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Diel Vertical Migration of an Invasive Calanoid Copepod, Eurytemora affinis, in Little Sturgeon Bay, Wisconsin

Alexandra N. Poli
Lawrence University

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I Hereby Reaffirm the Lawrence University Honor Code
Alexandra Poli

Abstract

Eurytemora affinis, a calanoid copepod, is known to be a versatile, prolific invader of freshwater ecosystems across the globe. It has recently been documented in the Laurentian Great Lakes, including in Little Sturgeon Bay, an embayment of Lake Michigan. One survival mechanism that could make *E. affinis* a successful invader is diel vertical migration (DVM), a behavior in which animals move to different lakes depths at different times of day in order to avoid predation. Much is known about DVM of *E. affinis,* but primarily from studies in marine and brackish systems. Our goal was to investigate how *E. affinis* responds to its new, non-native freshwater environment, and to make inferences about its invasive success. During the summer of 2014, samples were taken at Little Sturgeon Bay twice on four days—once at noon and again at night. Samples were collected at one-meter intervals from one nearshore site and one offshore site. Body size and darkness of different life-stages of *E. affinis* were evaluated to determine stage-dependent differences in visual predation risk. Abundance of *E. affinis* was determined at each depth of each site to describe diel patterns of movement through the water column. Results show significant differences among life-stages in both length and visual area, but not our measure of darkness. Magnitude of DVM was greater near shore than in the offshore habitat. This may be a result of greater predation pressure near shore. The magnitude of DVM was also stage-dependent, with adults performing a more drastic migration than copepodites. This stage-dependency could be a result of differing visual predation risk, since copepodites are smaller than adults. The variety of DVM magnitudes exhibited for different life stages and environmental conditions support the notion that E. affinis is highly phenotypically plastic, making it a successful invader.

Introduction

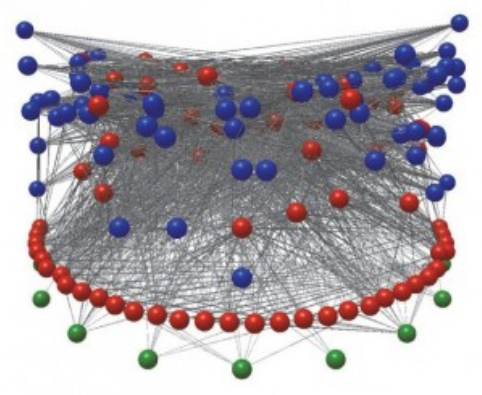


Figure 1. Example of a food web. Spheres represent organisms and lines describe who eats whom. Blue spheres are parasites, red spheres are free-living heterotrophs, and green spheres are primary producers. Image from Dr. Jennifer Dunne et al. (2013)

It is romantic, yet shortsighted, to think of an ecosystem as static and pristine—an archaic system of organisms interacting in the same way year after year. In reality, ecosystems are incredibly dynamic. Ecologists use food webs to describe relationships between different organisms: lines describing who eats whom cross and tangle between species (Figure 1). The result is a highly complex set of relationships, interactions, and interdependencies. The slightest manipulation of a single component of a food web can result in a cascade of changes. For example, elimination of a keystone predator (such as a wolf) could mean an increase in the abundance of its prey (elk), which could over-graze and diminish certain plant species (sage grass). Similarly, the elimination of a food source like sage grass could warrant a ricocheting response moving up through trophic levels (levels of the food web). Human activities, especially pollution and facilitating the introduction of invasive species, play a huge role in altering food webs.

Invasive species, or those that are introduced to a new (non-native) environment, are of particular interest to humans and ecologists for a variety of reasons. First, invasive species are frequently introduced as a result of human activity. Second, the introduction of a new organism into the food web of an ecosystem yields the potential for vast change in food web structure. Third, these changes are potentially harmful ecologically and/or economically (Mills et al. 1994). Oftentimes, an invasive species survives exceedingly

well in a new environment because its main predator or competitor from its previous environment is absent. Alternatively, the invasive species may be better equipped than native species to cope with certain conditions such as toxic algae blooms. As a result, the invasive organism can out-perform and out-compete native species (Dodson 2005).

Behaviors such as migration and patch selection are excellent indications of how a species fits into its environment. These behaviors are often survival strategies driven by the presence of certain pressures such as predation, food requirements, and metabolic efficiency. As a result, populations of organisms inhabit spaces where they are able to maximize their chance of survival and reproduction and minimize predation risk and metabolic costs (Mangel and Clark 1988). Through the study of behaviors such as migration, we may better understand how an invasive species responds to the pressures and challenges of its new environment.

DVM: Diel Vertical Migration

Diel vertical migration, or DVM, is a massive, population-wide daily migration performed by zooplankton and fish populations in aquatic systems across the globe (Dodson 2005). This study focuses primarily on DVM of zooplankton populations. The most common DVM behavior pattern for zooplankton is to spend daylight hours at deeper depths and move to shallower areas at night (Figure 2). This pattern is typically repeated on a diel, or 24-hour, cycle.

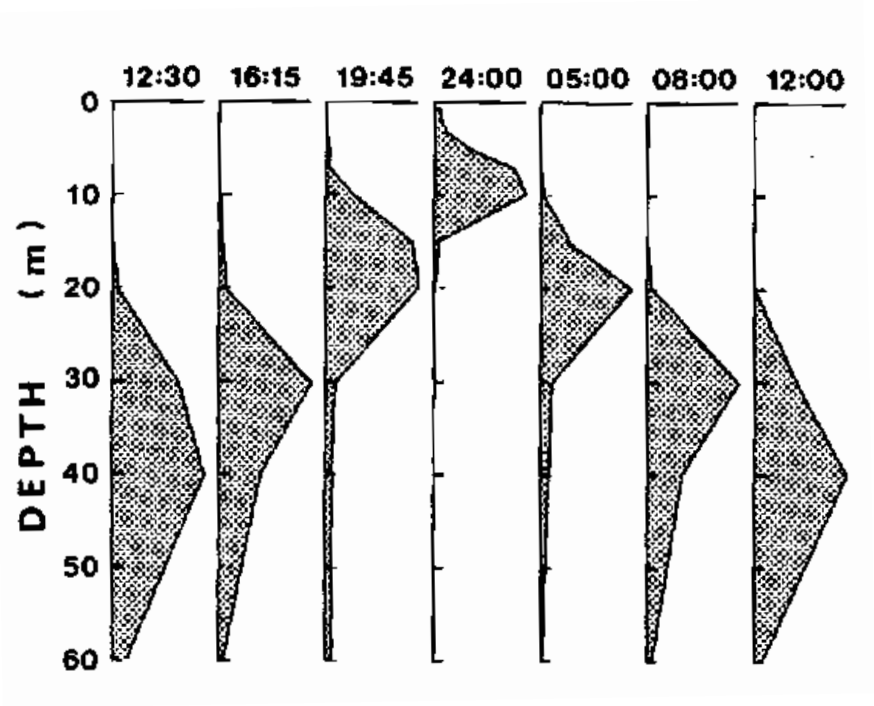


Figure 2. An example of the "normal" diel vertical migration pattern of zooplankton, adapted from Wolf & Mort, 1986: The vertical distribution of a population of cladoceran *Daphnia hyalina* in a lake at six times throughout a single day.

DVM can be a costly behavior, so it is only beneficial to a zooplankton population when certain pressures are present. Zooplankton that migrate to deeper waters during the day spend less time feeding at shallower depths where their food source, phytoplankton, has greater access to light, and therefore higher productivity. Migration is metabolically costly as well, since zooplankton inhabiting deeper, cooler waters show slower growth and lower fecundity rates (Dodson 1990). In addition, zooplankton eggs, which are carried by females, take longer to develop at colder temperatures (Lampert 1989).

Despite its apparent costs, DVM is an adaptive behavior that allows zooplankton populations to select habitat patches that will provide optimal foraging, reproductive, and survival opportunity. During the day, the optimal patch for zooplankton may be deep and out of sight of potential visual predators. At night, though, risk of visual predation is lower near the surface. As a result, zooplankton move near the surface where foraging

opportunity is greatest (Lampert 1989). Stated simply, migration is the behavior of a species in response to the changing location of optimal habitat.

Several theories exist to explain DVM. These theories identify a variety of drivers that contribute to the changing location of optimal habitat, making DVM a favorable behavior for zooplankton. These theories may be lumped into two categories: biotic and abiotic. Biotic theories emphasize the importance of living things such as predators and food as drivers of DVM, while abiotic theories examine the roles of non-living aspects such as ultraviolet radiation avoidance and temperature. Drivers may be further classified as either proximate or ultimate. Proximate drivers act as cues that zooplankton detect warning them of the presence of ultimate drivers—the factors that determine the suitability and survivability of a habitat. These drivers may also be classified as static—remaining the same between day and night—or dynamic, changing on a diel basis. (Table 1; Williamson 2011).

Table 1. Leading theories of DVM, proximate cues, ultimate drivers, nature of the drivers, and generalized response of herbivorous zooplankton to each driver (Williamson 2011).

Theory	Proximate drivers	Ultimate drivers	Nature of the drivers	Zooplankton response
UV avoidance	UV radiation	Photodamage, mortality	Dynamic	Down by day
Visual predation	Visible light, kairomones	Predation mortality	Dynamic	Down by day
Temperature	Temperature	Growth and reproduction	Structural	Up at any time
Food	Food	Survival, growth, and reproduction	Structural	Up or down at any time

Abiotic Drivers

Ultraviolet Radiation: During the day, the amount of light penetrating a lake decreases with increased depth. The rate at which irradiance decreases, or the extinction coefficient, varies by lake transparency (Figure 3). A lake may be less transparent if it is turbid, containing many suspended particles, or is highly productive, containing a high concentration of phytoplankton or a layer of blue-green algae covering its surface. At

night, though, very little light penetrates the water column. Because the light profile of a lake varies so drastically on a daily basis, it is considered a dynamic driver of DVM.

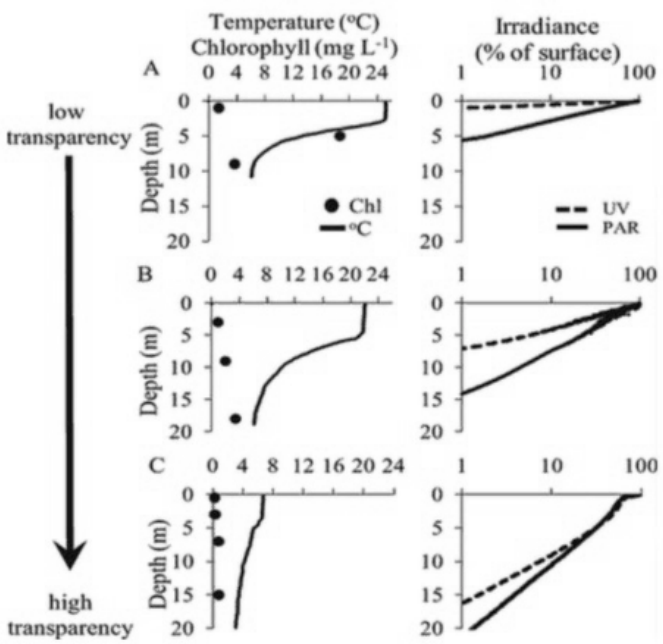


Figure 3. Sample temperature, chlorophyll, and light profiles of three lakes of differing transparencies (Williamson 2011).

Light is recognized as the primary proximate cue used by zooplankton to assess predation risk (Forward 1988). This should come as no surprise, since a well-lit zooplankton is more easily seen and captured by a visual, or 'seeing,' predator than a poorly-lit zooplankton. Experiments have confirmed that UV radiation enhances fish predation on zooplankton in UV-transparent systems (Leech et al. 2009).

In addition to increased predation risk, UV radiation is potentially damaging to aquatic organisms. UV-B rays in particular can negatively affect growth, reproduction, and survival of zooplankton (Leech and Williamson 2000). In cases of prolonged exposure to UV-B radiation, short UV wavelengths can damage DNA and membranes, leading to zooplankton mortality (Williamson et al. 1994, 1999).

Temperature: The temperature of a lake is typically warmest near the surface when a lake is stratified (Figure 2). Temperature stratification is observed when temperatures remain effectively the same through shallow lake depths and then rapidly decrease with

increasing depth. The depth at which temperature most quickly decreases is called the thermocline. Below the thermocline, temperatures remain nearly constant until the bottom of the water column. This vertical temperature profile is maintained in fresh water because less dense, warmer water floats near the surface and is, in turn, further warmed by the sun. A lake is said to be isothermal if it is the same temperature from top to bottom. An isothermal lake is more easily mixed by wind or water currents because waters with similar temperatures have similar densities, and therefore there is very little resistance to mixing based on density differences. Shallow lakes are more likely than deeper lakes to be stirred by wind, and are therefore more often isothermal (Dodson 2005).

Though lake temperature profiles may change seasonally, they rarely change drastically between a single day and night. The range of temperatures experienced from the top to the bottom of a stratified lake is much greater than any diel temperature variation. For this reason, temperature is generally considered a static driver of DVM (Williamson 2011). Zooplankton that spend more time in warmer surface waters experience higher rates of growth and reproduction (Orcutt and Porter 1983, Stich and Lampert 1984, Leibold 1989) while zooplankton that spend time at deeper, colder depths incur harsh metabolic costs (Pangle and Peacor 2006, Pangle et al. 2007). The costs that accompany cold water and benefits that accompany warm water provide incentive for zooplankton to spend time in shallower, warmer depths.

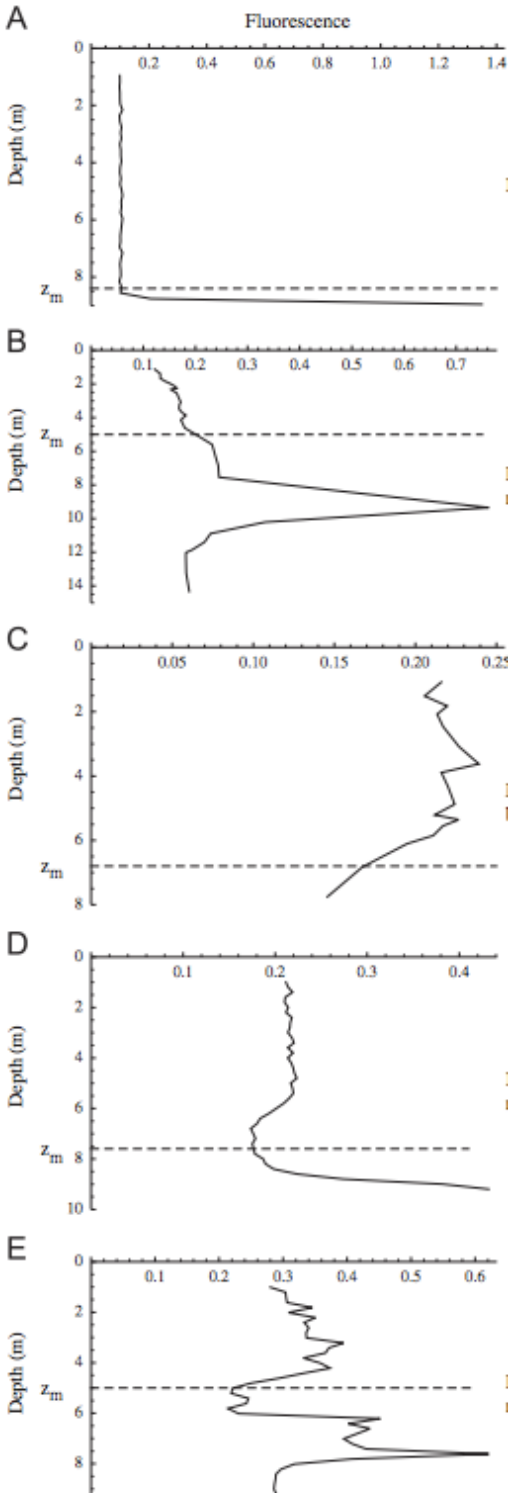


Figure 4. Examples of differing phytoplankton vertical profiles in five stratified lakes. Vertical distributions are Chlorophyll *a* fluorescence profiles. Z_m is the depth 1ºC cooler than the surface temperature. (Mellard et al 2011)

Biotic Drivers

Food Availability: Zooplankton feed on phytoplankton (e.g. algae and photosynthetic prokaryotes), so the vertical distribution of phytoplankton is an important factor for zooplankton habitat selection. While the vertical phytoplankton profile may be variable in a single lake over time, it generally does not change consistently on a diel basis. Therefore, food availability is considered a static driver of DVM (Williamson 2011).

Phytoplankton are hypothesized to exist where there is adequate light and nutrient supply, but these conditions vary between lakes (Reynolds 1984). Because of this variation, a diverse array of phytoplankton vertical distributions has been observed in lakes (Figure 4).

In a well-mixed lake, phytoplankton may be evenly distributed throughout the water column (Mellard et al. 2011). In a stratified lake, though, phytoplankton is

typically found throughout the shallower waters near the surface, or may congregate to form a deep chlorophyll maximum (DCM). The DCM, or peak abundance of phytoplankton, may be located deep in the water column if there is high light penetration (Figure 4B, E). A DCM may only persist in a stratified lake while there is little mixing and sufficient light penetration to support photosynthesis at deeper depths (Fee 1976).

When phytoplankton is limiting at certain depths of a lake, zooplankton will congregate in areas with high phytoplankton density despite possible high predation risk (Jonsen and Jakobsen 1987).

Predation: Predation is widely accepted by limnologists as the most important driving force behind DVM. Both field studies (Zaret and Suffern 1976) as well as experimental studies (Stich and Lampert 1981, Bollens and Frost 1991) have documented zooplankton migrating to deeper waters during light hours to avoid predators. Further evidence that predation heavily motivates DVM lies in the fact that migration amplitude increases in the presence of predators or their karimones (chemical compounds released by predators and detected by prey) (Gliwicz 1986, Leech et al. 2009).

Modeling predation risk the impact of predation on DVM has been of particular interest to several researchers for decades (Gerritsen & Stricckler 1976, Williamson 1993, De Robertis 2002). Predation risk, or the probability that an individual of a prey species will be killed by a predator, may be explained as the product of prey vulnerability and density risk (Williamson 1993).

$$PR = PV * DR$$

$PR = predation\ risk$
$PV = prey\ vulnerability$
$DR = density\ risk$

Density risk is a function of predator density and overlap between predator and prey populations. Thus, density risk is system-dependent. Prey vulnerability, on the other hand, is species and life stage-dependent. It includes all the prey-specific characteristics that influence the ability of the predator to detect, capture, and kill/ingest prey (Williamson 1993).

In the case of visual predation, prey vulnerability is of particular interest since it may vary according to physical attributes of the prey. Larger prey are at greater risk of predation by visual predators since they may be seen from farther distances at lower light levels (Figure 5; De Robertis 2002).

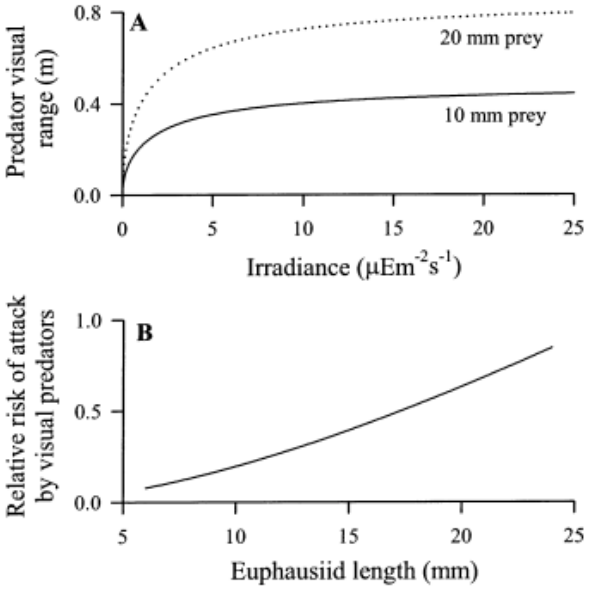


Figure 5. (De Robertis 2002) Vulnerability to visual predators depends on light intensity and prey size. A) Modeled dependence of the visual range at which a fish can detect two sizes of zooplankton prey (10 and 20 mm). B) Risk of attack by visual predators as a function of zooplankton prey size

Consequences of DVM

In theory, DVM by zooplankton results in a reprieve for phytoplankton from constant grazing and limits the size of the grazing zooplankton population. During daylight hours when zooplankton spend time at deeper depths, phytoplankton are offered temporary refuge from zooplankton grazing. Algae growth during this time leads to overall higher population productivity (Reichwaldt et al. 2004). In addition, spending time at deeper, colder depths limits zooplankton population growth. A smaller zooplankton population is unable to graze as intensely on phytoplankton, allowing the

phytoplankton population to be more productive (Loose & Dawidowicz 1994). While some research suggests that phytoplankton productivity may benefit from nutrient transport facilitated by zooplankton undergoing DVM (Sommer et al. 1986), others suggest that zooplankton performing DVM displace necessary nutrients such as nitrogen below the photic zone (the depth of water that is exposed to sufficient sunlight for photosynthesis to occur), resulting in limited phytoplankton production (Longhurst and Harrison 1988, Dodson 2005).

In reality, the effects of DVM on nutrient cycling and phytoplankton productivity are highly variable and case-specific. Different phytoplankton community compositions, degrees of nutrient limitation, and differences in zooplankton community and grazing all factor into how DVM impacts phytoplankton dynamics (Haupt et al. 2009). The relationship between DVM and phytoplankton dynamics is not one-size-fits-all.

The issue of zooplankton-phytoplankton community interactions is further complicated by the impact of ontogeny on zooplankton food preference. Zooplankton may face distinct challenges depending on their size and life stage. Different life stages of a single species may act vastly different—consuming different food, avoiding different predators, and performing DVM with differing magnitudes—in response to these distinct challenges (Werner & Gilliam 1984). Such ontogenetic changes in DVM have been documented in Eurytemora affinis—the copepod that is the focus of this study (Holliland et al. 2012).

Eurytemora affinis

Identification: Adult *Eurytemora affinis* typically measure around 1.2-1.3mm in length and have a number of morphological traits allowing it to be easily distinguished

from other calanoid copepod species (Torke 2001). Adults and copepodites (juvenile copepods that have not yet reached sexual maturity) have a unique caudal ramus that is over three times as long as it is wide (Figure 6). On the posterior end of the caudal ramus are five obvious setae. Mature adults display distinct sexual characteristics. Adult females exhibit two lateral metasomal wings—one on each side of the genital region. Adult males have "hooked" antennae used for grasping the female during mating and an enlarged fifth leg. The species passes through 6 naupliar and 5 copepodite stages before reaching sexual maturity (Poppe 1880).

Life History and Ecology: The *E. affinis* life cycle includes four major stages: egg, nauplius, copepodite, and adult (Figure 6). An individual may live up to 73 days after hatching, of which the juvenile (nauplius and copepodite stages combined) stage lasts between 11 and 37 days. Eggs develop over 1 to 14 days, but the development time is temperature-dependent. Eggs develop slowly at 5 ºC and rapidly at 22 ºC. If conditions are unfavorable, eggs may enter a dormant stage. These dormant, or diapausing, eggs can remain viable up to 18 years (Torke 2001).

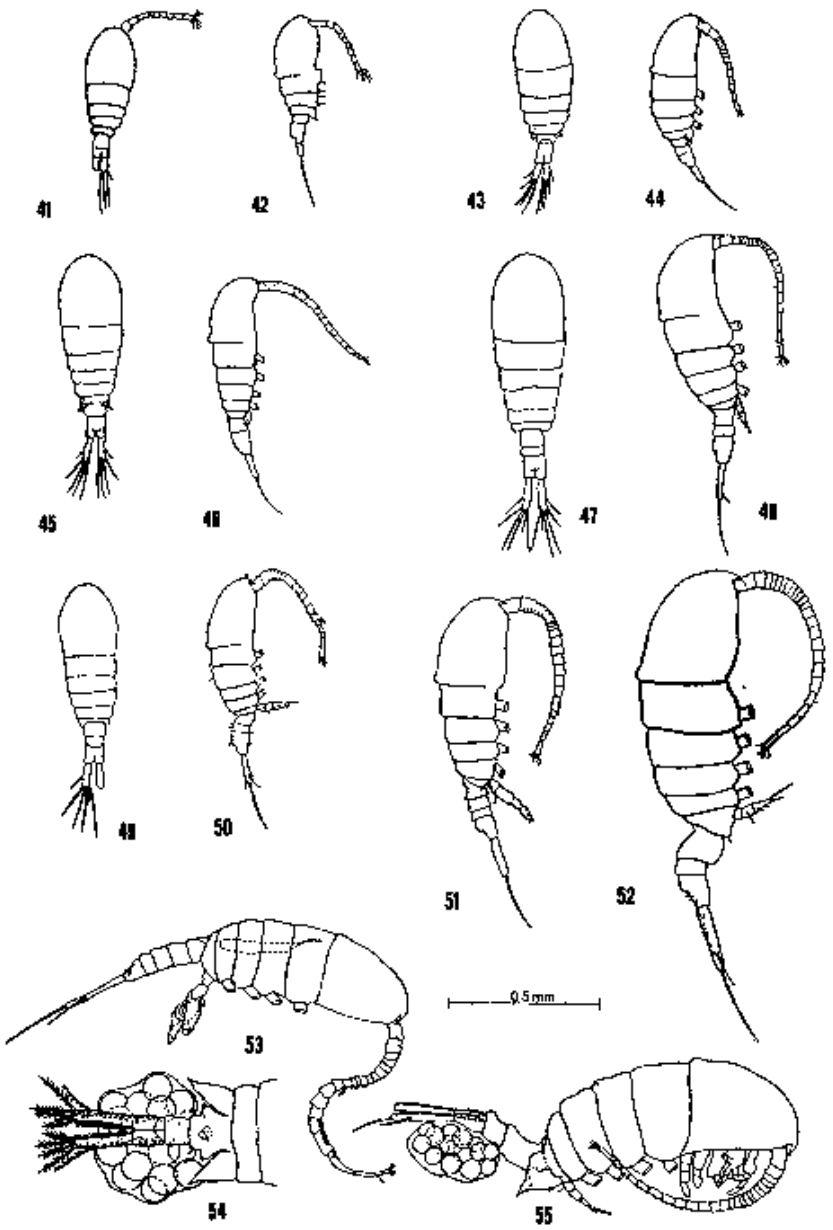


Figure 6. *Eurytemora affinis* (Poppe 1880). Copepodite Stage I (41-42); Copepodite Stage II (43-44), Copepodite Stage III (45-46), Copepodite Stage IV (female: 47-48, male: 49-50), Copepodite Stage V (female: 51, male: 52), adult male: 53, adult female, 54, 55.

In Lake Michigan, *E. affinis* spends the winter and spring as diapausing eggs and is far more abundant during the summer and fall (Torke 2001). During the peak seasons when eggs exit diapause, *E. affinis* is epibenthic, meaning it primarily inhabits the sediment of and the water immediately above the bottom of bodies of water (Evans and

Stewart 1977). Their epibenthic location is not static, though. *Eurytemora affinis* populations have been observed to undergo diel vertical migration (DVM) and diel horizontal migration (DHM) in both salt and freshwater systems around the globe (Hough & Naylor 1992, Almén et al 2014).

Invasive Success: On a global scale, *E. affinis* has a cosmopolitan distribution. This means that it inhabits a broad range that extends across the Northern Hemisphere. *Eurytemora affinis* is found in temperate regions of Asia and Europe, and from subtropical to subarctic regions of North America (Lee 2000). Its native range includes brackish and saltwater regions of the North American Atlantic coast, Pacific coast, western European coast, and parts of Asia (Torke 2001). In the past, *E. affinis* was documented in only a few freshwater habitats including coastal and oxbow lakes. Within the past 70 years, though, *E. affinis* populations have extended beyond their native saline and brackish waters to invade several freshwater habitats across the Northern Hemisphere (Lee 2000).

Eurytemora affinis is a particularly good invader for a variety of reasons. First, it is euryhaline, meaning that it tolerates a wide range of salinities. This eurytolerance (or wide tolerance) allows *E. affinis* to survive in both brackish and freshwater habitats. In addition, it is a generalist grazer and is able to consume a variety of phytoplankton, including the cyanobacteria *Microcystis* and *Nodularia,* which secrete the toxins microcystin and nodularin, respectively (Kozlowsky-Suzuki et al 2003, Kozlowsky et al 2002).

Tolerance to a wide range of phytoplankton and salinities alone, though, does not account entirely for its successful invasions. Mounting evidence suggests that the

"evolvability," or strong selection of phenotypic traits and rapid evolution following invasion, of *E. affinis* is the most important quality allowing the species to invade freshwater habitats independently across the globe (Lee 2003). Among these new freshwater habitats is the Laurentian Seaway, which extends from the Atlantic Ocean to the Laurentian Great Lakes (Figure 7).

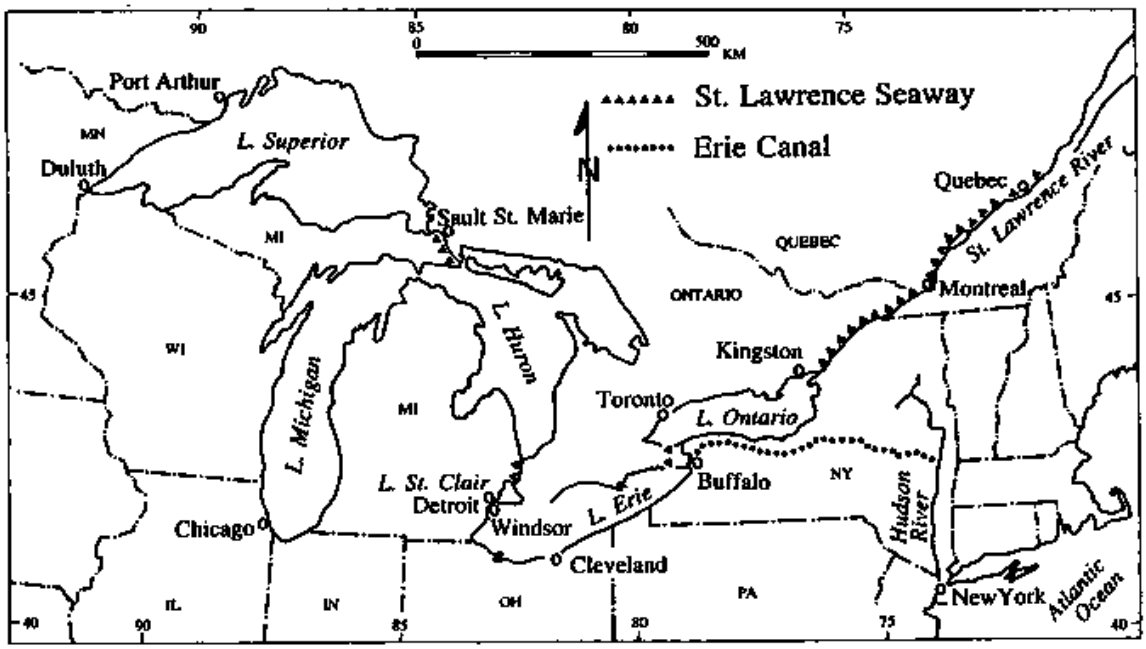


Figure 7. A map of the Laurentian Great Lakes including the St. Lawrence Seaway. Taken from Mills et al. 1994.

Introduction to and Establishment in the Laurentian Great Lakes: Since the completion of the St. Lawrence Seaway in 1959 extending from the Atlantic Ocean to Lake Ontario, at least 43 nonindigenous species have been introduced to the Great Lakes. Of these invasions, over 70% are attributed to the discharge of ballast water from ocean-faring vessels (Grigorovich et al. 2003). *Eurytemora affinis* is most likely among the species introduced into the Great Lakes in this manner, since it was first recorded in Lake Ontario in 1959, the same year as the seaway completion. In the years following, *E.*

affinis was found subsequently in Lake Erie in 1961 and then in Lake Michigan in 1964 (Mills 1993).

In Lake Michigan, *E. affinis* is commonly found in high abundance during the summer and early fall, but is difficult to find during winter and spring months. *Eurytemora affinis* is currently a wide-spread, well-established member of zooplankton communities across the shores of the Great Lakes, having been found in Milwaukee Harbor, coastal waters of southeastern Lake Michigan, and in the littoral and plankton communities of Green Bay (Torke 2001). In 1977, Gannon and Brickner (1982)found that within Green Bay, *E. affinis* was most abundant near Big Bay de Noc and Sturgeon Bay. Little Sturgeon Bay, which is located approximately 10 miles from Sturgeon Bay, is the sample site for this study.

Ecological Trends in Green Bay

The same qualities that make *E. affinis* a successful invasive species—tolerance to a wide range of salinities, rapid evolvability, and generalist grazing tendencies—make it particularly well-suited for changing conditions in Green Bay.

Green Bay is the largest and one of the most productive embayments in the Laurentian Great Lakes (Bertand et al. 1976). There exists a strong trophic gradient from the southern end of the bay near the mouth of the Fox River to the northern opening of the bay into Lake Michigan. The southern section is most eutrophic, with high phytoplankton growth. The middle and upper bay are more diluted with increasing distance from the river (Richman et al. 1984).

The zebra mussel, *Dressenia polymorpha*, which was introduced to the Laurentian Great Lakes in the 1980s, has dramatically changed the Green Bay ecosystem (Vanderploeg et al 2002*)*. *Dressenia polymorpha* is able to filter large volumes of water, resulting immediately in increased water clarity and decreased algal abundance (MacIsaac et al 1992, Lavrentyev et al 1995). Following the zebra mussel invasion of the Great Lakes, this trophic gradient from the inner bay to the outer bay has not changed. However, the phytoplankton community structure in Green Bay has significantly shifted. Most notably, cyanobacteria, or blue-green algae, now dominates with *Microcystis* being the most dominant phytoplankton taxon in the summer (DeStasio et al. 2014). *Microcystis* is known to form large-scale blooms that have large impacts on aquatic communities. *Microcystis* secretes a noxious compound, microcystin, which may kill aquatic organisms or become concentrated in top predators of an ecosystem through trophic transfer and bioconcentration (Smayda 1977).

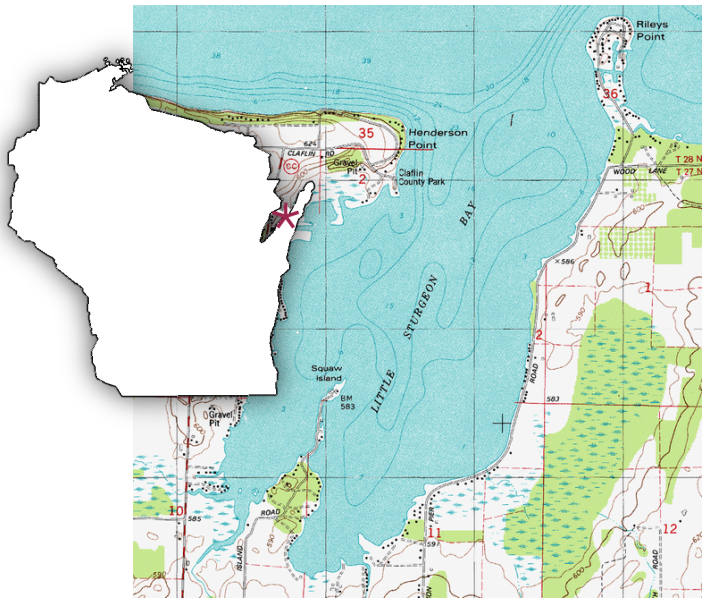


Figure 8. Left: the state of Wisconsin with a star designating the approximate location of Little Sturgeon Bay. Right: map of Little Sturgeon Bay

Little Sturgeon Bay

Little Sturgeon Bay, a small embayment located about halfway up the eastern enclosure of Green Bay, is the study site and is in the midst of the ecological shifts discussed above (Figure 8). Little Sturgeon Bay is approximately 1 mile long and 0.5 mile wide with a maximum depth of ~4.5 meters. The majority of the shoreline is undeveloped or residential, though there is a state park located near the western shore of the mouth of the bay. The near-shore habitat varies from rocky on the eastern side to weedy on the western side (personal observation).

Importance and Purpose of Study

Given the recent ecological changes in Green Bay—the invasion of zebra mussels and shift to cyanobacteria dominance in the phytoplankton community—it is vital that we understand how the ecosystem is changing. Changes to phytoplankton community structure most drastically and immediately affect zooplankton, since zooplankton consume phytoplankton. Negative changes in zooplankton populations may consequently impact the fish that consume them, resulting in potential economic hardships for humans.

Eurytemora affinis is of particular interest for Green Bay since it has proven to be a particularly versatile, robust copepod in its success as an invader. In addition, *E. affinis* is able to successfully feed on and in the presence of cyanobacteria (Koski et al. 1999). Considering the recent shift in Green Bay towards cyanobacteria dominance*, E. affinis* will certainly have a competitive advantage over other zooplankton that are less successful in the presence of cyanobacteria. However, differences in predation pressure could offset those advantages. Understanding how *E. affinis* survives in the face of

predation pressure therefore is critical for understanding recent and potential future changes in the zooplankton communities.

This study investigates the DVM of *E. affinis* in Little Sturgeon Bay. In particular, I ask whether *E. affinis* performs DVM in both offshore and near shore habitats. In addition, I assess whether life-stage affects predation risk through analysis of the visual susceptibility of life-stages of *E. affinis*. If there are differing predation risks associated with life-stage, I predict that more susceptible life-stage groups will perform DVM with greater magnitude than less susceptible life-stage groups.

By studying the migration of *E. affinis*, we may better understand how it is adapting to its non-native environment in Green Bay. Because migration is an adaptive response to the pressures that an ecosystem poses to zooplankton, better understanding of the migration helps us to comprehend the impact that biotic and abiotic drivers have on the survival of *E. affinis*.

Methods

Field Methods

A variety of methods were used to obtain information about zooplankton distribution and their vertical environment in Little Sturgeon Bay. Physical & chemical data, zooplankton, and chlorophyll samples were collected on 15 Jul, 24 Jul, 07 Aug, and 14 Aug 2014. Zooplankton and chlorophyll samples were collected twice on each sampling date: once around 12:00 and again around 22:00. Physical & chemical data readings were taken once at noon each sampling day.

Figure 9. A map of Little Sturgeon Bay with letters designating approximate locations of sampling sites. A) LS-Dock (44.8438 °N, 87.5592 °W), nearshore sampling site and B) LS-E (44.8427 °N, 87.5452 °W), offshore sampling site.

Two sites, one near shore and one offshore, were selected as sampling locations. The first site, LS-E, is located near the middle of the bay (Figure 9; 44.8427 °N, 87.5452 °W). The second site, LS-Dock, is near shore (Figure 9; 44.8438 °N, 87.5592 °W). At LS-E, zooplankton samples, chlorophyll samples, and physical & chemical data was taken at one-meter intervals through the water column, which was approximately 4m deep. At LS-Dock, only zooplankton samples were collected. On 15 Jul and 24 Jul samples were collected at 0m, just below the surface of the water. On 07 Aug and 14 Aug, samples were taken at 0m and at 2m, near the benthos.

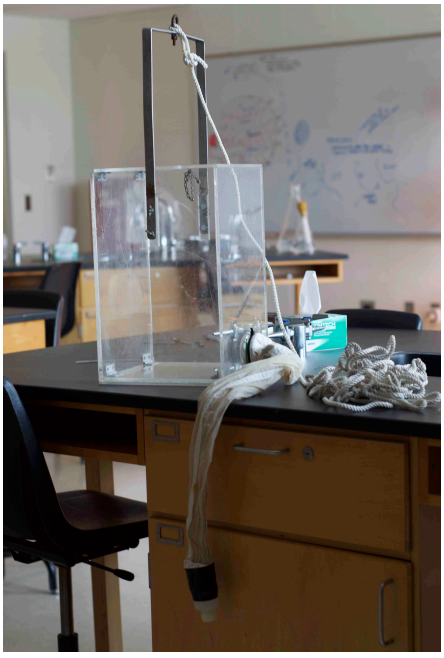


Figure 10. Schindler Trap

Zooplankton samples were collected using a Schindler trap with a 60um mesh (Figure 10). Three samples were obtained at each depth in descending order (0m first, 4m last) as to avoid mixing the water column. Samples were stored in 500 mL plastic Nalgene bottles, labeled, and preserved in a 4% formaldehyde solution.

Chlorophyll samples were collected using a water pump at 1m depth intervals at LS-E. Samples were obtained at both sampling times (noon and midnight) for all four days. Water was collected in 4L opaque jugs and promptly placed on ice. Water samples were kept refrigerated in the lab until chlorophyll analyses could be performed no later than the day following collection.

Physical & chemical data for each 1m depth interval at LS-E were obtained at noon on each sampling day. A Hydrolab Multisonde 5 was used to measure temperature, dissolved oxygen (DO, both mg/L and %saturation), pH, conductivity, oxidative-reductive potential (ORP), and total dissolved solids (TDS). A Li-Cor Model 1000 light meter with both air and underwater 2-π flat quantum sensors was used to measure light penetration for 15 July 2014, 24 July 2014, and 14 August 2014. A Secchi disk (0.20 m diameter) was used in its place on 07 August to measure the depth of light penetration.

Table 2. Summary of sampling methodology across dates and sites. Sample types: z = zooplankton, p= phytoplankton, h = hydrolab (physical/chemical data) l = light meter, s = secchi disk

	LS-E										LS-Dock			
	0m		1m		2m		3m		4m		0m		2m	
	Day	Night	D	N	D	N	D	N	D	N	D	N	D	N
15 Jul 2014	z p h l	z p	z p h l	z p	z p h l	z p	z p h l	z p	z p h l	z p	z	z		
24 Jul 2014	z p h l	z p		z p		z p		z p		z p	z	z		
07 Aug 2014	z p h s	z p		z p		z p		z p		z p	z	z	z	z
14 Aug 2014	z p h l	z p		z p		z p		z p		z p	z	z	z	z

Lab methods

Chlorophyll-a Analysis: In laboratory, Chl-*a* concentration for each depth sample was measured using acetone extraction protocol (Wetzel & Likens 1991). Replicate water samples from one-meter intervals were filtered onto GF/C filter paper, ground using a mortar and pestle, and extracted in 90% alkaline acetone. The resulting liquid-pulp mixture was centrifuged to separate particulate matter. A spectrophotometer was then used to determine absorbance at the Chl-*a* wavelength.

Counting zooplankton: A Folsom sample splitter was used to subsample zooplankton samples in the laboratory, resulting in half or quarter of the total sample (Figure 11). The decision of how many times to split the sample was made based on the apparent density of the sample—denser samples were subsampled twice while sparse samples were split once. The sample half or quarter was filtered using an 80 micrometer mesh cup and rinsed with water. The filtration residue was rinsed into a circular Ward counting wheel, and observed using a dissecting microscope. Two subsamples were counted from each jar. The following categories of zooplankton were counted in each sample:

Figure 11. Folsom sample splitter

E. affinis……..	Male Female Female with eggs Copepodite
Leptodora	
Bythotrephes longimanus/Cercopagis p	
Mesocyclops edax	
Skistodiaptomus	
Leptodiaptomus	

E. affinis sexing and life-stage determination: While species identification is relatively straightforward for zooplankton, gender and life-stage determination is more difficult and time consuming. For the purpose of consistency, a series of guidelines were followed to place *Eurytemora* into appropriate life-stage and gender categories (Figure 12; Balcer et al. 1984).

Adult male:	Hooked antennae Enlarged 5th leg
Adult female:	Metasomal wings Urosome usually bent
Adult female + eggs:	Shows all adult female characteristics Carrying two egg sacs attached laterally to urosome
Copepodite:	Shows no sexual characteristics, (hooked antenna, metasomal wings), but shows species characteristics

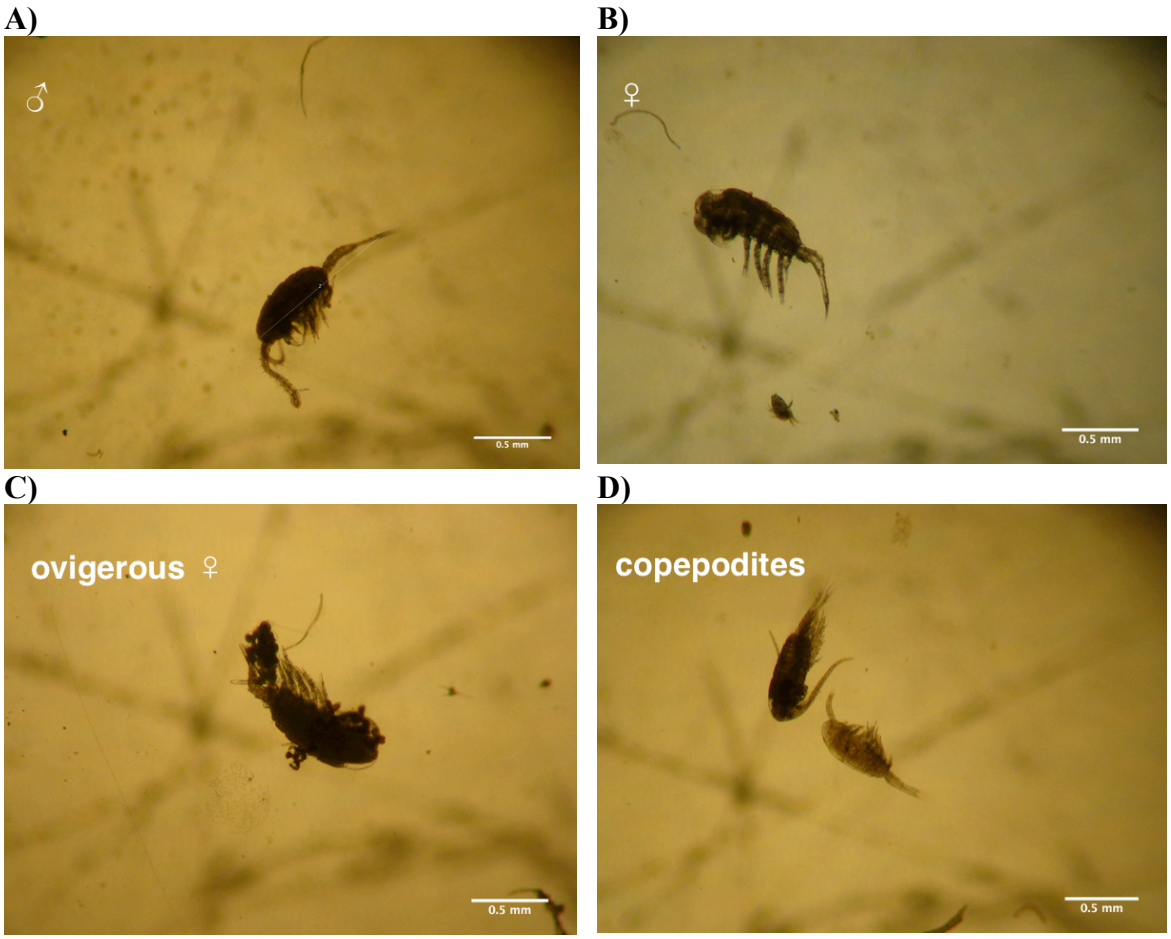


Figure 12. *E. affinis* life-stage groups A) adult male B) adult female C) adult ovigerous female D) copepodite

Image Capture: In order to determine whether there were significant size and darkness differences between sexes and life-stages of *E. affinis*, microscopic images were taken and analyzed. At least 30 organisms of each sex and life-stage were isolated from zooplankton samples, separated into petri dishes, and photographed. A Sony Handycam Model HDR-HC9 camcorder was mounted onto the eyepiece of a dissecting microscope (Figure 13). In an attempt to control for consistent lighting, images were taken with consistent room lighting and the light intensity of the microscope was turned to the same level for each photo. To control for appropriate scale, zoom settings on both the camera and the microscope were kept consistent throughout the photography process. Finally, a stage micrometer was photographed prior to the copepods to calibrate the image measurements.

Figure 13. Sony Handycam HDR-HC9 camcorder mounted to a dissecting microscope

Image Analysis: ImageJ 1.48v was used to analyze images of copepods (ImageJ, Rasband 1997). Each copepod was analyzed for length, area, and mean gray value. The length of each copepod was defined as the distance between the anterior end (near the eye) to the tip of the caudal ramus, excluding caudal setae (Figure 14). The area of each copepod was determined to be the entire body excluding antennae, legs, and caudal rami (Figure 14). Metasomal wings on females and egg sacs on oviparous females were included in area measurements. Mean gray value was determined using the same area outline. Mean gray value is defined as the "sum of the gray values of all the pixels in the selection divided by the number of pixels." It is determined in color photos by converting

each pixel to grayscale using the following formula: gray = (0.299*red) + (0.587*green) + (0.114*blue) (Ferreira & Rashbad 2010).

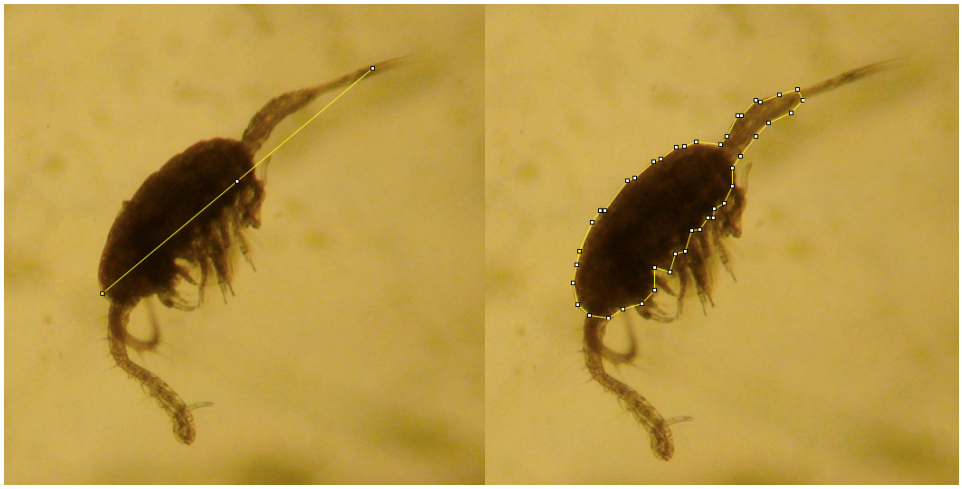


Figure 14. Length (left) and area measurements performed using imageJ

Data analysis

ImageJ was used to analyze photographs of copepods. Microsoft excel was used to perform transformations on data sets and create physical and biological profiles. The PAleontological STastics software program (PAST, Hammer et al. 2001) was used to create histograms, boxplots, and perform Pearson correlation, Analysis of Variance (ANOVA), Welch F, Levene's, and the Mann-Whitney pairwise test.

Physical quality comparison: Length, area, and mean gray value (MGV) were calculated separately for each animal photographed. Mean gray value was first converted to proportion darkness (for MGV, 0=black and 255=white) using darkness = (255-MGV)/255) and then transformed to arcsine(darkness). Histograms were compiled for each physical quality of each copepod group (*E. affinis* males, females, females carrying eggs, and copepodites) to test for normality of distributions. Based on the normality of histogram distributions, it was determined that parametric statistical tests were

appropriate. Average length, area, and arcsine(darkness) was calculated for each copepod group. An ANOVA was used to determine whether copepod group membership had a significant effect on physical qualities. If there was homogeneity of variance between copepod groups, Tukey's Pairwise comparison tests were used to determine which copepod groups were significantly different from the rest.

Vertical Distribution and DVM: Vertical distribution of zooplankton was described by calculating average organisms per liter of each species and life history stage at each depth. At each depth, either two or three zooplankton samples were collected on each day. Each sample was subsampled twice and counted as described above, resulting in four or six calculated zooplankton densities per depth. These densities were averaged, then corrected for volume to determine the number of organisms per liter at the given depth. The resulting data describes how the densities of zooplankton vary throughout the water column.

In addition to creating vertical profiles of zooplankton abundance, weighted mean depth (WMD) was calculated to more succinctly describe migration. WMD takes into account the abundances of zooplankton across all depths to calculate a mean depth for a population at a given time. A useful way to think about WMD is that it attempts to identify the "center" of a population at a given time. Tracking the diel changes in a zooplankton population's "center" gives us a good understanding of a population-wide migration. WMD is calculated using the following equation taken from Bollens and Frost 1989:

$$WMD = \frac{\sum n_i d_i}{\sum n_i}$$

$$n_i = abundance\ \left(^{\#}/_{L}\right)\ at\ depth\ interval\ i$$
$$d_i = midpoint\ of\ depth\ interval\ i$$

The difference in WMD between night and day samples, or ΔWMD, represents the direction and amplitude of migration. A positive ΔWMD signifies a negatively phototactic migration in which zooplankton move away from the sun during the day and to shallower depths at night. A ΔWMD value with a large magnitude represents a migration with large amplitude.

Results

Assessing Visual Predation Risk via Image Analysis

Analysis of photographs of *E. affinis* using ImageJ revealed that copepod length and area varied based on life-stage, but there was no difference in darkness among life-stage groups.

Copepod length differed between life-stage groups (Figure 15). Visual assessment of length histograms reveals fairly symmetrical distributions of length within each life-stage group (Appendix A1). However, Levene's test confirmed that variances were not homogeneous between groups (p=0.0127). Overall, there was a significant effect of life-stage on animal length based on the Welch F test ($F_{153,3}$=51.15 , p=2.64E-18). Females carrying eggs were significantly longer than all other stages, while males and females were longer than copepodites but not significantly different than each other (Mann-Whitney pairwise comparison test, P<0.01).

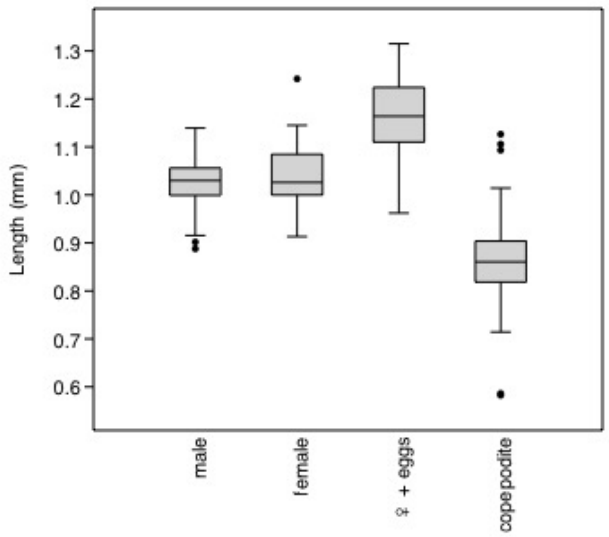


Figure 15. Boxplot comparing mean lengths of different *E. affinis* life-stage groups. Box represents the Interquartile Range (IQR), whiskers represent 1.5 times the IQR, and dots represent outliers. A Welch F test revealed a significant effect of life stage on copepod length (p=2.64E-18). Mann-Whitney comparison revealed significant differences between all life-stage groups except between adult males and females.

Similar to length, copepod area also differed between life-stage groups (Figure 16). Distributions of visual area of each life-stage are less symmetrical than copepod lengths, and variances are more heterogeneous (Appendix A2; Levene's test for homogeneity of variance, p=6.001E-06). Regardless, the Welch F test confirmed that there exists a significant effect of life-stage on area ($F_{144,3}$=136.3, p=1.556E-26). Visual area was significantly greatest for females carrying eggs, followed by females, males and then copepodites. Mann-Whitney pairwise comparisons indicated that there are significant differences between each life-stage group (P<0.01).

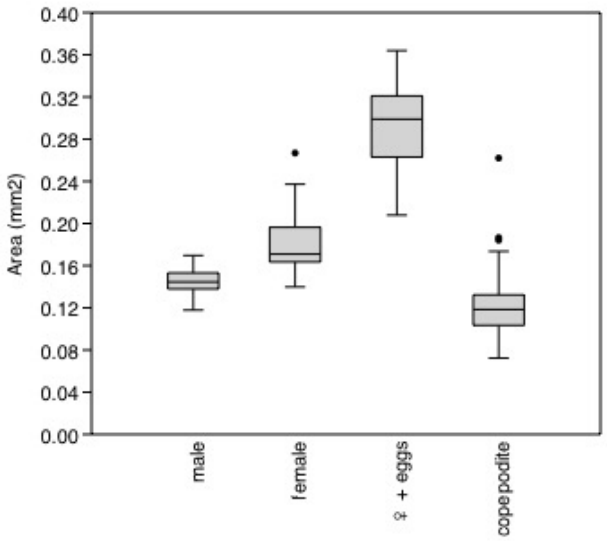


Figure 16. Boxplot comparing mean areas of different *E. affinis* life-stage groups. A Welch F test revealed a significant effect of life stage on copepod area (p=1.56E-26). Mann-Whitney comparison revealed significant differences between all life-stage groups.

Unlike length and area, copepod darkness does not differ between life-stage groups (Figure 17). Despite performing the arcsine transformation on percent darkness measurements, distributions of darkness measurements are not all symmetrical, and variances are heterogeneous (Appendix A3; Levene's test for homogeneity of variance,

p=0.006194). The Welch F test was unable to confirm an effect of life-stage on darkness ($F_{144,3}$=0.7856, p=0.5055).

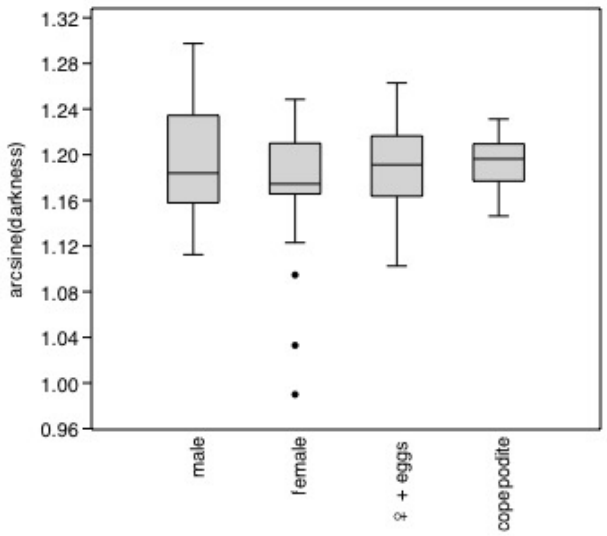


Figure 17. Boxplot comparing mean darkness values of different *E. affinis* life-stage groups. Darkness values are the arcsine(proportion darkness) calculated from mean gray value (MGV) using ImageJ. A Welch F test revealed no significant effect of life stage on copepod darkness (p=0.5055).

Offshore Conditions and Migration

Vertical Environmental Gradient: Physical, chemical and chlorophyll data taken at one-meter intervals offshore for each sampling date reveal a variety of vertical environmental gradients along the water column. Measurements such as light penetration, chlorophyll abundance, and temperature varied enough to create unique vertical environments on each sampling date.

Light Penetration: Incident light penetration measurements were variable across sampling dates, meaning that water transparency varied across sampling dates. Greatest incident light penetration was seen on 7 August 2014, meaning that the water was

clearest. The least incident light penetration was seen on the day when Little Sturgeon Bay was least transparent, 15 July 2014 (Figure 18).

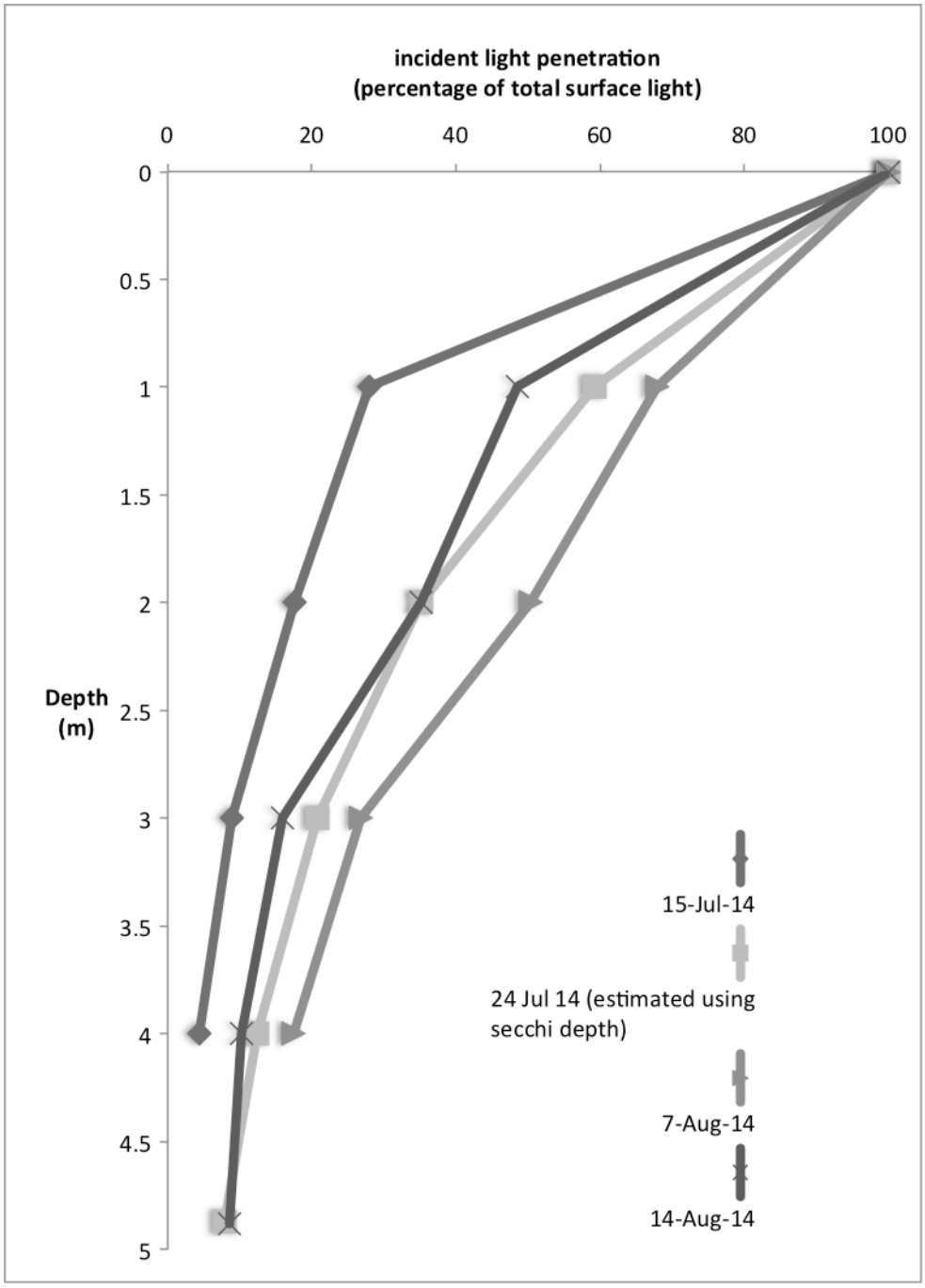


Figure 18. Incident light penetration (percentage of total surface light) in Little Sturgeon Bay across four sampling dates. 24 July 2014 incident light penetration was estimated using a Secchi depth measurement.

Temperature and Oxygen Conditions: There was a clear association between temperature and dissolved oxygen structure on each date. Temperature is the primary driver of density in freshwater lakes; therefore, thermal structure often affects chemical characteristics of the water, like dissolved oxygen.

15 July 2014: On July 15, Little Sturgeon Bay was isothermal in terms of both temperature and dissolved oxygen (DO). Water temperature hovered around 20 ºC all the way to the bottom, while DO ranged between 6 mg/L near the surface and 7 mg/L near the bottom (Figure 19A). Chl-*a* abundance peaked at 2m during both sampling times, but was overall more abundant during the day than at night (Figure 20A).

24 July 2014: Unlike on the first sampling date, Little Sturgeon Bay was stratified in terms of temperature and DO on July 24. Temperature readings began at 22 ºC at the surface of the lake. The thermocline was located somewhere between 2 and 3 m where the temperature dropped nearly 10 ºC in the span of one meter. Near the bottom of the bay, temperatures hovered around 8 ºC. The stratification trend was opposite for DO, which was 6 mg/L near the surface, then rapidly increased between 2 and 3m, and was nearly 9 mg/L near the bottom (Figure 19B).

Similar to July 15, Chl-*a* abundance peaked around 2m during both sampling times, with chlorophyll being more abundant during the day than at night. During the day, however, there was a second chlorophyll peak at 0m (Figure 20B).

07 August 2014: Temperature at the surface of the bay was 22 ºC and decreased slightly and steadily until the bottom, reaching a minimum temperature of 18 ºC. DO was highest in the first meter of the water column—beginning at 8 mg/L at 0m and peaking at

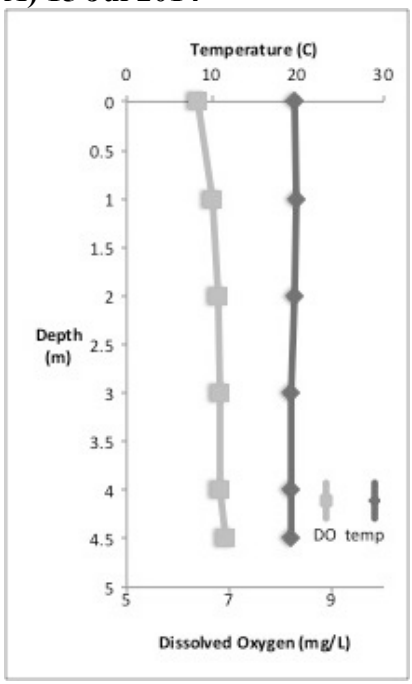
9 mg/L at 1m. DO levels sharply declined to 6 mg/L at 2m and hovered between 6 and 7 mg/L until the bottom (Figure 19C).

In comparison to the previous two sampling dates, Chl-*a* levels were much lower throughout the entire water column, ranging between 2 and 6 ug/L. During the day, Chl-*a* was most abundant at 4m, near the bottom of the bay. At night, however, Chl-*a* peaked between 2 and 3m (Figure 20C).

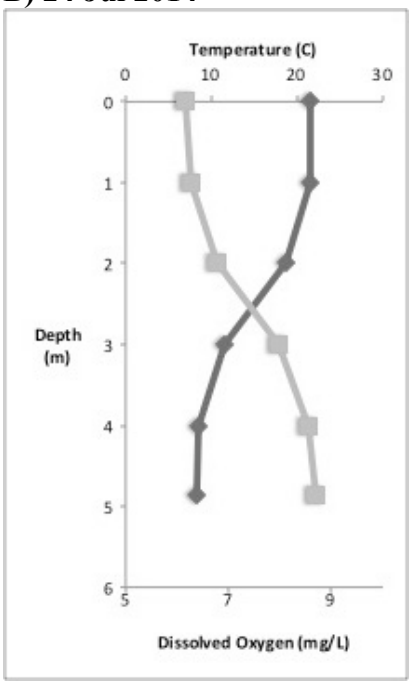
14 August 2014: Similar to the first sampling date, both temperature and DO were isothermal. The water was 20 ºC and the DO level ranged between 6 and 7 mg/L for the entire water column (Figure 19D).

Similarly, Chl-*a* levels were quite low, ranging between 1 and 5 ug/L, and fluctuated little between depths. However, the chlorophyll profile was opposite that of 07 Aug 2014. During the day, Chl-*a* was most abundant between 0 and 2m. At night, Chl-*a* levels peaked at 3m (Figure 20D).

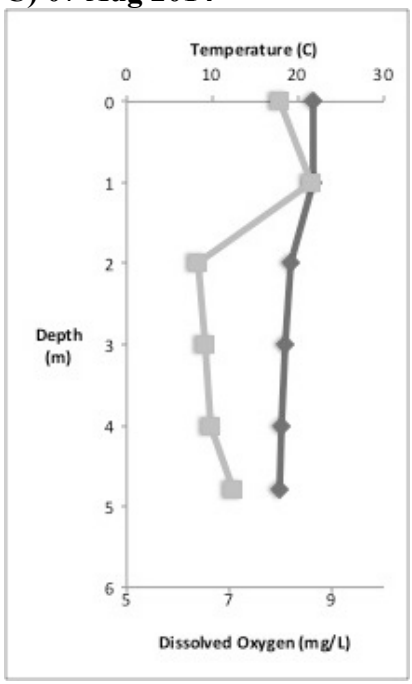
A) 15 Jul 2014



B) 24 Jul 2014



C) 07 Aug 2014



D) 14 Aug 2014

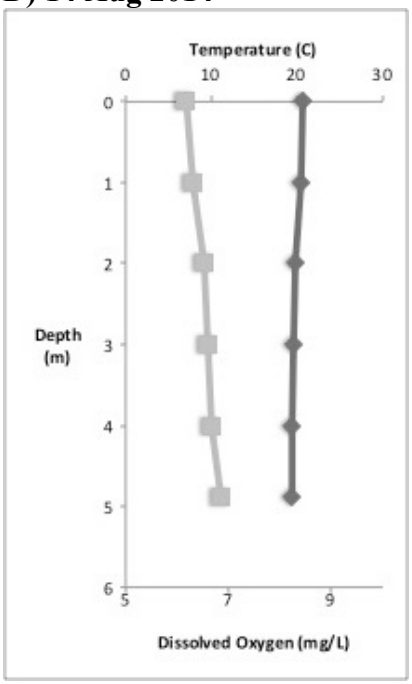
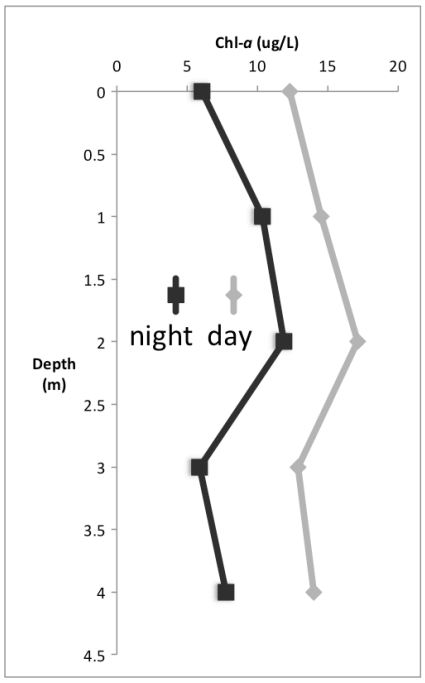
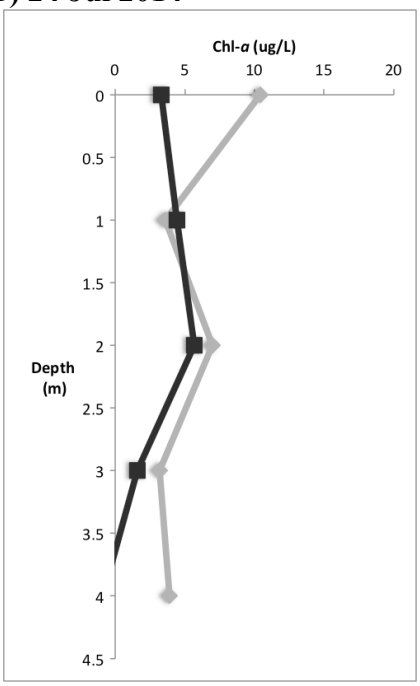


Figure 19. Temperature and Dissolved Oxygen profiles for Little Sturgeon Bay across four sampling dates (A) 15 July 2014 B) 24 July 2014 C) 07 August 2014 D) 14 August 2014)

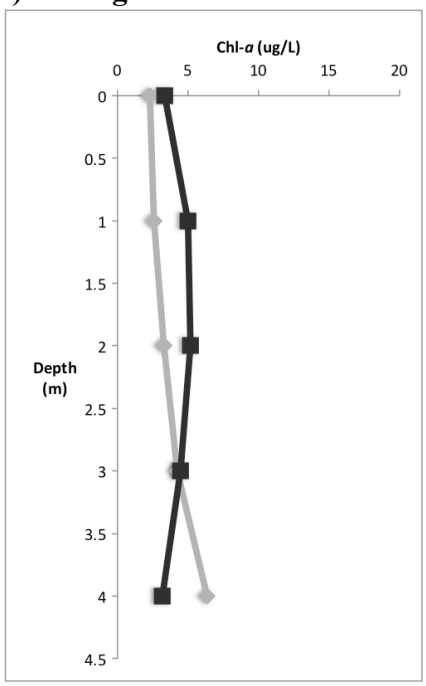
A) 15 Jul 2014



B) 24 Jul 2014



C) 07 Aug 2014



D) 14 Aug 2014

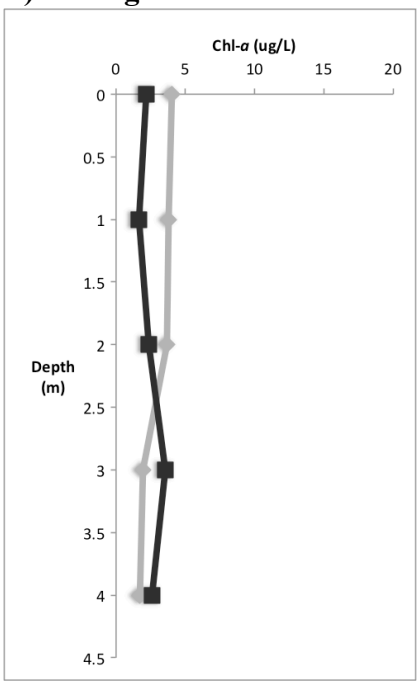
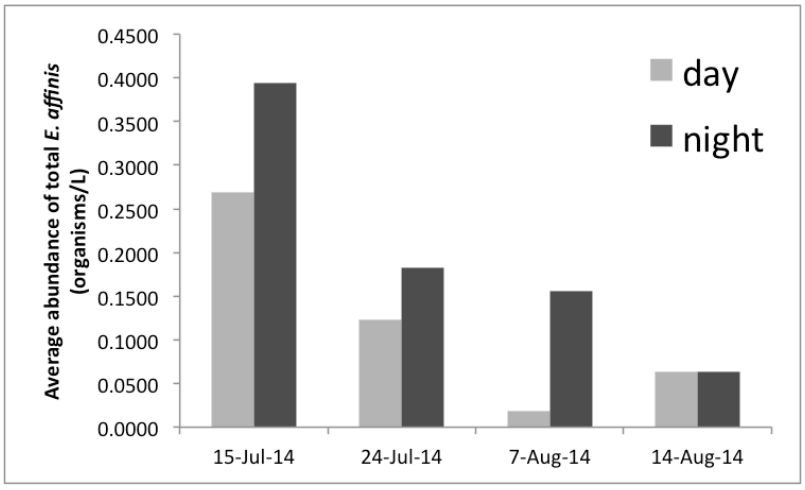


Figure 20. Chlorophyll *a* profiles for Little Sturgeon Bay during the day and at night across four sampling dates (A) 15 July 2014 B) 24 July 2014 C) 07 August 2014 D) 14 August 2014)

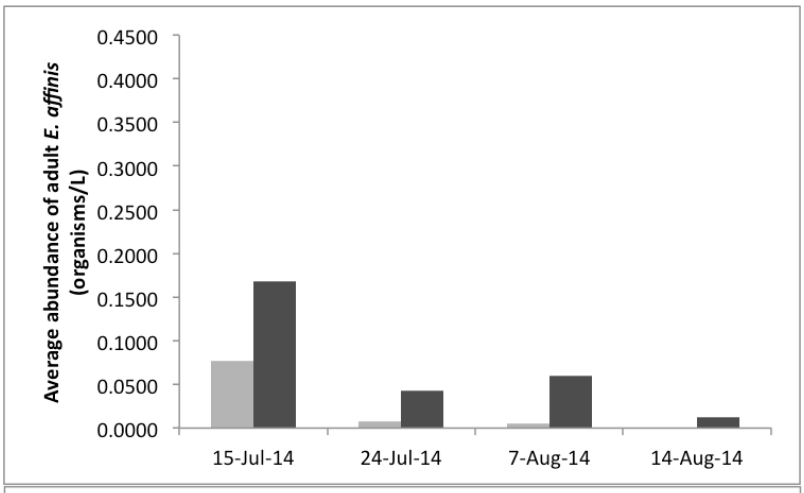
Offshore Vertical Distributions of E. affinis: For every date except 14 August 2014, *E. affinis* had a higher average abundance in the water column at night than during the day (Appendix A4; Figure 21A). However, there was little diel difference in zooplankton depth profiles, especially for 15 July 2014 and 24 July 2014 (Figure 22A, B). For these two sampling dates, *E. affinis* total abundance peaked at 2 and 4m during the day and at night. On 07 August 2014, *E. affinis* was most abundant at 4m during the day, but at 1m at night. In contrast, peak abundances switched on 14 August 2014, such that *E. affinis* was most abundant at 1m during the day and 4m at night.

Life-Stage Differences in DVM Offshore: The diel difference in average abundance of adult *E. affinis* was greater than the diel difference in average copepodite abundance (Appendix A4; Figure 21B, C). While copepodites were present at similar abundances in the water column during the day and the night (Figure 21C; Figure 23), adults were often absent or present in low abundance during the day and present in higher abundances at night (Figure 21B; Figure 23).

A)



B)



C)

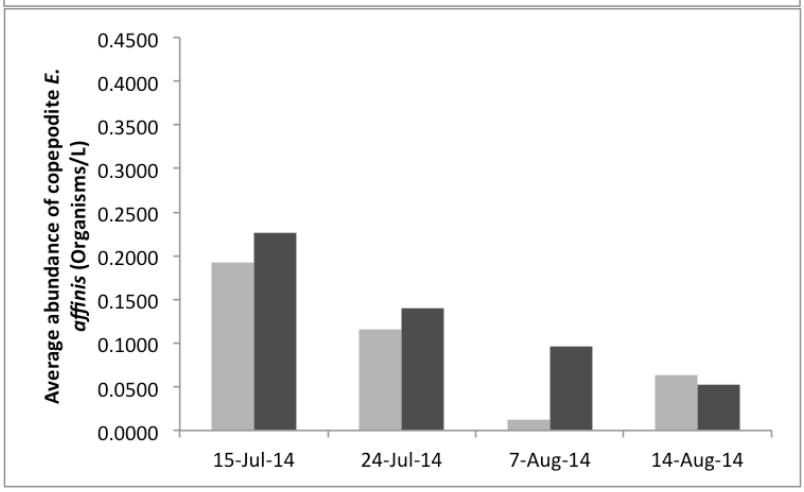


Figure 21. Average abundances (average of copepod abundances across 5 sampling depths) of A) total, B) adult, and C) copepodite *E. affinis* in Little Sturgeon Bay.

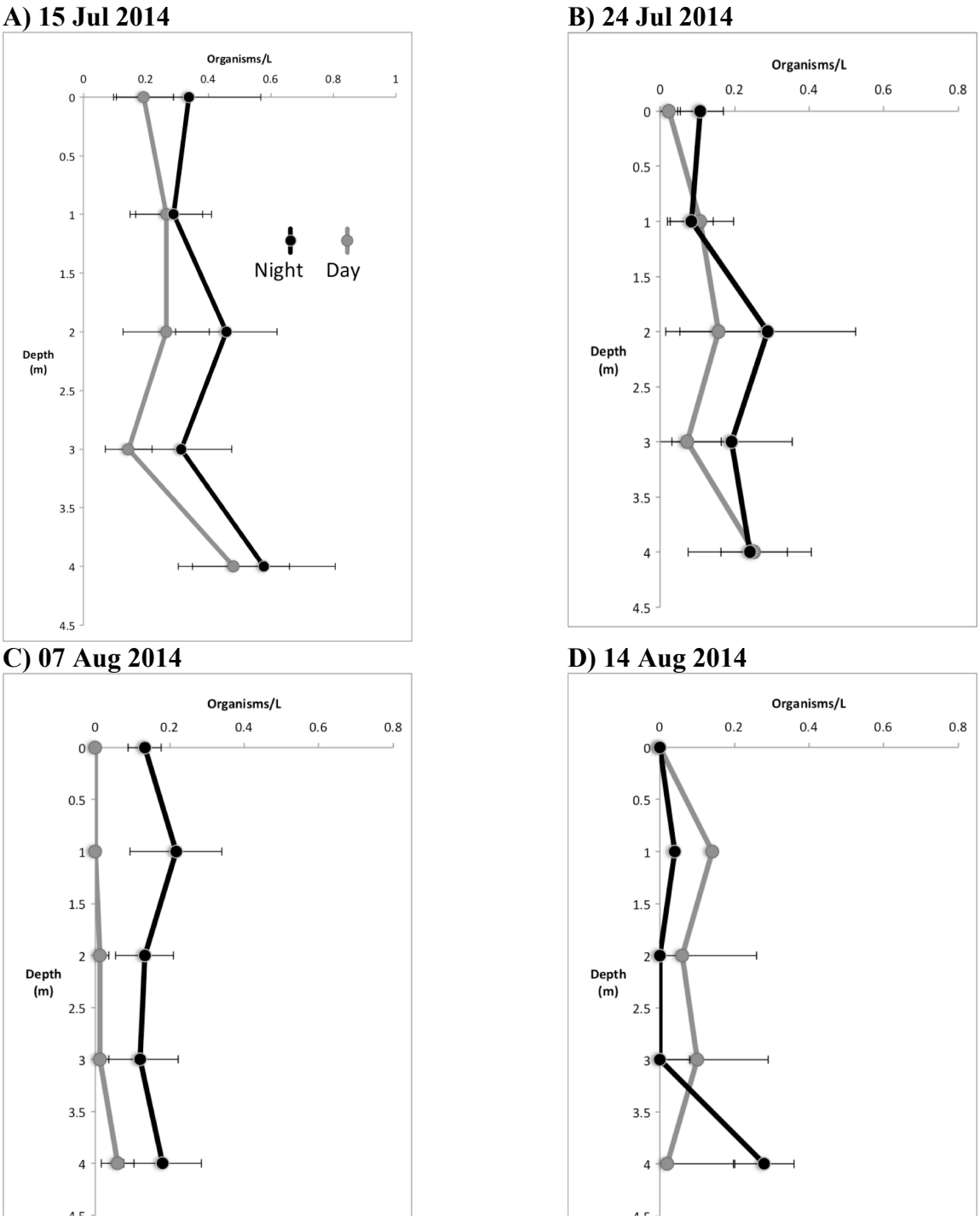


Figure 22. Depth profiles of total abundance of *E. affinis* in Little Sturgeon Bay during the day and at night for four sampling dates. Error bars represent ±1SEM.

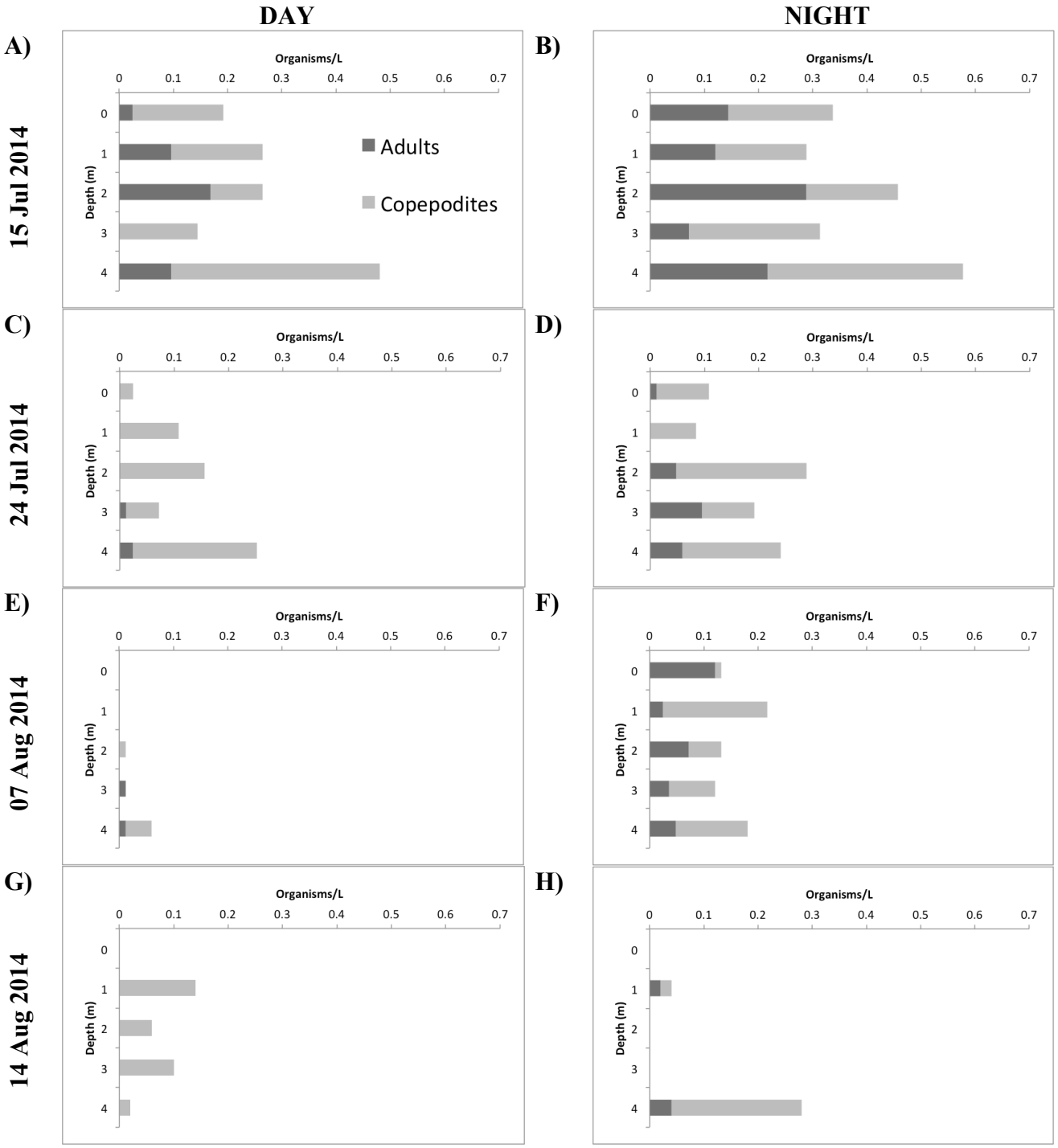
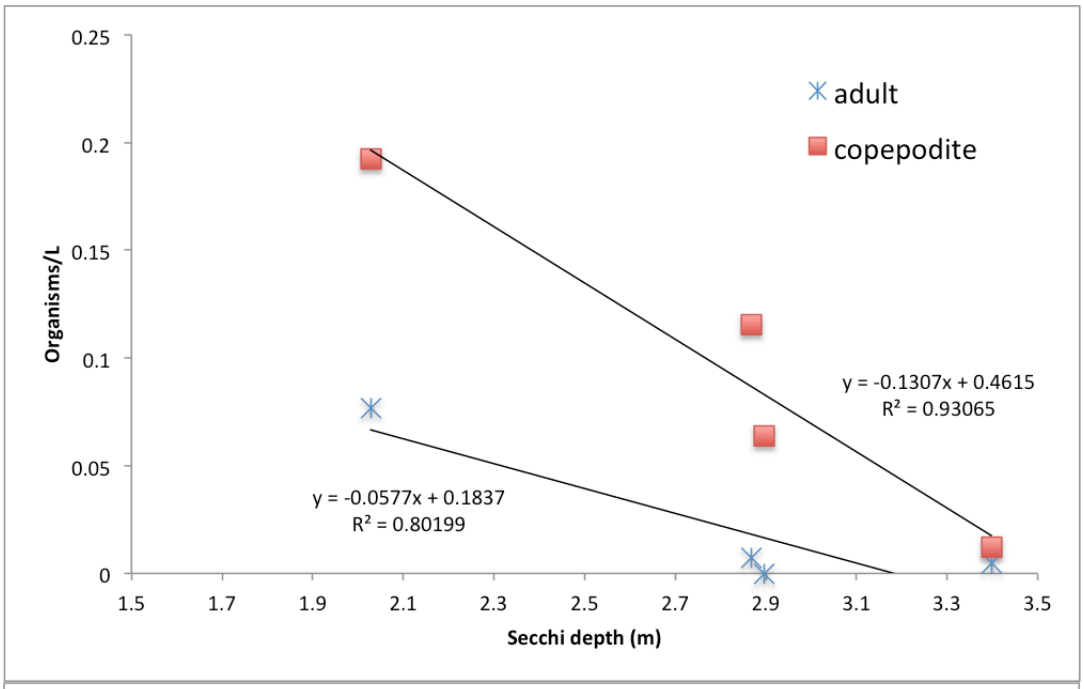


Figure 23. Depth profiles of abundance for *E. affinis* adults and copepodites during the day and at night for four sampling dates. 15 July 2014: A) day B) night, 24 July 2014: C) day D) night, 07 August 2014: E) day F) night, 14 August 2014: G) day H) night.

Correlation Between Water Transparency and Species Density: During the day, there was a strong, negative (but non-significant) correlation between water transparency and average adult density (r=-0.90, n=4, p=0.10) and a significant negative correlation between water transparency and average copepodite density (r=-0.96, n=4, p=0.04) (Figure 24A). At night, there were negative relationships between water transparency and adult density (r=-0.77, n=4, p=0.23) and between water transparency and average copepodite density (r=-0.80, n=4, p=0.20) (Figure 24B).

Examination of the best-fit lines reveals differing sensitivities of copepod densities to water transparency. During the day, copepodite density was more sensitive to water transparency than adult density, as evidenced by higher magnitude of the slope of the line of best fit (slope(day, adults)=-0.0577; slope(day, copepodites)=-0.1307) (Figure 24A). At night, copepodites were overall more abundant than adults regardless of water transparency, as evidenced by the higher intercept in the line of best fit (y-intercept(copepodite, day)=0.4214; y-intercept(adult, day)=0.329). However, the slopes of the lines of best fit were nearly identical, suggesting that adults and copepodites were equally sensitive to water transparency at night (slope(night, adults)=-0.0923; slope(night, copepodites)=-0.1047) (Figure 24B).

A)



B)

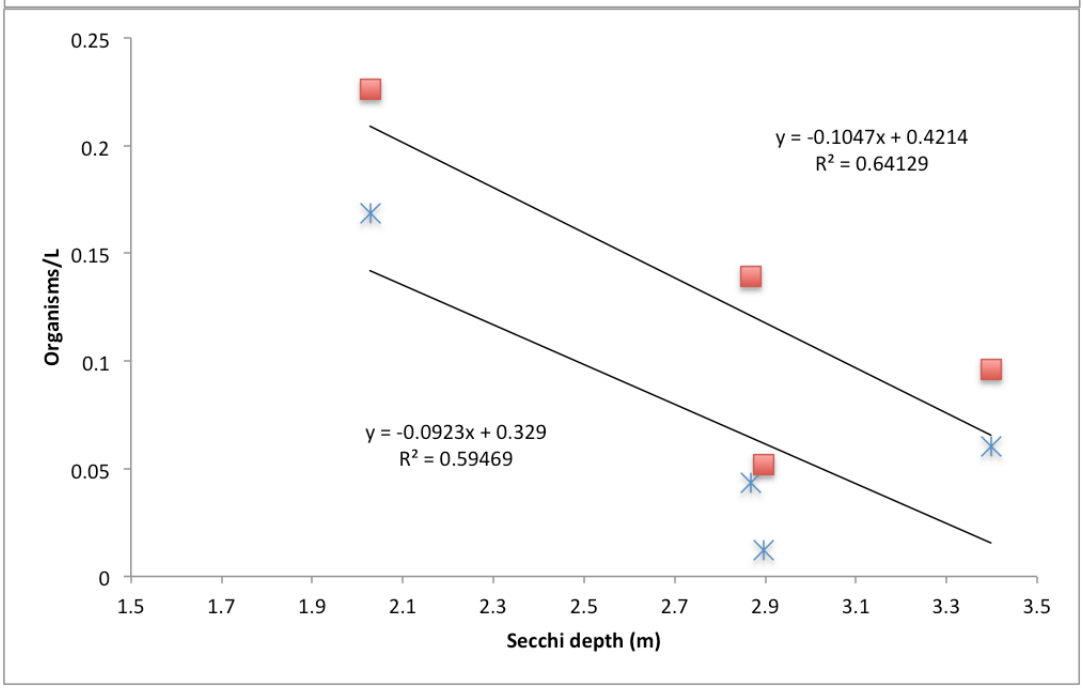
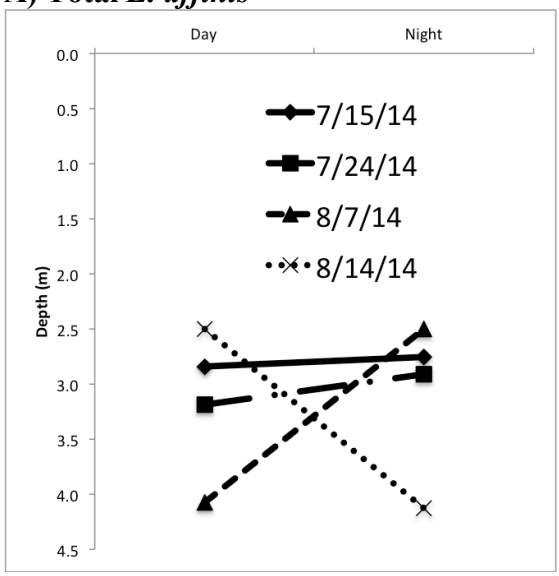


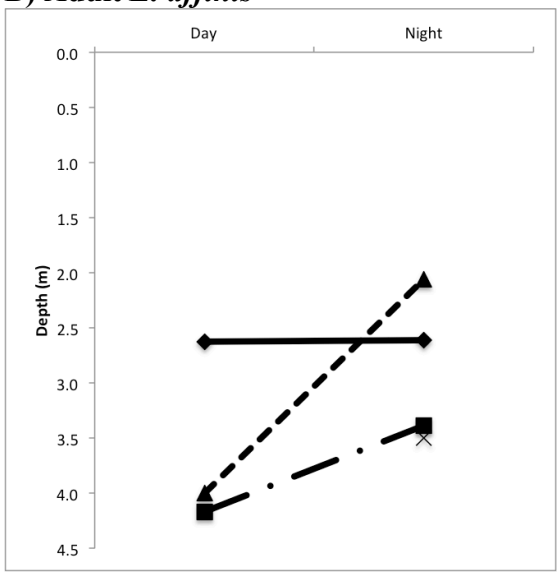
Figure 24. Relationship between water transparency (described by Secchi depth) and *E. affinis* adult and copepodite average densities in the water column during the day (A) and at night (B). Equations represent lines of best fit and R^2 represent goodness-of-fit assessments. Pearson's r(adults, day)= -0.90; r(copepodites, day)=-0.96; r(adults, night)=-0.77; r(copepodites, night)=-0.80.

Describing Migration Offshore: Assessment of diel changes in weighted mean depth (WMD) indicates that *E. affinis* performed diel vertical migrations, albeit small ones, at the offshore site on each day sampled. Each migration recorded was negatively phototactic (moving down, away from light, during the day and up at night) except on 14 August 2014, which was positively phototactic (Figure 25A). Adults tended to exhibit a more pronounced migration than copepodites, having larger changes in weighted mean depth (ΔWMD) than copepodites on 24 July 2014 and 07 August 2014 (Figure 25B, C; Table 3). Because no adults were observed in the water column during the day on 14 August 2014, it is not possible to calculate a change in WMD. This absence is presumably due to adults undergoing extreme DVM on that date since they were observed in the water less than 12 hours later during the night sampling (Figure 23H, Table 3)

A) Total *E. affinis*



B) Adult *E. affinis*



C) Copepodite *E. affinis*

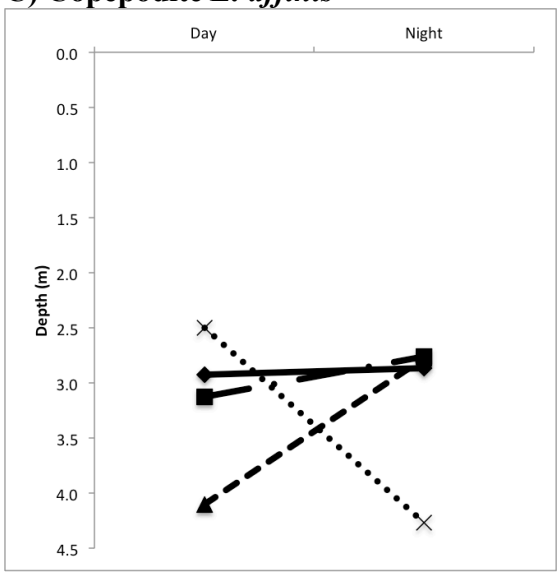


Figure 25. Diel changes in calculated Weighted Mean Depth (WMD) offshore for total (A), adult (B), and copepodite (C) *E. affinis* across four sampling dates

Table 3. Weighted Mean Depths (WMD) for total *E. affinis*, adults, and copepodites during the day and at night. ΔWMD represents the change in WMD between sampling times. Positive ΔWMD signifies a positively phototactic migration.

Total *E. affinis*	**7/15/14**	**7/24/14**	**8/7/14**	**8/14/14**
WMD (Day)	2.839	3.186	4.071	2.500
WMD (Night)	2.756	2.908	2.500	4.125
ΔWMD	0.083	0.278	1.571	-1.625
Adults				
Day	2.625	4.167	4.000	--
Night	2.614	3.389	2.060	3.500
ΔWMD	0.011	0.778	1.940	--
Copepodites				
Day	2.925	3.125	4.100	2.500
Night	2.862	2.759	2.775	4.269
ΔWMD	0.063	0.366	1.325	-1.769

Near Shore Distributions

Eurytemora affinis were generally more abundant near shore than at the offshore site (Appendix A4). For the first two sampling dates, near shore abundances were only measured at 0m. For the last two sampling dates, abundances were measured at both 0m and 2m. Since it is ineffective to calculate WMD when abundances are only known from one or two depths, I instead examined diel differences in abundances of *E. affinis* at each depth independently as an indication of near shore migration behavior.

The first two sampling dates both provide evidence that *E. affinis* is more abundant near the surface of the near shore site at night than during the day. On 15 July 2014 at 0m, *E. affinis* adults were significantly more abundant at night ($\bar{x}$=0.48, SD=0.30) than during the day ($\bar{x}$=0.15, SD=0.21) (p=0.03) (Figure 26A). Copepodites were also significantly more abundant at night ($\bar{x}$=1.18, SD=0.55) than during the day ($\bar{x}$=0.38, SD=0.48) at 0m (p=0.01) (Figure 26B). Similarly, on 24 July 2014, adults were significantly more abundant at night ($\bar{x}$=0.77, SD=0.54) than during the day ($\bar{x}$=0.02,

SD=0.04) at 0m (p=0.02) (Figure 26C). Finally, copepodites were also more abundant at night ($\bar{x}$=0.55, SD=0.29) than during the day ($\bar{x}$=0.17, SD=0.15) (p=0.02) (Figure 26D).

On 07 August 2014, *E. affinis* adults were significantly more abundant at both depths at night (0m, day: $\bar{x}$=0.20, SD=0.11; 0m, night: $\bar{x}$=3.42, SD=0.69; p<<0.01; 2m, day: $\bar{x}$=1.15, SD=0.85; 2m, night: $\bar{x}$=3.42, SD=0.62; p=0.006) (Figure 26E). However, the population distribution switched between day and night. A greater proportion of *E. affinis* adults were found at 2m during the day, but the population was split almost evenly between depths at night. Copepodites were significantly more abundant at 0m at night, but were more abundant at 2m during the day (0m, day: $\bar{x}$=1.70, SD=0.85; 0m, night: $\bar{x}$=6.40, SD=1.20; p<<0.01; 2m, day: $\bar{x}$=20.42, SD=3.63; 2m, night: $\bar{x}$=7.60, SD=2.15; p=0.002) (Figure 26F). During the day, a greater proportion of copepodites were found at 2m than at 0m. At night, similar to the adults, the population distribution of copepodites was split nearly evenly between depths.

On 14 August 2014, there were diel differences in copepod abundances for both copepodites and adults, but only one of these differences reached the level of significance. At 0m, adults were significantly more abundant in the water column at night ($\bar{x}$=2.12, SD=0.53) than during the day ($\bar{x}$=0.06, SD=0.08) (p=0.004) (Figure 26G). At 2m, adults had a higher mean abundance at night ($\bar{x}$=1.20, SD=1.17) than during the day ($\bar{x}$=0.24, SD=0.16). Similarly, copepodites were more abundant at 0m and 2m at night than during the day (0m, day: $\bar{x}$=0.40, SD=0.21; 0m, night: $\bar{x}$=0.76, SD=0.24; 2m, day: $\bar{x}$=0.36, SD=0.24; 2m, night: $\bar{x}$=0.74, SD=0.62) (Figure 26G, H).

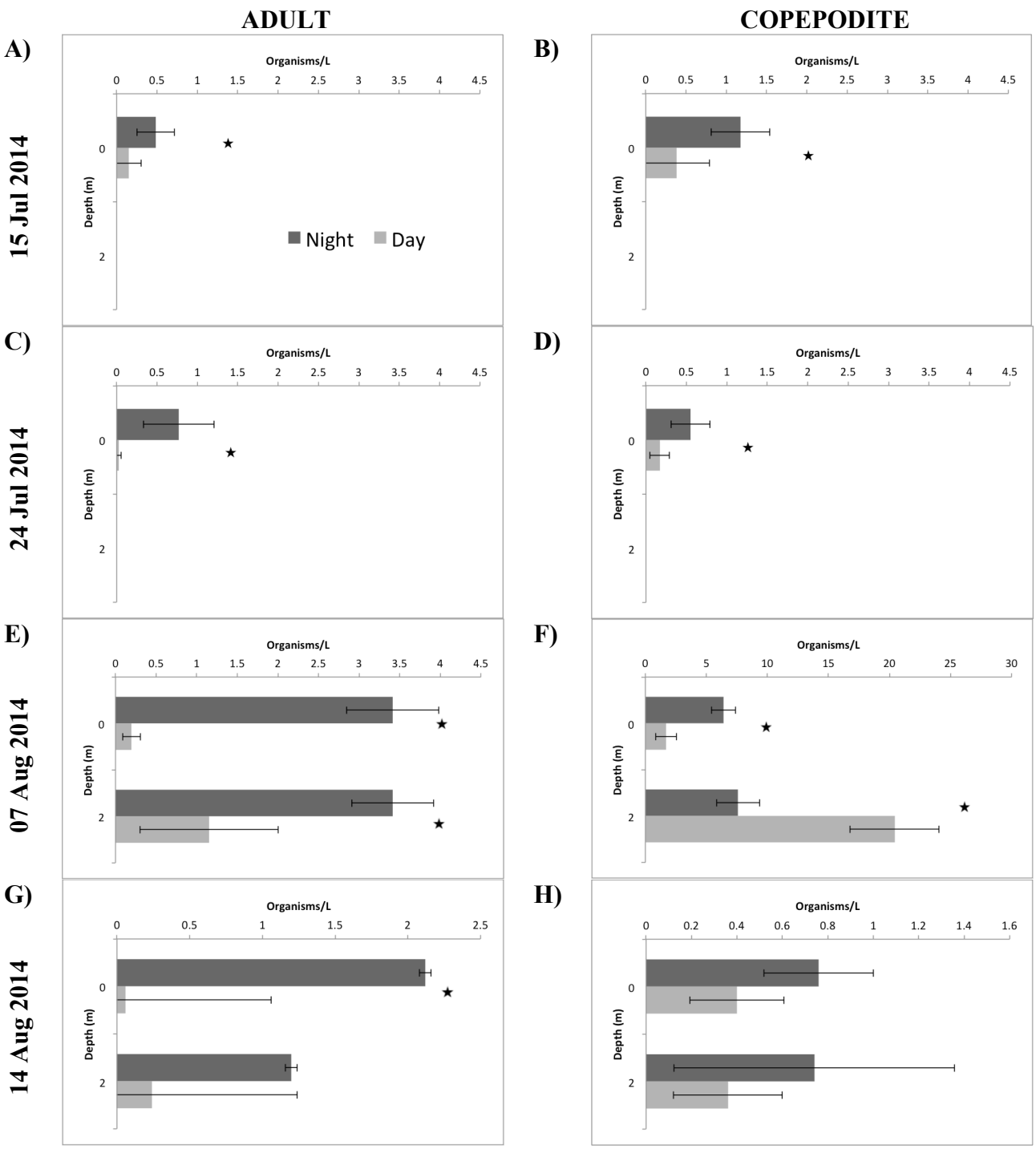


Figure 26. Nearshore abundances of *E. affinis* adults and copepodites during the day and at night. Error bars represent ±SEM. Stars designate significant differences.

Discussion

Results of our field study demonstrate that *E. affinis* performs DVM in Little Sturgeon Bay both near shore and offshore. The amplitude of this migration differs between sampling dates, sites, and life stages of the species. *Eurytemora affinis* was found in much higher abundance and performed a more drastic migration at the near shore site. Vertical migration did occur offshore, but was smaller in amplitude and quite variable between sampling dates. Across most sampling sites and dates, *E. affinis* adults migrated greater distances than did copepodites. The difference in migration amplitude may be explained by differences in predation risk among life-stages of *E. affinis*. Copepodites are shorter in length and smaller in area than adults, making them less visible to predators and therefore less likely to migrate as far.

Do Eurytemora affinis migrate?

Offshore DVM: I hypothesized that if *E. affinis* performed DVM offshore, there would be differences in weighted mean depth of the species from day to night. This prediction was realized, since WMD varied on a diel basis for each sampling date. However, the distance and direction of the migration was quite variable between sampling dates. Our understanding of the variability of the diel changes in WMD may be enriched by careful examination of each migration in the context of the biotic and abiotic environmental factors for each sampling date.

Little Sturgeon Bay provides a highly variable vertical environmental gradient. Little Sturgeon Bay is shallow, reaching a little over 4m at its deepest point. For this reason, it is susceptible to frequent and sudden mixing when winds are strong. Strong water currents entering Little Sturgeon Bay from Green Bay may result in internal

seiches, which also mix the lake—breaking down thermal and chemical stratification. In our system there is no clear relationship between the presence of physical and chemical lake stratification and copepod migration amplitude.

Little Sturgeon Bay showed similar thermal stratification on 24 July 2014 and 07 August 2014. Yet, the migration amplitude on 07 August was approximately 5.5 times greater than on 24 July (Figure 25). Similarly, DO also seems to have no bearing on migration amplitude. These results are in agreement with previous studies where *E. affinis* has been documented to occupy a wide range of physical and chemical conditions on a daily basis (Almén et al. 2014).

To understand why migration at the offshore location varied in amplitude and direction between sampling dates, we should also consider biotic factors such as food availability and predation. Though little data were collected on the abundance or diversity of zooplankton predators in Little Sturgeon Bay, planktivorous larval fish were occasionally caught in the Schindler Trap. In the classic model of DVM, zooplankton occupy deeper depths during the day to avoid visual predators like the larval fish noted above (Lampert 1989). Higher food availability at shallower depths is an incentive to migrate upwards at night. Alternatively, if food is not scarce in deeper waters, zooplankton may not need to migrate upward at night.

For this study, Chl-*a* abundance was used as a surrogate measurement for food abundance because Chl-*a* is produced by phytoplankton, the food of zooplankton. Chl-*a* profiles and abundances varied across sampling dates. Samples from 15 July 2014 showed a Chl-*a* profile that was consistently high across all depths and sampling times—ranging between 5 and 20 ug L^{-1} (Figure 20A). This amount of chlorophyll indicates that

the bay was mesotrophic to eutrophic in nature at that time, with fairly abundant food for copepods (Wetzel 2001). In a scenario like this with little food limitation, we would expect to see less intense zooplankton DVM. For both 15 and 24 July 2014, days with little food limitation, the amplitude of *E. affinis* DVM at the offshore site, represented by ΔWMD, was small (Figure 25).

In contrast, results from both 7 and 14 August 2014 showed Chl-*a* limitation at certain depths during different times of day. On 7 August 2014, Chl-*a* levels were much lower overall, indicating oligotrophic conditions but peaked at 4m during the day and at 2m at night (Wetzel 2001) (Figure 20C). In response, *E. affinis* performed a negatively phototactic migration with greater amplitude than the previous two sampling dates (Figure 25). On 14 August 2014, Chl-*a* levels were similarly low throughout the water column, but were highest in the shallowest two meters during the day and the deepest meter at night (Figure 20D). As a result, *E. affinis* performed a positively phototactic migration (Figure 25). In summary, it is likely that *E. affinis* performs DVM at the offshore site in response not to physical or chemical conditions, but rather to food limitation paired with predation.

Near Shore DVM: The near shore habitat of Little Sturgeon Bay, like most shallow lakes, presents a different set of resources and challenges to zooplankton than the offshore habitat. The near shore habitat was notably weedy, as it was filled with submerged macrophytes (personal observation). In general, predation on zooplankton by planktivorous fish is lower in habitats rich in macrophytes, since macrophytes provide a "hide-out" for zooplankton from their visual predators (Jeppesen et al. 1998). However, weedy, near shore habitats also act as a refuge for planktivorous fish from piscivorous

fish (fish that eat smaller fish). As a result, planktivorous fish congregate in shallow, macrophyte-rich near shore habitats (Turner and Mittelbach 1990).

DVM was trickier to quantify near shore for this study, since only two depths were sampled. It was therefore futile to attempt to estimate the "center" of the near shore zooplankton population by calculating WMD. Instead, differences in diel abundances at each depth were assessed to determine whether DVM occurred. Because this location was only approximately 2.4m deep, our sampling represented the majority of the vertical locations where copepods could occur.

The Diurnal Deficit: One of the most striking trends of *E. affinis* abundance in Little Sturgeon Bay is the diel difference in total abundance of organisms in the water column. *Eurytemora affinis* was consistently more abundant in the water column at night than during the day (Figure 21). This "diurnal deficit" has been documented in a variety of DVM studies on a variety of zooplankton (Kikuchi 1930, Hutchinson 1967, Bollens and Frost 1989, De Stasio 1993). Diurnal deficit in other zooplankton studies has been attributed to sampler avoidance (Omorie and Hamner 1982), migration into sediments during the day (Hart 1975), or horizontal movement (Moen and Langeland 1989).

In the case of *E. affinis* in Little Sturgeon Bay, it is likely that the disparity between day and night abundances is due largely to its vertical migration into or near the bottom sediment of the bay during the day. *Eurytemora affinis* is epibenthic, meaning it inhabits the water just above the sediment at the benthos, or the bottom of a lake (Evans and Stewart 1977). It is likely that the Schindler Trap sampler used in this study was not able to adequately sample close enough to the benthos to account for epibenthic organisms, resulting in an incomplete estimate of total zooplankton abundance in the

water column during the day.

While the diurnal deficit observed in this study may indicate an incomplete representation of total zooplankton abundance during the day, it also provides evidence that DVM is occurring. Greater abundances of *E. affinis* in the water column at night indicate that some have migrated out of the epibenthic zone, which is, in itself, a vertical migration.

Does Life-Stage Affect Predation Risk?

A major reason for zooplankton DVM is to reduce mortality through predator avoidance (Zaret and Suffern 1976, Lampert 1993). In the presence of fish karimones, zooplankton become more sensitive to changes in light intensity, inducing strong photobehavior in phenotypically plastic zooplankton (Ringelberg et al. 1991). Predation can lead to short-term genetic selection, favoring phenotypically plastic, migrating individuals over non-migrating individuals (Haney 1988).

If DVM is a mechanism for avoiding visual predation, then zooplankton size is important. Prey detection by visual predators depends on prey visibility, which increases with size (De Robertis et al. 2002, Hays 1995). If there are differences in copepod size between life-stage groups of *E. affinis*, then we should expect to find ontogenetic differences in DVM.

In Little Sturgeon Bay, life-stage groups were significantly different sizes. In particular, *E. affinis* copepodites were significantly shorter and had a smaller area than adults (Figure 15, Figure 16). Based on this size difference, copepodites should be less visible, and therefore less vulnerable to visual predation than adults.

Does Differing Predation Risk Lead to Different Migration Trends?

If differing visibility leads to differing predation risk between zooplankton life-stage groups and DVM is a behavior to avoid predation, then we expect to see differing DVM patterns between adults and copepodites. In particular, it is expected that adults, which face greater predation risk than copepodites, should have a more pronounced pattern of migration (Hays 1995, De Robertis et al. 2000). Our results are consistent with these expectations, since adults showed DVM of a greater magnitude at both the offshore and near shore site (Figure 25, Figure 26).

If visual predation risk differs ontogenetically, we also expect to see differing sensitivities of copepod densities to water clarity between life-stage groups. This expectation is also supported by our results, since copepodite density was significantly negatively correlated to water transparency during the day. There was also a negative, but non-significant, relationship between adult density and water transparency. The magnitude of the relationship between adult density and water transparency was not as great as that with copepodites (Figure 24A). The difference in these relationships suggests that visual predation risk is consistently higher for adult *E. affinis* than copepodites, discouraging adults from entering the water column even when water transparency is low. Alternatively, copepodites suffer less predation risk when water transparency is low and may inhabit the water column in high density.

The presence of a diel deficit provides additional evidence that zooplankton migrates up, out of benthic sediments at night. Differences in diel deficit suggest that life stage groups perform DVM with differing magnitudes. The diel deficit for *E. affinis* adults was proportionally larger than the deficit for copepodites at both the offshore and

near shore sites, indicating that a greater proportion of *E. affinis* adults spend daylight hours in the epibenthic zone than copepodites. Subsequently, a greater proportion of *E. affinis* adults leave the epibenthos and enter the water column at night to perform DVM than do copepodites.

Mechanisms Leading to Differing DVM Patterns

If DVM reflects individual zooplankton survival strategy rather than schooling or flocking behavior, we expect to see ontogenetic differences in DVM (De Robertis 2002). Predation risk differs ontogenetically and, to maximize survival probability, an individual of a particular life stage group should adopt a DVM pattern that most effectively minimizes predation risk without imposing unbearable metabolic costs. It is possible that zooplankton alter DVM amplitude throughout their life to maximize fitness based on immediate predation risk and resource availability.

It is also possible that the difference in DVM magnitude between life stage groups is a result of short-term genetic selection. In an environment with many visual predators, predation can lead to short-term genetic selection within a population, shifting a partially migrating zooplankton population towards a migrating population (Haney 1988). It is likely a combination of these factors—plasticity of behavior and short-term genetic selection—that lead to population-wide DVM in Little Sturgeon Bay.

Importance & Conclusions

In summary, we found that *E. affinis* performs DVM in Little Sturgeon Bay. Adults, which are more susceptible to visual predation, perform DVM with greater magnitude than copepodites. This greater magnitude of migration is indicated by larger

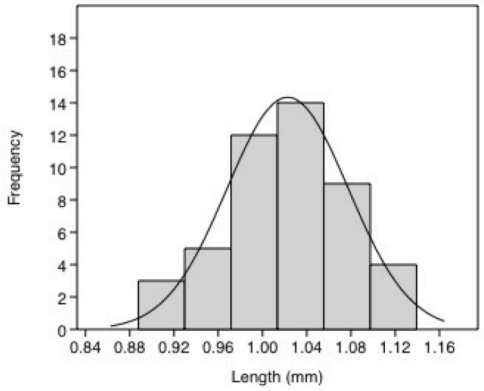
diel differences in weighted mean depth as well as diel differences in total abundance in the water column. It appears that offshore, negatively phototactic DVM occurs only when food is not limited. When food is scarce, *E. affinis* track food regardless of increased predation risk.

The fact that *E. affinis* migrates in Little Sturgeon Bay reveals a few clues about its success as an invader within this non-native habitat as well as other freshwater environments. Little Sturgeon Bay provides an incredibly variable and dynamic vertical environment that may switch between stratified and mixed in a short period of time (Figure 19). Chl-*a* abundance is also quite variable through the summer months. In the span of two months, Chl-*a* levels in Little Sturgeon Bay vary enough to suggest that it can be considered a eutrophic and an oligotrophic system at different times (Figure 20). DVM likely allows *E. affinis* to thrive in this highly variable environment. The variation in amplitude of *E. affinis* DVM across sampling dates suggests that the population is highly phenotypically plastic and individuals can change their migration behavior to best survive the rapidly changing conditions in Little Sturgeon Bay.

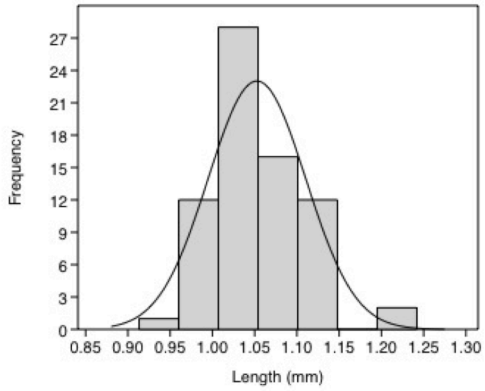
Genetic studies have revealed that *E. affinis* is highly phenotypically plastic, allowing the species to inhabit a range of marine, brackish, and freshwater environments (Lee 2000). The ability of *E. affinis* to tolerate a wide range of environments makes it a particularly good invader. The range of DVM amplitudes between life stage groups and sampling dates in Little Sturgeon Bay also indicates phenotypic plasticity within our study system. It is possible that plasticity of behavior, and not just morphology, allows *E. affinis* to be a successful invader in Little Sturgeon Bay as well as freshwater systems across the world.

Appendix

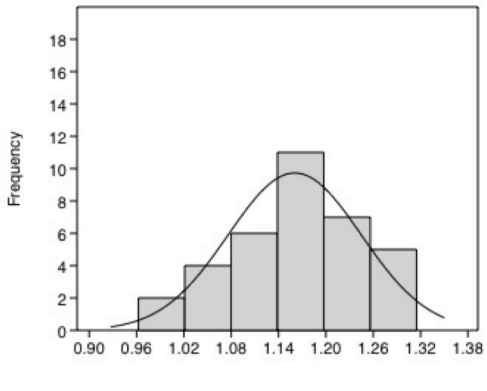
A) ♂



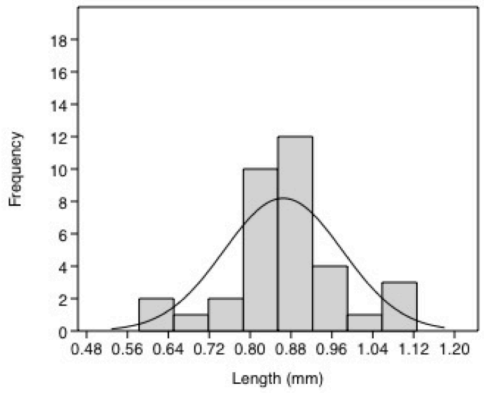
B) ♀



C) ♀ + eggs

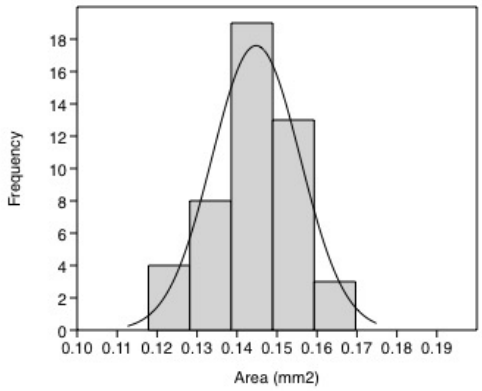


D) copepodite

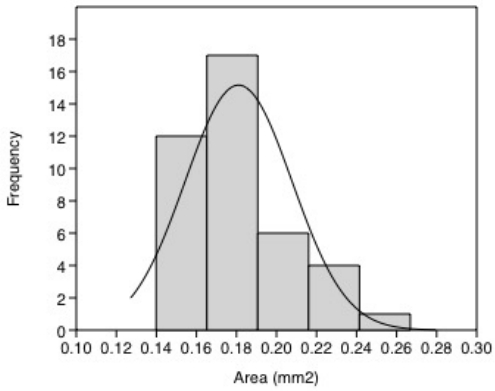


Appendix A1. Length distributions of life-stage groups (A) adult male N=47 B) adult female N=40 C) ovigerous female N=35 D) copepodite N=35) of *E. affinis* in Little Sturgeon Bay.

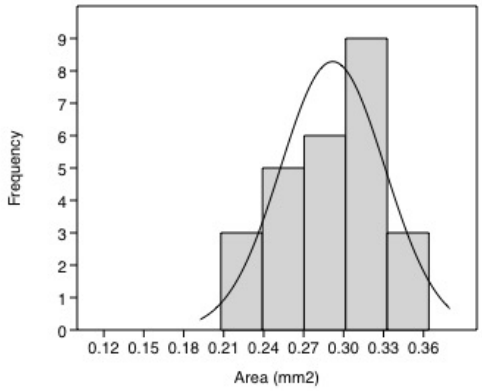
A) ♂



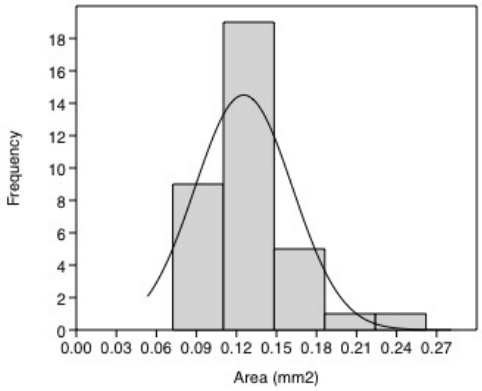
B) ♀



C) ♀ + eggs

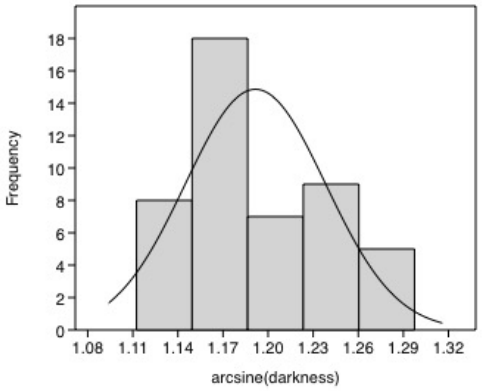


D) copepodite

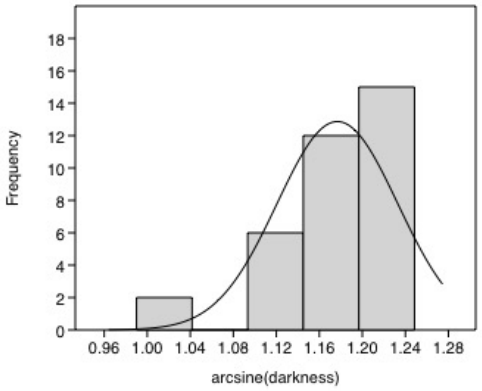


Appendix A2. Area distributions of life-stage groups (A) adult male N=47 B) adult female N=40 C) ovigerous female N=35 D) copepodite N=35) of *E. affinis* in Little Sturgeon Bay.

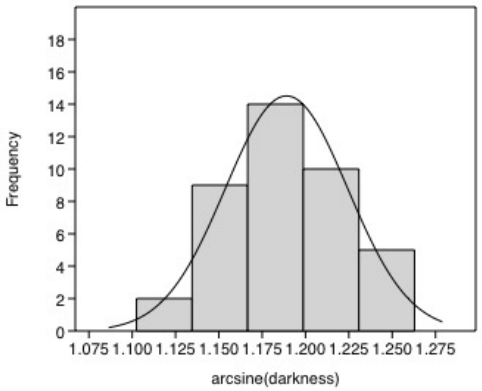
A) ♂



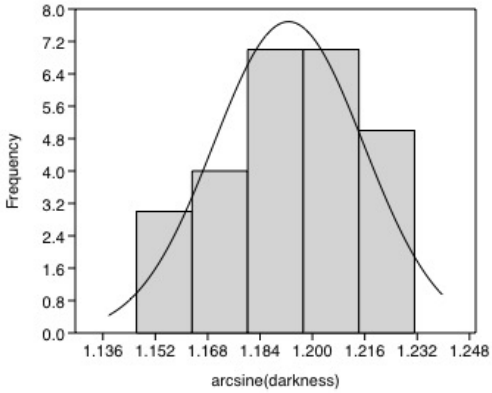
B) ♀



C) ♀ + eggs



D) copepodite



Appendix A3. Darkness distributions of life-stage groups (A) adult male N=47 B) adult female N=40 C) ovigerous female N=35 D) copepodite N=35) of *E. affinis* in Little Sturgeon Bay. Darkness measurements are the arcsine(proportion darkness), as determined by ImageJ.

Appendix A4. Average abundance of total, adult, and copepodite *E. affinis* in the water column near shore and offshore on four sampling dates. Average abundance is the mean of abundances at each depth.

	Average abundance (organisms/L)					
OFFSHORE	**Total**		**Adult**		**Copepodite**	
	Day	**Night**	**Day**	**Night**	**Day**	**Night**
15-Jul-14	0.2694	0.3945	0.0770	0.1684	0.1924	0.2261
24-Jul-14	0.1227	0.1828	0.0072	0.0433	0.1155	0.1395
7-Aug-14	0.0185	0.1563	0.0048	0.0601	0.0120	0.0962
14-Aug-14	0.0640	0.0640	0.0000	0.0120	0.0640	0.0520
NEAR SHORE						
	Day	**Night**	**Day**	**Night**	**Day**	**Night**
15-Jul-14	0.5283	1.6597	0.1469	0.4845	0.3814	1.1752
24-Jul-14	0.1924	1.3229	0.0241	0.7697	0.1684	0.5532
7-Aug-14	11.7351	10.4152	0.5893	1.8581	11.0586	6.9996
14-Aug-14	0.5300	2.4100	0.1500	1.6600	0.3800	0.7500

Appendix A5. Diel changes in average abundance of *E. affinis*. Changes were calculated by subtracting average day abundance from average night abundance shown in Appendix A4.

	Diel change in average abundance (organisms/L)		
OFFSHORE (LS-E)	**Total**	**Adult**	**Copepodite**
15-Jul-14	0.1251	0.0914	0.0337
24-Jul-14	0.0601	0.0361	0.0241
7-Aug-14	0.1379	0.0553	0.0842
14-Aug-14	0.0000	0.0120	-0.0120
NEAR SHORE (LS-DOCK)			
15-Jul-14	1.1314	0.3376	0.7938
24-Jul-14	1.1305	0.7457	0.3849
7-Aug-14	-1.3199	1.2688	-4.0590
14-Aug-14	1.8800	1.5100	0.3700

Literature Cited

Almén, A. K., Vehmaa, A., Brutemark, A., Engström-Öst, J. (2014) Coping with climate change? Copepods experience drastic variations in their physiochemical environment on a diurnal basis. *Journal of Experimental Marine Biology and Ecology*. 460: 120-128.

Balcer, M. D., Korda, N. L., and Dodson, S. I. (1984) *Zooplankton of the Great Lakes: A guide to the identification and ecology of the common crustacean species*. The University of Wisconsin Press, Ltd, Madison.

Bertand, G., Lang, J., and Ross, J. (1976) The Green Bay Watershed: Past, Present, Future. *University of Wisconsin Sea Grant Institute*. Madison, WI, USA.

Bollens, S. M. and Frost, B. W. (1991) Diel vertical migration in zooplankton—rapid individual-response to predators. *Journal of Plankton Research*. 13: 1359-1365.

DeRobertis, A. (2002) Size-dependent visual predation risk and the timing of vertical migration: an optimization model. *Limnology and Oceanography.* 47(4): 925-993.

DeStasio, B. T., Schrimpf, M. B., Cornwell, B. H. (2014) Phytoplankton communities in Green Bay, Lake Michigan after invasion by dreissenid mussels: increased dominance by cyanobacteria. *Diversity*. 6(4): 681-704.

Dodson, S. I. (1990) Predicting diel vertical migration of zooplankton. *Limnology and Oceanography.* 35(5): 1195-1200.

Dodson, S. I. (2005) *Introduction to Limnology*. McGraw-Hill, New York.

Dunne JA, Lafferty KD, Dobson AP, Hechinger RF, Kuris AM, et al. (2013) Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biol.* 11(6): e1001579.

Evans, M. S. and Stewart, J. A. (1977) Epibenthic and benthic microcrustaceans (copepods, cladocerans, ostracods) from a nearshore area in southeastern Lake Michigan. *Limnology and Oceanography*. 22(6): 1059-1066.

Fee, E.J. (1976) Vertical seasonal distribution of chlorophyll in lakes of Experimental-Lakes-Area, Northwestern Ontario implications for primary production estimates. *Limnology & Oceanography.* 21: 767–783.

Ferreira T and Rasband WS. (2010-2012) "ImageJ User Guide — IJ 1.46", imagej.nih.gov/ij/docs/guide/.

Forward, R. B. (1988) Diel vertical migration: Zooplankton photobiology and behavior. *Oceanography Marine Biology Annual Review*. 26: 361-393.

Gannon, J. E., Brickner, K. S. (1982) Zooplankton community composition in Green Bay, Lake Michigan. *United States Environmental Protection Agency*.

Gerritsen, J. and Strickler, J. R. (1976) Encounter probabilities and community structure in zooplankton: a mathematical model. *Journal of Fish Research Board of Canada.* 34: 73-82.

Gliwicz, M. Z. (1986) Predation and the evolution of vertical migration in zooplankton. *Nature.* 320: 746-748.

Grigorovich, I. A., Colautti, R. I., Mills, E. L., Holeck, K., Ballert, A. G., MacIsaac, H. J. (2003) Ballast-mediated animal introductions in the Laurentian Great Lakes: Retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences.* 60: 740–756.

Hammer, O., Harper, D. A. T., Ryan, P. D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*. 4(1):9pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Haupt, F. et al. (2009). Daphnia diel vertical migration: implications beyond zooplankton. *Journal of Plankton Research*. 31(5): 515-524.

Holliland, P. B., Ahlbeck, I., Westlund, E., and Hansson, S. (2012) Ontogenetic and seasonal changes in diel vertical migration amplitude of the calanoid copepods *Eurytemora affinis* and *Acartia* spp. in a costal area of the northern Baltic proper. *Journal of Plankton Research.* 0(0):1-10.

Hough, A. R. and Naylor, E. (1992) Endogenous rhythms of circatidal swimming activity in the estuarine copepod Eurytemora affinis (Poppe). *Journal of Experimental Marine Biology and Ecology.* 161(1): 27-32.

Johnsen, G. H., and P. J. Jakobsen. 1987. The effect of food limitation on vertical migration in Daphnia longispina. *Limnology & Oceanography*. 32: 873–880.

Koski, M., Engström, J., Viitasalo, M. (1999). Reproduction and survival of the calanoid copepod *Eurytemora affinis* fed with toxic and non-toxic Cyanobacteria. *Marine Ecology Progress Series 186:*187-189.

Kozlowsky-Suzuki, B., Lehtinimi, M., Engstrom-Ost, J., Karjalainen, M., and Viitasalo, M. (2002). Fate of cyanobacterial toxins in the pelagic food web: transfer to copepods or to fecal pellets? *Marine Ecology*. 241:13-21.

Kozlowsky-Suzuki, B., Karjalainen, M., Lehtiniemi, M., Engstrom-Ost, J., Koski, M., and Carlsson, P. (2003). Feeding, reproduction and toxin accumulation by the copepods *Acartia bifilosa* and Eurytemora affnis in the presence of the toxic

cyanobacterium *Nodularia spumigena*. *Marine Ecology Progress Series*. 249: 237-249

Lampert, W. (1989). The adaptive significance of diel vertical migration of zooplankton. *Functional Ecology*. 3(1): 21-27.

Lavrentyev, P. J., Gardner, W. S., Cavaletto, J. F., and Beaver, J. R. (1995**)** Effects of the zebra mussel (*Dreissena polymorpha* Pallas) on protozoa and phytoplankton from Saginaw Bay, Lake Huron. *Journal of Great Lakes Research.* 21: 545–557

Lee, C. E. (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations." *Evolution*. 54(6): 2014-2027.

Lee, C. E., Remfert, J. L., and Gelembiuk, G. W. (2003) Evolution of physiological tolerance and performance during freshwater invasions. *Integrated Comparative Biology*. 43(3): 439-449.

Leech, D. M., Boeing, W. J., Cooke, S. L, Williamson, C. E. and Torres, L. (2009) UV-enhanced fish predation and the differential migration of zooplankton in response to UV radiation and fish. *Limnology and Oceanography*. 54: 1152-1161.

Leech, D. M. and Williamson, C. E. (2000) Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecological Applications*. 10: 1530-1540.

Leibold, M. A. (1989) Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. *American Naturalist*. 134: 922–949

Loose, C. J. and Dawidowicz, P. (1994) Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology*. 75: 2255-2263.

Longhurst, A. R., and Harrison, W. G. (1988) Vertical nitrogen flux from the oceanic photic zone by diel migrant zooplankton and nekton. *Deep-Sea Research*. 35: 881–889

MacIsaac, H. J., Sprules, W. G., Johannsson, O. E., and Leach, J. H. (1992**)** Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. *Oecologia*. *92*: 30–39.

Mangel, M., Clark, C., McNamara, J., and Houston, A. (1988) Dynamic models in behavioural and evolutionary ecology. *Nature.* 332(6159): 29034.

Mellard, J. P., Yoshiyama, K., Litchman, E., and Klausmeier, C. A. (2011) The vertical distribution of phytoplankton in stratified water columns. *Journal of Theoretical Biology*. 269: 16-30.

Mills, E. L., Leach, J. H., Carlton, J. T., and Secor, C. L. (1993) Exotic species in the Great Lakes: A history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research*. 19(1):1-54.

Mills, E. L., Leach, J. H., Carlton, J. T., and Secor, C. L. (1994) Exotic species and the integrity of the Great Lakes. *BioScience*. 44(10): 666-676.

Orcutt, J. D., and Porter, K. G. (1983) Diel vertical migration by zooplankton: Constant and fluctuating temperature effects on life history parameters of Daphnia. *Limnology & Oceanography*. 28: 720–730.

Pangle, K. L., and Peacor, S. D. (2006) Non-lethal effect of the invasive predator Bythotrephes longimanus on Daphnia mendotae. *Freshwater Biology*. 51: 1070–1078.

Pangle, K. L., Peacor, S. D., and Johannsson, O. E. (2007) Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology.* 88: 402–412.

Poppe, S. A. (1880) Ueber eine neue Art der Calaniden-Gattung Temora, *Baird.Abh. naturw. Ver. Bremen*. 7: 55-60.

Rasband WS. (1997-2012) ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, imagej.nih.gov/ij/.

Reichwaldt, E. S., Wolf, I. S. and Stibor, H. (2004) The effect of different grazing patterns resulting from diel vertical migration on phytoplankton growth and composition: a laboratory experiment. *Oecologia*. 141: 411-419.

Reynolds, C.S. (1984) *The Ecology of Freshwater Phytoplankton*. Cambridge University Press

Richman, S., Sager, P. E., Banta, G., Harvey, T. R., and De Stasio, B. T. (1984**)** Phytoplankton standing stocks, size distribution, species composition and productivity along a trophic gradient in Green Bay, Lake Michigan. *Internationale Vereinigung fuer Theoretische und Angewandte Limnologie Verhandlungen*. *22*: 460–469.

Sommer, U., Gliwicz, Z. M., Lampert, W. et al. (1986) The peg-model of seasonal succession of planktonic events in fresh waters. *Archiv fur Hydrobiologie*. 106: 433-471.

Smayda, T. J. (1997) Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology & Oceanography*. 42: 1137–1153.

Stich, H. B. and Lampert, W. (1981) Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature*. 293: 396-398.

Stich, H. B. and Lampert, W. (1984) Growth and reproduction of migrating and nonmigrating Daphnia species under simulated food and temperature conditions of diurnal vertical migration. *Oecologia.* 61: 192–196.

Torke, B (2001) The distribution of calanoid copepods in the plankton of Wisconsin Lakes. *Hydrobiologia*. 453: 351-365.

Vanderploeg, H. A., Nalepa, T. F., Jude, D. J., Mills, E. L., Holeck, K. T., Liebig, J. R., Grigorovich, I. A., and Ojaveer, H. (2002**)** Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Canadian Journal of Fisheries Aquatic Science*.

Werner, E. E. and Gilliam, J. F. (1984) The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology, Evolution, and Systematics.* 15:393-425.

Wetzel, R. G. (2001) *Limnology: Lake and River Ecosystems*. Academic Press, San Diego.

Wetzel, R. G. and Likens, G. E. (1991) *Limnological Analyses*. Springer, New York.

Williamson, C. E. (1993) Linking predation risk models with behavioral mechanisms: identifying population bottlenecks. *Ecology*. 74(2): 320-331.

Williamson, C. E., Fischer, J. M., Bollens, S. M., Overholt, E. P., Breckenridge, J. K. (2011) Toward a more comprehensive theory of zooplankton diel vertical migration: Integrating ultraviolet radiation and water transparency into the biotic paradigm. *Limnology & Oceanography*. 56(5): 1603-1623.

Williamson, C. E., Zagarese, C. H., Schulze, P., Hargreaves, B. and Seva, J. (1994) The impact of short-term exposure to UV-B radiation on zooplankton communities in north temperate lakes. *Journal of Plankton Research*. 16: 205-218.

Wolf, H.G. & Mort, M.A. (1986) Interspecific hybridization underlies phenotypic variability in Daphnia populations. *Oecologia*, 68: 507-511.

Zaret, T. M. and Suffern, J.S. (1976) Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology & Oceanography*. 21: 804-813.