Larsen, S., Lennon, J.T., Read, J.S., Sadro, S. and Saros, J.E. 2015a. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, **18**, 376–389, https://doi.org/10.1007/s10021-015-9848-y.

Solomon, C.T., Jones, S.E., Weidel, B.C., Buffam, I., Fork, M.L., Karlsson, J., Larsen, S., Lennon, J.T., Read, J.S., Sadro, S. and Saros, J.E. 2015b. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, **18**, 376–389.

Spotte, Stephen. 2007. Bluegills: Biology and Behavior. American Fisheries Society, USA.

Thomas, J.D. 1997. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biology*, **38**, 1–36.

Vadeboncoeur, Y., Lodge, D.M. and Carpenter, S.R. 2001. *WHOLE-LAKE FERTILIZATION EFFECTS ON DISTRIBUTION OF PRIMARY PRODUCTION BETWEEN BENTHIC AND PELAGIC HABITATS*.

Vander-Zanden, M.J. and Vadeboncoeur, Y. 2002. *FISHES AS INTEGRATORS OF BENTHIC AND PELAGIC FOOD WEBS IN LAKES*.

Webb, P. W. 1982. Locomotor patterns in the evolution of actinopterygian fishes. *American Zoologist*, *22*(2), 329-342.

Werner, E.E. and Hall, D.J. 1974. Optimal Foraging and the Size Selection of Prey by the Bluegill Sunfish (Lepomis macrochirus). *Source: Ecology*, **55**, 1042–1052.

Werner, E.E., Gilliam, J.F., Hall, D.J. and Mittelbach, G.G. 1983. *An Experimental Test of the Effects of Predation Risk on Habitat Use in Fish*.

Werner, E.E., Hall, D.J. and Kellogg, W.K. 1988. ONTOGENETIC HABITAT SHIFTS IN BLUEGILL: THE FORAGING RATE-PREDATION RISK TRADE-OFF’. *Ecology*, **69**, 1352–1366.

Wimberger, P. H. (1994). Trophic polymorphisms, plasticity, and speciation in vertebrates. *Theory and application in fish feeding ecology*, *1*.

Yavno, S. and Fox, M.G. 2014. Morphological plasticity of native and non-native pumpkinseed sunfish in response to habitat type. *Evolutionary Ecology Research*, **16**, 373–395.

Zelditch, M., Swiderski, D., Sheets, D.H. and Fink, W. 2004. *Geometric Morphometrics for Biologists : A Primer*.

TABLES

Table 1. Summary of lake characteristics and sampling for survey lakes. DOC is the mean dissolved organic carbon concentration.

Lake	Latitude	Longitude	Area (ha)	Max depth (m)	DOC (mg/L)	Watershed	No. Fish collected	Capture method(s) used*
Bay	46.24418	-89.49733	67.3	12.2	7.4	Great Lakes	30	FN, A
Birch	46.21767	-89.83838	204.8	13.7	10.9	Mississippi	29	FN, E
Crampton	46.20965	-89.47368	25.9	18.5	5	Great Lakes	30	FN
Found	45.95051	-89.45320	136	6.4	6.2	Mississippi	30	E
Hummingbird	46.24368	-89.50587	0.8	7.6	24.5	Great Lakes	30	FN, A
Little Crooked	46.15085	-89.69511	62	9.1	4.1	Mississippi	30	E
Lost	45.96554	-89.48312	218.1	6.1	4.0	Mississippi	30	FN, E
McCullough	46.19831	-89.57043	89.4	8.2	14.3	Great Lakes	30	FN
Muskellunge	45.95180	-89.37989	109.3	5.8	6.7	Mississippi	30	E
Oxbow	46.19967	-89.49964	212	13.4	11.1	Great Lakes	30	FN, E
Papoose	46.18476	-89.80266	171	19.8	6.5	Mississippi	30	FN
Red Bass	46.19825	-89.66405	10.9	6.7	18.9	Great Lakes	30	FN
Squaw	45.88447	-89.99458	298	6.4	23.9	Mississippi	28	FN

Towanda	45.93854	-89.70771	56.3	8.2	5.3	Mississippi	30	FN, E

***FN= fyke-net, A= angling, E= electrofishing.**

Table 2. Multivariate analysis of covariance for bluegill shape data. Significant results are bold.

	Df	**SS**	**MS**	**Rsq**	**F**	**Z**	**Pr(>F)**
CSize	1	0.04901	0.07900	59.0470	59.0470	9.5052	**0.0010**
DOC	1	0.01140	0.01837	13.7332	13.7332	6.5652	**0.0010**
Watershed	1	0.02795	0.04505	33.6748	33.6748	8.2264	**0.0010**
Lake	11	0.09654	0.15564	1.0000	1.0000		0.5005
CSize:Watershed	1	0.00076	0.00122	0.9111	0.9111	-0.0270	0.5070
CSize:DOC	1	0.00250	0.00403	3.0153	3.0153	2.9277	**0.0020**
CSize:Watershed:DOC	1	0.00172	0.00277	2.0683	2.0683	2.0417	**0.0220**
Residuals	399	0.33115	0.000830	0.53383			
Total	416	0.62032					

FIGURES

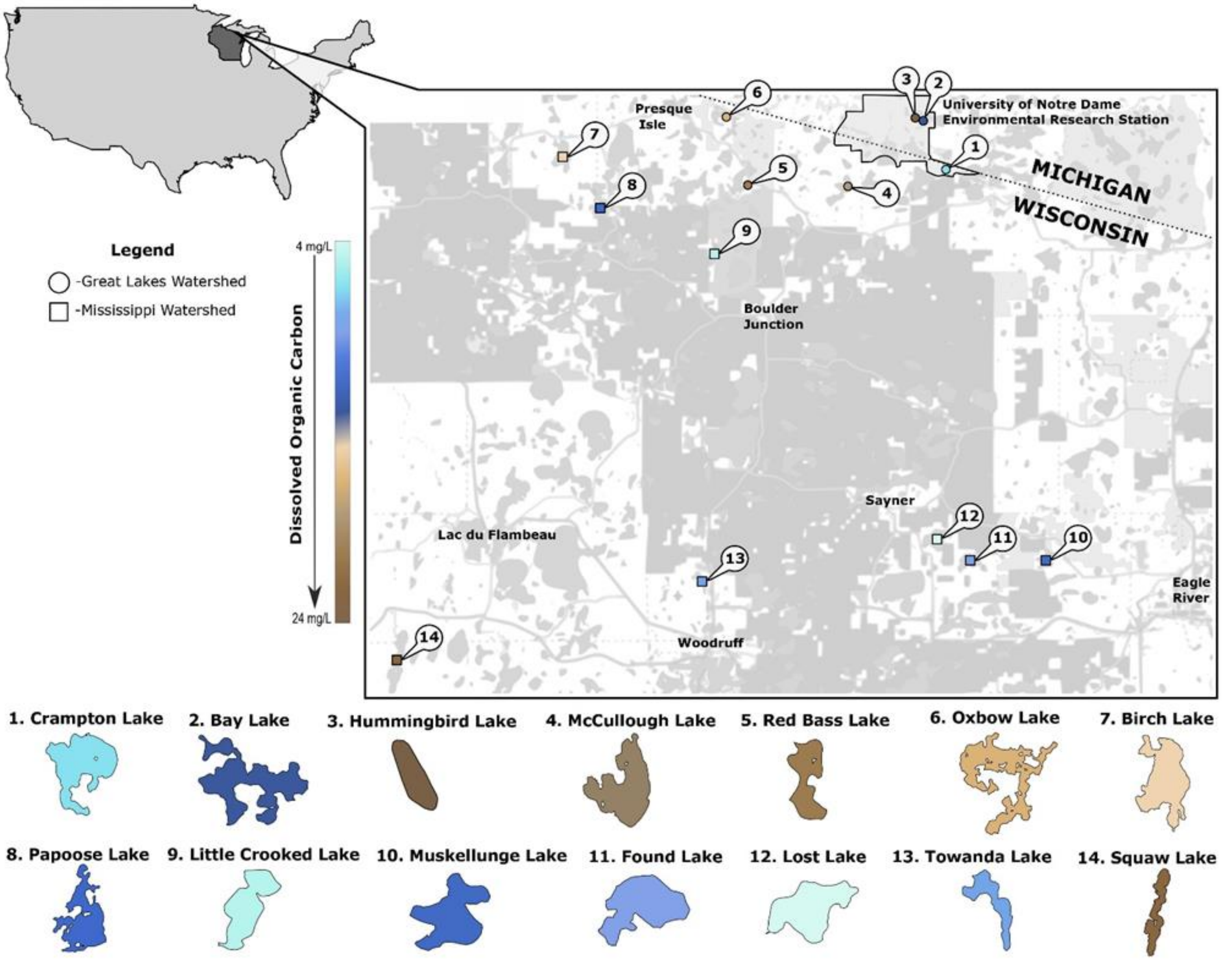

Legend
-Great Lakes Watershed
-Mississippi Watershed
Dissolved Organic Carbon
4 mg/L
24 mg/L
Presque Isle
University of Notre Dame Environmental Research Station
MICHIGAN
WISCONSIN
Boulder Junction
Sayner
Lac du Flambeau
Eagle River
Woodruff
1
2
3
4
5
6
7
8
9
10
11
12
13
14
1. Crampton Lake
2. Bay Lake
3. Hummingbird Lake
4. McCullough Lake
5. Red Bass Lake
6. Oxbow Lake
7. Birch Lake
8. Papoose Lake
9. Little Crooked Lake
10. Muskellunge Lake
11. Found Lake
12. Lost Lake
13. Towanda Lake
14. Squaw Lake

Figure 1. Map of sample lakes. Location of each sample lake throughout Wisconsin and Michigan is shown along with which watershed they belong to. Corresponding images of each lake colored by DOC concentration are also depicted.

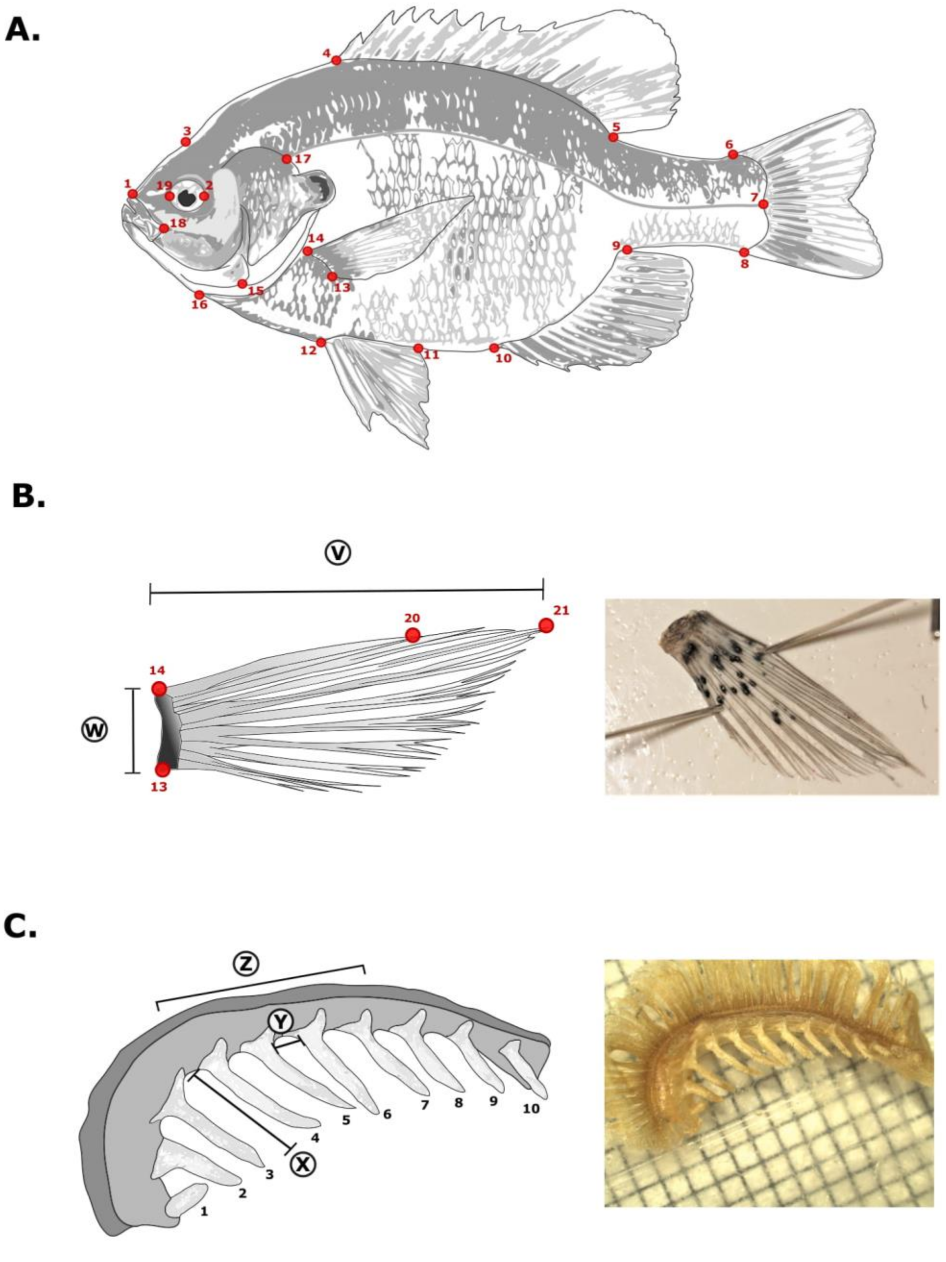


Figure 2. Landmark positions and meristic measurements for bluegill sunfish**. A.** Full body landmark positions**. 1.** Dorsal tip of premaxillary. **2**. Right-centered edge of eye. **3.** Forehead above center of eye. **4**. Dorsal fin anterior insertion point. **5**. Dorsal fin posterior insertion 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 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 measurements. **X**. Raker length measurement. **Y**. Raker spacing measurement. **Z**. Rakers 4-7 and spaces between them were those used in analyses.

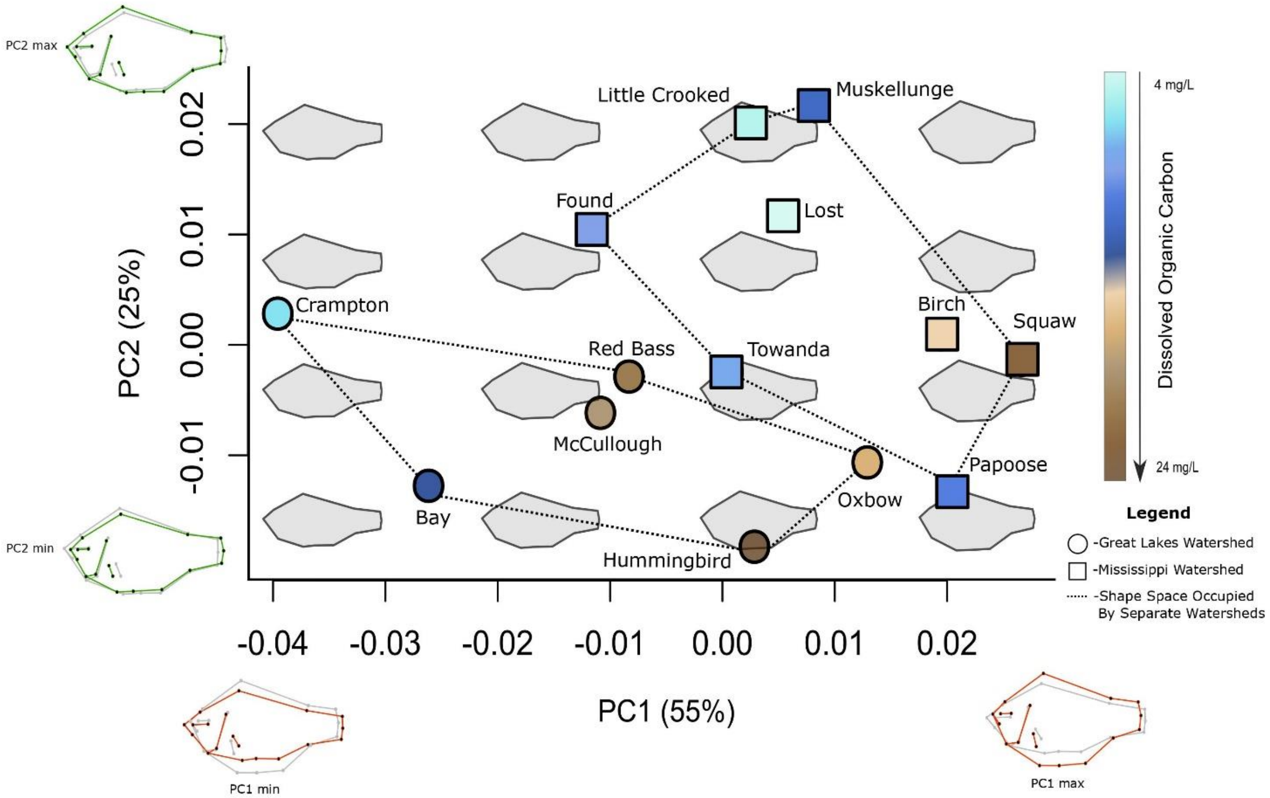


Figure 3. Variation in bluegill shape across lakes is related to dissolved organic carbon (DOC) concentration. Points indicate the mean fish shape from each lake. Fish shapes are plotted in the background for reference. PC1 is positively correlated with shorter, deeper body shape, a more vertical pectoral fin insertion angle and a smaller eye diameter. PC2 is positively correlated with head size and a more posterior pectoral fin position.

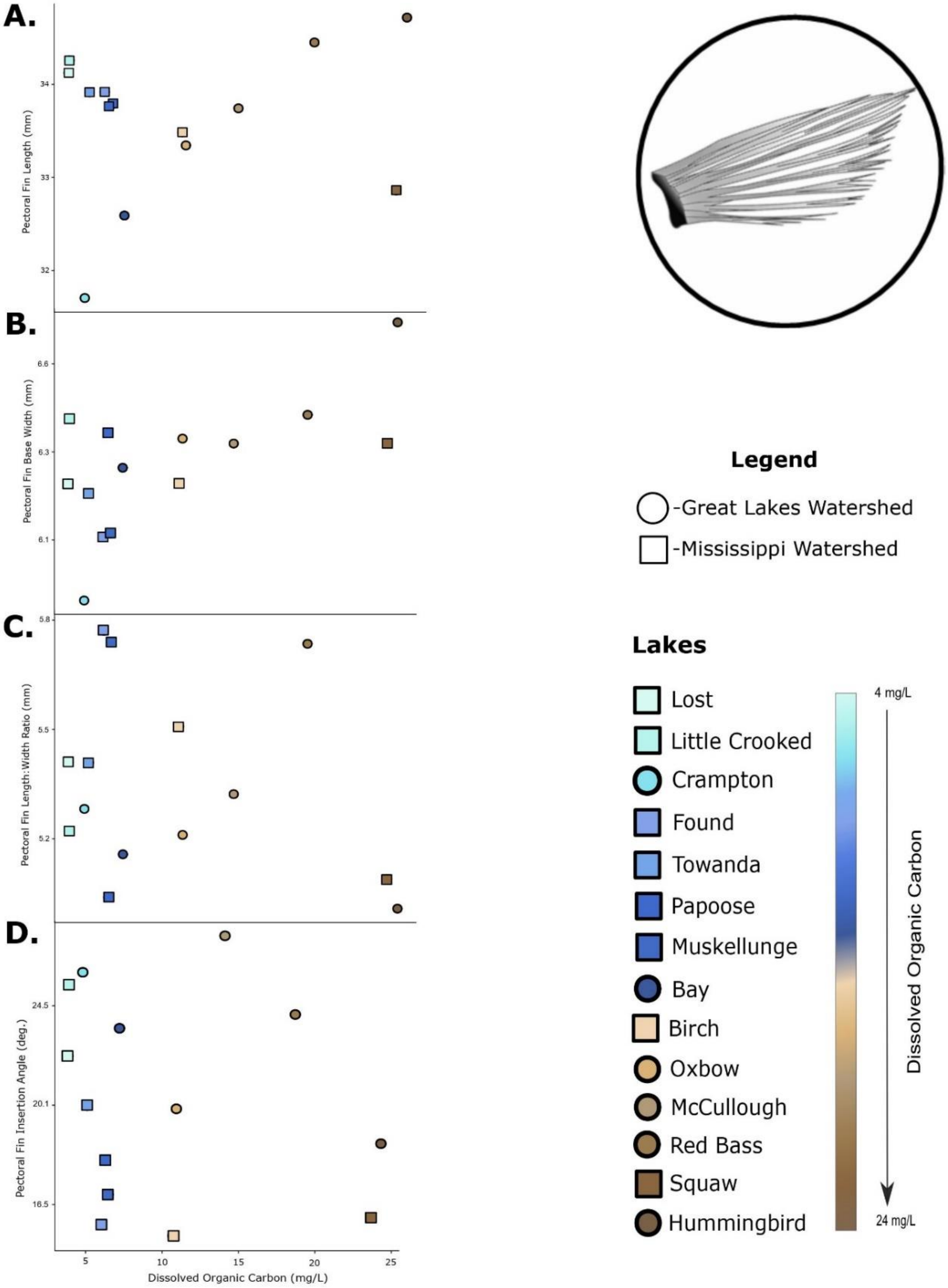


Figure 4. Variation in pectoral fin traits across DOC concentrations within two different watersheds. Points indicate the size-corrected, mean fitted values for each lake. **A**. Pectoral fin length. **B**. Pectoral fin width at base. **C**. Pectoral fin aspect ratio. **D**. Pectoral fin angle of insertion.

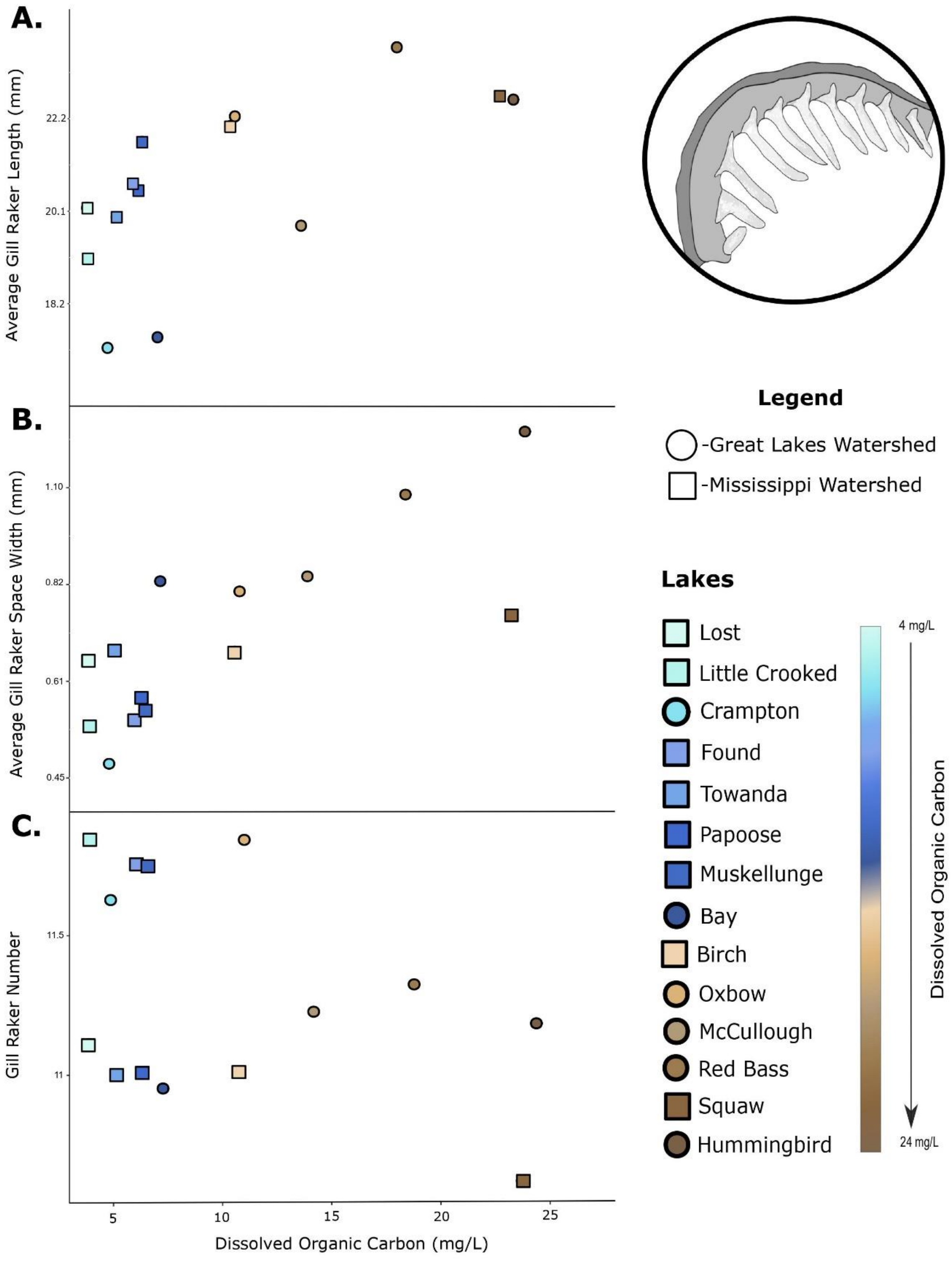


Figure 5. Variation in gill raker traits along the DOC gradient across two watersheds. Points indicate the size-corrected, mean fitted value for each lake. **A**. Gill raker length. **B**. Gill raker spacing. **C**. Gill raker number.

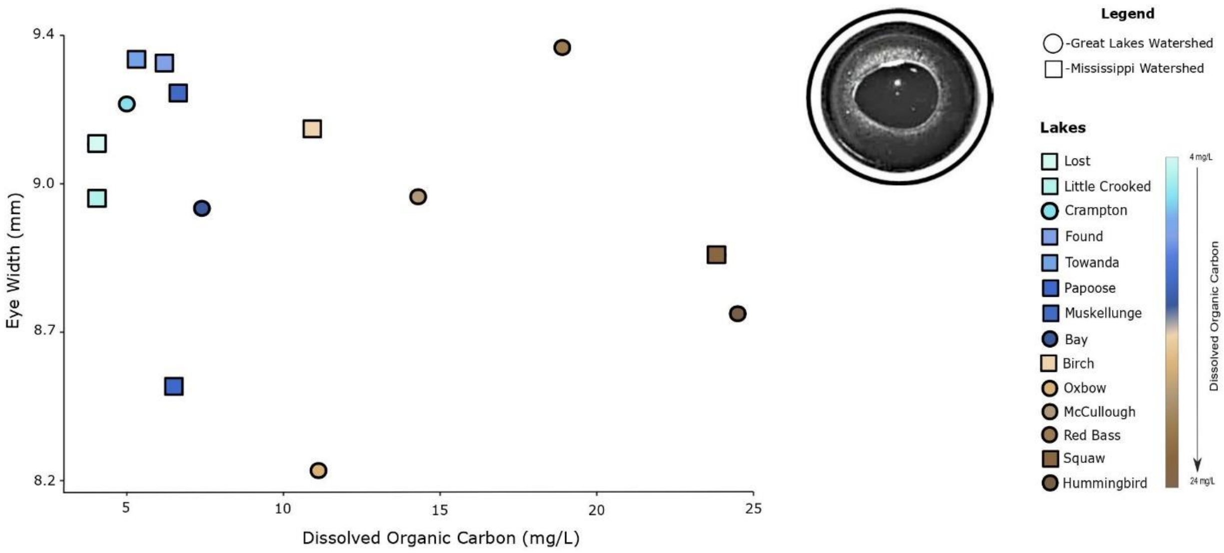


Figure 6. Variation in eye widths along the DOC gradient across two watersheds. Points indicate the size-corrected, mean fitted value for each lake.

APPENDIX I: Supplementary Tables and Figures

TABLES

Table S1. Univariate analysis of covariance for pectoral fin length data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	20.0118	5.6053	7.6317	3.570	**0.00788**
DOC	5.4528	2.2365	7.1524	2.438	**0.04417**
Basin	2.1689	0.9004	7.9947	2.409	**0.04261**
DOC:Basin	-0.8837	0.3798	7.8753	-2.327	**0.04889**

Table S2. Univariate analysis of covariance for pectoral fin base width data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	1.50049	0.28115	7.6317	5.337	**0.000511**
DOC	0.14447	0.11284	7.1524	1.280	0.235106
Basin	0.04564	0.04506	7.9947	1.013	0.337881
DOC:Basin	-0.02005	0.01904	7.8753	-1.053	0.321110

Table S3. Univariate analysis of covariance for pectoral fin length: base width ratio data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	1.543134	0.311060	10.610387	4.961	**0.000477**
DOC	0.044367	0.126187	10.237163	0.352	0.732271
Basin	0.025390	0.049919	10.479322	0.509	0.621552
DOC:Basin	-0.009105	0.021248	10.201550	-0.429	0.677181

Table S4. Univariate analysis of covariance for pectoral fin angle of insertion data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	3.55759	0.91393	9.99080	3.893	**0.003**
DOC	0.01725	0.37481	10.01195	0.046	0.964
Basin	-0.03034	0.14746	10.01774	-0.206	0.841
DOC:Basin	-0.03396	0.06335	10.06013	-0.536	0.604

Table S5. Univariate analysis of covariance for gill raker length data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	2.05699	0.31370	10.0035768	6.557	**6.3e-05**
DOC	0.35372	0.12867	10.0087641	2.749	**0.0204**
Basin	0.11548	0.05066	10.0101904	2.280	**0.0456**
DOC:Basin	-0.03803	0.02178	10.0205737	-1.746	0.1109

Table S6. Univariate analysis of covariance for gill raker spacing data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	-0.21825	0.69326	9.94882	-0.315	0.75941
DOC	1.09259	0.28434	9.97412	3.843	**0.00327**
Basin	0.25127	0.11194	10.00491	2.245	**0.04860**
DOC:Basin	-0.13664	0.04812	10.06848	-2.840	**0.01744**

Table S7. Univariate analysis of covariance for gill raker number data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	11.0592	2.2776	9.9786	4.856	**0.00067**
DOC	0.3048	0.9343	10.0118	0.326	0.75098
Basin	0.1588	0.3679	10.0523	0.432	0.67512
DOC:Basin	-0.1100	0.1582	10.1358	-0.695	0.50261

Table S8. Univariate analysis of covariance for eye width data.

	Estimate	**Std. Error**	**Df**	**t-Value**	**Pr(>| t |)**
Intercept	2.1992732	0.1901351	10.0035768	11.567	**4.11e-07**
DOC	-0.0087295	0.0779440	10.0087641	-0.112	0.913
Basin	0.0044360	0.0306618	10.0101904	0.145	0.888
DOC:Basin	-0.0006235	0.0131631	10.0205737	-0.047	0.963

FIGURES

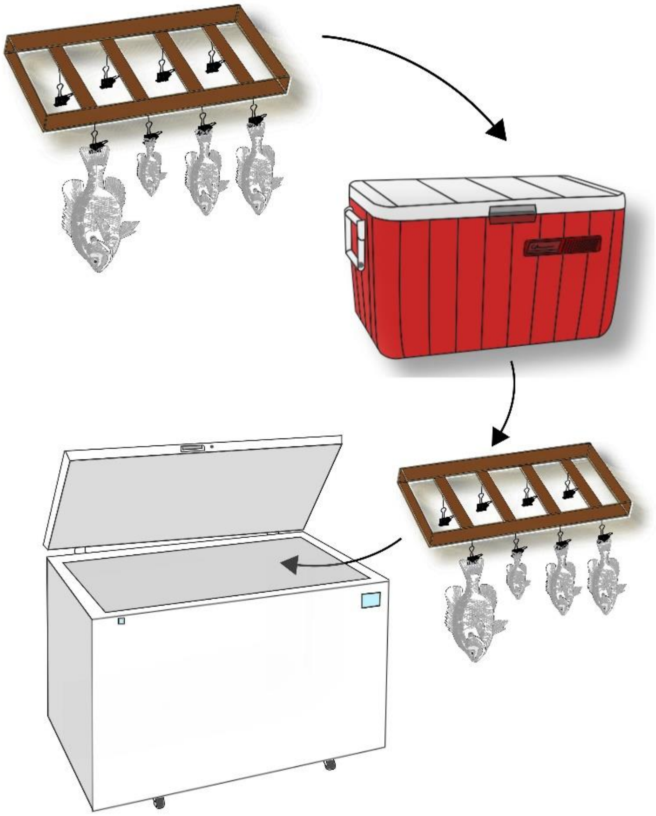
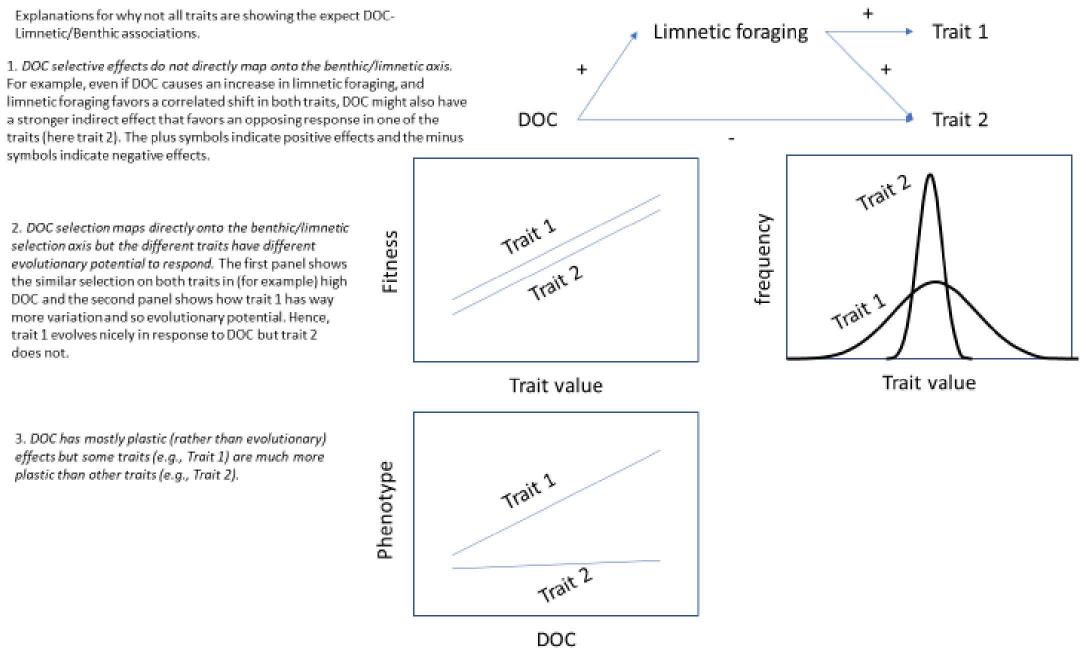


Figure S1. Fish hanging rack method. Used to avoid bending during freezing for more accurate geometric morphometrics analysis.



Explanations for why not all traits are showing the expect DOC-Limnetic/Benthic associations.

1. *DOC selective effects do not directly map onto the benthic/limnetic axis.* For example, even if DOC causes an increase in limnetic foraging, and limnetic foraging favors a correlated shift in both traits, DOC might also have a stronger indirect effect that favors an opposing response in one of the traits (here trait 2). The plus symbols indicate positive effects and the minus symbols indicate negative effects.

2. *DOC selection maps directly onto the benthic/limnetic selection axis but the different traits have different evolutionary potential to respond.* The first panel shows the similar selection on both traits in (for example) high DOC and the second panel shows how trait 1 has way more variation and so evolutionary potential. Hence, trait 1 evolves nicely in response to DOC but trait 2 does not.

3. *DOC has mostly plastic (rather than evolutionary) effects but some traits (e.g., Trait 1) are much more plastic than other traits (e.g., Trait 2).*

Figure S2. Possible explanations for why traits are not demonstrating expected result.

Developed by Andrew Hendry.

GENERAL CONCLUSION

As inputs of DOC into north-temperate lakes may continue to increase it is critical that we try to understand the impact it will have on freshwater ecosystems (Monteith et al., 2007). The majority of the primary literature on DOC effects tends to focus on primary production rather than those considering the impacts on higher trophic levels such as fish (Jones 1992; Ask *et al.* 2009b; Solomon *et al.* 2015). Further, no other study has considered the relationship between Bluegill intra-population trophic polymorphisms and increasing concentrations of DOC. The results of this thesis confirm a complex relationship between DOC and freshwater ecosystems and suggests further investigation in order to understand how increases will impact feeding and locomotor traits of fish populations in the future.

Using geometric morphometrics analyses along with univariate analyses revealed that fish from high DOC lakes tended to have deeper, shorter bodies. Furthermore, pectoral fin lengths differed with DOC (although the direction differed between watersheds) and gill rakers were longer and more widely spaced at high DOC, while number of gill rakers did not vary. This shows that while DOC does affect bluegill body shape and other select trophic traits, an increase in concentration does not directly lead to a distinct ‘pelagic’ phenotype (Ehlinger and Wilson, 1988).

This thesis provides a foundation and emphasizes the need for further research investigating the influence of DOC on higher level consumers in lake food webs. Future studies should examine Bluegill from specifically littoral and limnetic areas of the lake, as well as examine them on a temporal scale. While this study did not allow for the examination of individual fish diets, it would be useful to know exactly what the fish are eating in high DOC lakes even though the literature provides us with an idea of the primary and secondary producer composition (Vander Zanden and Vadeboncoeur 2002, Karlsson et al. 2009, Finstad et al. 2014, Craig et al. 2015). It would also be beneficial to explore these dynamics across a temporal scale as well as special. It is clear that gaining a true understanding of the relationship between DOC and freshwater systems will be challenging however it must be undertaken in order for aquatic ecologists to discern our future in a browning world.

REFERENCES

Ask, J., Karlsson, J., Persson, L., Ask, P., Pä, P., Byström, P., Byström, B. and Jansson, A.M. 2009. Whole-Lake Estimates of Carbon Flux through Algae and Bacteria in Benthic and Pelagic Habitats of Clear-Water Lakes. *Ecology*, **90**, 1923–1932.

Craig, N., Jones, S.E., Weidel, B.C. and Solomon, C.T. 2015. Habitat, not resource availability, limits consumer production in lake ecosystems. *Limnology and Oceanography*, **60**, 2079–2089, https://doi.org/10.1002/ln.

Ehlinger, T.J. and Wilson, D.S. 1988. Complex foraging polymorphism in bluegill sunfish. *Proc. Nail. Acad. Sci. USA*, **85**, 1878–1882.

Finstad, A.G., Helland, I.P., Ugedal, O., Hesthagen, T. and Hessen, D.O. 2014. Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**, 36–43, https://doi.org/10.1111/ele.12201.

Jones, R.I. 1992. Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator. *Hydrobiologia*, **229**, 73–91.

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L. and Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, 506–509, https://doi.org/10.1038/nature08179.

Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopécek, J. and Vesely, J. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540, https://doi.org/10.1038/nature06316.

Solomon, C.T., Jones, S.E., Weidel, B.C., Buffam, I., Fork, M.L., Karlsson, J., Larsen, S., Lennon, J.T., Read, J.S., Sadro, S. and Saros, J.E. 2015. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems*, **18**, 376–389.

Vadeboncoeur, Y., Lodge, D.M. and Carpenter, S.R. 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats.

Vander Zanden, M.J. and Vadeboncoeur, Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes.