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Regional coexistence and local dominance in *Chaoborus*:
species sorting along a predation gradient

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Erica Ann Garcia

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REGIONAL COEXISTENCE AND LOCAL DOMINANCE IN *CHAOBORUS*: SPECIES SORTING ALONG A PREDATION GRADIENT

By

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ABSTRACT

REGIONAL COEXISTENCE AND LOCAL DOMINANCE IN *CHAOBORUS*: SPECIES SORTING ALONG A PREDATION GRADIENT

By

Erica Ann Garcia

The non-random distributional patterns of species across the landscape may be a function of dispersal and local species sorting based on the traits of the species involved. In aquatic systems in particular, the direct and indirect effects of predators are major factors limiting species distributions and abundances. However, while a number of taxonomic groups have been shown to vary in their distribution based on the presence or absence of fish, far less is known about the distributional patterns of species across a landscape that varies in the intensity of fish predation (due either to variation in fish density or the degree to which organisms have a refuge from fish). In my dissertation research, I used four species of phantom midge larvae (*Chaoborus*) found in southwestern Michigan, to examine how different species' traits are functionally related to species sorting along natural and experimental gradients in predation.

Through field surveys of 17 lakes and ponds, a long term data set in one lake and empirical work in cattle tanks and replicate ponds at the Experimental Pond Facility at the Kellogg Biological Station, I found that species traits such as pigmentation, size, oviposition habitat selection (OHS) and diel vertical migration (DVM) are key in how *Chaoborus* species sort across the landscape. This is due to the fact that the four *Chaoborus* species in this study varied relative to these traits and I found that this variation was related to differences in vulnerability to fish. Thus, *Chaoborus* species' trait variation together with variation across the landscape in fish predation intensity lead to the observed patterns of *Chaoborus* species' regional coexistence and local dominance.

Overall, this research highlights the importance of considering environmental gradients (versus simply presence or absence of a factor) when examining the distribution and abundance of organisms.

For my parents, Guillermo and Vicki Garcia

And for Abuelita, te quiero mucho

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My lab mates Tara Darcy-Hall, Nate Dorn, Chris Steiner and Jeremy Wojdak helped get me started and kept me going. Chris showed me the ways of the zooplankton and Nate the ways of fish, I am indebted to them both for their mentorship, friendship and help in the field. I also appreciated the moral and intellectual support of Tara and Jeremy. I couldn't have imagined life in the Mittelbach lab without them. And then there was one...and I gained new lab mates, Sarah Emery, Greg Houseman and Rich Smith, thanks for taking me in.

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CHAPTER 1

INTRODUCTION

Local communities are composed of a subset of the regional species pool that can disperse to a locality, survive abiotic conditions and persist given the local biotic interactions (e.g. competition, predation) (Urban 2004). Historically, much of community ecology theory has focused on local interactions (competition and predation; Tilman 1982, Leibold 1996) independent of regional influences (colonization and extinction; MacArthur and Wilson 1967). However, in the last decade, community ecologists have acknowledged the metacommunity concept as an important way to better understand the linkages between regional and local factors in structuring communities.

Four paradigms of the metacommunity approach have been described and include two that assume local sites differ only with respect to the species composition at a given point in time (patch-dynamic and neutral) and two that assume local sites are heterogeneous and therefore different species are favored at different locales (species sorting and mass effects) (Leibold et al. 2004). In a recent meta-analysis, Cottenie (2005) observed that habitat heterogeneity and species sorting dynamics were dominant processes structuring communities. He also found that the type of dispersal (passive versus active) was an important determinant of metacommunity type and passive dispersers as well as active dispersers in marine and lake habitats were strongly related to environmental dynamics.

In my research, I examined the distribution and abundance of active dispersing lake and pond species in the genus *Chaoborus* (Diptera: Chaoboridae) and used the species sorting approach to determine the relative importance of environmental factors known to vary across the landscape (e.g. fish planktivory, productivity, pH, etc) in structuring this species assemblage.

Chaoborus background

The larvae of the phantom midge *Chaoborus* are important members of many freshwater food webs. They are distributed worldwide with 12 described species in North America (Sæther 1970) and are often the dominant invertebrate predators found in the plankton of lakes and ponds. *Chaoborus* are omnivorous, gape-limited, ambush predators of small to medium sized zooplankton (e.g., Moore et al. 1994, Swift and Fedorenko 1975, Pastorok 1981). In north temperate lakes, species of *Chaoborus* reveal patterns in distribution across the landscape, strongly suggesting the importance of fish predation and possibly other factors such as, interspecific interactions, water transparency, temperature, and nutrient levels (Pope et al. 1973, von Ende 1979, Lamontagne et al. 1994, Wissel et al. 2003). Here, I focus on the four *Chaoborus* species found in southwestern Michigan: *C. americanus*, *C. punctipennis*, *C. flavicans*, and *C. albatus*.

Figure 1.1. *C. americanus*. Photograph by C. Steiner.

Dissertation synopsis

In my dissertation research, I used a combination of field surveys of about 20 lakes and ponds, a long term data set in one lake, experimental work in mesocosms and two large-scale predator manipulations in ponds, to elucidate the processes that explain the non-random distributional pattern of the *Chaoborus* species assemblage.

In Chapter 2 I asked: What environmental factors are important to the distributional pattern of the *Chaoborus* species assemblage? My approach was to first document the pattern in *Chaoborus* species distribution and abundance in nature, across aquatic systems that varied in the level of fish planktivory, productivity, area, depth, conductivity, pH, temperature and dissolved oxygen. I then compared these results to the changes in *Chaoborus* community composition in response to a documented decrease and then increase in planktivore density over 16 years within a single lake. I found that the level of fish planktivory, in addition to environmental variables correlated to the level of fish planktivory, (i.e. lake area and depth; Tessier and Woodruff 2002) are important factors related to the distribution and abundance of *Chaoborus* species across the landscape.

In Chapter 3 I asked: Do species of *Chaoborus* exhibit directed dispersal (oviposition habitat selection; OHS) and can this explain the presence/absence patterns of *Chaoborus* species across the landscape? I tested the hypothesis that the *Chaoborus* species most vulnerable to predation by fish (*C. americanus*) will exhibit OHS but that species that are less vulnerable to fish predation (*C. punctipennis* and *C. flavicans*) will not. The results from one experiment supports this hypothesis but in a second experiment all species of *Chaoborus* were observed to show no discrimination between sites that were fishless and those with fish cues. The discrepancy between these two experiments may be explained by strong site fidelity of *C. americanus*.

In Chapter 4 I asked two questions: 1) How does predator density affect *Chaoborus* species composition and relative abundance? and 2) Is the pattern of prey choice by an important planktivorous fish, the bluegill sunfish (*Lepomis macrochirus*), consistent with the observed pattern of *Chaoborus* species sorting across the predation gradient? I investigated the first question in two pond experiments where I examined *Chaoborus* species sorting along an experimental gradient in bluegill density. I also experimentally tested bluegill prey choice for the four *Chaoborus* species found in southwestern, MI. The results of these experiments indicate that 1) the gradient in fish density can lead to clear species sorting within the species assemblage that is consistent with distributional patterns observed in nature, and 2) that larval body size, transparency, and diel vertical migration (DVM) behavior are important traits determining the vulnerability of different *Chaoborus* species to planktivorous fish such as the bluegill.

Table 1.1. *Chaoborus* species studied and their associated traits

Species	4th instar length (mm)	Transparent	OHS	DVM	References
C. americanus	10-13		X		Carter and Kwik 1977, vonEnde 1979
C. flavicans	9-12.7	X		X	Dawidowicz 1990, Luecke 1986, Tjossem 1990
C. punctipennis	7.5-9.5	X		X	Gonzalez and Tessier 1997, Gonzalez 1998
C. albatus	7-9.4	X		X	Tjossem 1990

Note: References point to selected papers on the change in the intensity of the DVM behavior associated with species experiencing a new environment (fish-fishless or fishless-fish).

CHAPTER 2

CHAOBORUS SPECIES DISTRIBUTION AND ABUNDANCE ACROSS NATURAL GRADIENTS IN PLANKTIVORY

Abstract

Species traits are functionally related to the determinants of species distributions and development and maintenance of community structure. Here, I present data on the distribution and abundance of four species of *Chaoborus* (Diptera: Chaoboridae) that vary in species traits (i.e. pigmentation, diel vertical migration (DVM) behavior and size) that are important to coexisting with fish predators. I examine the spatial and seasonal pattern of the *Chaoborus* species assemblage in 1) a field survey of 11 lakes and ponds that vary in environmental factors such as the level of fish planktivory, pH, productivity, area and depth, and 2) a long term data set from Wintergreen Lake that experienced large changes in the levels of fish planktivory but varied little with respect to other environmental variables. *Chaoborus americanus* is the largest species and does not exhibit DVM behavior, only occurred in ponds without fish. *Chaoborus albatus* is relatively small and does exhibit DVM behavior, was only found in shallow lakes and ponds with fish. *Chaoborus punctipennis* is also small and exhibits DVM behavior, was found in all lakes and ponds with fish; and *C. flavicans* is relatively large and exhibits DVM behavior, was found in both fish and fishless lakes and ponds. The results of this study clearly document the importance of fish predators as drivers of distribution and abundance in this assemblage.

Introduction

The distribution of species across the landscape may be a function of dispersal and local species sorting based on the traits of the species involved. The influence of particular species traits is especially apparent when a group of closely related species shows a clear distributional pattern across a strong environmental gradient. The presence of planktivorous fish has been considered to be a key factor contributing to the distribution and abundance of *Chaoborus* (Diptera: Chaoboridae) across a landscape of lakes and ponds (e.g. Pope et al. 1973, von Ende 1979, and Wissel et al 2003). The larvae of the phantom midge *Chaoborus* are important members of many freshwater food webs as they are frequently the dominant invertebrate predators in these systems. They are often the largest organisms in the plankton and are gape-limited, ambush predators of small to medium sized zooplankton (e.g., Moore et al. 1994, Swift and Fedorenko 1975, Pastorok 1981). Species traits such as small size and vertical migration (DVM) behavior, where larvae are found in the water column at night and then migrate into the sediments or deeper water during the day, are considered to be effective strategies that allow some species to co-occur with fish (Dawidowicz et al. 1990, Tjossem 1990, Berendonk et al. 2003). Of the 12 described *Chaoborus* species in North America (Sæther 1970), four inhabit lakes and ponds in southwestern Michigan: *C. americanus*, *C. punctipennis*, *C. flavicans*, and *C. albatus*. These four species vary widely in morphology from the relatively large, opaque *C. americanus* (4th instar length 10-13 mm) to the similar-sized but transparent *C. flavicans* (4th instar length 9-12.7 mm) to the small, transparent *C. punctipennis* (4th instar length 7.5-9.5 mm) and *C. albatus* (4th instar length 7-9.4 mm) (Cook 1956, Sæther 1970).

The objective of this study was to document the spatial and seasonal pattern of the *Chaoborus* species assemblage in southwestern Michigan and to determine the effect of environmental variables, particularly the level of fish planktivory, on species distribution and abundance. Although other studies have examined patterns of *Chaoborus* distribution most have only sampled during the day (Pope et al. 1973), or collected data from single visits to each lake or pond (Wissel et al. 2003) and few have directly looked at the effect of fish predation. While fish planktivory varies across the well-established environmental gradient in lentic freshwater habitats so do other environmental factors (Wellborn et al. 1996, Tessier and Woodruff 2002). Therefore, I also made use of a unique opportunity to examine *Chaoborus* abundances in a single lake that varied in level of fish planktivory over a 16-year period due to the extinction and purposeful reintroduction of fish species. Here, I present data on *Chaoborus* species presence/absence gathered from multiple surveys and experiments as well as data on the *Chaoborus* species assemblage from a field survey of 11 ponds and lakes that were sampled throughout the growing season of one year. In addition, I include a long-term data set on fish and *Chaoborus* dynamics to examine how *Chaoborus* community composition responded to a gradual decrease and then increase in planktivore density over 16 years within a single lake that varied little in abiotic conditions through time.

Methods

Field Survey

I conducted a field survey of 11 lakes and ponds located within a 100 km radius of the W. K. Kellogg Biological Station (KBS, Hickory Corners, Michigan, USA). All ponds contained water year-round at least two years prior to the study (personal observation). These systems were chosen to encompass a gradient in levels of planktivory (see Tessier and Woodruff 2002) and productivity (total phosphorus). Pond surface area and depth were measured on 25 June 2004; lake surface area and depth were collected from the literature (Table 2.1, Tessier and Woodruff 2002, Cáceres and Tessier 2004, Mittelbach et al. 2006).

Ponds and lakes were sampled every 2-4 weeks in 2004, beginning in mid-May and ending in early September. *Chaoborus* were collected one hour after sunset by taking three vertical tows through the entire water column with a 30 cm diameter, 500-micron plankton net, at the deepest point in each lake. In ponds, *Chaoborus* were collected during the day using a 2-L plastic pitcher dipped just below the pond surface and then poured through a 153-micron sieve. The dipping was repeated two times per sample and each sample was taken from equally spaced locations along a qualitative transect that started ~0.5 m in from the pond edge to the center of each pond for a total of three 8-L samples. *Chaoborus* were preserved in 95% ethanol, later identified to species

Table 2.1: Means and ranges (in parentheses) of limnological variables measured in the study ponds and lakes over the survey period

Pond/Lake	Latitude	Longitude	Surface area (m^2)	Max Depth (m)	Temp (°C)	Dissolved oxygen (mg/L)	Conductivity (μS/cm)	pH
Lux9	42°28'17.5" N	85°27'43.6" W	782	0.7	21.42 (19.46-26.24)	4.19 (0.79-10.88)	0.033 (0.014-0.047)	5.97 (5.77-6.29)
Lux10	42°28'15.7" N	85°27'40.4" W	6240	2	24.22 (21.85-27.84)	5.83 (3.85-8.75)	0.020 (0.018-0.022)	6.63 (6.02-7.12)
Lux16	42°28'27.1" N	85°27'50.2" W	300	0.7	20.36 (18.64-23.91)	2.97 (0.63-5.78)	0.022 (0.020-0.027)	6.15 (5.78-6.47)
Lux18	42°28'42.0" N	85°27'34.6" W	3525	1.3	22.81 (20.64-26.24)	4.93 (2.59-11.48)	0.023 (0.005-0.032)	6.24 (5.86-6.65)
Circle	42°23'34.1" N	85°22'50.4" W	1666	3	23.12 (21.18-25.99)	4.99 (3.98-6.84)	0.280 (0.241-0.334)	7.65 (7.33-7.85)
Horseshoe	42°23'33.6" N	85°22'49.2" W	484	0.5	21.76 (17.76-25.59)	2.69 (1.44-4.06)	0.099 (0.077-0.121)	6.57 (6.32-6.77)
Wintergreen	42°23'51.7" N	85°23'6.7" W	150000	6	19.78 (18.83-20.56)	4.11 (2.21-7.20)	0.410 (0.372-0.442)	7.95 (7.74-8.30)
Lawrence	42°26'27.1" N	85°21'2.5" W	50000	11.5	14.74 (14.32-15.39)	4.93 (3.02-6.98)	0.477 (0.467-0.485)	7.91 (7.90-7.93)
Duck	42°24'30.8" N	85°22'54.9" W	40000	3	22.53 (20.62-24.29)	4.71 (2.11-7.69)	0.146 (0.126-0.173)	7.26 (6.96-7.53)
Three Lakes 2	42°21'6.4" N	85°25'58.1" W	220000	10	17.13 (16.43-18.14)	5.59 (2.80-7.49)	0.440 (0.433-0.446)	8.05 (8.00-8.10)
Three Lakes 3	42°21'16.1" N	85°25'30.4" W	150000	4	22.21 (19.71-23.40)	5.62 (2.95-9.38)	0.421 (0.410-0.435)	7.95 (7.78-8.20)

Table 2.1: Continued

Pond/Lake	Total phosphorus (μg/L)	Total Chl *a* (μg/L)	<35 μ m Chl *a* (μg/L)	Piscivores	Planktivores
Lux9	127.80 (93.46-196.39)	41.16 (3.26-77.16)	12.27 (0.33-36.89)	**	**
Lux10	79.02 (24.18-205.58)	9.45 (3.50-19.93)	2.99 (0.75-6.35)	**	**
Lux16	300.80 (163.84-440.49)	82.42 (19.89-145.38)	12.53 (0.99-30.60)	**	**
Lux18	63.54 (48.70-86.42)	9.25 (4.71-16.31)	4.45 (0.52-11.75)	**	**
Circle	39.11 (31.88-50.43)	5.61 (3.24-9.33)	2.05 (0.51-4.02)	**	**
Horseshoe	71.56 (53.92-101.86)	18.30 (3.37-37.96)	5.96 (0.86-13.18)	**	**
Wintergreen	51.63 (42.67-62.19)	30.02 (16.84-51.12)	5.40 (1.16-12.00)	Ms	Lg, Lm
Lawrence	12.97 (11.40-14.66)	2.93 (1.17-6.60)	0.96 (0.01-3.97)	Ms	Lm
Duck	26.85 (25.44-28.38)	4.68 (3.95-5.74)	1.38 (1.04-1.86)	**	Lg, Nc, Nh
Three Lakes 2	24.78 (19.70-30.44)	6.95 (2.97-16.38)	1.61 (0.42-4.89)	Ms	Lg, Lm, Nh
Three Lakes 3	22.50 (16.46-34.51)	3.59 (1.79-5.80)	1.35 (0.33-3.10)	Ms	Lg, Lm, Nh

Notes: Dominant fish were determined by seining and minnow traps. Species abbreviations: Lm = ***Lepomis macrochirus***, Lg = ***L. gibbosus***, Ms = ***Micropterus salmoides***, Nc = ***Notemigonus crysoleucas***, Nh = ***Notropis heterodon***

** There were no piscivores/planktivores in this pond/lake.

(Cook 1956, Sæther 1970) and measured under 40X magnification using a digitizer tablet and software (SigmaScan Pro Version 4.01; SPSS Inc., 1987).

Pond water (1 L) was collected at each sample point along the transect using 500 ml polyethylene bottles and lake water (1 L) was collected with an integrated tube sampler (5 cm diameter plastic tubing) that sampled the entire water column at the deepest point of each lake. These water samples were immediately placed on ice in a cooler, for later analysis of chlorophyll a (chl *a*) and total phosphorus (TP). The water collected for the chl *a* analysis was divided in two and half was filtered onto Gelman A/E glass fiber filters (Gelman Sciences, Ann Arbor, MI USA) to measure total chl *a* (an approximate for algal biomass). The other half was first filtered through a 35 µm mesh and then filtered onto A/E filters to measure the "edible" size fraction. Algal size is known to be an important feeding constraint and the <35 µm fraction represents a commonly used measure of "edible algae" (Mittelbach et al. 2004) based on known size preferences of many filter feeding zooplankton (Sterner 1989). Chl *a* was then extracted from the filters overnight in cold 95% ethanol and measured using narrow band fluorometry (Welschmeyer 1994). Pond and lake water samples for TP measurement were frozen and later analyzed using spectrophotometry and standard methods (Wetzel and Likens 1991). Water temperature, dissolved oxygen, pH, and conductivity were also measured in each pond and lake every time that *Chaoborus* were sampled, using a Hydrolab® multi-probe (MiniSonde 4a). For the ponds these measures were taken at two points, one about 0.5 m away from shore and the other near the center of each pond and these values were averaged. For the lakes, abiotic measurements were sampled at the lake surface and then at every meter until I reached the lake bottom and these values were averaged for the following analyses.

Additionally, I explored the abundance of *Chaoborus* species in a long-term data set (1989-2004) from Wintergreen Lake (Mittelbach et al 1995, 2006). Wintergreen Lake experienced a gradual decrease and then increase in planktivore density over a 16-year period due to the elimination of the two dominant fish species (largemouth bass: *Micropterus salmoides* and bluegill sunfish: *Lepomis macrochirus*), followed by the subsequent intentional reintroduction of both species. For detailed methods on zooplankton collection and estimation of planktivore densities see Mittelbach et al. (2006).

Statistical analyses

All data presented on *Chaoborus* species abundance includes combined counts of III and IV instars. I used multivariate analyses to determine the important factors influencing *Chaoborus* species distribution and abundance using the field survey data. Similarities among surveyed sites based on environmental factors (e.g. water temperature, lake/pond area and depth, pH, conductivity, dissolved oxygen, total and <35 μm chl *a*) and changes in *Chaoborus* community composition were visualized using non-metric multi-dimensional scaling (NMDS) based on Bray-Curtis distances of the seasonal mean *Chaoborus* species abundances. All multivariate procedures were performed using PC-ORD (v4.25, McCune and Mefford 1999) and followed methods outlined by McCune and Grace (2002).

For the long-term data set, linear regression was used to examine the relationship between *Chaoborus* species abundance and planktivore density. Yearly mean *Chaoborus* species abundance and planktivore density were $\log_{10}$ (x+0.00039 (one half the lowest density)) and $\log_{10}$ (x) transformed, respectively to meet assumptions of the analyses. Regression analyses were performed using Systat 11.0 (SPSS Inc., 2004).

Results

Chaoborus species distributions showed remarkably clear separation when aquatic systems were divided into fish and fishless environments and arranged along a gradient of lake depth (Fig. 2.1). *Chaoborus americanus* was only found in ponds without fish; *C. albatus* was only found in shallow lakes and ponds with fish (2-6 m depth); *C. punctipennis* was found in all lakes and ponds with fish; and *C. flavicans* was found in both fish and fishless lakes and ponds (Fig. 2.1). A summary of the means and ranges for several environmental variables measured in each lake and pond is given in Table 2.1. With regard to relative abundance, *C. americanus* was by far the most abundant species in all fishless ponds and showed relatively little change in density across the sampling period (Fig. 2.3). *Chaoborus punctipennis* dominated or co-dominated the *Chaoborus* assemblage of all lakes with planktivorous fish and its density remained steady or increased through the growing season (Fig. 2.2). *Chaoborus albatus* and *C. flavicans* were more intermittent in their distributions than *C. americanus* or *C. punctipennis* and were generally rarer than the other two species (Figs. 2.2 and 2.3). Importantly, there was no evidence for any seasonal partitioning of these environments by the four *Chaoborus* species (Figs. 2.2 and 2.3).

An NMDS ordination of the abundance of the four *Chaoborus* species converged on two axes, with the final solution representing a cumulative 94% of the variation in the dataset (the two axes accounted for 79% and 15% of the variation, respectively). In the NMDS plot (Fig. 2.4) there is a clear separation of two groups along axis 1, which is strongly correlated with conductivity and pH (Pearson's r^2=0.58 and 0.60). The separation of sites along axis 2 is strongly related to pH, depth and area (Pearson's r^2=0.51, 0.54, and 0.87, respectively). Thus, *C. americanus* abundance is associated with ponds that are shallow, have small areas, low conductivity and are somewhat acidic (Fig. 2.4, lower right). *Chaoborus punctipennis* and *C. flavicans* abundances on the other hand, are positively related to deep lakes with large areas, high conductivity and neutral to slightly basic pH (Fig. 2.4, upper left). *Chaoborus albatus* abundance was linked to shallow lakes with small surface areas (Fig. 2.4, mid-left).

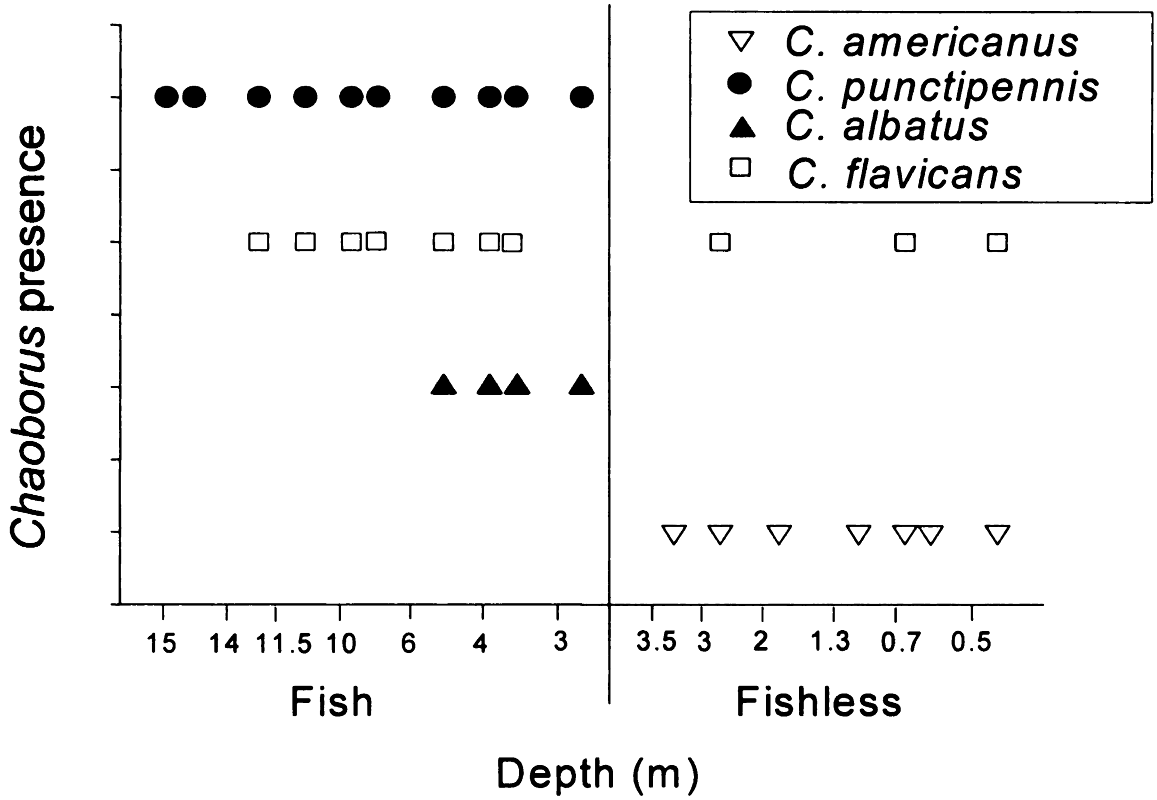

Figure 2.1. Presence of four *Chaoborus* species in 17 lakes and ponds that vary in presence/absence of fish and depth.

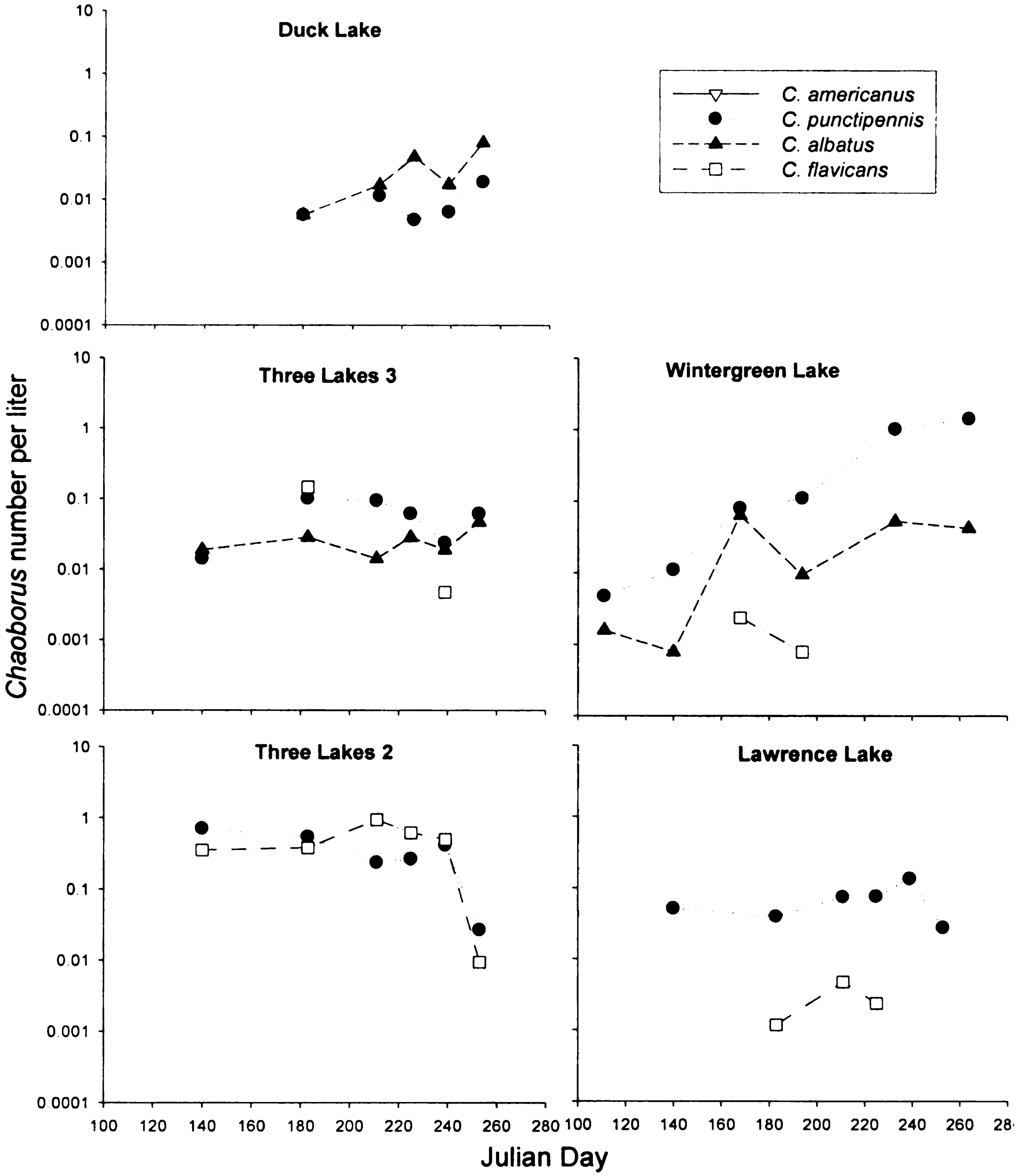

Figure 2.2. Seasonal dynamics of *Chaoborus* densities in five southwestern Michigan lakes (mean densities based on three samples per date).

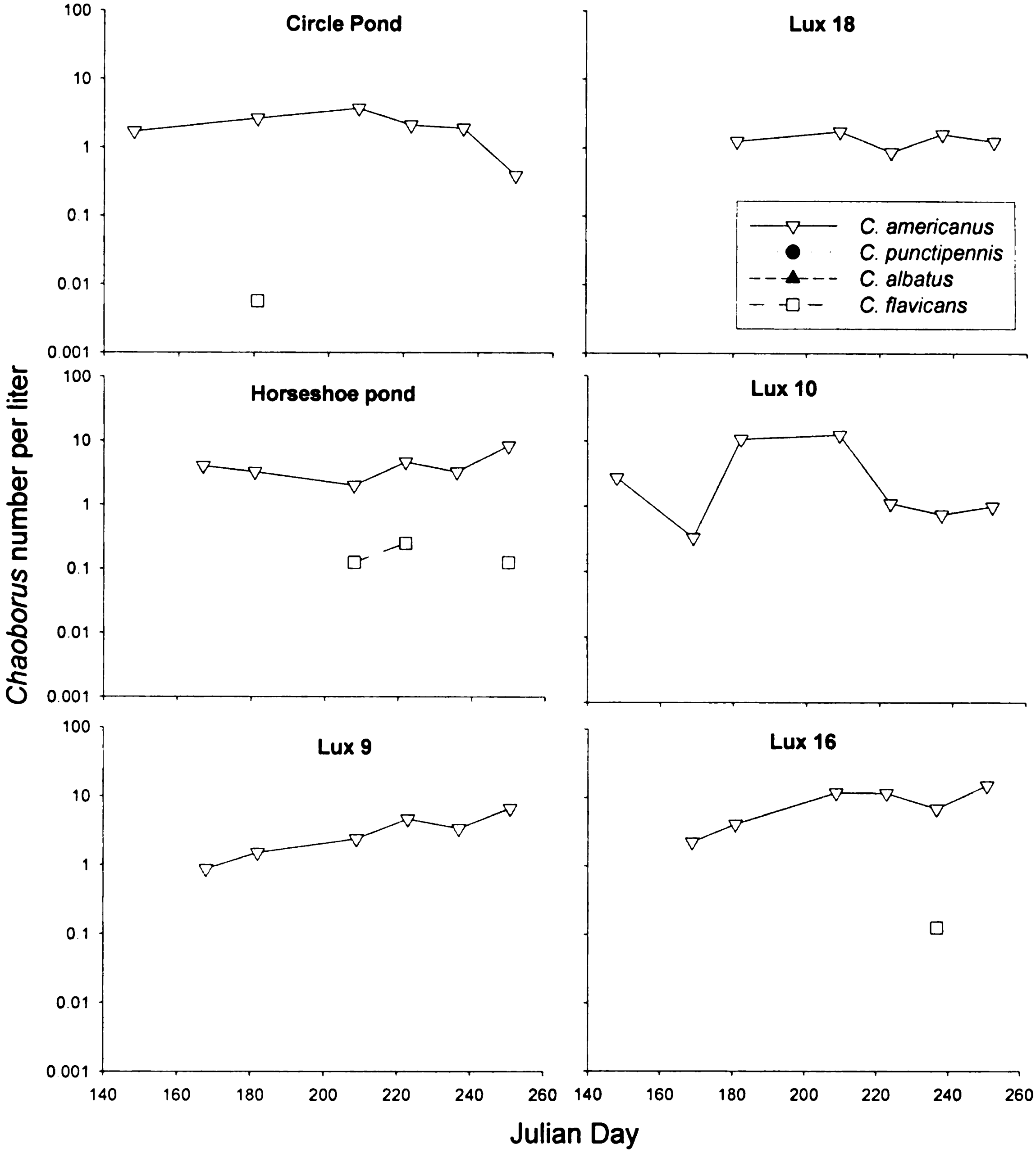

Figure 2.3. Seasonal dynamics of *Chaoborus* densities in six southwestern Michigan fishless ponds (mean densities based on three samples per date).

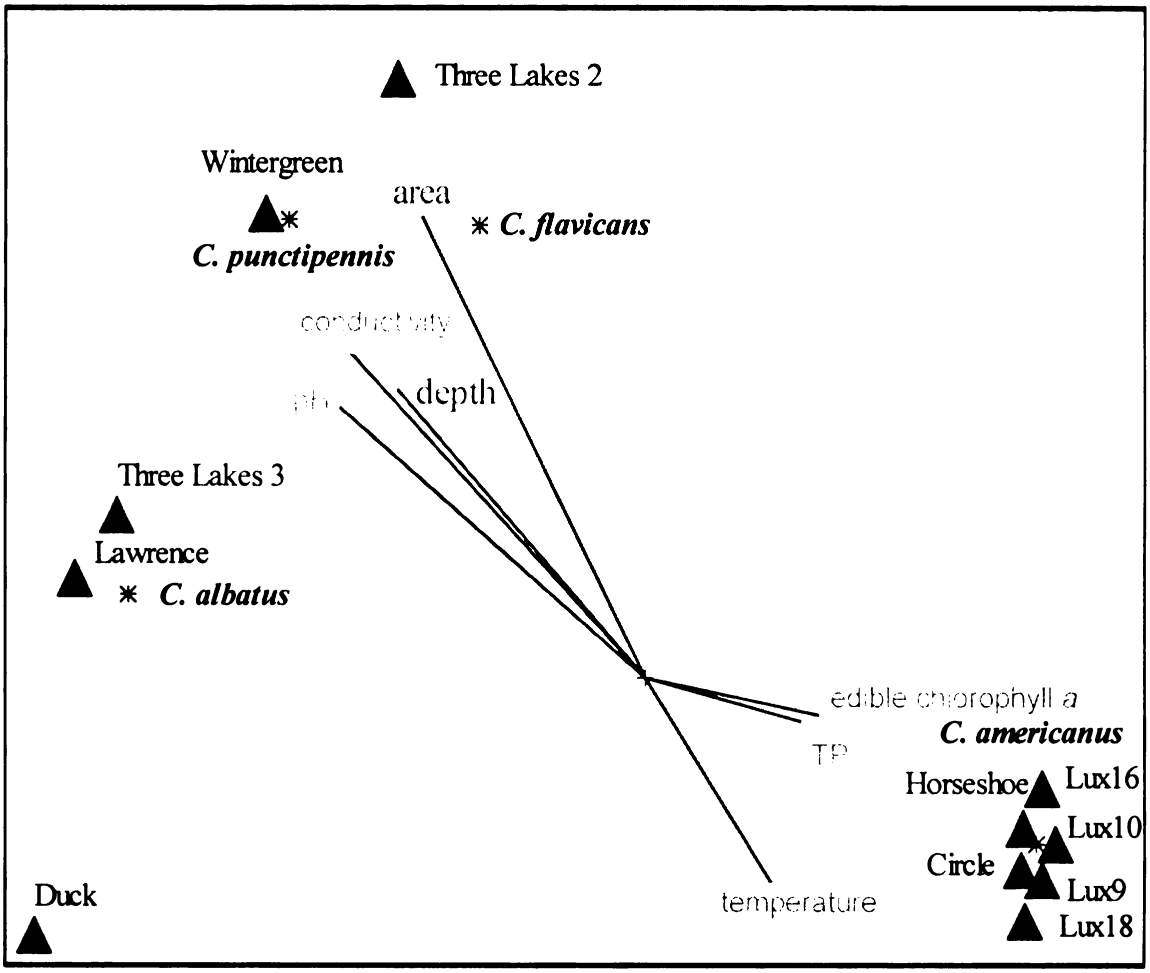

Figure 2.4. Association of 5 lakes and 6 ponds based on NMDS ordination of *Chaoborus* species abundances. The angle and length of the joint plot lines indicate the direction and strength of the relationship of environmental variables with the ordination scores. Asterisks represent the centroids for each *Chaoborus* species.

The field patterns indicate that *C. punctipennis* tends to dominate in all systems with planktivorous fish and that *C. albatus* might benefit under conditions of increased planktivory while *C. flavicans* should be neutral to changes in planktivore density. Looking at a 16-year record from Wintergreen Lake, where planktivore densities have varied across two orders of magnitude due to the elimination and reintroduction of fish species (Mittelbach et al. 2006), shows responses consistent with these expectations. *Chaoborus punctipennis* was consistently the dominant *Chaoborus* species in the lake and planktivore density had no significant effect on its density (Fig. 2.5; $r^2=0.08$, $p=0.36$). *Chaoborus albatus* responded positively to increased planktivore density (Fig. 2.5; $r^2=0.31$, $p=0.046$) and planktivore density had no significant effect on *C. flavicans* density (Fig. 2.5; $r^2=0.08$, $p=0.36$).

Discussion

Fish presence or absence is a major factor determining the distribution of many aquatic taxa in lakes and ponds (Wellborn et al 1996). My survey of chaoborid distributions clearly documents the influence of fish on the distribution and abundance in this assemblage. *Chaoborus americanus* was present only in fishless systems and in these systems it was always the numerical dominant. In fact, it was rare to find any other *Chaoborus* species co-occurring with *C. americanus*. *C. punctipennis*, conversely, occurs only with fish.

Previous observational studies have observed similar patterns in *Chaoborus* distribution where *C. americanus* was found only in fishless water bodies and *C. punctipennis* was positively correlated to the presence and sometimes intensity of fish planktivory (von Ende 1979, Ramcharan et al. 2001, Wissel et al. 2003). Such patterns may be the result of the direct effect of fish predation or could be a consequence of the indirect effects of fish on the shared zooplankton prey. Planktivorous fish feed selectively on the largest prey including *Chaoborus* (Garcia, Chap.4). Thus, the absence of *C. americanus* in fishless systems could be due to size selective predation by fish since

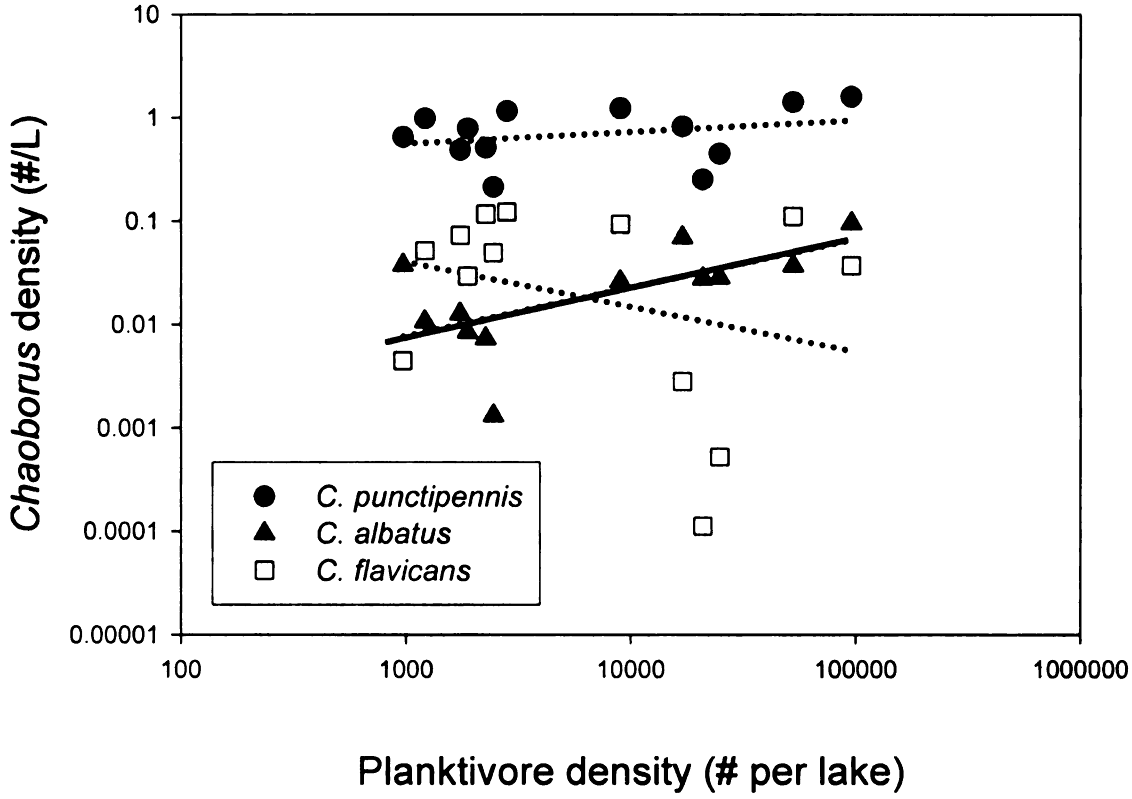

Figure 2.5. *Chaoborus* density in Wintergreen Lake as a function of variation in planktivore density over 16 yrs. Lines are the fitted linear regressions for each species (the solid line is significant, $p<0.05$). Planktivore densities determined by mark/recapture (see Mittelbach et al. 2006 for details).

C. americanus is the largest of the four chaoborid species. However, planktivory may also indirectly affect *Chaoborus* competitive interactions through changes in their zooplankton resources. There is abundant evidence that fish shift the size structure of the zooplankton community towards smaller-bodied prey (Brooks and Dodson 1965, Gliwicz and Pijanowska 1989). Because *Chaoborus* are gape-limited predators, this change in prey size structure may be advantageous for the smaller-bodied *Chaoborus* species (*C. punctipennis* and *C. albatus*) and thereby enhance recruitment of these species. However, there was no change in *C. punctipennis* dominance and abundance in Wintergreen Lake despite >2-fold changes in mean cladoceran body length and species composition related to the gradual decrease and then increase in planktivore density through time (Mittelbach et al. 2006). In addition, the *Chaoborus* species differ in DVM behavior, *C. punctipennis*, *C. flavicans* and *C. albatus* exhibit DVM behavior, whereas *C. americanus* does not (Berendonk et al. 2003). Thus, *C. americanus* is especially vulnerable to planktivorous fish (Garcia, Chap. 4) and a strong case can be made for the importance of the direct effect of fish predation in excluding the *C. americanus* from environments with fish.

Chaoborus flavicans does not seem to discriminate between habitats with and without fish (Fig. 2.1) and this is supported by the literature as well (Pope et al 1973, González and Tessier 1997, Wissel et al. 2003). Instead, Wissel et al. (2003) show that the abundance of *C. flavicans* was higher in lakes that were relatively small and that had elevated levels of dissolved organic carbon (DOC). My NMDS ordination shows a somewhat contradictory pattern, in that *C. flavicans* abundance was positively related to lake area, as well as conductivity and higher pH (Fig. 2.4). Lake area may increase or decrease the intensity of planktivory. Therefore, direct manipulations of planktivore density are needed to sort out their effects on *C. flavicans* abundance.

Chaoborus albatus showed a very clear pattern of only being found in shallow lakes with fish. This result indirectly implies that the distribution of this species may be dependent not just on the presence of fish predators but on the level of fish predation pressure. That is, environmental factors such as area and depth are considered to be related to the intensity of fish planktivory; shallow lakes tend to support higher levels of fish planktivory, due to the lack of a hypolimnentic refuge and because piscivores are oftentimes absent (Tessier and Woodruff 2002). In addition, I observed that within Wintergreen Lake, where lake area, depth and other environmental variables remained relatively consistent but planktivory changed dramatically over a 16-year period, the abundance of *C. albatus* increased significantly with increasing fish planktivory (Fig. 2.5).

From the field pattern and field survey we see that *C. americanus* never co-occurred with *C. punctipennis* or *C. albatus*, but was often found together with *C. flavicans* (Fig. 2.1). This indicates that competition and possibly predation by larger *Chaoborus* species could be another factor contributing to the pattern in the *Chaoborus* species assemblage across the landscape. von Ende (1979) observed that late instars of *C. americanus* will prey on the smaller *C. punctipennis* and proposes this as a possible mechanism excluding *C. punctipennis* from systems where *C. americanus* is present. In addition, the low incidence of coexistence between species of *Chaoborus* found in prior studies has been attributed to competitive exclusion since there is substantial overlap in resource use between species of *Chaoborus.* Thus, when coexistence is observed it is often ascribed to differences in phenology (Roth 1968, Carter and Kwik 1976, von Ende 1982, Sardella and Carter 1983). However, in my field survey, I found little evidence of seasonal partitioning of the environment by the different *Chaoborus* species. Instead, the four species showed similar patterns of abundance across the season, at least in the III and IV instar stages measured in this study. Thus, there was no clear evidence for coexistence via temporal resource partitioning. Other possibilities such as differences in depth distributions, prey selection, etc. remain to be explored.

The results of this observational study provide significant insight into the importance of fish predation in patterning the *Chaoborus* assemblage, although the exact mechanism by which sorting occurs remains unknown. *Chaoborus* species may exhibit oviposition habitat selection and thus select or avoid environments with fish. Alternatively, dispersal and oviposition may be random, and distribution patterns may be generated by differential mortality and species sorting. This can be tested by direct experimental manipulation of fish densities across a landscape of ponds and lakes.

CHAPTER 3

OVIPOSITION HABITAT SELECTION AND LARVAL PERFORMANCE

Abstract

The distribution of organisms across the landscape may be a function of random dispersal followed by non-random, site-specific mortality (species sorting). Alternatively, species may show directed dispersal and habitat selection. In southwestern Michigan, four species of *Chaoborus* (Diptera: Chaoboridae) show pronounced differences in their distribution in relation to variation in fish density. Here, I examine whether *Chaoborus* species that differ in their vulnerabilities to fish predators, discriminate between potential oviposition sites based on the presence of fish and fish cues. As their vulnerabilities would suggest, *C. americanus*, but not *C. punctipennis* or *C. flavicans*, showed evidence of oviposition habitat selection for fish-free sites in a field mesocosm experiment. However, in a second experiment, *C. americanus* did not avoid ovipositing in habitats with fish cues. This conflicting result may be due to possible strong site fidelity in this species. Oviposition habitat selection, together with direct consumption by fish and possibly larger *Chaoborus* species, as well as competitive interactions between species of *Chaoborus* appear to be important mechanisms influencing the distribution of *Chaoborus* species across the landscape.

Introduction

It is widely appreciated that the presence of predators can directly and/or indirectly affect the abundance and distribution of prey species, particularly in aquatic communities (Kerfoot and Sih 1987, Zaret 1980, Wellborn et al. 1996). In response to predators, prey have evolved many adaptations to reduce their mortality risk such as diel vertical migration behavior, morphological changes, and chemical defenses (Kerfoot and Sih 1987). While these adaptations are important factors structuring communities, the question of whether they lead to the distributional patterns of prey is unclear (Binckley and Resetarits 2003).

A growing body of evidence suggests that anurans and some insects can use chemical signals produced by predators to assess the risk of predation in a habitat (Blaustein 1999, Resetarits 2001, Åbjörnsson 2002). Oviposition habitat selection (OHS) in response to fish predators can strongly affect individual fitness and this may be an important mechanism generating the presence/absence patterns of prey abundance (Blaustein 1999, Resetarits 2001). Natural selection should favor females that avoid ovipositing in habitats where risk of predation for their offspring is high (Blaustein et al. 2004). Differences in vulnerability to fish predation, due to prey adaptations or prey size, may select for OHS behavior for some prey species and not for others.

In this study, I examined whether OHS in response to fish predators accounts for the distribution of *Chaoborus* (Diptera: Chaoboridae) species across a landscape of fish and fishless ponds. Most studies examining the processes influencing the distributional pattern of *Chaoborus* species across habitats have focused on the direct effects of fish predators (von Ende 1979, Wissel et al. 2003); empirical tests investigating indirect (non-lethal) effects are rare.

Larvae of the phantom midge *Chaoborus* are common inhabitants of the plankton of North American lakes and small ponds. Of the 12 described *Chaoborus* species in North America (Sæther 1970), four are found in southwestern Michigan: *C. americanus*, *C. punctipennis*, *C. flavicans*, and *C. albatus*. All *Chaoborus* species develop through four aquatic larval instars before becoming pupa, followed by a short lived, non-feeding flying adult stage where females lay one egg raft per lifetime (Moore 1986, Borkent 1979). Egg rafts may contain over a hundred eggs (Sæther 1997). *Chaoborus* larvae are gape-limited, ambush predators of small to medium sized zooplankton (e.g., Moore et al. 1994, Swift and Fedorenko 1975, Pastorok 1981) and are vulnerable to predation by planktivorous fish (Garcia, Chap.4). These four species vary widely in morphology from the relatively large, opaque *C. americanus* (4th instar length 10-13 mm) to the similar-sized, but transparent *C. flavicans* (4th instar length 9-12.65 mm) to the small, transparent

C. punctipennis (4th instar length 7.5-9.5 mm) and *C. albatus* (4th instar length 7-9.4 mm) (Cook 1956, Sæther 1970). *C. punctipennis, C. albatus*, and *C. flavicans* are most often found in lakes with fish and exhibit diel vertical migration (DVM) as an adaptive response to the presence of fish in these habitats (Dawidowicz et al 1990, Tjossem 1990). *Chaoborus americanus* is generally observed in fishless ponds and does not exhibit DVM behavior (Berendonk et al. 2003). Also, *C. americanus* rarely coexists with other species of *Chaoborus* (Pope et al. 1973, von Ende 1979, Wissel et al. 2003). The lack of co-occurrence of *C. americanus* and other species of *Chaoborus* may be the result of interspecific competition or intraguild predation, or may reflect differences in oviposition habitat selection. *Chaoborus americanus* is the most vulnerable of the four species to fish predation (Garcia, Chap. 4). Therefore I hypothesized that: (1a) *C. americanus* will exhibit oviposition habitat selection because risk of predation is high for their progeny, but (1b) *C. flavicans, C. punctipennis*, and *C. albatus* will not exhibit OHS because they have evolved a behavioral response to the presence of fish; and (2) the presence of *C. americanus* in a habitat will exclude the other three species.

I tested these hypotheses in two similar experiments. The first was an outdoor mesocosm experiment allowing ovipositing *Chaoborus* to select between large tanks with free-swimming fish, caged fish (fish cue) and controls without fish. The second was an enclosure (bag) experiment conducted in a fishless reservoir, where *Chaoborus* could choose between bags with a single caged fish (fish cue), two caged fish (double fish cue) and controls without fish.

Methods

The two experiments were conducted at the W. K. Kellogg Biological Station (KBS), Experimental Pond Facility (Hickory Corners, MI). Experiment 1 (mesocosm experiment) was performed from June-September 2004 in 24, aquatic mesocosms (300 L, 1 m diameter, cattle tanks). On 28 May 2004, the mesocosms were acid-washed, filled with low nutrient well water, and equipped with a free floating fish cage (2 mm mesh, 25 cm diameter). The experimental design consisted of four treatments: 1) control (without fish), 2) high productivity with no fish, 3) fish cue (one caged fish), and 4) fish (one free-swimming fish), each replicated six times. Phosphorus (as potassium phosphate) and nitrogen (as ammonium nitrate) were added to the water column of all tanks to bring nutrient concentrations to levels commonly observed in local lakes and ponds (Darcy-Hall 2006). Target nutrient supply concentrations were 25 (for control, fish and fish cue tanks) and 100 (for high productivity tanks) µg/L total phosphorus (TP), with nitrogen added in a 50:1 molar ratio. Nutrients were added the day the tanks were filled and every 10 days after that throughout the experiment to maintain approximate target nutrient concentrations.

A diverse algal inoculum collected from eight local ponds was introduced into each tank two days after they were filled. Ten snails (*Physa* sp.) were also added to each tank to regulate periphyton growth on the bottom and sides of the tank. A week later, a diverse zooplankton inoculum collected from the same eight local ponds was introduced into each tank after samples were filtered to remove all potential predators of *Chaoborus*. Zooplankton collected from these same ponds were added every ten days throughout the experiment to maintain prey populations. The tanks were placed in 2 rows separated by 1.5 m, with treatments and replicates randomly assigned. Bluegill sunfish (*Lepomis macrochirus)* (32-35 mm SL) collected from one of the experimental ponds were added to the appropriate treatments on 22 June 2004. This bluegill stocking density (~1.3 bluegills/m^2) is within the natural range of bluegill densities found in this region (Mittelbach 1988). Tanks were covered with fiberglass screen lids until fish were added and thereafter were left uncovered, open to colonization. The presence and number of *Chaoborus* egg rafts were checked daily throughout the experiment, although egg rafts could not be identified to species. *Chaoborus* larvae were sampled weekly with a dip net (0.25 m diameter, 250-micron mesh), by two sweeps around the circumference of the tank, just under the surface and again at the bottom. A water column sample was also taken using a round sieve (50 cm diameter, 500-micron). *Chaoborus* larvae were counted, identified to species and promptly returned to their original tank. All sampling equipment was washed with well water and a power nozzle between tanks to reduce risk of contamination. On 15 September 2004, fish were removed and all tanks were drained completely and filtered through a sieve (500-micron). *Chaoborus* larvae were preserved in 75% ethanol, later enumerated under 40X magnification, and identified to species. Water temperature, dissolved oxygen, pH, and conductivity were measured in each tank every time that *Chaoborus* were sampled, using a Hydrolab® multi-probe (MiniSonde 4a).

Experiment 2 (bag experiment) was performed from July-September 2005, in a fishless reservoir using 1200-liter polyethylene "bag" enclosures (1m diameter, 1.5m deep), sealed at their bottoms and suspended in the water column from floating frames. Bags were equipped with the free floating fish cages used in the mesocosm experiment, and covered with fiberglass screens until fish introduction. To explore effects of fish cue, three treatments were employed: 1) control (without fish), 2) fish cue (one caged fish) and 3) double fish cue (two caged fish). Each treatment was replicated six times and randomly assigned for a total of 18 enclosures. Bags were filled by pumping water from the reservoir through a 153-micron zooplankton net to remove large zooplankton and invertebrate predators. A diverse zooplankton inoculum collected from six local ponds was introduced into each bag after samples were filtered to remove all potential predators of *Chaoborus*. A month later, 1 August 2005, bluegill sunfish (54-70 mm SL) from one of the experimental ponds were added to the appropriate treatments. The bluegill stocking densities (1 and 2 bluegills/m^2) are within the natural range of bluegill densities found in this region (Mittelbach 1988). Bags were then left uncovered, open to colonization by ovipositing *Chaoborus*. On 26 September 2005, the bags were sampled by taking five vertical tows through the entire water column of each bag with a 30 cm diameter, 500-micron plankton net. *Chaoborus* were preserved in 75% ethanol, measured and identified to species (Cook 1956, Sæther 1970) under 40X magnification using a digitizer tablet and software (SigmaScan Pro Version 4.01; SPSS Inc., 1987). Additionally, water temperature, dissolved oxygen, pH, and conductivity were measured using a Hydrolab® multi-probe (MiniSonde 4a).

Statistical Analyses

Examination of treatment effects for the mesocosm experiment and the bag experiment was performed using repeated measures ANOVA (rmANOVA) and one-way ANOVA, respectively. For the mesocosm experiment, *Chaoborus* species abundances per replicate were averaged across two consecutive sampling dates. In addition, values for the experiments were $\log_{10}$ (x+(one half the lowest observed density); mesocosm = .0017 and bag = 0.0016)) transformed to meet assumptions of the analyses. Statistics were performed using Systat 11.0 (SPSS Inc., 2004).

Results

Mesocosm experiment

The first *Chaoborus* egg raft appeared (in a fish cue tank) one week after the start of the experiment and egg rafts were found at least once in all treatments throughout the experiment. *Chaoborus americanus*, *C. punctipennis* and *C. albatus* larvae first appeared in the control and high productivity treatments two weeks into the experiment. The rmANOVA revealed a significant treatment effect only for *C. americanus* ($p=0.007$, $F_{3,20}=5.32$, between subjects effect), where *C. americanus* abundance was higher in the fishless treatments (Fig. 3.1, control and high productivity). Because there were no significant effects of time or time by treatment interactions, the experimental data are shown as *Chaoborus* density averaged across all sampling dates within each of the four treatments (Fig. 3.1). Closely reexamining the fishless treatments showed that if *C. americanus* was present in the tank then the other two *Chaoborus* species were not (Fig. 3.2a) and if *C. flavicans* was present in a tank then *C. punctipennis* was not (Fig. 3.2b).

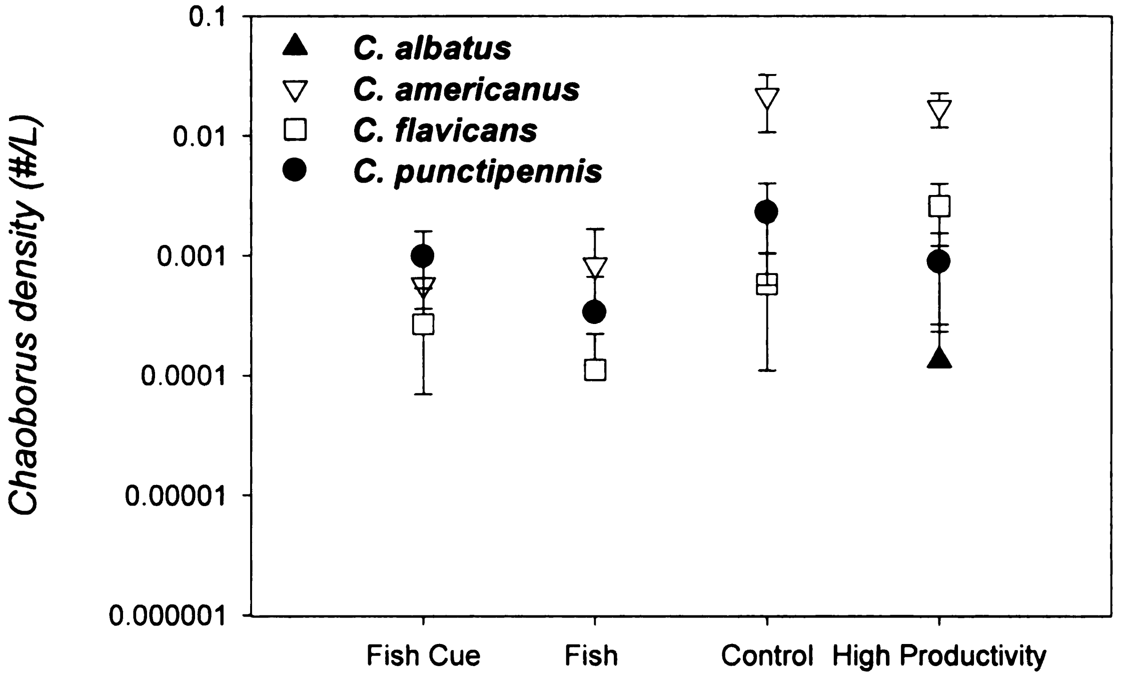

Figure 3.1. Effects of fish cue, fish, and high productivity on mean *Chaoborus* density (number per liter ± 1 SE) averaged across all sampling dates.

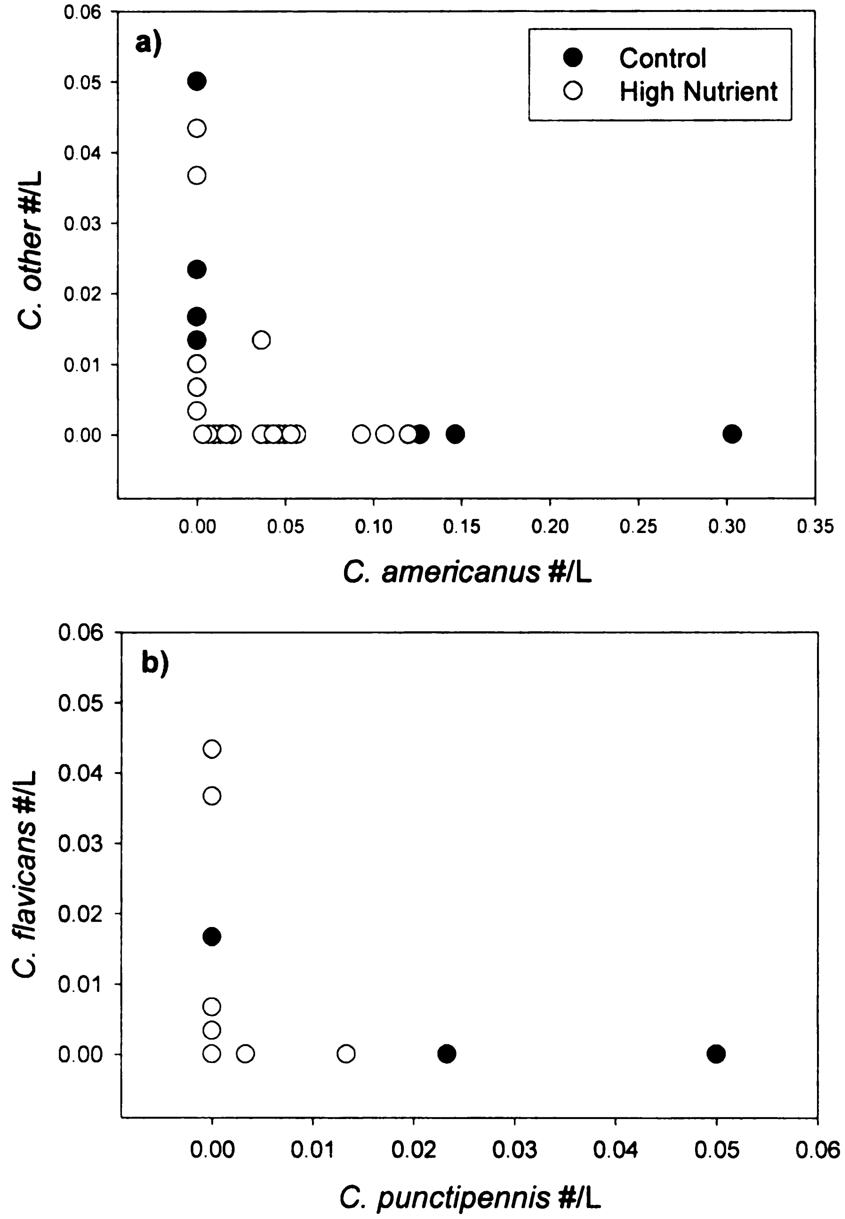

Figure 3.2. *Chaoborus* density (number per liter) in the fishless treatments of the mesocosm experiment. Each point is a sampling date for an individual tank. (*C. other* includes *C. punctipennis* and *C. flavicans*).

Lastly, *Chaoborus* failed to pupate in the treatments with fish, but pupa of all three *Chaoborus* species were found in the fishless treatments.

Bag experiment

This experiment differed from the mesocosm experiment in three main ways: 1) the environmental matrix surrounding the treatments, 2) scale (bags were deeper and wider than the tanks), and 3) the focus on the effect of the intensity of fish cue on *Chaoborus* oviposition decisions. The bags were placed in a reservoir where *C. americanus* was present, whereas in the mesocosm experiment the tanks were in a mowed field at the experimental pond facility. The first egg rafts appeared two days after the start of the experiment in at least one bag of each treatment. *Chaoborus americanus* was observed in all treatments, *C. flavicans* was found in the double fish cue and control bags and *C. punctipennis* was observed in one of the double fish cue bags (Fig. 3.3). There was only a significant effect of the intensity of fish cue on *C. americanus,* where *C. americanus* abundance was highest in the double fish cue and control treatments (Fig. 3.3; p=0.04, $F_{2,15}$= 4.23, one-way ANOVA).

Discussion

Several recent studies have observed oviposition habitat selection (OHS) in response to risk of predation (Blaustein 1999, Blaustein et al. 2004, Rieger et al 2004). Blaustein (1999) suggested that the evolution of this behavior is most likely under the following conditions: 1) immature stages are highly vulnerable to the predator; 2) predator density is highly variable among patches; 3) prey have few lifetime reproductive events; and 4) predator distribution among patches is largely fixed from the time the female oviposits until offspring leave the patch. *Chaoborus americanus* meets all of the conditions that should favor OHS and in the mesocosm study I found evidence that *C. americanus* exhibited OHS. However, when the fish-free and fish cue treatments were

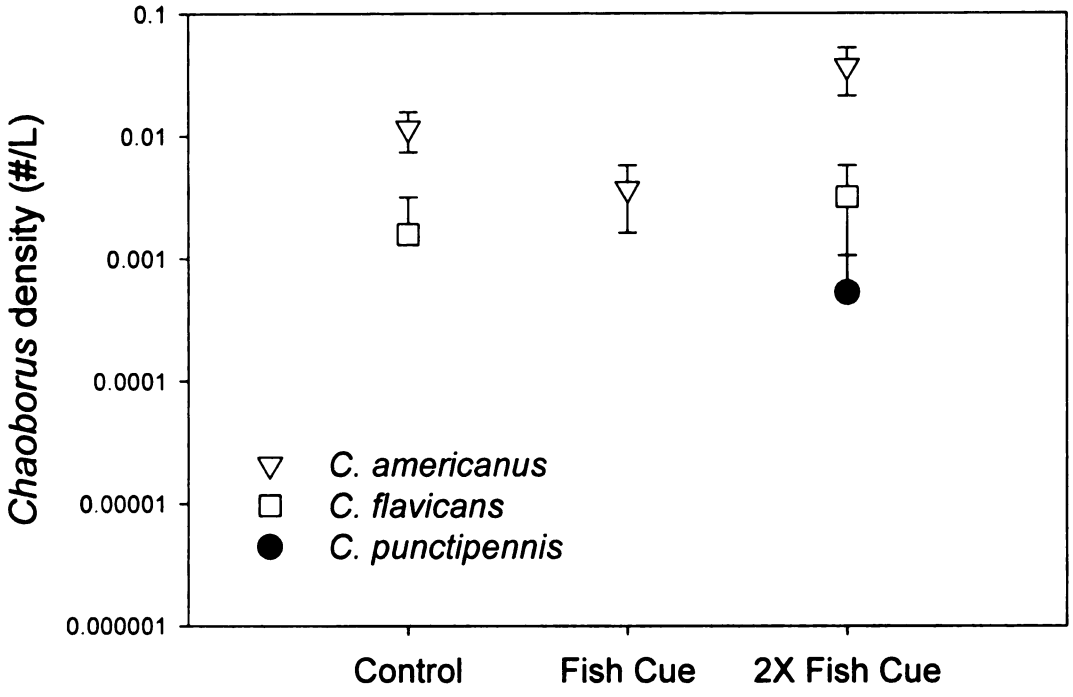

Figure 3.3. Effects of fish cue on mean density (number per liter ± 1 SE) of three *Chaoborus* species on the final day of the bag experiment.

located in bags suspended in a fish-free reservoir that supported a population of *C. americanus*, *C. americanus* did not discriminate between fish cue and fishless treatments. *Chaoborus punctipennis* and *C. flavicans* do not meet the condition of high larval vulnerability to the predator (Dawidowicz et al 1990, Tjossem 1990) and did not avoid fish or fish cue treatments in either experiment.

Berendonk (1999) found a similar pattern where the *Chaoborus* species most vulnerable to fish predation avoided ovipositing in containers with fish kairomones (*C. crystallinus* and *C. obscuripes,* both Old World pond-dwelling species) and the less vulnerable *C. flavicans* was not observed to discriminate between fish kairomone and control containers. These results support the suggestion that the avoidance of ovipositing in habitats with fish by certain *Chaoborus* species is based on chemical and not visual cues. Berendonk and Bonsall (2002) also found that *C. crystallinus* avoided ovipositing in barrels with caged fish, but observed that this oviposition preference decreased with distance from the source population. McPeek (1989) reported that *Enallagma* damselflies from fishless lakes could not distinguish between experimentally manipulated fish and fishless ponds and he attributed this lack of a response to the damselflies strong philopatry. These studies suggest several possible explanations for the contrasting results of the bag experiment. The first possibility assumes that female *C. americanus* may have been dispersing from distant source populations and thus may have reached a critical point and had to oviposit regardless of the suitability of the habitat. However, the bag experiment was conducted in a habitat with a population of *C. americanus* and hence dispersal distance would have been minimal. The second and third, more likely explanations may be that *C. americanus* exhibits strong site fidelity, and/or that *C. americanus* could not detect (or misinterpreted) the fish cue in the bags because the matrix was "fish free".

In the mesocosm experiment, I observed no coexistence between the *Chaoborus* species. In particular, when *C. americanus* was present, all other species were absent.

This pattern is consistent with patterns observed in nature, where *C. americanus* is often the only *Chaoborus* species in fishless ponds (Pope et al. 1973, Garcia Chap.2). von Ende (1979) hypothesized that predation by *C. americanus* on smaller species of *Chaoborus* may be one of the mechanisms preventing coexistence among these species. If this is the case, *C. punctipennis* and *C. flavicans* may not select oviposition sites based on fish cues, but might instead use habitat area, lake depth, or some other factor that was not considered in this present study.

In this study, I show that oviposition habitat selection may contribute to the distributional pattern of *C. americanus* occurrence across the landscape. However, from these experiments OHS does not contribute to the distributional patterns of *C. punctipennis* and *C. flavicans*. Instead, direct consumption by fish and possibly larger *Chaoborus* species as well as competitive interactions between species of *Chaoborus* may be the dominant factors influencing *C. punctipennis* and *C. flavicans* distribution; this remains to be investigated both empirically and theoretically. Distribution and abundance patterns at the community scale can result from two distinct mechanisms; random dispersal followed by non-random, site-specific mortality (species sorting) and oviposition habitat selection (Binckley and Resetarits 2005). For active dispersing organisms such as *Chaoborus* examining potential filters at the colonization stage is an important first step in understanding how patterns in species abundance are created.

CHAPTER 4

CHAOBORUS SPECIES SORTING ALONG A PREDATION GRADIENT

Abstract

Variation in the intensity of predation pressure across the well-known environmental gradient of lentic freshwater habitats from small, ephemeral ponds to large, permanent lakes is a key ecological interaction important in the development and maintenance of aquatic community structure. Here, I examined *Chaoborus* (Diptera: Chaoboridae) species sorting along an experimental gradient in bluegill sunfish (*Lepomis macrochirus*) density. In addition, I tested bluegill prey choice for the four species of *Chaoborus* found in Southwestern, MI. I found that a gradient in fish density can lead to clear species sorting of four *Chaoborus* species, and that this response is consistent with distributional patterns observed in nature. *Chaoborus americanus* was most abundant in the fishless ponds, *C. flavicans* was neutral in response to fish and *C. punctipennis* was most abundant in the high fish biomass ponds. Furthermore, fish size selectivity and differences in *Chaoborus* species traits (i.e. pigmentation, DVM behavior and size) were all observed to contribute to the pattern of *Chaoborus* abundance and distribution.

Introduction

The pattern of species turnover along ecological gradients can reveal factors potentially important in determining their abundance and distribution. Ecologists have long recognized the environmental gradient of lentic freshwater habitats from small, ephemeral ponds to large, permanent lakes as a critical axis along which aquatic communities are organized (Wellborn et al. 1996, Stoks and McPeek 2003). Across this gradient, species from many freshwater taxa sort out according to physical factors (e.g. pond drying) and biotic interactions (e.g. predation; Wellborn et al. 19996, Werner and McPeek 1994, Stoks and McPeek 2003). For example, the intensity of fish planktivory may vary across gradients in lake size, from high levels in shallow lakes without piscivores, to medium levels in shallow lakes with piscivores, to low levels in deep lakes with piscivores and a refuge. Many species of plankton separate out across this gradient in lake type (Tessier and Woodruff 2002). However, while the correspondence between species distributions and environmental gradients may suggest causal relationships, the true test of these relationships depends on experimentally manipulating the hypothesized causal factors.

In north temperate lakes, species of the phantom midge *Chaoborus* (Diptera: Chaoboridae) reveal consistent distribution patterns across the landscape (Garcia, Chap. 2), that strongly suggest the importance of fish predation and possibly other factors known to vary across the landscape such as, interspecific interactions, water transparency, temperature, and nutrient levels (Pope et al. 1973, von Ende 1979, Lamontagne et al. 1994, Wissel et al. 2003). Most of the above observational studies of *Chaoborus* distributions have focused on a single species or a single habitat type and few have directly measured the abundance of the predators presumed to drive the pattern of species turnover. A powerful way to examine the role of fish predation in determining species distributions in this and other systems is to establish an experimental gradient in fish density and use regression analysis examine the response of the *Chaoborus* species assemblage to this gradient (Wellborn et al. 1996). This experimental design provides invaluable information, vital to the development of simulation models, and can be used to make predictions for new systems (e.g., Cottingham et al 2005 and references therein).

Here I examine *Chaoborus* species sorting across an experimental gradient of bluegill sunfish *(Lepomis macrochirus)* density. I asked two questions: 1) How does predator (fish) density affect *Chaoborus* species composition and relative abundance? and 2) Is the pattern of prey choice by bluegill consistent with the observed pattern of *Chaoborus* species sorting across the predation gradient? I tested the first question in two field experiments in ponds in southwest Michigan. I tested the second question in a prey preference experiment conducted in a large, outdoor mesocosm.

Methods

Study Organisms

The larvae of the phantom midge *Chaoborus* are distributed worldwide and are common inhabitants of North American lakes and small ponds. All species develop through four instars, pupate and become non-feeding flying adults that lay one egg raft per lifetime (Moore 1986, Borkent 1979). They are planktonic, gape-limited, ambush predators of small to medium sized zooplankton (e.g., Moore et al. 1994, Swift and Fedorenko 1975, Pastorok 1981). Of the 12 described *Chaoborus* species in North America (Sæther 1970), four are found in southwestern Michigan: *C. americanus*, *C. punctipennis*, *C. flavicans*, and *C. albatus*. These four species vary widely in morphology from the relatively large, opaque *C. americanus* (4th instar length 10-13 mm) to the similar-sized but transparent *C. flavicans* (4th instar length 9-12.65 mm) to the small, transparent *C. punctipennis* (4th instar length 7.5-9.5 mm) and *C. albatus* (4th instar length 7-9.4 mm) (Cook 1956, Sæther 1970). *C. americanus* is found in environments without fish and does not exhibit diel vertical migration (DVM) behavior. The other three species occur commonly with fish and vertically migrate; they are found in the upper waters of lakes at night and then migrate down into the sediments during the day (e.g., von Ende 1979, Tjossem 1990)

The bluegill sunfish is an important planktivore in small lakes throughout the eastern United States (e.g., Mittelbach and Osenberg 1993, Mittelbach et al. 2006, Werner et al. 1977, 1978). They are diurnal, size selective, and prey preferentially on large zooplankton such as *Chaoborus* and *Daphnia* (Mittelbach 1981, Turner and Mittelbach 1990).

Fish Gradient Experiments

The experiments were performed in a series of ponds (each 30m diameter and 1.6m max. depth) located at the W. K. Kellogg Biological Station (KBS) Experimental Pond Facility in southwestern MI. These experimental ponds support an invertebrate fauna characteristic of small, mesotrophic ponds in southwestern Michigan (Garcia, Chap. 2) and all four *Chaoborus* species native to the region occur at the Experimental Pond site. In the first experiment, conducted May-Sept. 2003, I looked at the effects of planktivore density on the potential colonization and population growth of the four *Chaoborus* species. For the first experiment, ten ponds were drained in September 2002, all fish were removed, and the ponds remained dry through the winter. In late April/early May (28th-2nd) I filled the ponds with water from the same source (a fishless reservoir on site which contained some larvae of *C. americanus*) and then established a gradient in fish density by stocking adult bluegill from nearby Warner Lake on 16 May 2005 (Table 4.1A).

In a second experiment, conducted in summer 2005, I used a larger number of ponds (15) and allowed *Chaoborus* and other invertebrate populations to establish in the ponds for a year before adding fish. The 15 experimental ponds were drained in September 2003 and remained dry through the winter. In May 2004, the ponds were filled with water from the same fishless reservoir used in Experimental 1 and were left undisturbed (and fishless) for one year. I established a gradient in fish density by stocking adult bluegill from nearby Wintergreen Lake on 24 May 2005 (Table 4.1B). For both experiments, ponds were assigned haphazardly to the gradient in fish biomass. The standard length (SL) of each fish added was measured (Experiment 1: range 30-75mm SL; Experimental 2: range 100-145mm SL) and total fish biomass per pond was calculated with a length-weight regression using a subset of the fish collected that were not used in the experiments. Stocked bluegill biomasses (Experiment 1: ~0.07-8 g/m^2; Experiment 2: ~0.2-4 g/m^2) were within the range found in nearby lakes (Mittelbach and Osenberg 1993).

Bluegill grew and reproduced in the ponds in both years. To determine final fish biomass in each pond at the end of the experiments, I first captured adult and young of the year (YOY) bluegill with a beach seine (23 x 1.8m, 3.2-mm mesh; two seines per pond), then drained the pond and removed the remaining fish. All adult bluegill were collected and measured. A random sample of 40 adults were weighed and measured to generate length-weight regressions to calculate biomass. In addition, a majority of YOY bluegill were collected and weighed. Final fish biomass per pond (adults plus YOY) was used in the analyses. Similar results were found using initial bluegill biomass or the mean of initial and final biomass.

In Experiment 1, *Chaoborus* and other zooplankton were sampled once before fish addition and then every 10-14 d thereafter for 17 weeks. *Chaoborus* and other zooplankton were collected one hour after sunset by taking three Schindler trap samples (18.5 L, 60-micron net) at the deepest point in each pond. In Experiment 2, *Chaoborus* were sampled once prior to the start of the experiment and then weekly for four weeks, and then every three weeks thereafter as changes in *Chaoborus* species composition slowed. I used vertical tows with a large-mesh plankton net to collect *Chaoborus* in Experiment 2 as opposed to the Schindler trap used in Experiment 1, as I wanted to collect a greater number of *Chaoborus* per sampling date than I was able to collect in Experiment 1. *Chaoborus* were collected one hour after sunset by taking three vertical tows through the entire water column with a 30 cm diameter, 500-micron plankton net, at the deepest point in each pond. Zooplankton were collected once midway through Experiment 2 (day 50) one hour after sunset by taking three vertical tows through the entire water column with a 30 cm diameter, 153-micron plankton net, at the deepest point in each pond. Samples were preserved in 95% ethanol and later enumerated and measured under 40X magnification using a digitizer tablet and software (SigmaScan Pro Version 4.01; SPSS Inc., 1987). *Chaoborus* and cladocerans were identified to species, copepods to suborder (i.e., calanoids and cyclopoids) and rotifers were also counted. Water temperature, dissolved oxygen, pH, and conductivity were measured in each pond every time that *Chaoborus* were sampled, using a Hydrolab® multi-probe (MiniSonde 4a).

Statistical Analyses

For each fish gradient experiment, I examined the effect of fish biomass on *Chaoborus* species abundance for the period where *Chaoborus* densities had stabilized (Experiment 1: days 56-123; Experiment 2: days 50-112), using repeated measures ANOVA (rmANOVA), grouping fish biomass into three categories; zero fish biomass, medium biomass (Experiment 1:2300-3200 g; Experiment 2:500-1250 g) and high biomass (Experiment 1:5700-12,050 g; Experiment 2:3250-5850 g). For each pond, *Chaoborus* species abundances were averaged across three Schindler trap samples per date (Experiment 1) or across three vertical tows per date (Experiment 2) and then $\log_{10}$ (x+(one half the lowest observed density); Experiment 1 = 0.00393 and Experiment 2 = 0.00442)) transformed to meet assumptions of the analyses. Linear regression was used to examine: 1) the relationship between *Chaoborus* species density (Experiment 1: mean of sampling days 56-123; Experiment 2: mean of sampling days 50-112) and final fish biomass, and for Experiment 2 only, 2) the relationship between final fish biomass and number of days post fish addition that *C. americanus* was present in the water column of each pond.

Prey Preference Trials: Non-refuge and refuge

Eight feeding selectivity experiments using 1000-L cattle tanks were performed in 2005 to determine bluegill preference for three of the four *Chaoborus* species common to southwestern Michigan and occurring in the fish gradient experiments (*C. albatus* was too rare to use in the feeding trials). Cattle tanks were acid-washed and filled with well water prior to the initiation of the experiments. *C. punctipennis* and *C. flavicans* were collected from two nearby lakes where they are common (Little Mill and Bristol) and *C. americanus* was collected from two nearby ponds (Lux 16 and Pond A). Field collections of *Chaoborus* and other zooplankton were left overnight in 16 liter buckets with the lids on to eliminate most of the non-*Chaoborus* zooplankton and then filtered through a 1 mm sieve the next day. All three species of *Chaoborus* were stocked into a 1000-L cattle tank that was covered with 1-mm mesh fiberglass window screening between trials to prevent colonization by other organisms. To maintain sufficiently large populations of the three *Chaoborus* species, the cattle tank was restocked 24 hours prior to the start of each trial. *Chaoborus* species abundance prior to the start of each feeding trial was determined by sampling the entire water column of the cattle tank with 2 vertical tows using a 30 cm diameter, 500-micron mesh net. For each trial, five randomly chosen bluegill from a pool of 50 bluegill, ranging in size from 59-89 mm SL, were starved for 24 h and then placed in the *Chaoborus* stocked tank. Bluegill were allowed to feed for 10 min to minimize the effect of prey depletion. At the end of each trial, bluegill were removed, measured, and stomach flushed using deionized water from a 20 cc syringe. *Chaoborus* sampled pre-trial and bluegill stomach contents were preserved in 95% ethanol, identified to species, counted, and measured under 40X magnification using a digitizer tablet and software (SigmaScan Pro Version 4.01; SPSS Inc., 1987). Bluegill preference for prey type i was calculated using the Manly-Chesson index:

$$\alpha_i = (d_i / E_i) \,/ \sum_{j=1}^{k} (d_j / E_j)$$

where i = 1, 2, ..., k and where k is the number of prey types, d_i is the number of prey type i in the diet summed across all five bluegill, and E_i is the density of prey type i in the cattle tank prior to fish addition (Chesson 1978, 1983, Manly 1974). Prey types that are consumed in proportion to their abundance in the environment (i.e. no preference) have $\alpha_i = 1/k$, for this experiment k = 3. Prey types that are selected for have $\alpha_i > 1/k$ and prey types selected against have $\alpha_i < 1/k$.

Bluegill selectivity for the three species of *Chaoborus* was analyzed using the mean α of eight trials by species versus $1/k = 0.33$ in a one-sample t-test. The difference in *Chaoborus* size distributions in the environment versus bluegill diet was analyzed using a two-sample Kolmogorov-Smirnov test.

To examine whether migratory behavior and the presence of a refuge from fish predation would affect selectivity of the three *Chaoborus* species, a 5 mm mesh plastic screen was placed at half the depth of the cattle tank one hour before sunset on the day before each trial for a total of four trials. *Chaoborus* species abundance in the cattle tank was measured prior to the addition of the refuge with 2 vertical tows. The abundance of *Chaoborus* above the refuge (non-migratory) was measured the morning after refuge addition with 2 vertical tows that sampled the water column above the refuge. For each trial five bluegill were added to the cattle tank using the same methods as in the non-refuge feeding trials but were allowed to feed for only 8 minutes. At the end of each trial bluegill were removed, measured, and stomach flushed using deionized water from a 20 cc syringe. *Chaoborus* sampled pre-trial and bluegill stomach contents were preserved in 95% ethanol, identified to species, counted, and measured under 40X magnification using a digitizer tablet and software (SigmaScan Pro Version 4.01; SPSS Inc., 1987). Bluegill preference for each prey type was calculated using the Manly-Chesson index.

Bluegill selectivity for the three species of *Chaoborus* was analyzed using the mean α of four trials by species versus $1/k = 0.33$ in a one sample t-test. The difference in *Chaoborus* size distributions above the refuge versus bluegill diet and above the refuge versus the entire cattle tank was analyzed using a two-sample Kolmogorov-Smirnov test. The effect of the refuge was analyzed using the mean proportion of *Chaoborus* by species found above the refuge versus a random distribution of *Chaoborus* species in a one-sample t-test. All analyses were performed using Systat 11.0 (SPSS Inc., 2004).

Results

Fish Gradient Experiments: Species sorting and colonization

The results of Experiment 2 are presented first, as the larger numbers of *Chaoborus* collected in this experiment provide the clearest picture of *Chaoborus* species sorting along the bluegill density gradient. The results of Experiment 1 support the patterns observed in Experiment 2 and are presented second.

In Experiment 2, the four *Chaoborus* species showed dramatically different responses to fish predation. At the start of the experiment (prior to fish introduction) *C. americanus* was present at a high density in all the ponds and the other three *Chaoborus* species were absent (Fig. 4.1). This pattern is consistent with the observed species distributions in nature (Garcia, Chap. 2). *C. americanus* remained abundant in ponds without fish throughout the experiment, declined slightly in the medium fish biomass ponds, and declined dramatically in the high fish biomass ponds (Fig. 4.1a). The differential response between the fish biomass categories but overall general decline through time was supported by the rmANOVA, which showed a significant treatment and time effect ($p=0.04$, $F_{2,12}=4.20$, between subjects; $p=.002$, $F_{2,24}=7.94$, within subjects time effect, rmANOVA). The other three species of *Chaoborus* first appeared in the ponds on sampling day 14. *C. punctipennis* density increased through time (leveling off by day 84) and this increase was most noticeable in the high fish biomass ponds (Fig. 4.1b; $p=0.003$, $F_{2,12}=9.82$, between subjects, and $p=0.003$, $F_{2,24}=7.41$, within subjects time effect, rmANOVA). *C. flavicans* density also increased through time in all of the ponds but there were no significant differences between fish biomass categories (Fig. 4.1c; $p>0.05$, for all within and between subjects fish biomass effects and time by biomass interactions, rmANOVA). *C. albatus* density increased through time in the medium and high fish biomass ponds but differences between fish biomass categories were not significant (Fig. 4.1d; $p>0.2$, for all within and between subjects fish biomass effects and time by biomass interactions, rmANOVA).

Looking at *Chaoborus* densities averaged over the last three sampling dates showed that *C. americanus* density significantly declined with final fish biomass across all ponds (Fig. 4.2a; $r^2=0.45$, $p=0.006$). *C. punctipennis* and *C. albatus* showed the opposite pattern, as both showed a strong, positive response to final fish biomass (*C. punctipennis,* Fig. 4.2b; $r^2=0.71$, $p<0.001$; *C. albatus,* Fig. 4.2d; $r^2=0.39$, $p=0.013$). Final fish biomass had no significant effect on *C. flavicans* density although there was a slight

Table 4.1. Fish gradient experimental design in A) Experiment 1 and B) Experiment 2, with initial and final fish biomass.

A)

POND	2	15	17	16	6	8	3	4	11	18
# of bluegill added	0	0	0	10	40	100	500	500	500	1200
Initial fish biomass (g)	0	0	0	47	189	418	2358	3363	2861	5658
Final fish biomass (g)	0	0	0	2346	3170	5720	6334	8528	9156	12037

B)

POND	8	11	14	17	9	3	10	12	5	15	6	1	16	13	18
# of bluegill added	0	0	0	0	2	4	4	6	6	12	12	20	20	40	40
Initial fish biomass (g)	0	0	0	0	153	264	293	398	360	767	713	1017	1299	2351	2678
Final fish biomass(g)	0	0	0	0	557	712	802	1236	5485	3434	7603	3288	5832	5264	5426

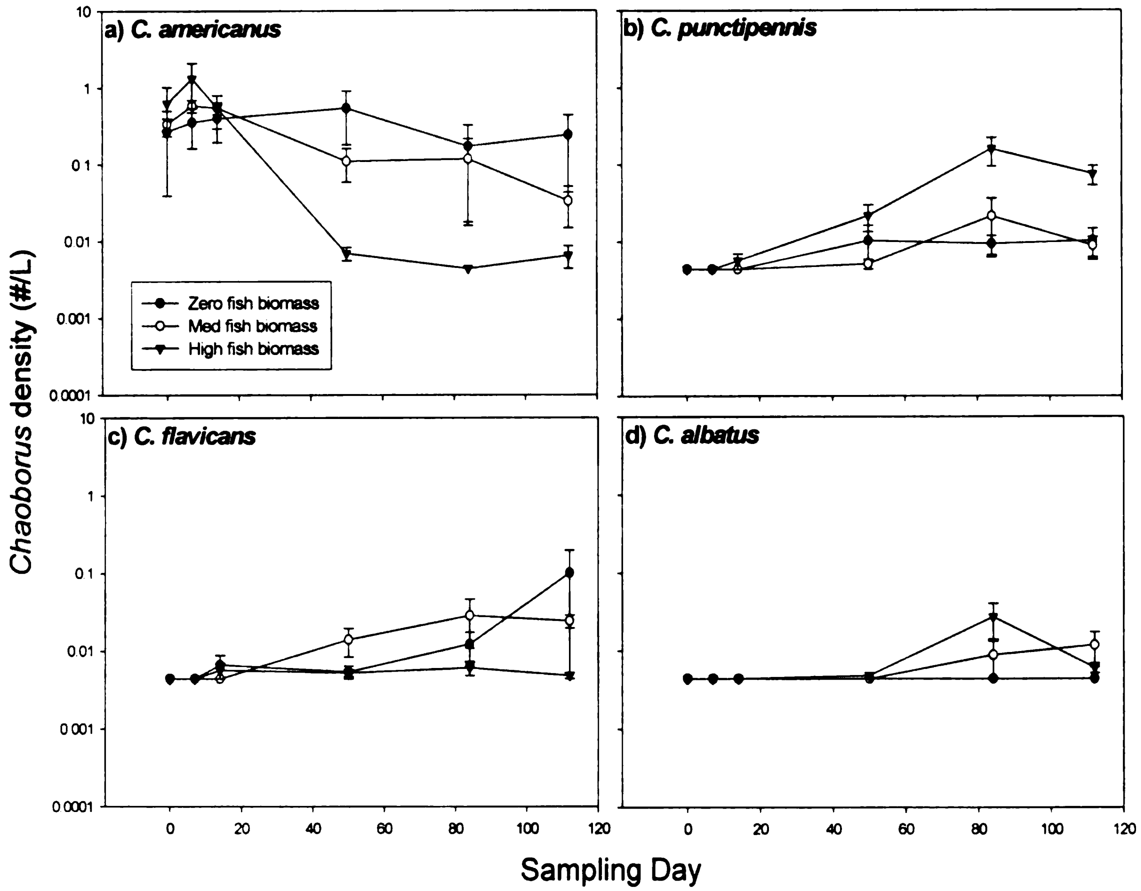

Figure 4.1. Change in *Chaoborus* density (number per liter) through time in Experiment 2 grouped into three fish density categories. Time is the sampling day of the experiment (0-112, May-September 2005). The filled circles are the means (± 1 s.e., n=4) for the zero fish biomass ponds, the open circles are the means (± 1 s.e., n=4) for the medium fish biomass (500-1250 g) ponds, and the filled triangles are the means (± 1 s.e., n=7) for the high fish biomass (3250-5850 g) ponds.

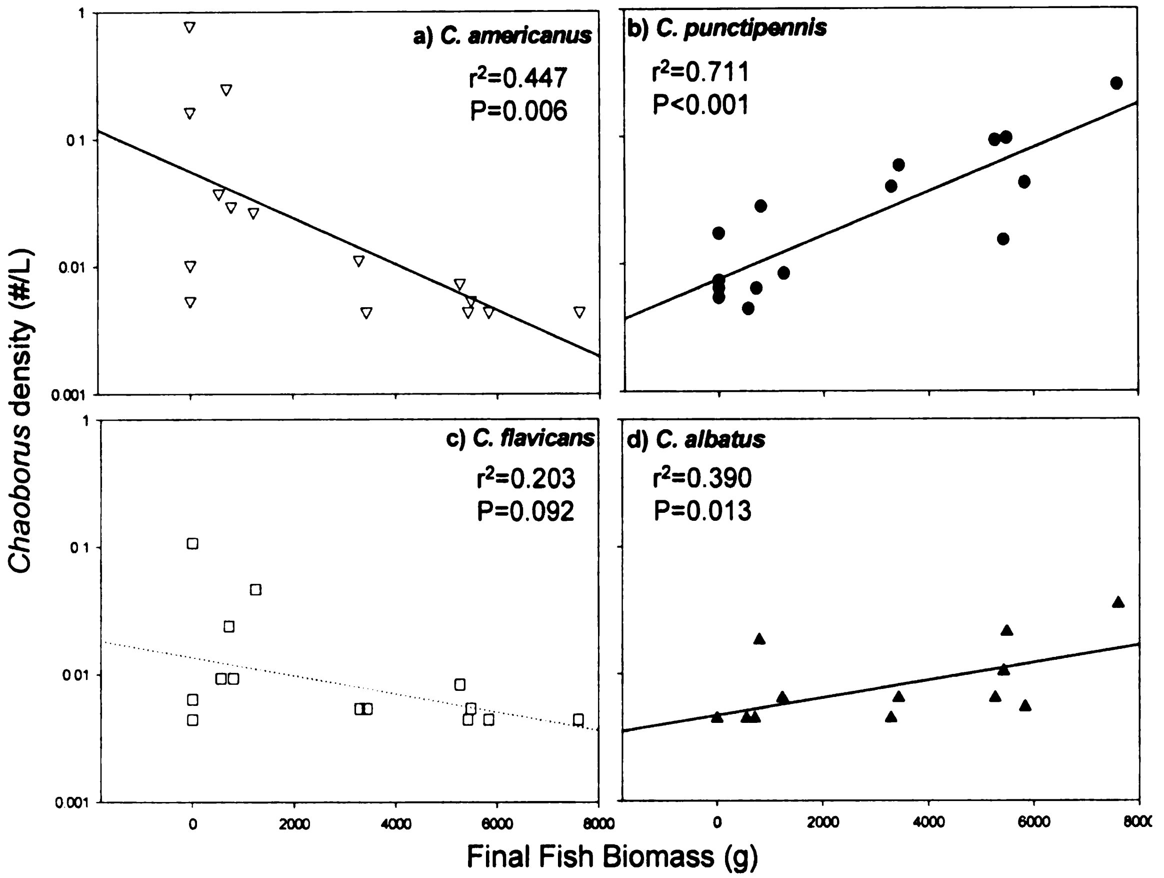

Figure 4.2. *Chaoborus* density (number per liter) in Experiment 2 averaged across the last three sampling dates as a function of final fish biomass. Lines are the fitted linear regressions for each species (solid lines are significant, $p<0.01$) and each point represents a pond.

negative trend (Fig. 4.2c; $r^2=0.2$, $p=0.09$). Final fish biomass also had a significant negative effect on the number of days *C. americanus* was present in the ponds (Fig. 4.3; $r^2=0.65$, $p<0.0001$).

In Experiment 1, the *Chaoborus* species assemblage responded similarly to the gradient in fish biomass as in Experiment 2, although the results were less striking due to the lower number of *Chaoborus* individuals sampled. *C. americanus* was observed in seven of the ten ponds at low densities prior to fish introduction when compared to the densities of *C. americanus* in the ponds at the start of Experiment 2 (Figs. 4.1a and 4.4a). These initial density differences are not surprising given that *C. americanus* had a full year to colonize the ponds in Experiment 2 and only a couple weeks in Experiment 1. *C. americanus* densities declined rapidly in the medium and high fish biomass ponds, but increased in abundance in the fishless ponds throughout the experiment (Fig. 4.4a: $p=0.01$, $F_{2,7}=8.96$, between subjects biomass effect, rmANOVA). Final *C. americanus* abundances were similar in fishless ponds in the two experiments. *C. flavicans* was present in three of the ten ponds prior to fish introduction and showed no significant changes in density through time or across fish treatments (Fig. 4.4c; $p>0.2$, for all within and between subjects fish biomass effects and time by biomass interactions, rmANOVA). *C. punctipennis* first appeared in the ponds on sampling day 10 and then increased through time. As in Experimental 1, this increase was most noticeable in the high fish biomass ponds, although there were no significant differences (Fig. 4.4b; $p>0.2$, for all within and between subjects fish biomass effects and time by biomass interactions, rmANOVA). *C. albatus* densities increased through time only in the high fish biomass ponds but there were no significant differences between fish biomass categories (Fig. 4.4d; $p>0.2$, for all within and between subjects fish biomass effects and time by biomass interactions, rmANOVA).

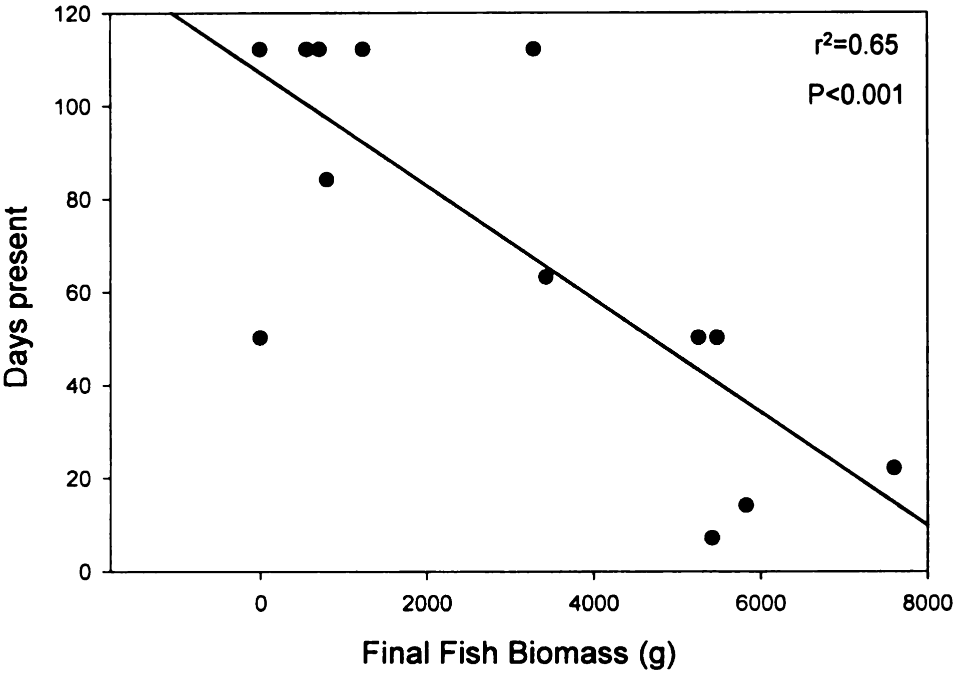

Figure 4.3. Number of days *C. americanus* was present in the water column in Experiment 2 as a function of final fish biomass. The solid line is the fitted linear regression ($p<0.01$) and each point represents a pond.

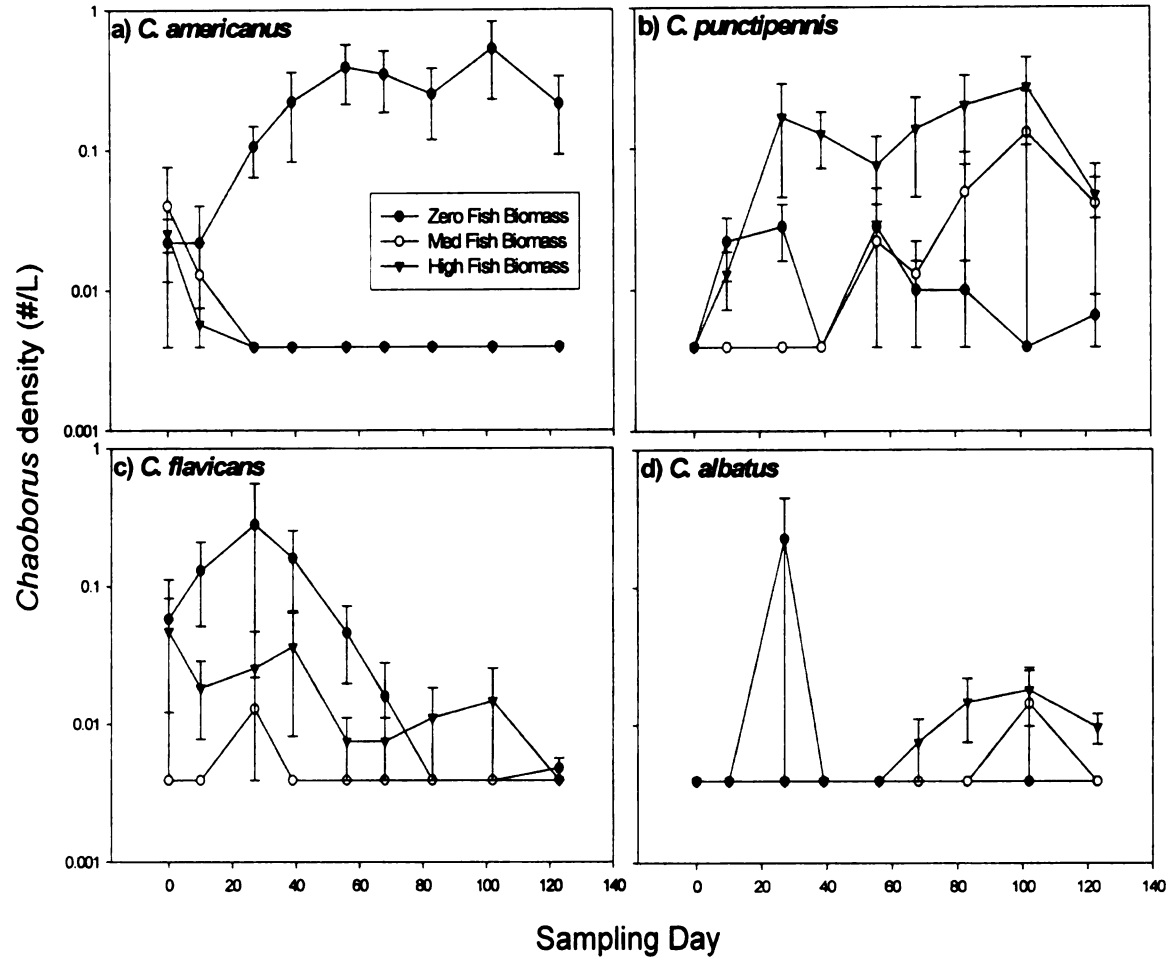

Figure 4.4. Change in *Chaoborus* density (number per liter) through time in Experiment 1 grouped into three fish biomass categories. Time is the sampling day of the experiment (0-123, May-September 2003). The filled circles are the means (± 1 s.e., n=3) for the zero fish biomass ponds, the open circles are the means (± 1 s.e., n=2) for the medium fish biomass (2300-3200 g) ponds, and the filled triangles are the means (± 1 s.e., n=5) for the high fish biomass (5700-12,050 g) ponds.

Looking at *Chaoborus* densities averaged over the last five sampling dates showed that *C. americanus* was present only in the ponds without fish (Fig. 4.5a; $r^2=0.45$, $p=0.03$). *C. punctipennis* and *C. albatus* again showed the opposite pattern, as both showed a positive response to final fish biomass (*C. punctipennis*, Fig. 4.5b; $r^2=0.33$, $p=0.09$; *C. albatus*, Fig. 4.5d; $r^2=0.73$, $p=0.002$), but final fish biomass had no significant effect on *C. flavicans* density (Fig. 4.5c; $r^2=0.02$, $p=0.74$). Thus, the combined results from both experiments found that *C. americanus* showed an overall negative response to increasing planktivore abundance, whereas *C. punctipennis* and *C. albatus* showed positive responses, and there was no trend for *C. flavicans*.

Prey Preference Trials: Non-refuge and refuge

In the absence of a refuge, bluegill tended to prefer *C. americanus* (Fig. 4.6a, $p=0.10$, $t_7=1.87$), whereas *C. punctipennis* was strongly selected against (Fig. 4.6a, $p=0.01$, $t_7=-3.52$), and there was no preference for or against *C. flavicans* (Fig. 4.6a, $p=0.843$, $t_7=-0.21$). In five out of eight feeding trials, the size distribution of all *Chaoborus* (*C. punctipennis*, *C. flavicans*, and *C. americanus*) eaten by the bluegill was significantly larger than that found in the environment (Fig. 4.7a, $p<0.06$). When size-selection was analyzed within species, *C. americanus* and *C. punctipennis* were larger in the diet than in the environment ($p<0.06$) but this was not true for *C. flavicans*.

The presence of a refuge did not alter the pattern of species selection (Fig. 4.6b) and few *C. flavicans* were found above the refuge indicating that they vertically migrated ($p=0.02$, $t_3=-6.38$). In all four trials, the size distribution of the *Chaoborus* assemblage (*C. punctipennis, C. flavicans*, and *C. americanus*) found above the refuge was smaller than the size distribution found in the entire water column before adding the refuge ($p<0.03$); indicating that the larger *Chaoborus* moved below the refuge. This result was largely due to the influence of *C. americanus*. A comparison of the size distributions by species showed that *C. americanus* was the only species whose mean size above the refuge was smaller than in the absence of the refuge ($p<0.01$). As in the non-refuge experiment, fish

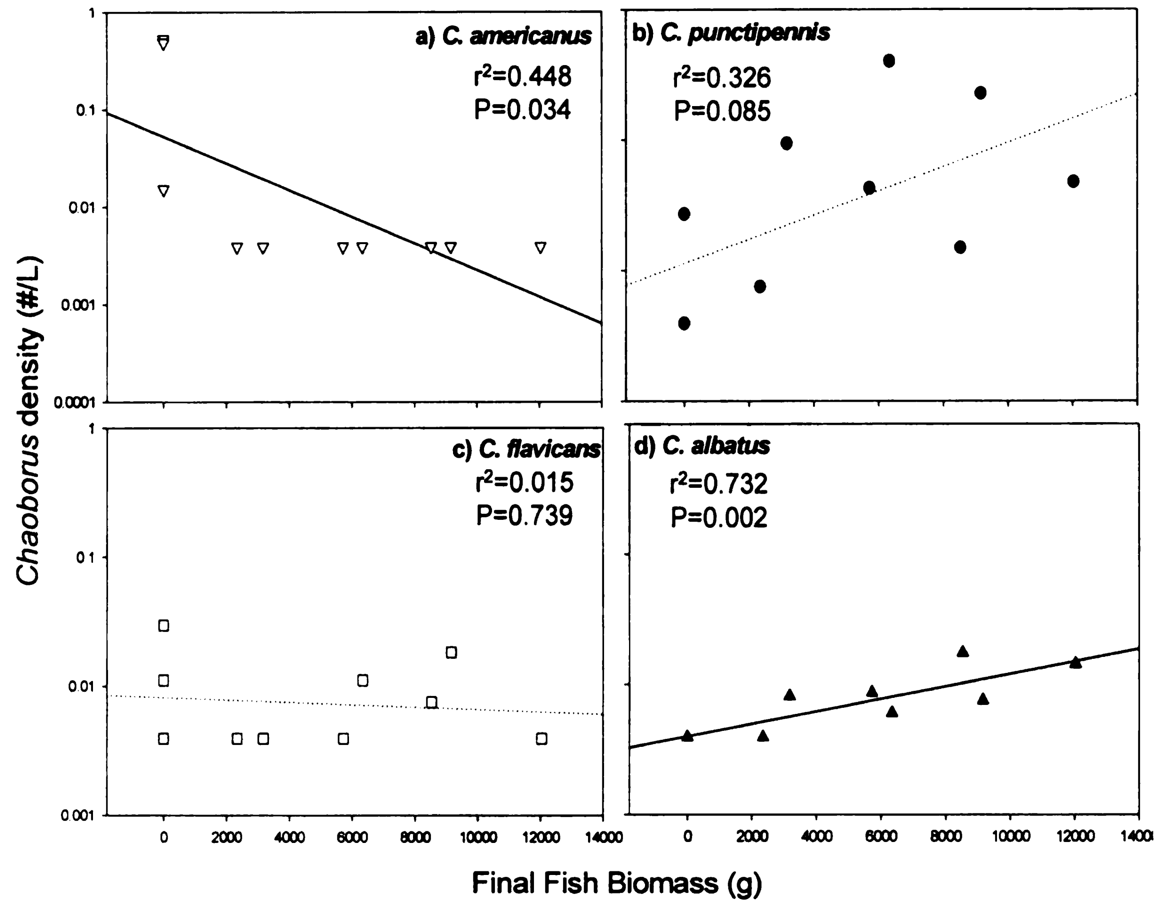

Figure 4.5. *Chaoborus* density (number per liter) in Experiment 1 averaged across the last five sampling dates as a function of final fish biomass. Lines are the fitted linear regressions for each species (solid lines are significant, p<0.01) and each point represents a pond.

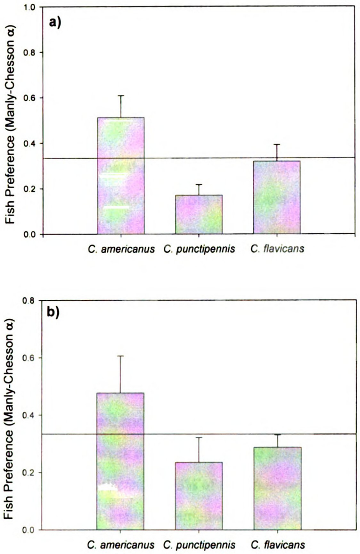

Figure 4.6. Average preference (Manly-Chesson α ± 1 s.e.) of Bluegill for three species of *Chaoborus* in a) 8 non-refuge feeding trials and b) 4 refuge feeding trials. The line indicates no preference.

were positively size selective. The size distribution of *Chaoborus* eaten was larger than the size distribution of *Chaoborus* found above the refuge (and therefore available to the fish) (Fig. 4.7b, $p<0.06$). This result was significant for *C. americanus* and *C. punctipennis* ($p<0.05$), but not for *C. flavicans*.

Discussion

It is widely appreciated that the presence of predators can affect the abundance and distribution of prey species (Kerfoot and Sih 1987, Zaret 1980). However, how variation in predator abundance may affect prey species sorting and colonization is less well understood. In this study, I show that manipulating fish density can lead to clear species sorting within an assemblage of four *Chaoborus* species, and that this response is consistent with distributional patterns observed in nature. Turner and Mittelbach (1990) report similar shifts in the *Chaoborus* species assemblage when they added 400 bluegill to one of the KBS experimental ponds. Prior to fish addition, *C. americanus* and *C. flavicans* were present in the pond and *C. americanus* made up 95% of the *Chaoborus* population. However, within a week of adding fish, these species disappeared. Sometime later in the experiment, *C. punctipennis* (>95% of the *Chaoborus* population) and *C. albatus* appeared in the pond. Turner and Mittelbach (1990) were unable to document the time course of species replacement because their daytime sampling missed censusing the vertically migrating *C. punctipennis* and *C. albatus* when they first appeared in the pond. An important observation from the current study (which employs a regression design as opposed to simple fish presence/absence) is that the pattern in the *Chaoborus* species abundance depends quantitatively on fish density.

Many observational studies have found that *C. americanus* only occurs in fishless habitats (Garcia, Chap. 2, Wissel et al. 2003). von Ende (1979) did observe one instance of a lake with only a single species of fish that also contained *C. americanus*. He stated that, "...although the exclusion of *C. americanus* by fish appears to be a fairly general phenomenon, the intensity of predation depends on the interaction of the characteristics

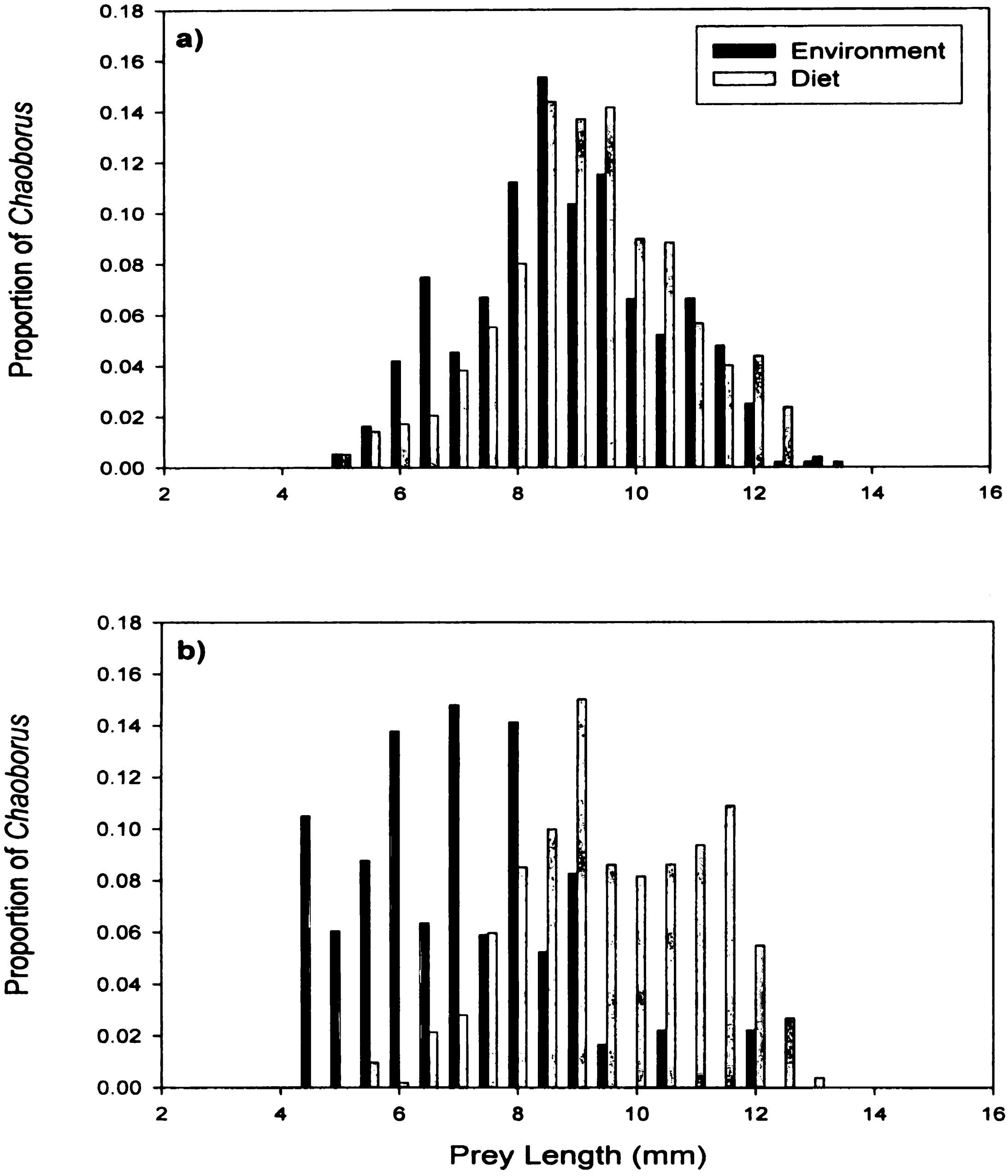

Figure 4.7. Frequency distribution of *Chaoborus* lengths (includes *C. punctipennis*, *C. flavicans*, and *C. americanus*) found in the environment (gray bars) and fish diet (black bars) in a) non-refuge feeding trials (n=8 trials) b) refuge feeding trials (n=4 trials).

of the lake, the *C. americanus* population, and the fish species." In my experimental gradients, *C. americanus* was quickly eliminated in the high fish biomass ponds but in Experiment 2, *C. americanus* was able to persist in ponds with medium fish biomass (Figs. 4.1a, 4.3, and 4.5a). This result was not observed in the first experiment, likely due to the fact that *C. americanus* densities, prior to fish addition, were much lower and therefore more vulnerable to local extinction.

Unlike *C. americanus*, most studies find that *C. punctipennis* commonly coexists with fish (von Ende 1979, Ramcharan et al. 2001, Carter et al. 1980, Wissel et al. 2003, Garcia, Chap. 2). Not only was *C. punctipennis* found in all the experimental ponds with fish, but recruitment of *C. punctipennis* was particularly favored in the high fish biomass ponds (Figs. 4.1b and 4.5b). This result is supported by Wissel et al. (2003), where they found that *C. punctipennis* was positively correlated with abundance and presence of fish. They also observed that *C. punctipennis* tended to reach higher densities in shallow lakes which makes sense because shallow lakes tend to support higher levels of fish planktivory (Tessier and Woodruff 2002). Ramcharan et al. (2001) also found that *C. punctipennis* was positively associated with high planktivory.

The increase in *C. punctipennis* and *C. albatus* density with increasing final fish biomass may be explained by the elimination of *C. americanus* in those systems (Fig. 4.2a and b). Little is known about *C. albatus* because it is a rare species (Garcia, Chap. 2), but von Ende (1979) observed that late instars of *C. americanus* will prey on *C. punctipennis* and he posits this as the mechanism excluding *C. punctipennis* from systems where *C. americanus* is present. Here I propose another possible mechanism, where recruitment of *C. punctipennis* and possibly *C. albatus* is favored in environments with fish due to the indirect effect of fish on their shared zooplankton prey. Many studies have found that in the presence of fish the zooplankton community shifts toward greater dominance by small-bodied zooplankton (Vanni 1987, Brooks and Dodson 1965). Since *Chaoborus* are gape-limited predators and *C. punctipennis* and *C. albatus* are the smallest of the four species found in this region, recruitment of these two species would be expected to be favored in environments with abundant small sized zooplankton. When I examined mean zooplankton size in ponds grouped into the three fish biomass categories I found a significant negative effect of high fish biomass on mean zooplankton size ($p<0.05$, one-way ANOVA). Further, the lack of coexistence between *C. americanus* and *C. punctipennis* may be a result of a combination of the mechanism proposed here and that of von Ende (1979).

The neutral response of *C. flavicans* to my experimental bluegill gradient is also supported in the literature and from a survey of local lakes and ponds (Pope et al 1973, González and Tessier 1997, Garcia, Chap.2). Wissel et al (2003) found that *C. flavicans* presence was negatively correlated to lake area, positively correlated with DOC levels and had no relationship with the presence or absence of fish. In my survey I found that *C. flavicans* was found in both ponds without fish and often in greater abundance in lakes with fish. Berendonk et al. (2003) found that *C. flavicans* is a comparatively "new" lake lifestyle species and this could be related to its neutral response. They state that at least three shifts have occurred between pond and lake lifestyles for *Chaoborus* species and that this shift is often accompanied by a decrease in larval body size, and associated with the evolution of DVM behavior. Although *C. flavicans* does exhibit DVM behavior this may be a plastic response to fish chemicals because *C. flavicans* has shown a marked decrease in migratory behavior when not exposed to fish chemicals (McQueen et al. 1999, Tjossem 1990). It may be that coexistence with fish is due to *C. flavicans*'s transparency and DVM behavior. In addition, *C. flavicans*'s potential ability to modify its migration behavior and its large size may enable it to persist in the larger prey size environments of fishless habitats but also be why it is not positively associated with high fish biomass like *C. punctipennis*.

While a number of studies report *Chaoborus* in bluegill diets (e.g., Mittelbach 1981, Turner and Mittelbach 1990), no one has specifically examined foraging preference by bluegill (or other fish species) for different species of *Chaoborus*. The prey preference experiments reported here indicate that larval body size and DVM behavior are important traits determining the vulnerability of different *Chaoborus* species to bluegill, and these traits should therefore influence the distribution of *Chaoborus* species across the fish density gradient (Berendonk et al. 2003). The observation that large (III and IV instar) *C. americanus* may have vertically migrated while smaller (I and II instar) *C. americanus* did not was a surprising result and has not been found in previous studies. This may be another possible mechanism by which *C. americanus* was able to persist in the medium fish biomass ponds. Another surprising result was the lack of preference for *C. flavicans* in the non-refuge feeding trials because both *C. americanus* and *C. flavicans* should be preferred because of their large body size relative to the other *Chaoborus* species. The lack of preference for *C. flavicans* may be understandable in the context of the refuge feeding trials since *C. flavicans* exhibited DVM behavior in response to the refuge. Thus, selection for *C. americanus* and not *C. flavicans* may be more to do with the combination of pigmentation and large size in *C. americanus* (Stenson 1980). Duffy et al. (2005) found strong evidence of bluegill preferring *Daphnia* that were pigmented due to infection by a parasite over similarly sized *Daphnia* that were not infected and thus not pigmented.

The experiments presented here illustrate that the processes responsible for the distributional pattern of *Chaoborus* species in nature is more involved than the simple presence or absence of a fish predator. They also suggest that *Chaoborus* species traits such as DVM behavior, size and pigmentation, are functionally related to the determinants of species distributions and development and maintenance of community structure across the gradient. Aquatic systems vary in size, depth and the presence and abundance of piscivores and this variation is related to differences in the intensity of fish planktivory (Tessier and Woodruff 2002). Future work on the effect of gradients in the density of competitors and predators on species sorting are needed because such datasets are readily useful for incorporation into simulation models of food web dynamics (Cottingham et al. 2005). This will increase the applicability of such models to natural systems and enhance our understanding of factors that are important to the development and maintenance of community structure.

LITERATURE CITED

Åbjörnsson, K., C. Brönmark, and L. Hansson. 2002. The relative importance of lethal and non-lethal effects of fish on insect colonisation of ponds. Freshwater biology 47:1489-1495.

Binckley, C. A. and W. J. Resetarits Jr. 2003. Functional equivalence of non-lethal effects: generalized fish avoidance determines distribution of gray treefrog, Hyla chrysoscelis, larvae. Oikos 102:623-629.

Berendonk, T. U. 1999. Influence of fish kairomones on the ovipositing behavior of *Chaoborus* imagines. Limnology and Oceanography 44(2):454-458.

Berendonk, T. U., and M. B. Bonsall. 2002. The phantom midge and a comparison of metapopulation structures: Ecology 83:116–129.

Berendonk, T. U., T. G. Barraclough, and J. C. Barraclough. 2003. Phylogenetics of pond and lake lifestyles in *Chaoborus* midge larvae. Evolution 57(9):2173-2178.

Blaustein, L. 1999. Oviposition site selection in response to risk of predation: evidence from aquatic habitats and consequences for population dynamics and community structure. In: Wasser SP (ed) Evolutionary theory and processes: modern perspectives. Kluwer, Dordrecht, pp. 441-456.

Blaustein, L., M. Kiflawi, A. Eitam, M. Mangel, J.E. Cohen. 2004. Oviposition habitat selection in response to risk of predation in temporary pools: mode of detection and consistency across experimental venue. Oecologica 138:300-305.

Borkent, A. 1979. Systematics and bionomics of the species of the Subgenus Schadonophasma *Chaoborus* Chaoboridae Diptera. Quaestiones Entomologicae 15:122–256.

Borkent, A. 1981. The distribution and habitat preferences of the Chaoboridae Culicomorpha Diptera of the Holarctic region: Canadian Journal of Zoology 59:122–133.

Brooks, J. L. and S. I. Dodson 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Carter, J. C. H., M. J. Dadswell, J. C. Roff, and W. G. Sprules. 1980. Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern North America. Canadian Journal for Zoology 58:1355-1387.

Carter, J. C. H., J. K. Kwik. 1977. Instar succession, vertical distribution, and interspecific competition among four species of *Chaoborus*. Journal of Fisheries Research Board of Canada 34:113-118.

Chesson, J. 1978. Measuring preference in selective predation. Ecology 59(2):211-215.

Chesson, J. 1983. The estimation and analysis of preference and its relationship to foraging models. Ecology 64(5):1297-1304.

Cook, E. F. 1956. The Nearctic Chaoborinae (Diptera: Culicidae): Technical Bulletin of University Minnesota Agricultural Experiment Station 218:1-102.

Cottenie, K. 2005. Integrating environmental and spatial processes in ecological community dynamics. Ecology Letters 8(11):1175-1182.

Cottingham, K. L., J. T. Lennon, and B. L. Brown. 2005. Knowing when to draw the line: designing more informative ecological experiments. Frontiers in Ecology and the Environment 3(3):145-152.

Darcy-Hall, T. L. 2006. Oecologia (in press)

Dawidowicz, P., J. Pijanowska, and K. Ciechomski. 1990. Vertical migration of *Chaoborus* larvae is induced by the presence of fish. Limnology and Oceanography 35(7):1631-1637.

Duffy, M.A., S.R. Hall, A.J. Tessier and M. Huebner. 2005. Selective predators and their parasitized prey: Are epidemics in zooplankton under top-down control? Limnology and Oceanography 50(2):412-420.

Gliwicz, Z. M. and J. Pijanowska. 1989. The role of predation in zooplankton succession. In Sommer, U. (ed.), Plankton Ecology: Succession in Plankton Communities. Springer-Verlag, New York, pp.253-295.

González, M. J. 1998. Spatial segregation between rotifers and cladocerans mediated by *Chaoborus*. Hydrobiologia 387/388:427-436.

González, M. J. and A. J. Tessier. 1997. Habitat segregation and interactive effects of multiple predators on a prey assemblage. Freshwater Biology 38:179-191.

Kerfoot, W. C., and A. Sih. 1987. Predation: Direct and indirect impacts on aquatic communities: University Press of New England, Hanover, NH, USA.

Lamontagne, S., D. B. Donald and D. W. Schindler. 1994. The distribution of four *Chaoborus* species (Diptera: Chaoboridae) along an elevational gradient in Canadian Rocky Mountain lakes. Canadian Journal of Zoology 72:1531-1537.

Leibold, M.A. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence and diversity patterns in communities. American Naturalist 147:784-812.

MacArthur, R.H. & Wilson, E.O. 1967. The Theory of Island Biogeography. Princeton University Press, Princeton, NJ, USA.

Manly, B.F.J. 1974. A model for certain types of selection experiments. Biometrics 30:281-294.

McCune, G. and J. B. Grace. 2002. Analysis of Ecological Communities. MjM software Design, Gleneden Beach, Oregon, USA.

McCune, G. and M. J. Mefford. 1999. PC-ORD. Multivariate analysis of ecological data, v4.0. MjM Sotware Design, Gleneden Beach, Oregon, USA.

McPeek, M.A. 1989. Differential dispersal tendencies among Enallagma damselflies (Odonata) inhabiting different habitats. Oikos 56(2):187-195.

McQueen D.J., C. W. Ramcharan, E. Demers, N. D. Yan, L. M. Conforti, and A. Perez-Fuentetaja. 1999. *Chaoborus* behavioral responses to changes in fish density. Archiv Hydrobiolgie 145(2):165-179.

Mittelbach, G. G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62:1370-1386.

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

Mittelbach, G. G. and P. L. Chesson. 1987. Predation risk: indirect effects on fish populations, p.237-289 in W. C. Kerfoot and A. Sih, (eds.), Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, NH, USA.

Mittelbach, G. G. 1988. Competition among refuging sunfishes and effects of fish density on littoral zone invertebrates. Ecology 69(3):614-623.

Mittelbach, G. G. and C. W. Osenberg. 1993. Stage-structured interactions in bluegill: consequences of adult resource variation. Ecology 74:2381-2394.

Mittelbach, G. G., A. M. Turner, D. J. Hall, J. E. Rettig, and C. W. Osenberg. 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. Ecology 76:2347-2360.

Mittelbach, G. G. 2002. Fish foraging and habitat choice: a theoretical perspective, pgs. 251-266 in: P. J. B. Hart and J. D. Reynolds (eds.), Handbook of Fish and Fisheries, Blackwell Science.

Mittelbach, G. G., E. A. Garcia, and Y. Taniguchi. 2006. Fish reintroductions reveal smooth transitions between lake community states. Ecology 87(2):312-318.

Moore, M. V., 1986. Method for culturing the phantom midge, *Chaoborus* (Diptera: Chaoboridae), in a laboratory. Aquaculture 56:307-316.

Moore, M. V., N. D. Yan, and T. Pawson. 1994, Omnivory of the larval phantom midge (*Chaoborus* spp.) and its potential significance for freshwater planktonic food webs: Canadian Journal of Zoology 72:2055-2065.

Pastorok, R. A. 1981. Prey vulnerability and size selection by *Chaoborus* larvae. Ecology 62:1311-1324.

Pope, G. F., J. C. H. Carter, and G. Power. 1973. The influence of fish on the distribution of *Chaoborus* spp. (Diptera) and density of larvae in the Matamek River system, Québec. Transactions of the American Fisheries Society 102:707-714.

Ramcharan, C. W., N. D. Yan, D. J. McQueen, A. Pérez-Fuentetaja, E. Demers, and J. A. Rusak. 2001. Complex responses of *Chaoborus* to changes in fish populations. Archiv für Hydrobiologie Special Issue Advances in Limnology 56:81-100.

Resetarits, W .J., Jr. 2001. Colonization under threat of predation: non-lethal effects of fish on aquatic beetles, *Tropisternus lateralis* (Coleoptera: Hydrophilidae). Oecologia 129:155-160.

Rieger, J. F., C. A. Binckley, and W. J. Resetarits, Jr. 2004. Larval performance and oviposition site preference along a predation gradient. Ecology 85(8):2094-2099.

Sæther, O. A. 1970. Nearctic and Palearctic *Chaoborus*, Diptera, Chaoboridae. Fisheries Research Board of Canada Bulletin 57.

Sæther, O. A. 1972. Chaoboridae, Das Zooplankton der Binnengewässer: Die Binnengewässer. 26:257-281.

Sæther, O. A. 1997. Diptera Chaoboridae, Phantom Midges, pgs.149-161 in A. N. Nilsson, (ed). Aquatic Insects of North Europe-A Taxonomic Handbook Vol. 2. Apollo Books, Stenstrup, Denmark.

Stenson, J. A. E. 1980. Predation pressure from fish and two *Chaoborus* species as related to their visibility. In: Evolution and Ecology of Zooplankton Communities, American Society Limnology Oceanography Special Symposium 3 (Ed. W.C. Kerfoot), pp. 618-622. University Press New England, Hanover, NH, USA.

Sterner, R. W. 1989. The role of grazers in phytoplankton succession. In: Sommer, U. (ed.), Plankton ecology: succession in plankton communities. Springer-Verlag, New York, pp. 107-169.

Stoks, R. and M. A. McPeek. 2003. Predators and life histories shape *Lestes* damselfly assemblages along a freshwater habitat gradient. Ecology 84(6):1576-1587.

Swift M. C., and A. Y. Fedorenko. 1975. Some aspects of prey capture by *Chaoborus* larvae. Limnology and Oceanography 20:418-425.

Tessier, A. J., and P. Woodruff. 2002. Cryptic trophic cascade along a gradient of lake size. Ecology 83(5):1263-1270.

Tilman, D. 1982. Resource Competition and Community Structure. Princeton University Press, Princeton, NJ, USA.

Tjossem, S. F. 1990. Effects of fish chemical cues on vertical migration behavior of *Chaoborus*. Limnology and Oceanography 35: 1456-1468.

Turner, A. M., and G. G. Mittelbach. 1990. Predator avoidance and community structure: Interactions among piscivores, planktivores, and plankton: Ecology 71:2241-2254.

Urban, M. C. 2004. Disturbance heterogeneity determines freshwater metacommunity structure. Ecology 85(11):2971-2978.

Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology 68:624-635.

von Ende, C. N. 1979. Fish predation, interspecific predation, and the distribution of two *Chaoborus* species. Ecology 60: 119-128.

von Ende, C. N. 1982. Phenology of four *Chaoborus* species. Environmental Entomology 11:9–16.

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. Limnology Oceanography 39:1985-1992.

Werner, E. E. and D. J. Hall. 1988. Ontogenetic habitat shift in bluegill: the foraging rate-predation risk trade-off. Ecology 69:1352-1366.

Werner, E. E., J. F. Gilliam, D. J. Hall, and G. G. Mittelbach. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64:1540-1548.

Werner, E. E. and M. A. McPeek. 1994. Direct and indirect effects of predators on two anuran species along an environmental gradient. Ecology 75(5):1368-1382.

Wetzel, R. G. and Likens, G. E. 1991. Limnological Analyses. 2nd edition, Springer-Verlag, New York, pp. 89-92.

Wissel, B., N. D. Yan and C. W. Ramcharan. (2003) Predation and refugia: implications for *Chaoborus* abundance and species composition. Freshwater Biology 48(8):1421-1431.

Zaret, T. M. 1980. Predation and freshwater communities. Yale University Press, New Haven, CT, USA.