No change in life history of *Daphnia catawba* after 7 years of DOC differences in Long Lake

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Abstract

Lakes across the northern hemisphere continue to experience increased dissolved organic carbon (DOC) levels. How these increases will impact lake communities is uncertain, as DOC has multifaceted effects on lakes. To explore the possibility of DOC-directed evolution of *D. catawba*, we examined various life history characteristics of *D. catawba* from historically lower and higher DOC levels in otherwise similar halves of Long Lake. Clonal lines from each side were exposed to either no DOC or a high concentration of DOC for 21 days. We observed no differences in body width, body length, tail spine length, clutch size, time to maturity, and time to third instar between *D. catawba* of either historic DOC condition and either DOC treatment, suggesting no evolution in *D. catawba* after 7 years of 4 mg/L difference in DOC. It is possible that not enough time has passed for significant evolution to occur in *Daphnia*, but it is more likely that a 4 mg/L difference in DOC levels does not create strong enough selective pressure for evolutionary change in *D. catawba.*

Introduction

The conditions of many freshwater ecosystems are tightly linked to their surrounding terrestrial ecosystems. This interplay between freshwater and terrestrial habitats makes aquatic habitats vulnerable to anthropogenic increases and decreases in dissolved organic carbon (DOC), especially since most DOC input into freshwater systems comes from surface water, not ground water (Findlay et al. 2001). The mechanisms by which humans cause DOC changes are not completely understood, nor are their impacts on freshwater ecosystems. Human development and land cultivation are correlated with decreased dissolved organic matter compared to natural forests, partially due to riparian deforestation (Williams et al. 2016; France, Culbert and Peters 1996). Meanwhile, land tillage and other land management practices can directly increase freshwater DOC levels (Ren et al. 2016), while anthropogenic climate change increases DOC export from peat soils into freshwater systems (Freeman et al. 2001). In the past few decades, DOC concentrations in most lakes across Europe and North America have increased, including a doubling of the DOC in the Hudson River in New York (Monteith and Evans 2005, Solomon et al. 2015, Porcal et al. 2009). This trend is expected to continue into the future, increasing the importance of understanding the role of DOC in freshwater aquatic ecosystems (Hejzlar et al. 2003).

Changes in DOC have impacts on lake producers, especially phytoplankton. Increased DOC attenuation of light was found to decrease phytoplankton production by decreasing light available for photosynthesis (Fransner et al. 2019). On the other hand, DOC addition does provide stimulation to plankton production likely by increasing nutrients (Kelly et al. 2016, Hessen et al. 2004), and a full-scale DOC increase in a northern lake resulted in increased total food web productivity (Stuart Jones, personal communication). Dissolved organic carbon also explained 86% of variability in log-transformed benthic primary production in a collection of North American lakes (Godwin et al. 2014).

In addition to altering the productivity of lakes, there is much uncertainty about how changes in DOC further impact zooplankton such as *Daphnia*. *Daphnia* provide a crucial link between producers and consumers, both vertebrates and invertebrates, as well as top-down control on phytoplankton, making it critical to understand how changing DOC levels will affect *Daphnia*. Increased DOC was found to benefit *Daphnia,* increasing egg production and decreasing copper toxicity (Nova et al. 2019, Taylor et al. 2015). However, other studies found that increased Humic substrates, one type of DOC, decreased *Daphnia* fecundity as well as caused oxidative stress (Brouchnak and Steinberg 2013; Saebelfeld et al. 2017). *Daphnia* have been found to take up DOC directly only in marginal quantities, suggesting that DOC-linked changes are due to non-nutritional factors (Speas and Duffy 1998).

In addition to direct influences, changes in DOC have the potential to alter the selective pressure experienced by *Daphnia*. The DOC-linked decrease in phytoplankton productivity observed by Fransner et al. (2019) has the potential to decrease the ability of *Daphnia* to use phytoplankton as a food source. Decreased light levels caused by increased DOC could also decrease predation of *Daphnia* by fish, which are visual predators, potentially removing the selective pressure to respond to fish kairomones that induce smaller size and younger age at maturity in *Daphnia* (Beckerman et al. 2010). Another study showing that highly colored water decreased top-down control of fish on zooplankton size further supports this idea (Wissel et al. 2003). Darker water also contains more *Chaoborus* (Wissel et al. 2003), a size-limited predator whose kairomones induce larger *Daphnia* with higher age at maturity (Beckerman et al. 2010). *Daphnia* in high-DOC waters may face increased pressure to respond to these kairomones as *Chaoborus* numbers increase alongside DOC levels. This pressure would not be weakened by decreased predation as *Chaoborus* are not visual predators, but could be weakened by DOC attenuation of UV radiation, which can allow *Daphnia* to remain closer to the surface and out of the reach of lower-residing *Chaoborus* (Wissel and Ramcharan 2003, Boeing et al. 2003). The decrease in light provided by higher DOC may also limit the pressure towards smaller clutch sizes caused by fish predation (Gliwicz and Boavida 1996).

Previous studies have revolved largely around the short-term changes *Daphnia* and other zooplankton experience due to changing DOC levels and water color. While some research has focused on selective pressures *Daphnia* experience, little research has explored potential long-term effects of DOC on *Daphnia*. This study investigated life history characteristics of *Daphnia* *catawba* from high- and low-DOC sides of Long Lake, a north temperate lake in the United States, seven years after its manipulation to explore the possibility of DOC-induced evolution in *Daphnia* (Zwart et al. 2019). My hypothesis was that *D. catawba* from the high-DOC side of Long Lake have larger sizes and a greater age at maturity than those from the low-DOC side. I also hypothesized that *D. catawba* on the high-DOC side have larger clutch sizes due to both decreased predation by fish and larger body sizes.

Materials and Methods

Long Lake is located at the University of Notre Dame's Environmental Research Center in the upper peninsula of Michigan, a northern hardwood forest ecosystem. The research center is largely free of human impacts, and recreational activity on Long Lake is not permitted, making it a prime location for ecological field work. To create a large-scale experimental location for studies of DOC it was divided in 2012 with a polyester curtain to induce naturally higher levels of DOC on the east side of the lake as described in Zwart et al. 2016. Because the lake's inflow is from a stream entering the east side, this separation has resulted in DOC concentrations of 10.60 mg/L^3 on the east side and 6.54 mg/L^3 on the west side (Zwart et al. 2016). The east side has a thermocline depth of 1.89 m and a surface area of 4.22 ha (Zwart et al. 2016, Wilkinson et al. 2013, University of Notre Dame). The west side has a thermocline depth of 2.21 m and a surface area of 3.6 ha (Zwart et al. 2016, Wilkinson et al. 2013). Otherwise, each side of the lake has relatively similar light, weather, and limnological conditions, including similar 14 m basins on each side (University of Notre Dame).

I collected *Daphnia catawba* from at least 11 sites on either side of Long Lake using a vertical tow down at least 180 cm. Collected daphnids were sorted by site and held in groups of 5 in 50 mL centrifuge tubes. Clonal offspring from each site were reared in individual 50 mL centrifuge tubes. I randomly assigned individual clones either a high-DOC (20.3 mg/L^3) treatment or no-DOC treatment in order to maximize the likelihood of observing the effects of DOC on individual traits. The high-DOC concentration was chosen because it is known to be non-toxic to other *Daphnia* populations in nearby Hummingbird Lake (Kadjeski 2016, unpublished data). I made the DOC treatment using SuperHume (Eco Lawn and Garden; Burnsville, MN), a fertilizer derived from Leonardite shale containing 17% Humic acid, 13% Fulvic acid, and 4% Humic which has previously been found to share similar properties to natural DOC (Lennon et al. 2013). I determined DOC concentration using an average of two regressions using absorbance at 360 nm (Collier 1987 and Grieve 1985). All daphnids were held in COMBO solution and fed *Scenedesmus obbliquus* at a concentration of 200,000 cells/mL, determined using 95% transmittance 600 nm light (Kilham et al. 1998).

I measured the body length of each daphnid from the beginning of the tail spine to the top of the head through the eye. I measured tail spine length from the base to the tip and daphnid width from the middle of the digestive tract ventrally across the body. I determined clutch number by observing egg development, egg number, and the presence of offspring and carapaces. Maturity was considered to be reached at the first appearance of the first clutch. I made all measurements with LAS EZ software at 25x zoom on a Leica dissecting microscope (Leica Microsystems; Wetzlar, Germany) at least every two days from birth to 21 days.

I ran a two-way, type III ANOVA comparing side (east and west) and media (no-DOC and high-DOC) using RStudio 3.5.2 (RStudio; Boston, Massachusetts). A Shapiro-Wilk's test was used to check for normality of data. Non-normal data was transformed using the ideal transformation determined by Box Cox transformation. Body length was transformed to the 1/5 power and body width at 21 days was transformed to the -1/3 power for optimal normality. Trials were started with *D. catawba* from 11 sites from each side, but due to deaths, the east/high-DOC treatment had 3 replicates, the east/low-DOC treatment had 4 replicates, the west/high-DOC treatments had 5 replicates, and the west/low-DOC treatment had 4 replicates. Because of the unbalanced sample sizes, a type III ANOVA was used. A Levene test was used to ensure that data from each side and media had equal variance.

Results

The influence of historic DOC exposure and chronic high DOC exposure was compared for width, clutch size, body size, and tail spine size at maturity, the third instar, and the end of the trial (21 days). No significant difference in size was found for any variable between *Daphnia* from either historic DOC exposure regardless of chronic DOC exposure (Table 1, Figure 1). Additionally, no significant difference for any characteristic was found due to DOC exposure alone (Table 1, Figure 1).

Similar ANOVAs were also run on days to maturity and days to third instar. No significant difference was found between any of these variables regardless of past DOC exposure or chronic DOC exposure (Table 1, Figure 1).

Daphnia from the east side had longer body sizes at all stages of life, and those in no-DOC water reached maturity slower but the third instar faster (Table 2, Figure 1). Neither of these results is significant.

Discussion

This study investigated life history differences in *Daphnia catawba* from the east and west sides of Long Lake, which was artificially divided 7 years ago to create a natural high-DOC lake on the east side (Zwart et al. 2016). Daphnids from both sides of Long Lake showed no significant difference from each other in width, tail spine length, or body length at several points throughout the experiment. There was also no significant difference between DOC treatments among each side. Time to maturity, time to third instar, and average clutch size were also measured and showed no significant difference due to historic or chronic DOC levels. These results suggest that there has been no evolutionary change in *D. catawba* in either side of Long Lake due to DOC changes.

It is possible that not enough time has passed for evolution to have occurred in *D. catawba* due to DOC changes. I suggest that this study be repeated in the future to allow more time for evolutionary change. Even with an extended time frame, it is likely that there is not enough selective pressure to push evolution on the high-DOC side of Long Lake since the DOC difference between the high- and low-DOC sides is less than 5 mg/L. It has been theorized that a DOC threshold exists between 10 and 14 mg/L that greatly reduces consumer production (Solomon et al. 2015), so it is possible that the high DOC levels (10.60 mg/L) in the east side of Long Lake are not high enough to induce evolutionary change. This is a likely case as lake conditions can induce strong clonal selection on *Daphnia* in as few as ten weeks but none were observed after 7 years in Long Lake (Chislock et al. 2019). Furthermore, morphological characteristics have been found to have high heritability in other species of *Daphnia* so favorable physical characteristics would likely be evolutionarily selected for (Tian et al. 2019). Long Lake has recently been divided again to induce an even stronger DOC gradient (S. Jones, personal communication, July 4, 2019), so experimentation on *Daphnia* from the highest DOC section is recommended in about a decade. Finally, *Daphnia* have been found to not take up large amounts of DOC, but rather particulate organic carbon (Speas and Duffy 1998, Cole et al. 2006). This would suggest that most observed changes in *Daphnia* due to DOC are behavioral, not nutritional, which would likely require more extreme conditions to be noticeable.

More than 30% of daphnids at the beginning of the experiment did not survive the 21 days until the end. This greatly reduced the statistical power of this experiment by reducing us to five or fewer replicates for each treatment, so there is a possibility that evolution on the east side of Long Lake occurred but was not picked up by this experiment. Investigation into this abnormally high mortality could also prove fruitful. This study could also be affected by its sampling techniques. All sites on both sides were sampled with vertical tows to the same depth (around 180cm). Solely by coincidence, this is approximately the depth of the thermocline on the east side, while the thermocline on the west side is almost half a meter deeper (Zwart et al. 2016). This sampling could have resulted in *D. catawba* that react to fish differently from each side as fish push *Daphnia* into deeper waters (Larsson and Lampert 2012). Further studies should consider taking daphnids from a consistent height compared to the vertical stratification of the lake. Additionally, *Holopedium* were observed to have caused *Daphnia* mortality between collection and isolation, especially on the west side. This could have artificially selected for certain traits more strongly on the west than the east side and also required additional samples of the west side to be taken on a different day.

It is worth noting that daphnids from the east side of Long Lake, and especially those in no-DOC water, were consistently longer than those from the west side. Although this result is not statistically significant, it could suggest an evolutionary difference due to historic DOC exposure that a small sample size could not pick up and would be consistent with findings by Wissel et al. (2003). If *D. catawba* with historic DOC exposure were larger when reared without DOC, it could be due to a combination of historically decreased fish predation, which would allow for larger daphnids (Beckerman et al. 2010), and a removal of the potentially harmful effects of high DOC on reared daphnids found by Saebelfeld et al. (2017). I also found that daphnids reared in high-DOC water from the high-DOC side of Long Lake consistently had longer tail spines than daphnids in no-DOC water, albeit nonsignificantly. This could suggest that high DOC promotes tail spine growth in *D. catawba* with historic high-DOC exposure, though study with a larger sample size is needed. Tail spine size has 91% heritability in *D. pulicaria*, so this would not be unexpected in *D. catawba* (Tian et al. 2019). Finally, daphnids reared in no-DOC water reached maturity at a younger age than those reared in high-DOC water, regardless of historic DOC exposure. While these results are nonsignificant, they could point towards present genetic responses to DOC found in *D. catawba*, including DOC-induced changes of age of maturity and time between molts. If present, these changes could confound future studies attempting to tie decreased fish predation in high-DOC water to a higher age at maturity. However, it is unlikely that the latter of these has occurred, as time between molts has a heritability of 0 in *D. pulicaria* (Tian et al. 2019).

Despite the increase in DOC in waters across the northern hemisphere reported by many researchers (Monteith and Evans 2005, Solomon et al. 2015, Porcal et al. 2009), the lack of observed evolution in *Daphnia catawba* due to increased DOC is no reason for alarm. When global increases in DOC were first discovered, lake DOC across the UK was increasing by 0.2 mg/L/yr (Monteith et al. 2001). Meanwhile, the east side of Long Lake experienced a much faster increase in DOC but maintains a strong *Daphnia* population, suggesting that global DOC increases are unlikely to harm *D. catawba*. While much about the effects of DOC on *Daphnia* remains unknown, this experiment suggests that DOC increases as large as 4 mg/L may be unable to induce evolutionary changes in *Daphnia catawba.*

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Tables

Table 1. Type III two-way ANOVA values for life history measurements versus side and media.

VARIABLE	FSIDE	FMEDIA	FINT	PSIDE	PMEDIA	PINT
Width (Maturity)	1.0027	0.0818	0.0818	0.3364	0.7796	0.7796
Width (3rd Instar)	1.5843	1.3614	2.3540	0.2320	0.2659	0.1508
Width^{-3} (21 Days)	0.7746	1.0464	0.1562	0.3960	0.3264	0.6995
Length (Maturity)	0.2079	0.1177	0.4894	0.6565	0.7374	0.8286
Length (3rd Instar)	0.2716	0.3420	0.2826	0.6117	0.8563	0.9868
Length$^{1/3}$ (21 Days)	1.5995	0.0159	0.06398	0.2299	0.9014	0.8045
Spine (Maturity)	0.0753	0.9615	0.9615	0.7883	0.3461	0.3461
Spine (3rd Instar)	0.4233	0.5819	1.8730	0.5275	0.4603	0.1962
Spine (21 Days)	0.0629	1.2839	1.0594	0.8061	0.2739	0.3236
Days To Maturity	0.0038	0.4349	0.1334	0.9518	0.5220	0.7212
Days To 3rd Instar	1.0827	0.2683	0.0640	0.3185	0.6138	0.8044
Clutch Size (Avg)	0.0632	0.1864	1.0089	0.805	0.6736	0.3349

Table 2. Average life history measurements by side and media.

	East, no DOC	**East, high DOC**	**West, no DOC**	**West, high DOC**
Width (Maturity) (mm)	0.51 ± .03	0.51 ± .03	0.50 ± .02	0.49 ± .03
Width (3rd Instar) (mm)	0.56 ± .01	0.56 ± .01	0.56 ± .02	0.54 ± .02
Width (21 Days) (mm)	0.57 ± .02	0.58 ± .03	0.55 ± .05	0.57 ± .05
Spine (Maturity) (mm)	0.19 ± .05	0.23 ± .06	0.20 ± .03	0.20 ± .03
Spine (3rd Instar) (mm)	0.17 ± .04	0.22 ± .04	0.22 ± .06	0.20 ± .03
Spine (21 Days) (mm)	0.18 ± .03	0.22 ± .03	0.19 ± .04	0.19 ± .04
Length (Maturity) (mm)	1.40 ± .08	1.38 ± .01	1.37 ± .02	1.37 ± .10
Length (3rd Instar) (mm)	1.54 ± .08	1.54 ± .07	1.53 ± .05	1.52 ± .07
Length (21 Days) (mm)	1.60 ± .06	1.58 ± .08	1.52 ± .11	1.54 ± .09
Clutch Size	2.73 ± .45	2.45 ± .51	2.49 ± .34	2.60 ± .26
Days to Maturity	10.56 ± 1.2	11.52 ± 1.02	10.96 ± 2.09	11.23 ± 2.28
Days to 3rd Instar	17.98 ± 1.77	17.35 ± 1.44	16.92 ± 2.38	16.70 ± .45

Figures

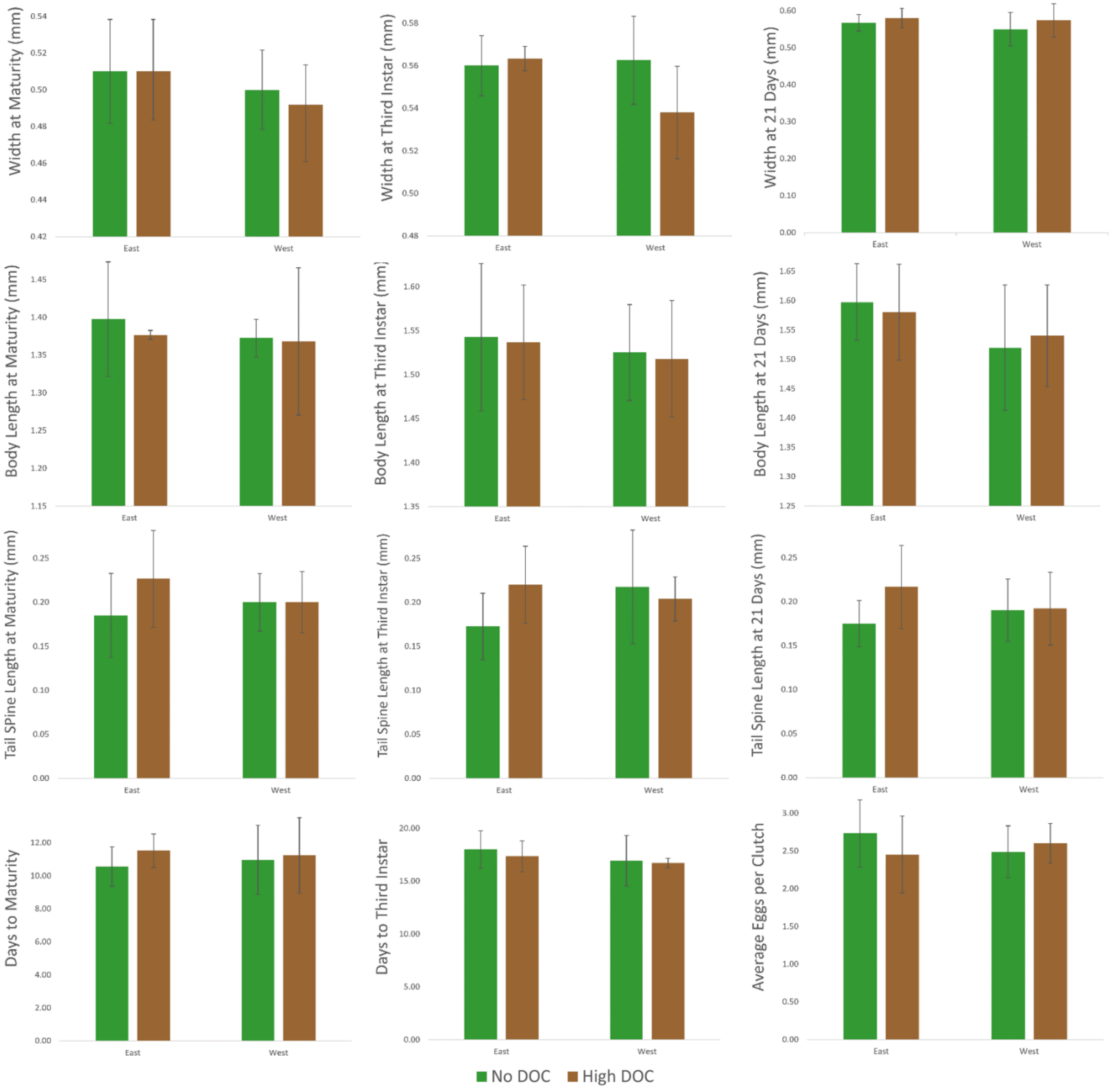
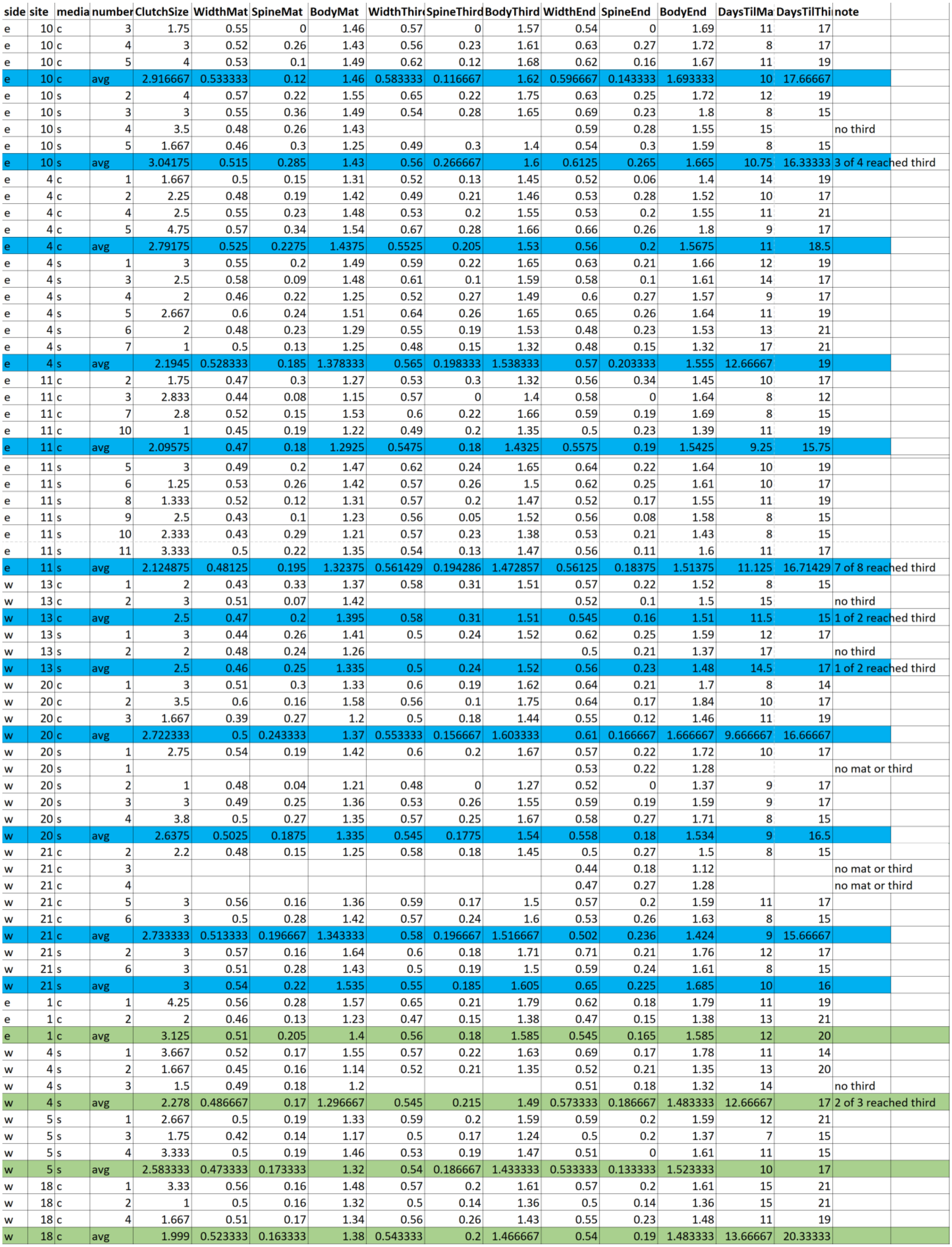


Figure 1. Historic and chronic DOC exposure has no effect on *Daphnia catawba* life history. *D. catawba* from east and west Long Lake were kept in high DOC or no DOC COMBO and measured for various life history characteristics over 21 days. *D. catawba* from the east side had longer body lengths at all stages of life, though not statistically. *D. catawba* in DOC water reached maturity later but the third instar faster, though not statistically.

Appendix A



side	site	media	number	ClutchSize	WidthMat	SpineMat	BodyMat	WidthThir	SpineThird	BodyThird	WidthEnd	SpineEnd	BodyEnd	DaysTilMa	DaysTilThi	note
e	10	c	3	1.75	0.55	0	1.46	0.57	0	1.57	0.54	0	1.69	11	17	
e	10	c	4	3	0.52	0.26	1.43	0.56	0.23	1.61	0.63	0.27	1.72	8	17	
e	10	c	5	4	0.53	0.1	1.49	0.62	0.12	1.68	0.62	0.16	1.67	11	19	
e	10	c	avg	2.916667	0.533333	0.12	1.46	0.583333	0.116667	1.62	0.596667	0.143333	1.693333	10	17.66667	
e	10	s	2	4	0.57	0.22	1.55	0.65	0.22	1.75	0.63	0.25	1.72	12	19	
e	10	s	3	3	0.55	0.36	1.49	0.54	0.28	1.65	0.69	0.23	1.8	8	15	
e	10	s	4	3.5	0.48	0.26	1.43				0.59	0.28	1.55	15		no third
e	10	s	5	1.667	0.46	0.3	1.25	0.49	0.3	1.4	0.54	0.3	1.59	8	15	
e	10	s	avg	3.04175	0.515	0.285	1.43	0.56	0.266667	1.6	0.6125	0.265	1.665	10.75	16.33333	3 of 4 reached third
e	4	c	1	1.667	0.5	0.15	1.31	0.52	0.13	1.45	0.52	0.06	1.4	14	19	
e	4	c	2	2.25	0.48	0.19	1.42	0.49	0.21	1.46	0.53	0.28	1.52	10	17	
e	4	c	4	2.5	0.55	0.23	1.48	0.53	0.2	1.55	0.53	0.2	1.55	11	21	
e	4	c	5	4.75	0.57	0.34	1.54	0.67	0.28	1.66	0.66	0.26	1.8	9	17	
e	4	c	avg	2.79175	0.525	0.2275	1.4375	0.5525	0.205	1.53	0.56	0.2	1.5675	11	18.5	
e	4	s	1	3	0.55	0.2	1.49	0.59	0.22	1.65	0.63	0.21	1.66	12	19	
e	4	s	3	2.5	0.58	0.09	1.48	0.61	0.1	1.59	0.58	0.1	1.61	14	17	
e	4	s	4	2	0.46	0.22	1.25	0.52	0.27	1.49	0.6	0.27	1.57	9	17	
e	4	s	5	2.667	0.6	0.24	1.51	0.64	0.26	1.65	0.65	0.26	1.64	11	19	
e	4	s	6	2	0.48	0.23	1.29	0.55	0.19	1.53	0.48	0.23	1.53	13	21	
e	4	s	7	1	0.5	0.13	1.25	0.48	0.15	1.32	0.48	0.15	1.32	17	21	
e	4	s	avg	2.1945	0.528333	0.185	1.378333	0.565	0.198333	1.538333	0.57	0.203333	1.555	12.66667	19	
e	11	c	2	1.75	0.47	0.3	1.27	0.53	0.3	1.32	0.56	0.34	1.45	10	17	
e	11	c	3	2.833	0.44	0.08	1.15	0.57	0	1.4	0.58	0	1.64	8	12	
e	11	c	7	2.8	0.52	0.15	1.53	0.6	0.22	1.66	0.59	0.19	1.69	8	15	
e	11	c	10	1	0.45	0.19	1.22	0.49	0.2	1.35	0.5	0.23	1.39	11	19	
e	11	c	avg	2.09575	0.47	0.18	1.2925	0.5475	0.18	1.4325	0.5575	0.19	1.5425	9.25	15.75	
e	11	s	5	3	0.49	0.2	1.47	0.62	0.24	1.65	0.64	0.22	1.64	10	19	
e	11	s	6	1.25	0.53	0.26	1.42	0.57	0.26	1.5	0.62	0.25	1.61	10	17	
e	11	s	8	1.333	0.52	0.12	1.31	0.57	0.2	1.47	0.52	0.17	1.55	11	19	
e	11	s	9	2.5	0.43	0.1	1.23	0.56	0.05	1.52	0.56	0.08	1.58	8	15	
e	11	s	10	2.333	0.43	0.29	1.21	0.57	0.23	1.38	0.53	0.21	1.43	8	15	
e	11	s	11	3.333	0.5	0.22	1.35	0.54	0.13	1.47	0.56	0.11	1.6	11	17	
e	11	s	avg	2.124875	0.48125	0.195	1.32375	0.561429	0.194286	1.472857	0.56125	0.18375	1.51375	11.125	16.71429	7 of 8 reached third
w	13	c	1	2	0.43	0.33	1.37	0.58	0.31	1.51	0.57	0.22	1.52	8	15	
w	13	c	2	3	0.51	0.07	1.42				0.52	0.1	1.5	15		no third
w	13	c	avg	2.5	0.47	0.2	1.395	0.58	0.31	1.51	0.545	0.16	1.51	11.5	15	1 of 2 reached third
w	13	s	1	3	0.44	0.26	1.41	0.5	0.24	1.52	0.62	0.25	1.59	12	17	
w	13	s	2	2	0.48	0.24	1.26				0.5	0.21	1.37	17		no third
w	13	s	avg	2.5	0.46	0.25	1.335	0.5	0.24	1.52	0.56	0.23	1.48	14.5	17	1 of 2 reached third
w	20	c	1	3	0.51	0.3	1.33	0.6	0.19	1.62	0.64	0.21	1.7	8	14	
w	20	c	2	3.5	0.6	0.16	1.58	0.56	0.1	1.75	0.64	0.17	1.84	10	17	
w	20	c	3	1.667	0.39	0.27	1.2	0.5	0.18	1.44	0.55	0.12	1.46	11	19	
w	20	c	avg	2.722333	0.5	0.243333	1.37	0.553333	0.156667	1.603333	0.61	0.166667	1.666667	9.666667	16.66667	
w	20	s	1	2.75	0.54	0.19	1.42	0.6	0.2	1.67	0.57	0.22	1.72	10	17	
w	20	s	1								0.53	0.22	1.28			no mat or third
w	20	s	2	1	0.48	0.04	1.21	0.48	0	1.27	0.52	0	1.37	9	17	
w	20	s	3	3	0.49	0.25	1.36	0.53	0.26	1.55	0.59	0.19	1.59	9	17	
w	20	s	4	3.8	0.5	0.27	1.35	0.57	0.25	1.67	0.58	0.27	1.71	8	15	
w	20	s	avg	2.6375	0.5025	0.1875	1.335	0.545	0.1775	1.54	0.558	0.18	1.534	9	16.5	
w	21	c	2	2.2	0.48	0.15	1.25	0.58	0.18	1.45	0.5	0.27	1.5	8	15	
w	21	c	3								0.44	0.18	1.12			no mat or third
w	21	c	4								0.47	0.27	1.28			no mat or third
w	21	c	5	3	0.56	0.16	1.36	0.59	0.17	1.5	0.57	0.2	1.59	11	17	
w	21	c	6	3	0.5	0.28	1.42	0.57	0.24	1.6	0.53	0.26	1.63	8	15	
w	21	c	avg	2.733333	0.513333	0.196667	1.343333	0.58	0.196667	1.516667	0.502	0.236	1.424	9	15.66667	
w	21	s	2	3	0.57	0.16	1.64	0.6	0.18	1.71	0.71	0.21	1.76	12	17	
w	21	s	6	3	0.51	0.28	1.43	0.5	0.19	1.5	0.59	0.24	1.61	8	15	
w	21	s	avg	3	0.54	0.22	1.535	0.55	0.185	1.605	0.65	0.225	1.685	10	16	
e	1	c	1	4.25	0.56	0.28	1.57	0.65	0.21	1.79	0.62	0.18	1.79	11	19	
e	1	c	2	2	0.46	0.13	1.23	0.47	0.15	1.38	0.47	0.15	1.38	13	21	
e	1	c	avg	3.125	0.51	0.205	1.4	0.56	0.18	1.585	0.545	0.165	1.585	12	20	
w	4	s	1	3.667	0.52	0.17	1.55	0.57	0.22	1.63	0.69	0.17	1.78	11	14	
w	4	s	2	1.667	0.45	0.16	1.14	0.52	0.21	1.35	0.52	0.21	1.35	13	20	
w	4	s	3	1.5	0.49	0.18	1.2				0.51	0.18	1.32	14		no third
w	4	s	avg	2.278	0.486667	0.17	1.296667	0.545	0.215	1.49	0.573333	0.186667	1.483333	12.66667	17	2 of 3 reached third
w	5	s	1	2.667	0.5	0.19	1.33	0.59	0.2	1.59	0.59	0.2	1.59	12	21	
w	5	s	3	1.75	0.42	0.14	1.17	0.5	0.17	1.24	0.5	0.2	1.37	7	15	
w	5	s	4	3.333	0.5	0.19	1.46	0.53	0.19	1.47	0.51	0	1.61	11	15	
w	5	s	avg	2.583333	0.473333	0.173333	1.32	0.54	0.186667	1.433333	0.533333	0.133333	1.523333	10	17	
w	18	c	1	3.33	0.56	0.16	1.48	0.57	0.2	1.61	0.57	0.2	1.61	15	21	
w	18	c	2	1	0.5	0.16	1.32	0.5	0.14	1.36	0.5	0.14	1.36	15	21	
w	18	c	4	1.667	0.51	0.17	1.34	0.56	0.26	1.43	0.55	0.23	1.48	11	19	
w	18	c	avg	1.999	0.523333	0.163333	1.38	0.543333	0.2	1.466667	0.54	0.19	1.483333	13.66667	20.33333	