The niche and morphology of the invasive *Daphnia lumholtzi* in a subtropical reservoir, twenty years after invasion

Jessica E. Beyer*[a], K. David Hambright[a]

[a] Program in Ecology and Evolutionary Biology, and Plankton Ecology and Limnology Laboratory, Department of Biology, University of Oklahoma, Norman, OK, USA

*Corresponding author: beyer@ou.edu

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Abstract

The establishment and persistence of invasive species are continuously shaped by resource availability, competition, and predation, yet most research is conducted immediately after invasion. Twenty years after *Daphnia lumholtzi* invaded Lake Texoma, OK-TX we uncovered seasonality in *D. lumholtzi* abundances and defensive morphology. Tail-spine growth was hyperallometric (faster than core body growth) in summer and fall, but allometric (growing at same rate as body) in winter. Head-spine growth declined with increasing body size, and was curvilinear in summer and fall, but linear in winter. Thus, we hypothesized that the selective predation pressure acting upon these traits is strongest in summer and fall and that the large plastic defensive spines allow persistence of *D. lumholtzi*. Additionally, we found that abundances of *D. lumholtzi* in Lake Texoma were best predicted by cyanobacteria concentration. However, as lab experiments have shown that *D. lumholtzi* does not have superior cyanobacteria tolerance, cyanobacteria may be serving as a proxy for another pressure shaping the abundance of *D. lumholtzi*, like a seasonal predator. The niche of this invasive species within Lake Texoma appears to be primarily limited by predation pressure, and further studies may illuminate whether this limiting factor is responsible for range limits within North America.

Introduction

Invasion ecology often treats systems as existing in a binary state, either before or after invasion by a particular species. A single point in time post-invasion is often used to describe the impact of an invasion, and invasive species are often treated as static once they establish in a new system. However, natural systems are always in flux, particularly when we consider that global change means that these species may be part of a rapidly changing ecosystem. Thus, the abiotic factors, predation, and competition that shape the establishment and persistence of non-native species may be changing over time. Ongoing characterization of the realized niche of these invasive species will allow more comprehensive understanding of the mechanisms underlying their persistence and impacts. For example, the enemy release hypothesis (ERH) posits that invasive species become established in new habitats where they no longer overlap with coevolved predators (Colautti et al., 2004).

Daphnia lumholtzi, a crustacean zooplankter, invaded the United States over the past twenty-five years (Sorensen and Sterner, 1992), with the source of these introductions likely being from Africa or Asia, but not Australia (Havel and Shurin, 2004). The primary introduction of *D. lumholtzi* occurred in the southern US (Frisch et al., 2012), with the first records occurring in Missouri in 1990 (Havel et al., 1995), Texas in 1991 (Sorensen and Sterner, 1992), and in Lake Texoma, OK-TX in 1991 (Work and Gophen, 1995). Following the initial introduction, molecular data show that there were possible later introductions across the US, coupled with spread from established populations (Frisch et al., 2012). The current distribution of *D. lumholtzi* stretches west to California (Havel and Shurin, 2004), north to Lake Erie (Muzinic, 2000) and Lake St. Clair (Tudorancea *et al.*, 2009), east to North Carolina (Finn et al., 2012), and south to Florida (Havel and Hebert, 1993). Throughout this period, researchers have focused in particular on understanding which biotic and abiotic factors drive *D. lumholzi* invasion success and abundances, as well as their interactions with native competitors and predators.

Previous research into abiotic drivers of *D. lumholtzi* invasion success has identified temperature, turbidity, and phosphorous concentration as key environmental characteristics. Temperature is positively associated with *D. lumholtzi* abundance and population growth rates in field-based observational studies (Havel et al., 1995; Work and Gophen, 1995; 1999a; Yurista et al., 2000; Lennon et al., 2001; Havens et al., 2012; East et al. 1999), and laboratory-based experimental studies (Work and Gophen, 1999b; Lennon et al., 2001; Yurista, 2004). Turbidity negatively affects *D. lumholtzi* population growth rates in the laboratory (Work and Gophen, 1999b) and abundances in lakes (Havens et al., 2012). However, *D. lumholtzi* may tolerate intermediate suspended sediment concentrations better than native *Daphnia* (Soeken-Gittinger et al., 2009). Additionally, phosphorous concentrations and the associated increase in primary productivity have been associated with both increased *D. lumholtzi* invasion success (Havel et al., 2005) and abundance (Havens et al., 2012), as well as decreased invasion success (Dzialowski and O'Brien, 2000). Composition of the primary producers may account for these contradictory findings, as differences in the tolerance of cyanobacteria have been found between *D. lumholtzi* and native *Daphnia* spp. (Pattinson et al., 2003; Fey and Cottingham, 2011).

Daphnia lumholtzi interact with native predators and competitors within their invasive range. Competition with native *Daphnia* may affect the abundance of *D. lumholtzi*, although the results from several studies are contradictory. Fey and Cottingham (2011) found that the outcome of competition experiments was mediated by temperature and algal composition, with warmer temperatures promoting *D. lumholtzi* and higher cyanobacterial abundances promoting the native *D. pulex.* On the other hand, in field experiments, Johnson and Havel (2001) found that the presence of *D. lumholtzi* had a negative effect on native *D. parvula* population growth rates during the late summer and fall, when *D. lumholtzi* occurs at high abundances. However, this competitive interaction was asymmetrical, with the presence of *D. parvula* not affecting *D. lumholtzi* population growth rates. East and colleagues (1999) found a negative relationship between the native *D. ambigua* and *D. lumholtzi* in Lake Okeechobee, Florida. Others have suggested that the coexistence of native *Daphnia* and *D. lumholtzi* may be mediated by the presence of invertebrate predators (Celik et al., 2002), with *Chaoborus* presence increasing *D. lumholtzi* abundances relative to native *Daphnia*.

Shortly after *D. lumholtzi* invaded Lake Texoma in 1991, their abundances in 1994 and 1995 were driven primarily by temperature (Work and Gophen, 1995), with peak abundances appearing in June and July. During this midsummer abundance peak, *D. lumholtzi* constituted a large fraction of the diet of the inland silverside (*Menidia beryllina*, hereafter *Menidia*), itself also a likely invader (Hubbs, 1982), suggesting that *D. lumholtzi* had been incorporated into the carbon flow in Lake Texoma (Lienesch and Gophen, 2001). Further, predation by *Menidia* on *D. lumholtzi* is size-selective, with smaller juveniles preying less readily on large *D. lumholtzi* (Lienesch and Gophen, 2005). Small changes in *D. lumholtzi* morphology may thus have large effects on survival and population growth if they shift individuals into the 'inedible' category of *Menidia*. If *Menidia* predation is driving *D. lumholtzi* morphology, we can make some predictions about relative strength of this selective pressure during the course of a year based on the life history and ontogeny of *Menidia* in Lake Texoma (Hubbs, 1982).

Interactions between predators and *D. lumholtzi* are mediated by the large, defensive head and tail spines of *D. lumholtzi*. In *D. lumholtzi*, growth of head and tail spines is plastic, and mothers produce offspring with larger head and tail spines in response to the presence of vertebrate and

invertebrate predator kairomones (Dzialowski et al., 2003), as well as increased temperature (Yurista, 2000) and increased cyanobacteria concentration (Whittington and Walsh, 2015). Temperature, in this case, is likely acting as a proxy cue for predation risk, as has been shown in other cladocerans (Miehls et al., 2013). Longer spines decrease the risk of predation from both vertebrates and invertebrates (Engel et al., 2014). However, as in other *Daphnia* species, predator identity beyond 'vertebrate' vs. 'invertebrate' matters (Herzog and Laforsch, 2013), and growth of head and tail spines does not protect against all invertebrate predators (e.g., *Leptodora kindtii*) (Effert and Pederson, 2006). In the presence of vertebrate predators, the inducible defenses of *D. lumholtzi* increase survivorship relative to native *Daphnia* (Engel and Tollrian, 2009). Similarly, the competitive interactions of *D. lumholtzi* and native *Daphnia* may be affected by the presence of the invertebrate predator *Chaoborus* sp., with the 'winner' being native *Daphnia* in the absence of *Chaoborus*, while *D. lumholtzi* wins in the presence of *Chaoborus* (Celik et al., 2002).

The goal of this study was to characterize the niche of *D. lumholtzi* within Lake Texoma, with comparisons to earlier work in Lake Texoma and other invaded lakes. We tested the hypothesis that environmental factors associated with *D. lumholtzi* abundance in other lakes and from earlier work in Lake Texoma are associated with current patterns of *D. lumholtzi* abundance in Lake Texoma. Our second hypothesis was that the morphology of *D. lumholtzi* reflects seasonal predation pressure by gape-limited predators. Given that the predominant predators of *D. lumholtzi* in Lake Texoma are likely gape limited with seasonal abundances, we hypothesized that growth of defensive traits (head and tail spines) would vary seasonally.

Methods

Lake Texoma is a large, dendritic reservoir (surface area = 360 km^2 at normal pool elevation) formed by the impoundment of the Red and Washita Rivers. The Red River arm is characterized as hypereutrophic with high turbidity (mean = 59 NTU) and moderately elevated salinity (mean = 1.7 ppt) (Sager et al., 2011). In contrast, the Washita River arm ranges from eutrophic to mesotrophic with low turbidity (average = 5 NTU) and low salinity (average = 0.30 ppt). The phytoplankton assemblage of Lake Texoma has been studied periodically and is generally characterized by green algae in the spring, cyanobacteria blooms in the summer (Threlkeld

1986), and, depending on the arm of the lake and local conditions, blooms of the toxigenic haptophyte, *Prymnesium parvum*, in the winter (Hambright et al. 2010, Hambright et al. 2015). Investigations of cyanobacterial toxins, which may have effects on the zooplankton assemblage, in Lake Texoma during two years have identified trace amounts of cylindrospermopsin, with abundances of *Cylindrospermopsis raciborskii* increasing from June, remaining elevated throughout the summer, and decreasing in October (Lillis et al. 2012, Teel et al. 2013). Other toxins produced by cyanobacteria, including anatoxin-a, microcystins, and saxitoxin were not present in the limited samples taken during these two years.

Over the course of a year (June 2010–July 2011), vertical, depth-integrated zooplankton tows with a Wisconsin-type net (350-μm mesh, aperture diameter 10.5 cm) were taken through the entire water column monthly at five pelagic sites on Lake Texoma, OK-TX (Fig. 1). Each zooplankton sample was initially fixed in 4% sugar-formalin and later transferred to 70% ethanol with 1% glycerol. These five pelagic sites span two watersheds, with the Red River entering the western edge of the lake and the Washita River entering the eastern portion. Environmental measurements, including temperature, salinity, phycocyanin concentration (a proxy for cyanobacterial abundances, Randolph et al., 2008), pH, and dissolved oxygen were taken concurrently with zooplankton sampling (for details, see Hambright et al., 2010, Hambright et al. 2015). Phycocyanin concentration was used as a proxy for total cyanobacterial abundances (Randolph et al., 2008), including cells that are too big (e.g., filamentous or colonial species) or too small (i.e., picoplankton) to be consumed by size-selective zooplankton such as *D. lumholtzi.*

From each zooplankton sample, we measured abundances of *D. lumholtzi* (females L^{-1}), as well as length of body, head and tail spines in mm of each female (Fig. 2). We measured individual *Daphnia* using a digital camera mounted on a dissecting microscope, paired with PlanktoMetrix software (Zohary et al., 2016). First, we calibrated PlanktoMetrix with a stage micrometer. Then the length of body, head and tail spines were measured (see Fig. 2) through the point-and-click interface. We measured 571 individuals collected throughout the year, although in some individuals, the tail spine had broken, and so the sample size was larger for head spine analyses (n = 571) than tail spines (n = 558). We divided these samples into three seasons: summer (June–August), fall (September–November), and winter (December–February). Only one individual

was collected in the spring (March–May), so that season was omitted from morphological analyses but included in abundance analyses. Additionally, we counted the number of native *Daphnia* spp. (primarily *D. mendotae* and *D. parvula*) in each sample to compare abundances of native and invasive *Daphnia*.

We investigated growth of head and tail spines using an allometric approach. In this framework, the growth of a trait (e.g., tail spine) is measured relative to body size growth by log-transforming both variables, and performing a linear regression on the log-transformed trait size against the log-transformed body size. If the slope of the relationship is equal to one, the trait is considered allometric, and the trait grows at the same rate as the body. If the slope of the relationship is significantly greater than one, the trait is considered hyperallometric, and the trait grows at a faster rate than the body. If the slope is less than one, the trait is considered hypoallometric, and the trait grows at a slower rate than the body.

To test for seasonal differences in the allometry of the head and tail spines, we used nonlinear regression, following the approach of Lagergren et al. (2007). For each season, we fitted the logarithmic form of the complex allometry function (Jolicoeur, 1989) in R (Version 3.2.4, R Core Team, 2016) as shown in Eq. 1, where A is a constant, C is the allometry exponent, and D is the curvature parameter.

$$\ln(y) = \ln(A) - C(\ln(x_{max}) - \ln(x))^D \qquad \text{Eq. (1)}$$

To test whether *D*, the curvature parameter, differed significantly from unity (which would indicate a curvilinear relationship and deviation from simple allometry), we constructed 95% confidence intervals for *D* using the *confint* function in the MASS package (Version 7.3-45, Venables and Ripley, 2002)) and determined whether each interval overlapped unity. If *D* was not significantly different from unity, we carried out a linear allometric analysis on the natural log-transformed trait and body lengths and reported the results of both analyses.

We used regression trees to test for associations between environmental variables and abundances of *D. lumholtzi* during this time period (June 2010–July 2011). One site/sample combination (Red River on 15 June, 2011) was excluded from our regression tree analysis because the measured abundance (39.5 individuals L^{-1}) was approximately 30 times greater than

the next highest abundance, resulting in a total of 69 measurements of *D. lumholtzi* abundance. Regression trees are well suited to this data set because they allow modeling of complicated interactions among predictors and nonlinear relationships between predictor and response variables (De'ath and Fabricius, 2000), both of which are common in aquatic ecology. For predictor variables, we used water temperature (˚C), salinity (practical salinity units, PSU), dissolved oxygen (mg L^{-1}), chlorophyll (µg L^{-1}), phycocyanin (proportional to µg L^{-1}), total nitrogen (µM), total phosphorous (µM), native *Daphnia* spp. density (females L^{-1}), and Secchi depth (m). We constructed a regression tree using recursive partitioning with the *ctree* function in the party package (Version 1.0-25, Hothorn et al., 2006). For each terminal node, we estimated the 95% confidence interval of the mean abundance of *D. lumholtzi* using bootstrap resampling (n = 9999)

Results

As body size of *D. lumholtzi* increased, so did length of head spines (Fig. 3). The relationship between head spine and body length was significantly curvilinear (D > 1) for *D. lumholtzi* collected during fall and summer (Table I). The relative growth of head spines declined with increasing body sizes. For *D. lumholtzi* collected in the winter, however, the relationship between head spine and body length was linear and allometric (head spines grew at the same rate as body sizes). There was substantially more variation in head spine size in *D. lumholtzi* collected during the winter (Fig. 3). Overall, for a given body size, head spines were smaller in the winter than in the summer or fall, as shown by the lower intercept value fitted to winter individuals (Table I).

In all seasons, we found a linear relationship between tail spine and body length (Table II), with tail spine length increasing with body length (Fig. 4). In the summer and fall, growth of tail spines was hyperallometric (growing at a faster rate than the body), whereas in winter, growth of tail spines was allometric (Table II). As with head spines, we found that for a given body size, tail spines were smaller in the winter than in the summer or fall (Fig. 4). Additionally, for *D. lumholtzi* of a given body size, the tail spine was longer than the head spine.

Phycocyanin was the only predictor variable included in the regression tree for *D. lumholtzi* abundances across all four seasons (Fig. 5). *D. lumholtzi* abundances were positively associated with phycocyanin concentrations, with the highest *D. lumholtzi* abundances (mean [95% CI]: 0.272 [0.125, 0.445]) associated with phycocyanin above 20.9 µg L^{-1} (Fig. 6). At low phycocyanin concentrations (<16.5), *D. lumholtzi* abundances were very low (0.022 [0.012, 0.034]). Intermediate phycocyanin concentrations were associated with intermediate *D. lumholtzi* abundances (0.092 [0.031, 0.154]). The sample that was excluded from the regression tree analysis as a high abundance outlier (39.47 females L^{-1}) had intermediate phycocyanin abundance (22.1 µg L^{-1}). Although these factors were not retained by the regression tree model, *D. lumholtzi* abundance appeared unimodally related to salinity, positively related to pH, and negatively related to Secchi depth (See supplementary material). We found no relationship between temperature and *D. lumholtzi* abundance.

Discussion

We set out to characterize factors associated with abundances and morphology of *D. lumholtzi* within Lake Texoma. We found that the single best predictor of *D. lumholtzi* abundances was a positive association with cyanobacteria abundances. Although temperature, salinity, Secchi depth, and other factors found to be important in previous studies were included in the model, none of them were included in the final regression tree. Additionally, we did not find a significant relationship between temperature and abundance of *D. lumholtzi* within Lake Texoma, contrary to earlier work in this lake (Work and Gophen, 1995). The positive association between cyanobacteria and *D. lumholtzi* within Lake Texoma suggests that *D. lumholtzi* persists during blooms of cyanobacteria that typically occur during the summer with warmer temperatures. However, unlike in Lake Okeechobee, we did not find a strong negative relationship between native *Daphnia* spp. and *D. lumholtzi* (Fig. A2). On one date, we recorded exceptionally high abundances (39.5 females L^{-1}) of *D. lumholtzi* at the western-most site on Lake Texoma. While this is an unusually high density, more extreme values have been recorded in other reservoirs, including a swarm with more than 10,000 individuals L^{-1} from Nolin Reservoir, Kentucky, USA (Beaver et al. 2018).

Two studies tested the allometry of head and tail spines in field-collected *D. lumholtzi*. Sorensen and Sterner (1992) measured head spine, tail spine, and body size in *D. lumholtzi* individuals collected from Fairfield Reservoir, TX from January to March 1991. There were differences between months in spine investment; increases in temperature were associated with increased growth of tail spines relative to body size. Yurista (2000) collected *D. lumholtzi* individuals from Kentucky Lake, KY from July to September. Within both populations, growth of head and tail spines were hyperallometric (Sorensen and Sterner, 1992; Yurista, 2000). However, these two studies found differential investment in the two spines. Yurista (2000) found that tail spines were both absolutely larger than head spines, and the relative growth rate of tail spines was higher than that of head spines. Sorensen and Sterner (1992) found that tail spines were absolutely larger, but that the growth rate of head spines was greater than that of tail spines.

Two other studies took alternative approaches that make the results difficult to directly compare to other published work. Work and Gophen (1995) measured head spine + head, tail spine, and body size in *D. lumholtzi* from February–July 1993 in Lake Texoma, OK-TX. They found positive relationships between head spine + head and body size, as well as between tail spine and body size. They found nonlinear relationships between some of these variables by relating spine size to body size squared, although without reporting regression equations, these claims are difficult to substantiate. For both head and tail spines, the proportion of total length increased throughout the season, suggesting a greater investment in spines from February to July within this subtropical reservoir (Work and Gophen, 1995). Schnake (2002) measured *D. lumholtzi* collected from Lake Taylorville, IL from May to December in 1993, 1994, 1999, and 2000. Measurements were transformed to proportion of total length before being arcsine transformed. These transformed proportional values varied across months and by site, and variation in head and tail spine investment was associated with many water quality variables, including dissolved solids, Secchi depth, temperature, dissolved oxygen, chlorophyll *a*, conductivity, and phosphate (Schnake, 2002).

The analytical methods employed in these four studies effectively assume linear scaling between traits and body size-squared and do not allow for rigorous testing of nonlinear relationships. However, tests of non-linear allometry in *D. lumholtzi* are important because a curvilinear

pattern could suggest that growth of spines is sustained only until the cost of producing the trait exceeds the benefits conferred by the trait. There is a cost of head and tail spine production in *Daphnia* (Spaak and Boersma, 1997; Boeing et al., 2005), and so individuals should only invest in this trait if the cost of growth is outweighed by the benefit. Thus, if there is a size refuge from predation, we would predict a curvilinear relationship between body size and size of defensive traits. For example, if large *D. lumholtzi* escape gape-limited predators, there would be no selective benefit to sustaining further spine growth. Thus, the rate of head spine growth may decline with increasing body size. There is evidence of nonlinear allometry in the defensive traits of other cladocerans, including length of head and tail spines of *Daphnia cucullata* (Lagergren et al., 2007), however no one has tested for nonlinearity in these scaling relationships in *D. lumholtzi.*

The defensive morphology of *D. lumholtzi* further allows their persistence during the summer and fall when predation pressure by young-of-year fishes is likely higher in Lake Texoma (Hubbs, 1982; Lienesch and Gophen, 2001). We found that growth of head spines in *D. lumholtzi* followed a curvilinear relationship with body size. Investment in head spines decreased as body sized increased. This pattern is consistent with the selection pressure on *D. lumholtzi* spine size being a small, gape-limited predator. Growth of tail spines was linear and hyperallometric in the summer and fall, but allometric in the winter. Earlier research has found growth of tail spines to be hyperallometric (Sorensen and Sterner, 1992; Yurista, 2000). Sorensen and Sterner measured individuals in the winter and spring (January–March), and Yurista collected primarily in the summer (July–early September, P. Yurista, personal communication). The seasonal differences in allometry of both head and tail spines suggest that the selective agent is itself seasonal.

Within Lake Texoma, several species fit the profile as small, gape-limited predators exerting seasonal pressure. Lake Texoma has two large-bodied invertebrate predators that can feed on *D. lumholtzi:* the cladoceran, *Leptodora kindti* (Holt et al., 1978), and the dipteran, *Chaoborus punctipennis* (Sublette, 1957). In laboratory experiments, *Leptodora kindti* prefers *D. lumholtzi* over the native *Daphnia pulex* and is not discouraged by its defensive spines (Effert and

Pederson, 2006), and thus is unlikely to be the predator shaping the nonlinear and seasonal allometry of head spines of *D. lumholtzi* within Lake Texoma.

Chaoborus punctipennis, on the other hand, is a size-selective predator of *D. lumholtzi* and laboratory experiments have shown that it is unable to consume *D. lumholtzi* with total lengths greater than 1.84 mm and head spines greater than 0.51 mm in length (Engel et al., 2014). Within other invaded lakes, *D. lumholtzi* performs diel vertical migration (Sorensen and Sterner, 1992; Williams and Pederson, 2004), although we do not know if this is the case in Lake Texoma. *Chaoborus punctipennis* migrates in the same way within Lake Texoma (Sublette, 1957), moving into the hypolimnion during the day to avoid vertebrate (fish) predators, and moving into the epilimnion during the night to feed. Thus, assuming *D. lumholtzi* is migrating as it does in other invaded lakes, it likely overlaps in depth preferences with *Chaoborus punctipennis* within Lake Texoma. Additionally, if *Chaoborus* abundances in Lake Texoma peak in late summer to fall, as they do in North Carolina (Celik et al., 2002), then the trend in head spine growth may reflect population densities of, and predation pressure by, *Chaoborus*. Our zooplankton sampling methodology was insufficient for accurately estimating *Chaoborus* abundance patterns from these samples (Persaud and Yan 2001), although future research could elucidate the potential for *Chaoborus* overlap with *D. lumholtzi*.

Within Lake Texoma, *Menidia* also consumes *D. lumholtzi* (Lienesch and Gophen, 2001), and cannot consume larger-spined *D. lumholtzi* (Lienesch and Gophen, 2001). Predation pressure by young-of-year fishes, including *Menidia*, would be seasonal, with highest abundances observed May through July (Hubbs, 1982). Evidence of non-linear growth of head spines and seasonal patterns in allometry lead us to hypothesize that size-selective predation on *D. lumholtzi* is carried out by a small, gape-limited predator, likely either *Chaoborus punctipennis* or *Menidia.*

Daphnia lumholtzi reach high abundances in eutrophic lakes during the summer, which makes them unique relative to native *Daphnia* that tend to peak in abundances in late spring. The higher temperature tolerance of *D. lumholtzi*, paired with their unique defensive morphology, allows them to exploit late summer resources in subtropical reservoirs. The relatively high abundances of *D. lumholtzi* observed during summers in the southern US (Havel et al., 1995; Work and

Gophen, 1995; Yurista et al., 2000; Havens et al., 2012; East et al. 1999) co-occur with native non-cladoceran grazers, including copepods, rotifers, and ciliates. Although tests of competition have focused on native *Daphnia*, the two do not temporally overlap in most invaded systems. Further work should be done to characterize the interactions between *D. lumholtzi* and the native grazers that are abundant during the summer peaks of *D. lumholtzi*, including copepods and rotifers (Hambright et al., 2010). Copepods are considered to be more tolerant of cyanobacteria than native *Daphnia* due to their selective feeding mechanisms (Sommer et al. 2001, Hambright et al. 2007). So there may be either direct or indirect competition between *D. lumholtzi* and copepods during the blooms of cyanobacteria that are associated with high abundances of *D. lumholtzi*. Although *D. lumholtzi* has a shared evolutionary history with cyanobacterial blooms, lab experiments do not support the hypothesis that they are more tolerant of toxigenic cyanobacteria than native *Daphnia* spp. (Pattinson et al., 2003; Fey and Cottingham 2011). Thus, the positive relationship we uncovered between cyanobacteria and *D. lumholtzi* abundances is likely a proxy for other ecological factors, likely predation pressure and temperature tolerance. As global change is predicted to lead to increases in both the frequency and duration of blooms of cyanobacteria (Paerl and Paul, 2012), we need further research to characterize the proximate causes of positive relationships between *D. lumholtzi* and cyanobacteria, as well as potential competitive interactions with native grazers.

Conclusions

Twenty years after invasion, we found the predictors of *Daphnia lumholtzi* abundances in a subtropical reservoir were different from initial predictors of establishment. Within our study, the best predictor of *D. lumholtzi* abundance was cyanobacterial abundance, which is counterintuitive as this species does not display superior cyanobacterial tolerance in laboratory experiments. However, our measurements of *D. lumholtzi* morphology during the same time period suggests a potential explanation for this paradox. The seasonal patterns in length of head and tail spines relative to body size lead us to hypothesize that a small, gape-limited predator is exerting seasonal selection pressure on *D. lumholtzi* within Lake Texoma. We suggest the plastic morphology of large spines in *D. lumholtzi* give it a competitive advantage relative to the native *Daphnia* spp. during the summer and fall. Thus, the underlying driver of *D. lumholtzi* abundance and persistence within Lake Texoma may be predation pressure.

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Data Archiving

Data will be made available on PANGAEA upon publication.

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Figures

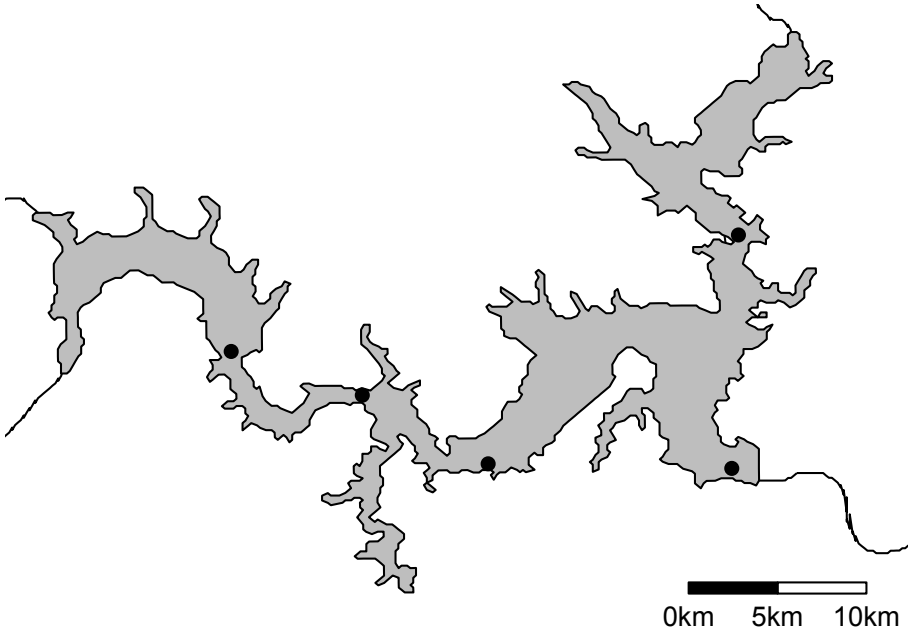


Figure 1- Map of Lake Texoma, OK-TX, with five sampling sites indicated by black circles.

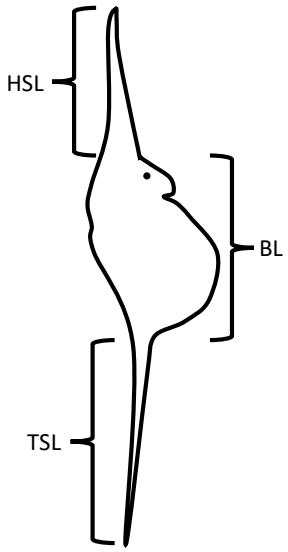


Figure 2- Diagram of measurements of *Daphnia lumholtzi* including head-spine length (HSL), body length (BL), and tail-spine length (TSL).

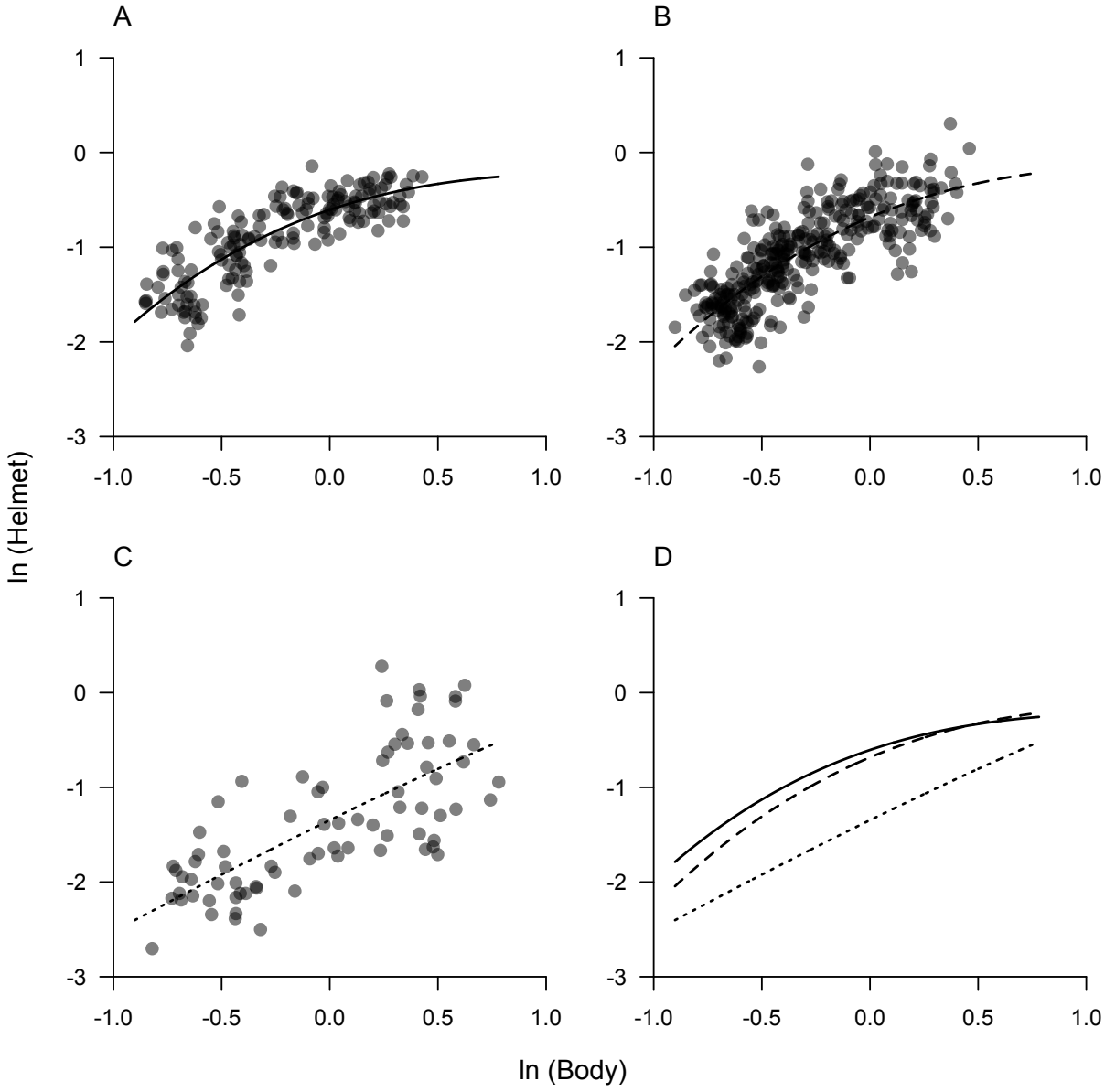


Figure 3- Relationship between log-transformed length of body and head spine by season: (A) Summer, (B) Fall, (C) Winter, (D) the same nonlinear regression fits from (A-C) with points removed for clarity. Lines represent nonlinear regression fit. All points are transparent grey to demonstrate overlap in points. Spring is not displayed because only one individual was collected during that period.

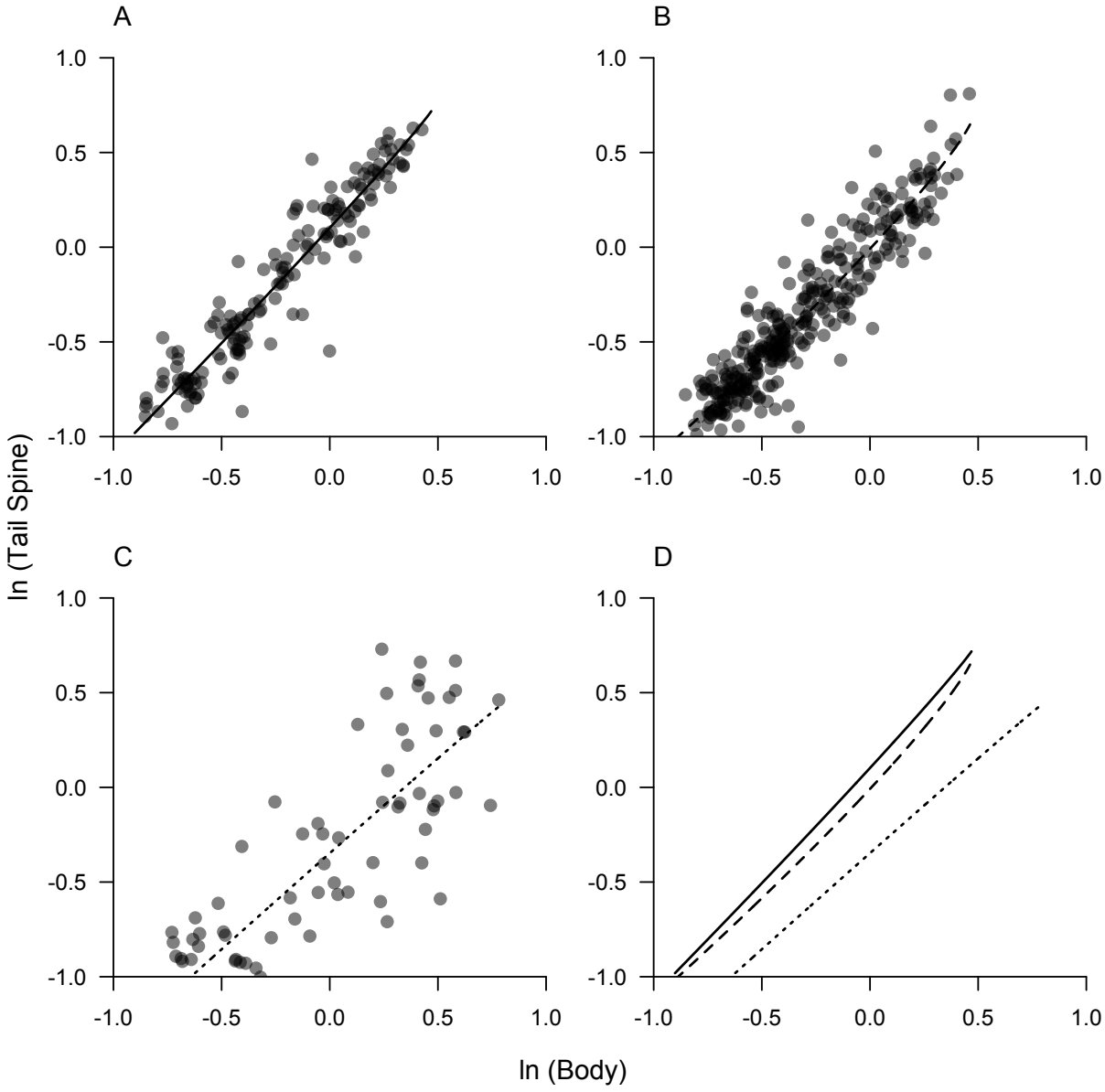


Figure 4- Relationship between log-transformed body and tail-spine size by season: (A) Summer, (B) Fall, (C) Winter, (D) the same nonlinear regression fits from (A-C) with points removed for clarity. Lines represent nonlinear regression fit. All points are transparent grey to demonstrate overlap in points. Spring is not displayed because only one individual was collected during that period.

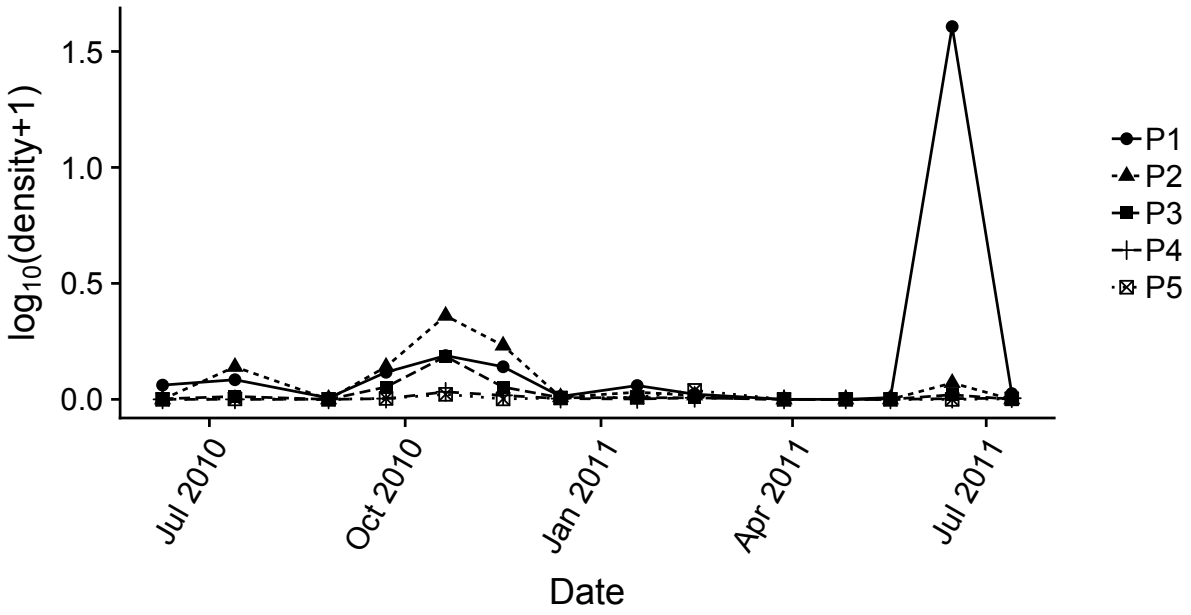


Figure 5- Density of *Daphnia lumholtzi* from June 2010 to July 2011. Densities are log(x+1) transformed for clarity. Each site is indicated by a different shape, as indicated by legend.

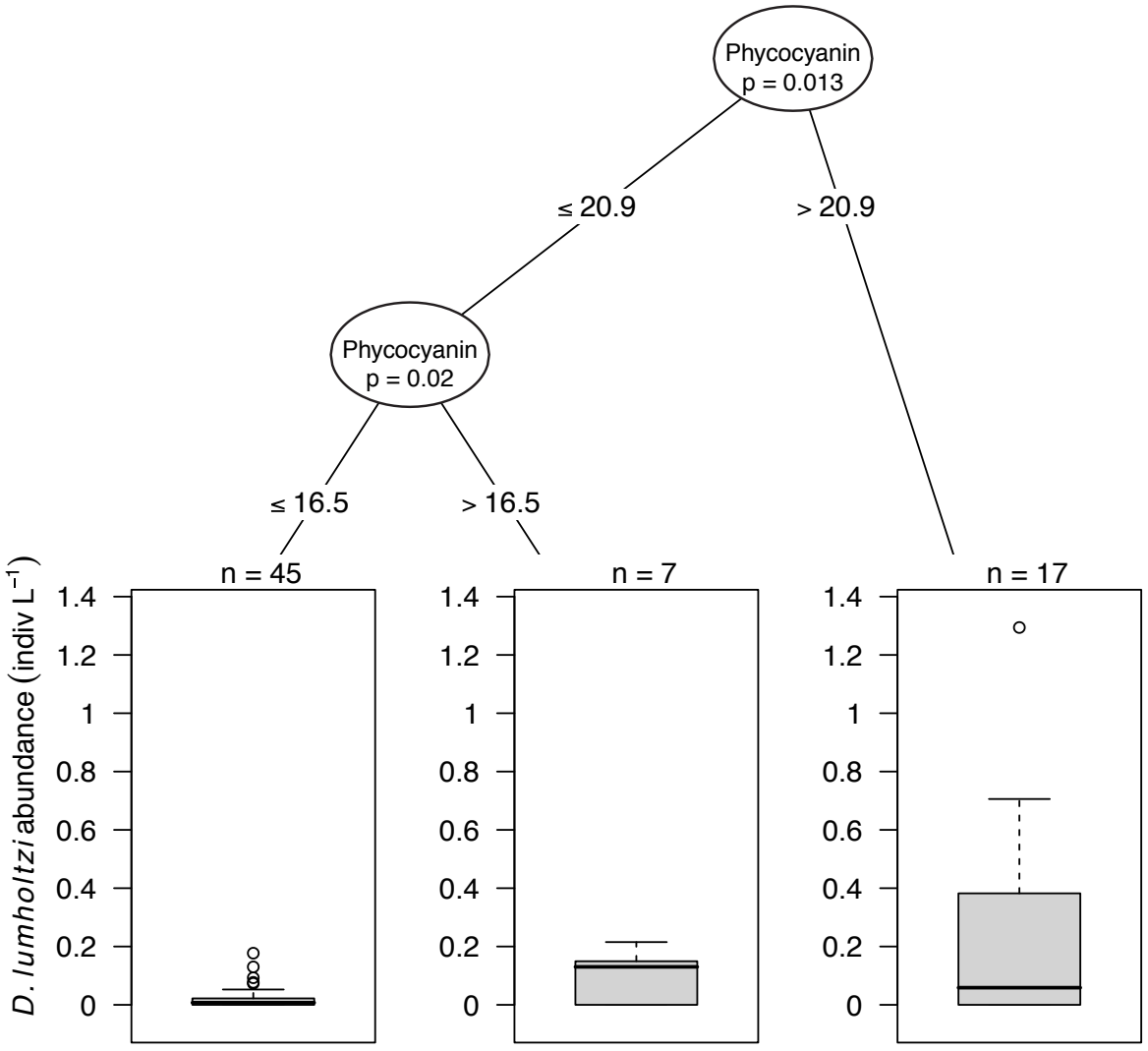


Figure 6- Regression tree for predicting *Daphnia lumholtzi* abundance across all seasons. Each internal node is labeled with the p-value of the conditional test of association of predictor variable and response variable (*D. lumholtzi* abundance). The three box and whisker plots show the median (center line), first and third quartiles (top and bottom of box), range (end of whiskers) of abundance of *D. lumholtzi* in the samples at each of three identified groups, with points identifying outliers. Each box and whisker plot is labeled with the number of samples in belonging to that group (n).

Tables

Table I- Parameter estimates ± standard error, and confidence intervals from nonlinear regression of ln-transformed helmet length against body length. Where the curvature parameter, *D*, is not significantly different from 1, results of linear regression of the same variables are also provided.

		Nonlinear regression					Linear regression		
Season	*n*	*A*	*C*	*D*	95% CI for *D*	Residual standard error	Slope	Intercept	95% CI for Slope
Summer	163	0.67 ± 0.037	0.78 ± 0.07	1.8 ± 0.26	(1.37, 2.33)	0.25			
Fall	331	0.67 ± 0.041	0.95 ± 0.07	1.7 ± 0.20	(1.32, 2.09)	0.29			
Winter	77	0.57 ± 0.14	1.0 ± 0.29	1.2 ± 0.43	(0.58, 2.06)	0.50	1.12 ± 0.12	–2.19 ± 0.85	(0.88, 1.4)

Table II- Parameter estimates ± standard error, and confidence intervals from nonlinear regression of ln-transformed tail- spine length against body length. Where the curvature parameter, *D*, is not significantly different from 1, results of linear regression of the same variables are also provided.

		Nonlinear regression					Linear regression		
Season	*n*	*A*	*C*	*D*	95% CI for *D*	Residual standard error	Slope	Intercept	95% CI for Slope
Summer	162	2.1 ± 0.16	1.3 ± 0.08	0.95 ± 0.10	(0.77, 1.1)	0.15	1.22 ± 0.03	–1.43 ± 0.22	(1.16, 1.29)
Fall	323	1.9 ± 0.14	1.3 ± 0.08	0.851 ± 0.08	(0.70, 1.0)	0.15	1.17 ± 0.03	–1.17 ± 0.17	(1.12, 1.22)
Winter	73	1.5 ± 0.29	0.99 ± 0.2	1.02 ± 0.30	(0.58, 1.7)	0.33	1.00 ± 0.08	–0.408 ± 0.58	(0.842, 1.17)