

**The ecology of aquatic macroinvertebrates:
Understanding interactions among drought, introduced fishes, and parasites**

By

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find that both the content and the form meet acceptable presentation standards
of scholarly work in the above mentioned discipline*

Abstract

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The Ecology of Aquatic Macroinvertebrates : Understanding interactions among drought, introduced fishes and parasites

Thesis directed by Associate Professor Dr. Pieter T. J. Johnson

Aquatic macroinvertebrates play key roles in structuring aquatic communities and provide a key link with the surrounding terrestrial environment through their metamorphosis from aquatic larvae to terrestrial adults. It is therefore important to understand how their distribution across a landscape shifts through time and in response to environmental change, such as prolonged drought. Concurrently, because relatively little is known about the parasites that use pond macroinvertebrates as hosts, I also explored the relative importance of factors affecting infection prevalence and parasite load within common invertebrate host taxa. For each year over four years, I sampled 36 ponds within the Bay Area of California, USA, to characterize the diversity and composition of aquatic macroinvertebrates and quantify the parasites that utilize these organisms as hosts. I specifically aimed to answer the following questions: (1) what are the relative influences of non-native fishes and hydroperiod in structuring communities? (2) How does the magnitude of such filters vary through time? And (3) how do host- and habitat-level factors combine to determine patterns of infection with larval dragonflies and damselflies? My results indicated that while fish play a dominant role in structuring the macroinvertebrate composition and richness, the strength of this effect was attenuated during a prolonged drought such that, by the last year of the study and the height of

California superdrought, fish had no detectable effect on the macroinvertebrate diversity or species composition. The parasite survey revealed six parasite taxa using macroinvertebrates as hosts with the majority infecting members of the Odonata (dragonflies and damselflies). The hierarchical generalized mixed model results suggested that the majority of variation in both infection prevalence and load was associated with site-level variables, such as water chemistry, and with an interaction between the presence of fish and host suborder. These findings suggest that infection probability for odonates is more closely linked to site-level factors than host-level factors though there are potential interactions between the two levels that must be considered.

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Chapter 1

Drought attenuates the impact of fish on aquatic macroinvertebrate diversity and species composition

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Introduction

In a recent synthesis of community ecology, Vellend (2010) challenged the field to identify generalizable processes governing community organization rather than producing system-specific results (see also Lawton 1999, Leibold et al. 2004, Simberloff 2004, and Chave 2013). This has contributed to an emphasis shift away from identifying specific factors that control the assembly and stability of local communities and toward understanding how communities are generally shaped by the contributions of both niche-based and neutral processes. The niche of a given species is defined through species interactions (intra- and interspecific), trait-by-habitat matching, and resource availability (Grinnell 1917, Elton 1927, Hutchinson 1978). Niche filters function to reduce the regional species pool (i.e., gamma diversity) down to a local species pool (i.e., alpha diversity) in a consistent, predictable fashion along environmental gradients (Chase and Myers 2011). Neutral processes are stochastic in nature and “neutral” in terms of which species derive benefits (Hubbell 2001). Whereas niche theory assumes each species has a well-defined niche space, neutral theory contends that communities are constructed based

on stochastic events involving both demographic (colonizations and extinctions) and spatial (dispersal priority effects) processes. Systems dominated by neutral processes tend to show little predictable pattern across environmental gradients (Chase and Myers 2011).

While there has been considerable debate on the relative importance of these two processes in shaping communities, emerging evidence illustrates that both niche and neutral factors can play significant roles in community structure (Cadotte 2007, Vergnon et al. 2009, Chase and Myers 2011, Stegen et al. 2012, Kalyuzhny et al. 2014, Vellend et al. 2014). Thus, rather than constituting alternative frameworks, niche and neutral factors represent opposing ends of a continuum, with most ecological communities likely falling somewhere along the spectrum. A key first step in determining where a community falls along the niche-neutral gradient involves testing the influence of hypothesized niche filters on species composition and diversity among assemblages that vary in the magnitude of particular filter (Chase and Myers 2011, Vellend et al. 2014). Chase (2010), for example, used experimental mesocosms to illustrate the importance of net primary productivity as a niche filter in aquatic systems. While both low and high productivity treatments had similar levels of local (alpha) richness over four years, mesocosms in the low productivity treatment had 33% fewer species at the regional level (gamma diversity) and thus much lower variation in species composition among replicates (lower beta diversity). These observations suggested that low productivity functioned as a ‘strong’ niche filter, leading to more consistent and predictable community structures relative to high productivity sites in which neutral forces manifested more strongly. Far less clear however is whether the factors that determine the relative degree of influence between niche and neutral processes remain stable over time and through environmental change.

Instead most studies have focused on solely identifying the factors driving the relative influence between niche and neutral processes.

In small aquatic ecosystems, one of the most dominant paradigms classifies systems according to two major niche filters represented by hydroperiod (e.g., ephemeral versus permanent) and the dominant predator type (e.g., fishes versus invertebrates) (Welborn et al. 1996, Crowder and Cooper 1982, Shurin et al. 2009). The effect of fish presence on invertebrate community structure has been extensively studied (McPeck 1990, McCauley et al. 2008, Chase et al. 2009, Knorp and Dorn 2014), leading to lower diversity, smaller average body size, and a shift in composition toward more cryptic species that are less vulnerable to fish predators (Dixon and Baker 1988, Pierce 1988, Batzer et al. 2000). Ponds that are more ephemeral in hydroperiod, which rarely sustain fish, often support higher invertebrate diversity and a greater fraction of large-bodied, active invertebrate predators. Despite the strength of this paradigm for understanding wetland communities (Ellis et al. 2011, Batzer 2013), comparatively little research has explored how major niche filters interact or change in magnitude over time, for which multi-year data are required.

In accordance with the consumer stress model (Menge and Sutherland 1987), different taxa often demonstrated variable responses to temporal changes in environmental quality (Menge and Sutherland 1987) thus the stability of a biological niche filter's strength may depend on its response to changing environments relative to the taxa it regulates. For example, the negative effects of fish presence on the invertebrate community can be significantly reduced with the addition of nutrients resulting in the formation of large, algal mats that function as invertebrate refugia (Diehl and Kornijów

1998), while the loss of macrophytic vegetation in pond systems can increase the strength of fish effects on local invertebrate communities (Diehl and Kornijów 1998). Because ecological communities and their associated habitats are dynamic over time, how identified niche filters vary in magnitude and relative importance represent important questions for community ecology.

To assess temporal variation in the form and magnitude of niche filters determining invertebrate diversity and species composition, we intensively sampled the aquatic macroinvertebrate communities of 36 wetlands in California between 2011 and 2014. Because this period corresponded with the onset of the recent megadrought (Griffin and Anchukaitis, 2014), it afforded a unique opportunity to evaluate how the strength of presumptive filters shifted in response to a strong environmental change. Using a mixed-modeling approach, we examined the effects of fish occurrence on both site-level alpha richness and site-level community composition, both within years and across the four-year study period. We expected that fish presence would act as a strong niche filter, predictably decreasing richness and altering the identity of taxa present, but that the magnitude of such effects would be altered by the annual effect of drought severity.

Methods

Field surveys – Each year between 2011 and 2014, we sampled 36 ponds distributed across Alameda and Santa Clara counties in the East Bay Region of California. These ponds, many of which were built to support livestock grazing are now managed on as part of regional or county parks. Each pond was sampled once between May and July to assess the biotic and abiotic characteristics. Using a YSI MDS 550 probe

held 30 cm below the surface, we measured pH, salinity, and total dissolved solids. We collected a water sample from each site to be brought back to the lab to calculate turbidity using a LaMotte 2020i turbidity meter and to assess the nutrient concentrations using standard methods (<http://snobear.colorado.edu/Kiowa/Kiowaref/procedure.html>). Water chemistry data and nutrient data were combined in principal component analysis (PCA) in order to reduce the dimensions of our variables. We kept all scores with an eigenvalue above 1. Pond perimeters were calculated using the tracking function on a handheld GPS unit (Garmin GPSMAP 60) as the researcher walked the shoreline of the pond. We categorized hydroperiod into two categories: permanent, for which the pond held water across all years of the study, and ephemeral, in which the pond went dry at least once during the duration of the study. Drought intensity was determined based on mean annual Palmer drought severity index (PDSI) (Palmer 1965). PDSI is calculated using both temperature and precipitation to calculate soil moisture supply and demand (Mishra and Singh 2010). PDSI can range from positive to negative infinity with 0 representing normal conditions, 4.00 represents extreme wet conditions and -4.0 represents harsh dry conditions (Mishra and Singh 2010). PDSI data used in this study were obtained from the National Oceanic and Atmosphere Administration (NOAA) for the California central coast drainage division. (<http://www7.ncdc.noaa.gov/CDO/CDODivisionalSelect.jsp#>).

To assess the biological community, we conducted 1-meter netsweeps inward from the pond edge to the shore every 10 meters along the pond perimeter using a D-frame dipnet (30.5 cm by 35.5 cm). The contents of each netsweep were placed into a sorting tray with a small amount of pond water. We identified all organisms larger than a copepod (i.e., macroinvertebrates) to family and collected a representative sample to be

further identified in the lab. Collected samples were identified to genus or lowest taxonomic level using an Olympus SZXC10 dissecting scope and taxonomic guides of aquatic invertebrates (Merritt and Cummins 1996, Thorp and Covich 2009). The resulting identification was used to construct a site-by-taxa presence-absence matrix. To assess the occurrence of fish at site, we relied on visual signs of fish or fishing activity, standardized dipnet sweeps, and 3 to 5 habitat-stratified seine hauls using two different seine lengths, a 91 by 183 and a 183 by 457 cm net.

Statistical analysis – To investigate the processes shaping macroinvertebrate communities and how they varied over time, we performed analyses at two levels: within-years and among-years. Our analyses assessed changes in either invertebrate richness, which was estimated as the number of unique macroinvertebrate taxa for each site-by-year combination (i.e., alpha diversity), or composition of species within a site (i.e., beta diversity). Within each year, we also calculated the total (gamma) diversity of macroinvertebrates among ponds that either supported fish or were fishless. We used rarefaction curves to assess the validity of our sampling protocol on regional gamma diversity using the *vegan* package in R statistical program (R Core Development Team 2008).

To model macroinvertebrate species richness, we used a generalized linear mixed effects model (GLMM) with a Poisson- distribution and log-link to predict local species alpha diversity the *glmer* function in the *lme4* (Bates et al. 2014) R package. As explanatory variables, we used the detection of fish (yes or no), pond perimeter (\log_{10} -transformed), hydroperiod (ephemeral, or permanent represented as 0 or 1, respectively), and the first two PCA score of water quality data (pH, conductivity, turbidity, dissolved

organic carbon, total nitrogen, and total phosphorus, see Appendix A for more information). For the within-year analysis, instead of running four separate GLMMS we utilized all the data and included sample year and site as random effects. By including year and site as random effects we were able to avoid violations of independence due to multiple year samples from the same site. For the among-year analysis, we included year as a fixed effect along with its interactions with hydroperiod and the presence of fish. Subsequently we also replaced the year factor with annual PDSI to determine whether drought-related data offered comparable explanatory power. For both the within-and among-year analyses, we initially constructed a model that included all main effects and targeted interactions between the effects of fish and pond perimeter based on prior research (Ryberg and Chase 2007). We then used likelihood-ratio tests to remove less-supported terms until we arrived at the best-supported model based on Akaike information criteria (AIC) values we considered any models with $2 \Delta AIC$ to be comparable.

To assess the drivers of macroinvertebrate community composition (beta diversity), we used the *mvabund* package in R (Wang et al. 2012). The *mvabund* package fits generalized linear models to each taxon in a taxa-by-site incidence matrix, including as output both an overall effect of each explanatory variable as well as taxon-specific responses. To help predict the occurrence of each invertebrate taxon (a binomial response), we included the same explanatory variables from the mixed model fitted for species richness. For the among-year analysis, we also included a fixed effect of year and interactions with hydroperiod and predator type, as described above. However, because *mvabund* does not allow inclusion of random effects, we did not include a random

effect for site. We chose to use *mvabund* to analysis our beta diversity over the approaches involving beta null deviances (Chase et al. 2011) due to the presence-absence nature of our data, for which the beta null deviance method does a poor job of capturing community composition patterns (Tucker et al. 2015). Finally, to test for spatial autocorrelation in community composition, we performed a Mantel test that examined whether the similarity in species composition between any two sites was related to their geographic distance. Specifically, we compared the Euclidean distance between each site pair with their Jaccard's dissimilarity index using the *vegan* package in R (Oksanen et al. 2007).

Results

Of the 36 sites sampled, 12 had fish whereas 24 were fishless. All sites maintained a consistent fish status throughout the course of the study (i.e., no sites lost or gained fish). Observed fish species – all of which were non-native warm-water game fish – included: *Lepomis macrochirus* (bluegill), *Lepomis cyanellus* (green sunfish), *Micropterus salmoides* (largemouth bass) and *Gambusia affinis* (mosquitofish). Nineteen sites were permanent, and 17 sites went dry at least once during our sampling period (ephemeral). In 2014, which was the third consecutive year of drought in California (Seager et al. 2014), five sites failed to hold water at all. We excluded these sites from the 2014 analysis, but kept them for years in which they held water. Of the fishless sites, seven were permanent, 17 were ephemeral. The first axis of the PCA for water chemistry data explained 41% of the variation of the data, with strong, positive loading from all the variables (Appendix A).

We identified 44 unique macroinvertebrate taxa spanning four phyla, 10 orders, and 17 families. Site-level alpha diversity varied from 4 to 24 species (mean species richness 12.35 ± 0.44) within a given year. Overall richness was dominated by coleopterans with 18 unique taxa representing three families, followed by odonates with 15 unique taxa from four families (Appendix B). Within years, local alpha diversity was negatively affected by fish occurrence (Poisson GLMM; AIC: 745.1, Fish presence coefficient = -1.29 ± 0.71 ; $z = -1.81$, $p = 0.04$). On average, sites with fish supported 23% fewer invertebrate taxa relative to sites without fish (Fig. 1A). We also found an interaction between pond perimeter and fish occurrence, such that increases in perimeter had a much stronger positive effect on diversity at fishless sites than at sites with fish (Poisson GLMM: fish-by-pond perimeter interaction coefficient = 0.36 ± 0.13 ; $z = 2.28$, $p = 0.02$) (see Appendix C, Fig 1C). The first axis of the water chemistry PCA also had a negative effect on alpha diversity (coefficient: -0.12 ± 0.03 ; $z = -3.37$, $p < 0.001$). Site hydroperiod did not have a significant effect on invertebrate diversity (coefficient: 0.01 ± 0.08 ; $z = 0.24$, $p = 0.82$).

Similarly, invertebrate community composition within years was best explained by fish presence, hydroperiod, and their interaction (overall model results: Predator: deviance = 266.50, $p = 0.001$, hydroperiod: deviance = 190.48, $p = 0.001$, predator: perimeter; deviance = 64.17, $p = 0.008$). The presence of fish had strong, negative effects on large-bodied and active invertebrates across multiple orders, including the Hemipterians (e.g., *Lethocerus americanus*: deviance = 29.03, $p = 0.001$, *Notonecta* sp.; deviance = 25.30, $p = 0.001$, and *Hespercorixa* sp.; deviance = 21.76, $p = 0.001$) as well as the larger Coleoptera (e.g., *Cybister* sp.: deviance = 19.53, $p = 0.001$). Increase in pond

hydroperiod also had a positive effects on large-bodied dragonfly larvae in the family Libellulidae (e.g., *Erythemis* sp.: deviance = 23.65, $p = 0.001$) as well as the potentially dispersal-limited taxa such as the Hemipteran, *Ranatra* sp.: deviance = 26.24, $p = 0.001$.

Among years, the presence of fish interacted strongly with year to determine alpha diversity (Year 2013 predator interaction coefficient = -0.41 ± 0.15 ; $z = -2.55$, $p = 0.007$, Year 2014 predator interaction: coefficient = -0.31 ± 0.16 ; $z = -1.97$, $p = 0.04$) (Fig. 1B). Thus, while fish presence had strong negative effect on alpha diversity in 2011 and 2012, it had no effect in 2013 and 2014 (Fig. 1 B). This weakening of the fish-niche filter was congruent with a progressive decline in the PDSI observed over the four-year sampling period, which decreased from 2.8 in 2011 to -6.23 PDSI in 2014. Incorporation of mean annual PDSI into the models of local richness provided a similar fit and explanatory power to treating year as a factor, again with a significant interaction between fish and PDSI ($\Delta AIC = 4.3$, predator type PDSI interaction: Estimated coefficient: 0.042 ± 0.01 ; $z = 2.38$ $p = 0.01$, conditional R^2 value; Year model: 0.45, PDSI model: 0.45) (Fig 2 Top). Correspondingly, in the analysis of invertebrate community composition among years, including an interaction term between year and fish introduces a novel significant interaction (fish-by-year interaction: deviance = 78.52, $p = 0.004$). This corresponded with the detection of previously absent taxa at fish sites in final two years of the survey, including *Notonecta* sp., *Cybister* sp., and *Lethocerus americanus*, resulting in an increase in fish site gamma diversity (Fig 3).

We found no evidence for spatial autocorrelation when using all years and sites (Mantel r : 0.1093, $p = 0.128$), looking at each year independently (Mantel r range: -0.04 – 0.10, p range: 0.11 – 0.64) or if invertebrate groups were divided into passive and

active-dispersing taxa based on published sources (Merritt and Cummins 1996, Thorp and Covich 2009) (Passive dispersers: Mantel r : 0.063, p = 0.07, Active dispersers: Mantel r : 0.05, p = 0.10).

Discussion

Ecologists have increasingly recognized the dual and concurrent roles of both neutral- and niche-based processes in structuring ecological communities (Adler et al. 2007, Pierce et al. 2007, Siepielski et al. 2010, Chase and Myers 2011, Vellend et al. 2014). Nonetheless, few studies have tested for temporal variation in the occurrence and magnitude of species-sorting mechanisms, which could be common in many systems. In this study, we show how the macroinvertebrate communities of ponds shift along the neutral–niche continuum over the course of a prolonged drought. In particular, our analysis revealed that the strength of the niche filter represented by fish presence was significantly eroded during a progressively worsening drought. While the occurrence of fish was the primary determinant of macroinvertebrate richness and species composition early in the study, these effects became non-detectable by the fourth year of sustained drought. These results could not be explained by spatial autocorrelation or a shift in harshness of the hydroperiod niche. Such findings highlight how even well-established ecological niche filters can interact with climate to create temporal variation in their influence on observed communities.

Consistent with previous literature, the presence of fish had a strong, negative effect on invertebrate diversity and was an important determinant of the invertebrate community composition (Diehl 1992, Williams 1997, McPeck 1998, Chase 2007, Chase

et al. 2009, Holomuzki et al. 2010, Hoverman et al. 2011). Within our study region, sites with fish supported, on average, 23% fewer taxa; conspicuously absent were active, large-bodied invertebrate taxa such as several hemipterans (*Notonecta* sp. and *Lethocerus* sp.) and dytiscid beetles. These observations parallel those from previous studies highlighting the strong inhibitory effects of fish on invertebrate abundance and overall richness (Batzner et al. 2000), which is often attributed both to reduced colonization and intense predation pressure (McPeck 1997). However, as the severity of the California megadrought worsened, falling to the lowest observed level of precipitation since 1974 (Seager et al. 2014), the differences between fish and fishless sites became markedly less pronounced. By the end of the study, both the richness and the composition of wetlands with and without fishes became largely indistinguishable, in part due to the addition of previously ‘filtered’ taxa to sites with fish, such as large-bodied hemipterans (e.g., *Belostoma* sp.) and coleopterans (e.g., *Cybister* sp.). This pattern could not be explained by the hydroperiod filter becoming harsher; although many of these ponds rely on winter rain events to recharge, we found no temporal change in the richness or composition associated with hydroperiod (but note that 10 ephemeral ponds failed to hold water in 2014).

The mechanisms through which drought altered the effects of fish on invertebrate communities could involve several direct and indirect pathways. Although the locations and identities of fish species observed over the sampling period remained consistent (i.e., there was no evidence of local extirpations or colonizations of fish), it is possible that drought-driven decreases in fish abundance or size distribution could have dampened the intensity of predation risk on sensitive macroinvertebrate taxa (Dorn and Cook 2015).

Alternatively, reduction in pond size is associated with numerous physiochemical changes that can have significant effects on fish behavior and microhabitat use (Magoulick and Kobza 2003). For instance, increases in littoral zone temperature can cause fish to forage in deeper microhabitats (Grossman and Ratajczak, 1998), thereby providing refuge to formerly ‘filtered’ taxa. A final possibility involves a progressive loss in the number of fishless sites over time. By 2014, five sites failed to hold water for the first time and 10 ponds held water for <1 month. This change in the spatial arrangement and number of ponds on the landscape could have caused dispersing invertebrates to colonize suboptimal habitats, such as those with fishes. Differentiation among these hypotheses would require a more in-depth study of how the interactions between fish and invertebrate shift in a changing habitat.

These findings illustrate the need for multi-year studies to understand dynamic shifts in the relative influences of neutral and niche processes on community structure. Temporal factors such as climate and disturbance can play important roles in the spatial distribution and intensity of niche filters, such that a “snapshot” approach may limit opportunities to broadly capture the dynamic nature of community assembly. This research also highlights the potentially positive roles of fish sites at the landscape scale; although often suboptimal for use by many invertebrate taxa, during severe droughts or environmental shifts they may nonetheless offer temporary refugia. Whether these invertebrates were successful in their development and able to further colonize additional sites was not measured, emphasizing the need for future work to shed light on temporal variation in the identity of potential niche filters and their intensity. Further insight can be gained by utilizing species-trait-by-environment matching to illustrate the expanse or

constriction of a species' niche over time and in response to environmental changes (Lavorel and Garnier 2002).

Figure Captions

Figure 1: Variation in alpha-level species richness between small ponds with fish and sites without fish. (A) Total effect of fish presence on species richness during the duration of the study. Presented is the mean richness \pm 1 standard error, estimated Fish present coefficient = -1.42 ± 0.70 , p-value=0.04 (B) The effect of fish presence on species richness across the four years of the study, mean \pm standard error. Predator type and year 2013 interaction: -0.44 ± 0.16 , p-value = 0.004, predator type and year 2014 interaction: -0.36 ± 0.16 , p-value = 0.02.

Figure 2: Top) The effect of mean annual Palmer's drought severity index (PDSI) on site level alpha diversity between fish and fishless sites. Predator type and PDSI interaction estimated coefficient: 0.047 ± 0.018 . Bottom) Monthly changes in PDSI values for central coast valley region ranging from January 2011 to December 2014.

Figure 3: Change in cumulative species presence (gamma diversity) between fish and fishless sites through time, 2011 Fish = 24, Fishless = 42 , 2012 Fish = 28 , Fishless = 42, 2013 Fish = 31 , Fishless = 37, and 2014 Fish = 34 , Fishless = 39.

Figure 1

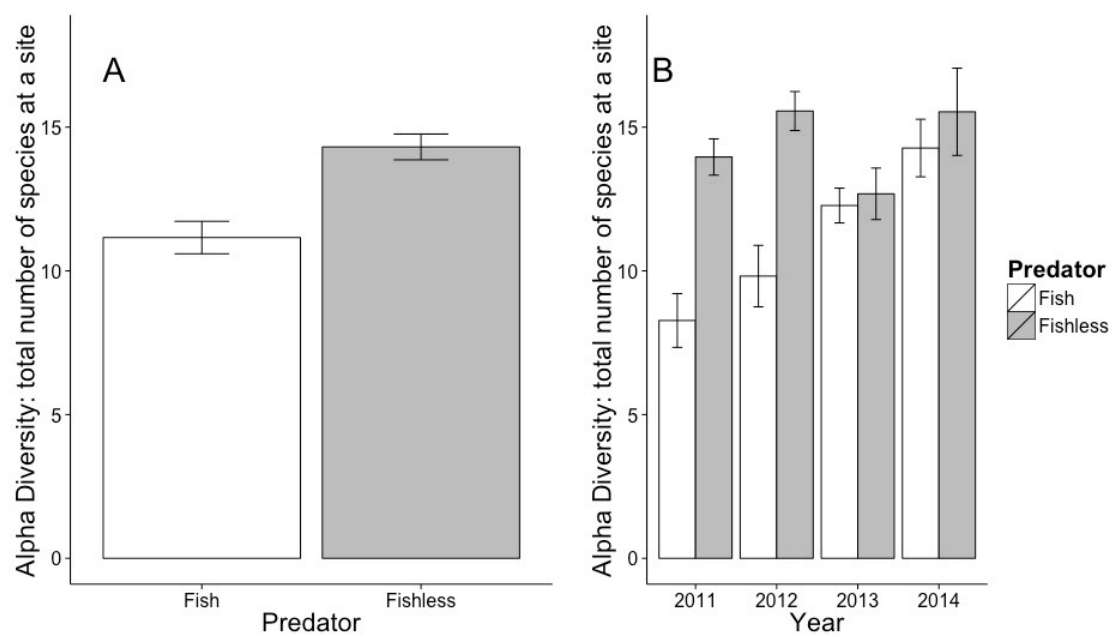


Figure 2

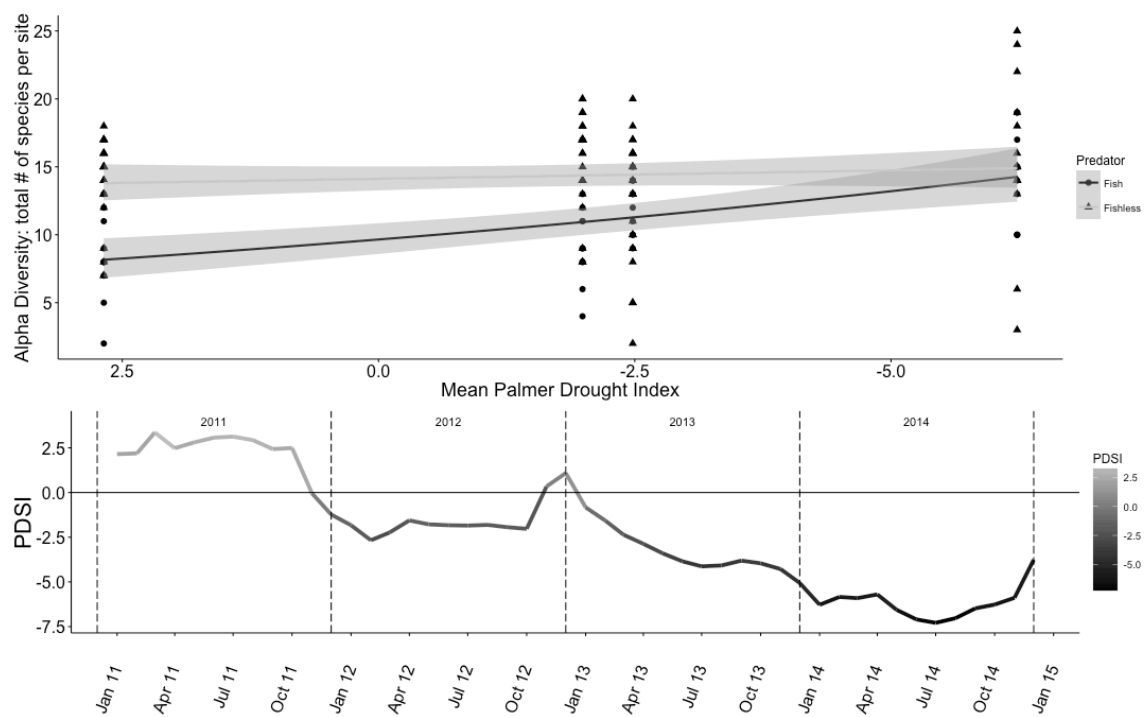
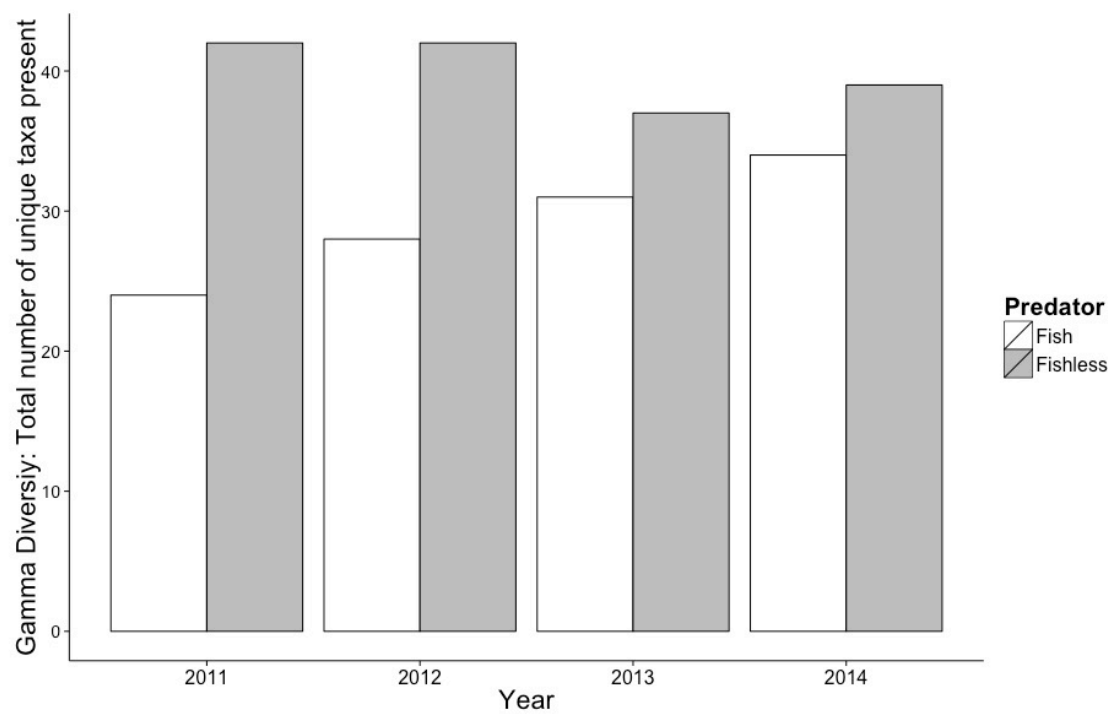


Figure 3



Chapter 2

Parasite richness and abundance within aquatic macroinvertebrate communities: testing the roles of host and environmental factors

Status: manuscript is being prepared for submission to *Freshwater Biology* pending input from the committee.

Introduction

A central goal in ecology is to understand the distribution of species across ecosystems through space and time (Simberloff 2004; Velland 2010). While major advances have contributed to a better understanding of the factors affecting community composition, particularly for well-described groups of plants and animals (Strong et al. 1984; Matthews 2012), we know considerably less about the distribution and structuring forces underlying more cryptic taxa, such as parasites (Mouritsen and Poulin 2002; Wood et al. 2007; Mihaljevic 2012; Hatcher et al. 2014). Despite ongoing research illustrating the influential roles of parasites on host communities (Rynkiewicz et al. 2015; Wood and Lafferty 2015), food web connectivity (Britton 2013; Poulin et al. 2013; Preston et al. 2014), and ecosystem energy flow (Wood et al. 2007; De Vos et al. 2016), recent estimates suggest that we have cataloged only a small fraction of the parasites found in most communities (Dobson et al. 2008; Wood and Johnson 2015). This knowledge gap has important implications both for understanding patterns of infectious disease and advancing the field of community ecology. For instance, Dobson et al. (2008) referred to parasites as the “hidden ‘dark matter’ that holds the structure of the [food] web together”.

Collectively, these studies emphasize the importance of further research to both characterize patterns of parasite diversity and understand the factors controlling infection patterns.

An added challenge inherent to the study of parasites and other symbionts involves the simultaneous identification of both the host-level factors affecting infection (the ‘host environment’) and factors operating beyond the scale of individual hosts (the ‘extra-host environment’). Thus, parasites with free-living stages, such as many multi-host parasites, must be able to adapt to factors in the extra-host environment as well as factors at the host level. As a result, parasites and their hosts can be considered a nested metacommunity: parasite assemblages form within individual hosts while host communities are spatially distributed across the landscape (Leibold et al. 2004; Mihaljevic 2012; Dallas and Presley 2014). While individual host attributes, such as body size, susceptibility, and age, have all been shown to affect parasite infection within a host (Pacala and Dobson 1988; Morand et al. 1996; Arneberg 2002; Hannon et al. 2015; Young et al. 2015), spatial and physical features of the surrounding environment will also affect the capacity of parasites to disperse between hosts and among sites. For example, in a study of flea parasitism on rodents in East Africa, Young et al. (2015) showed how both vegetation cover (extra–host environment effects) and body mass (host environment) jointly affected flea load. Decoupling host and habitat characteristics offers an opportunity not only to better understand the relative strength of factors structuring parasite metacommunities, but also to investigate the role of their interactions in driving parasite dynamics, including outcomes such as disease risk and infection intensity.

In community ecology, small pond ecosystems have been an influential system for exploring and understanding the forces affecting biodiversity and species composition (Werner and Hall 1976; Wilber 1987; Downing and Leibold 2002; Wellborn 2002; Chase et al. 2009; Johnson et al. 2013). Owing to their discrete boundaries and overall abundance, ponds offer an opportunity to sample replicate communities across a landscape (De Meester et al. 2005; Paull and Johnson 2011). Their diverse vertebrate and invertebrate assemblages also facilitate comparative studies among species that vary in dispersal ability, trophic position, and niche specificity (Wissinger 1992; Rundle et al. 2002; San Sebastian et al. 2015). Macroinvertebrates often play important ecological roles in ponds, constituting a major prey source for amphibians and fishes while functioning as top predators in fishless habitats (Schoenly 1991; Wellborn et al. 1996). In contrast to lakes, small ponds are often dominated by macroinvertebrates, which can comprise a major percentage of community biomass and - by virtue of their metamorphic transformation to flying adults - represent an important trophic linkage between aquatic and terrestrial environments (Benke and Huryn 2010; Preston et al. 2013).

Much less is known about patterns of parasitism within pond ecosystems, particularly within invertebrate hosts. While several studies have shown how ponds can harbor a diverse community of parasite species (Loy and Haas 2001; Preston et al. 2013), much of the previous work on freshwater parasites has focused on vertebrate hosts, such as fishes (Dogiel 1961) and amphibians (Johnson et al. 2013), or on snails (Richgels et al. 2013) and zooplankton (Caceres et al. 2014). Research efforts assessing and quantifying parasites within macroinvertebrate hosts, such as larval insects, remain relatively rare (Synder and Janovy 1996; Bolker and Janovy 2007; Kohler 2009). However, what studies

have been done suggest that, when present, freshwater parasites can have important effects on invertebrate host population dynamics, predator-prey interactions, and ecosystem function. In a study of stream communities, for instance, Kohler and Wiley (1992) demonstrated that population crashes of the dominant caddisfly driven by a microsporidian parasite resulting in periphyton and other grazers to increase in abundance in these streams in the years following the collapse (Kohler and Wiley 1997). Similarly, Mischler et al. (2016) found that high levels of trematode parasite infections in aquatic snails strongly affected nitrogen cycling at the pond ecosystem scale.

One of the most commonly reported parasite groups in ponds are helminth parasites with complex life cycles, such as digenetic trematodes (Johnson et al. 2013; Preston et al. 2013; Russell et al. 2015). Digenetic trematodes have a complex life cycle in which they infect multiple species of hosts, frequently involving gastropods as first intermediate hosts, macroinvertebrates or aquatic vertebrates as second intermediate hosts, and fishes, birds, mammals, or reptiles as definitive hosts (Schell 1985; Galaktionov and Dobrovolskij 2013). Transmission between host species often involves a combination of direct transmission via the production of free-living infectious stages, such as eggs, miracidia, and cercariae, as well as trophic transmission in which a host becomes infected by consuming an infected intermediate host. To increase the probability of trophic transmission, some trematodes cause morphological or behavior modifications in their hosts that increase their vulnerability to predators (Anderson and May 1978; Dobson and Hudson 1986; Johnson et al. 1999; Ponton et al. 2005; Johnson et al. 2006; Calhoun et al. 2013). For example, when infected with the trematode *Microphallus papillorobustus*, the brackish amphipod *Gammarus insensibilis* spends significantly

more time near the water's surface where it incurs greater risk of predation by fish, which is the definitive host for the trematode. High levels of infection have further been linked to population-level declines in female fecundity and male energy reserves (Ponton et al. 2005). While much of the work on parasites and food webs has focused on marine estuaries (Kuris et al. 2008), emerging evidence indicates that trematodes may also be highly influential in pond ecosystems. In small freshwater ponds in the Bay Area of California, for instance, Preston et al. (2013) showed that trematodes comprised a portion of the organismal biomass comparable to such insect orders as Odonata and Ephemeroptera. The trematodes exhibited high production-to-biomass ratios, suggesting that these parasites may play an important role in the movement of energy through pond food webs.

Digenetic trematodes in the genus *Haematoloechus* are common parasites in ponds that support true frogs in the family Ranidae (Bolek and Janovy Jr. 2007). These trematodes generally use freshwater snails in the genera *Physa* or *Gyraulus* as first intermediate hosts and aquatic invertebrates such as dragonfly and damselfly larvae as second intermediate hosts; ranid frogs become infected when they consume an infected intermediate hosts and function as the definitive host in which the parasite reproduces sexually (Snyder and Janovy 1996; Wetzel and Esch 1996; Bolek et al. 2007; Novak et al. 2013). While numerous studies have explored patterns of host specificity by *Haematoloechus* and variations in the life cycle (Snyder and Janovy 1996; Wetzel and Esch 1996; Bolek et al. 2007; Novak et al. 2013), less is known about the drivers of infection and how they vary geographically. Although parasites in the genus *Haematoloechus* have been documented across North America (Synder et al. 1996;

Goldberg et al. 1998; Bolek and Janovy 2007), Novak and Goater (2013) recently highlighted the potential for invasions by the American bullfrog, *Lithobates catesbeianus* to have expanded the range of *H. longiplexus* to the west coast of North America.

In this study, we explored patterns of parasite biodiversity within aquatic macroinvertebrate hosts and tested hypothesized drivers of parasite infection with a focus on the relative influence of host and habitat characteristics. Our study had two main objectives: first, we surveyed parasite richness and species composition within the macroinvertebrate communities of small ponds with the aim of deriving a better understanding of the types of parasites and how they varied between major host groups and among sites. Second, we focused on patterns of infection by the trematode group *Haematoloechus* sp. within larval odonates (dragonflies and damselflies) to quantify natural variation in infection prevalence and load. More specifically, we sought to test the relative influence of factors operating at two different scales: individual host characteristics, such as host species identity, body size, and habitat use, as well as site-level habitat characteristics, such as the presence of fishes, the density of odonate hosts, and abiotic aspects of water quality. To address these objectives, we surveyed 35 ponds in the Bay Area of California, USA, over two years and dissected a subset of the macroinvertebrate community to quantify their parasite load and richness. With a focus on trematodes in the genus *Haematoloechus*, we modeled both infection status and infection load using host and habitat characteristics to assess the relative importance of each group of factors. Given the ubiquity of pond ecosystems and the ecological importance of insects with aquatic larvae, studies of parasites in macroinvertebrates have

the potential to reveal both novel ecological drivers of disease dynamics as well factors controlling populations of vital group of organisms.

Materials and Methods

Field surveys – Parasite, host, and habitat data were collected in the summers of 2014 and 2015. We sampled 35 ponds distributed across Alameda and Santa Clara counties within the East Bay Region of California. These ponds, many of which were originally constructed to support livestock grazing, are often managed for mixed use as part of regional or county parks. Each pond was sampled twice between May and July to collect specimens and assess habitat level characteristics. We used a Garmin GPSMAP 642 hand held GPS unit to calculate pond perimeter and area by setting the track function while a researcher walked the perimeter. Salinity, pH, total dissolved solids, and conductivity were measured using a YSI MDS 550 water probe, which was held 150 mm underwater 5 meters from shore. A water sample was collected using an acid-washed Nalgene bottle and we tested the turbidity using a Lamotte 2020i turbidity meter. To reduce dimensionality in natural log-transformed values of turbidity, conductivity, and salinity, we used a principal components analysis (PCA) and retained axes with an eigenvalue greater than 1 (Jackson 1993). To determine the identity and abundance of different aquatic taxa, we conducted standardized, 1-m long net sweeps using a D-frame dipnet (30.5 cm by 35.5 cm) every 10 m around the shoreline. The contents of each sweep were transferred into a sorting tray, where we counted and identified snails and all arthropods. We specifically focused on members of the invertebrate orders Odonata, Ephemeroptera (mayfly), Hemiptera (true bugs), Coleoptera (beetles), and Malacostraca (amphipod) (for a full species list see Appendix E). Schell (1985) and Krull (1932) keys

were used to assist in identification. After all individuals were counted, we collected a subset of 10 individuals per taxon per site for further taxonomic identification and parasite examination. Lastly, we determined whether non-native fishes were presented based on visual observation, prior sampling of these sites, and capture during the dipnet surveys.

Parasite examination – To characterize patterns of parasite taxonomic richness and abundance, we dissected hosts by cutting along the lateral lines, removing the internal organs, and compressing organs between two slides for further examination under a compound microscope. The remaining exoskeleton was teased apart using forceps and carefully examined for endo- and ectoparasites under the dissecting scope on 10X magnification. Before dissection, host taxa were identified to genus using multiple keys (Merritt and Cummins 1996, Thorp and Covich 2009) or lowest possible taxonomic level and their total body length were measured using digital calipers to the nearest 0.01 mm. Isolated parasites were examined at higher magnification using an Olympus CX31 compound microscope and identified to lowest taxonomic level using (Merritt et al. 1996; Thorp and Covich 2009). We identified trematode (Platyhelminth) and gregarine (Apicomplexan) parasites to genus, whereas acanthocephalans, nematodes, and mites (Arthropoda) were identified to the lowest possible taxonomic unit. Species-level identifications were generally not possible given that many of these infections constitute larval stages, for which distinctive morphological features are often lacking. We estimated host specificity for each parasite as the total number of host taxa in which it occurred during the survey. We estimated parasite richness for each host taxon and for each site.

Host taxa characteristics – To better explore the drivers of *Haematoloechus* sp. infection, we collected information on odonate host species traits from Corbet (1975) and Corbet et al. (2006). Traits of interest for this study included voltinism (the number of generations per year) and foraging strategy (how and where hosts acquire food). We used voltinism as a proxy for development time, with bivoltinism, in which more than one generation occurs per year, representing higher growth rates relative to univoltinism, in which only a single generation occurs each year. Host taxa were assigned to a foraging strategy based on their method of prey capture: sprawlers (ambush prey while positioned on aquatic vegetation), burrowers (forage within the benthic sediment), and active hunters (actively traverse habitats in search of prey). Because our analysis focused on odonates, we indicated whether the specific taxon was a member of the damselfly suborder, Zygoptera, or the dragonfly suborder, Anisoptera.

Analysis – For *Haemaoloechus* sp. infections within odonates, which were among the most commonly observed host-by-parasite combination within sampled wetlands, we conducted additional analyses to assess the relative contributions of pond characteristics versus host characteristics in explaining infection prevalence (proportion of infected hosts), infection status of each individual (whether each host was infected), and the infection load (number of trematode metacercariae per host, including zero values). At the host individual level, we considered body length and voltine status as variables to help capture variation in growth rates and aquatic development time, which is likely to affect exposure risk. We included a fixed effect for suborder group due to the differences between transmission pathways between Zygoptera and Anisoptera : in the suborder Anisoptera, *Haematoloechus* sp. penetrates the organism passively by being drawn into

the brachial basket through the anal opening during gas exchange, whereas in Zygoptera, *Haematoloechus* sp. must first attach itself to the organism then makes it's way to the base of the Zygoptera's caudal gills and then penetrate it (Snyder and Janovy 1996)

At the pond level, we tested for effects of pond perimeter (\log_{10} -transformed), snail density, host density, fish presence, and the first two scores of the water chemistry PCA. Increases in snail or host density should enhance the probability of exposure to *Haematoloechus* sp., whereas increases in overall pond size will likely decrease infection risk by diluting the number of infectious stages per host (although larger ponds might also be more likely to support the necessary hosts in the life cycle or experience higher colonization rates). We included the water chemistry PCA to account for variation in water quality of the extra-host environment in which *Haematoloechus* sp. cercariae must find a host. Multiple characteristics of water quality can alter the lifespan of cercariae, including ion concentration and pH (Möller 1978; Pietrock and Marcogliese 2003; Paull and Johnson 2014). Finally, we included an interaction between the host suborder and fish presence based on the idea that infection risk by damselflies and dragonflies might be differentially affected by the presence of fishes, particularly in light of the differences in their transmission pathways (Johnson 1991; Brown and Robinson 2016).

Our analytical approach consisted of using generalized linear mixed models (GLMM) with the lme4 package (Bates et al. 2014) in R (glmer, glmer.nb) (R core Team 2015). We used binomial distributions for analyses of parasite prevalence and a negative binomial distribution for parasite load. Initially, we contrasted models that included random effects only: site (i.e., pond) and host taxonomic identity (i.e., species or lowest taxonomic resolution.) using intraclass correlation coefficients (ICC) to determine

the proportion of the variance within both groups (Gelman and Hill 2007). The ICC score ranges from 0-1 with a score of 1 indicating that all variation is contained within the corresponding group and a score of 0 indicating no variation within the group. Building from this approach, we incorporated a full model with the complete set of fixed effects associated with host and pond characteristics after testing for collinearity among predictors using variance inflation factors (VIF). VIF scores ranged from 1.04 to 1.22, suggesting a lack of any collinearity (O'Brien 2007). The best model was selected by dropping individual factors and performing a log-likelihood ratio test to compare model likelihoods. We assessed model assumptions by visually inspecting residual plots (Bolker et al. 2009).

Results

Parasite survey – In total, we sampled and dissected 1,801 macroinvertebrates representing 19 different genera and five orders of pond-dwelling insects and crustaceans (Table 1). We observed six different parasite taxa among examined hosts, including two digenetic trematodes (*Halipegus* sp. and *Haematoloechus*, sp.), an Apicomplexa gregarine (*Hoplorhynchus* sp.), a larval nematode, an acanthocephalan, and an ectoparasitic mite (Acari) (see Figure 1). Patterns of parasite richness differed considerably among host taxa. Among the five orders of arthropods, only three supported parasitic infections (Odonata, Coleoptera, and the Amphipod order, Malacostraca), with Odonata harboring the majority of parasite diversity (five parasite species). Coleoptera and Malacostraca were only infected with a single parasite, *Hoplorhynchus* sp. and an acanthocephalan, respectfully (see Table 2 for infection status by host taxon). Malacostraca infections were restricted to 5.7% of the examined sites (2/35) and had a

mean infection prevalence of 4.6 ± 4.4 SE %. Odonate infections were present at 82.9% (29/35) of sites with prevalence ranging from 0 to 75% with a mean of 21.7 ± 4.6 SE %. *Halipegus* sp. and *Haematoloechus* sp. were found in odonate taxa only; *Halipegus* sp. occurred only in Anisoptera while *Haematoloechus* sp. occurred in 8 out of the 9 genera in Anisoptera and 2 out of the 5 genera in Zygoptera. *Hoplorhyncus* sp. occurred in two orders, Coleoptera and Odonata, while the unidentified larval nematode infection occurred in both suborders of Odonata.

Among odonates, which were the most commonly infected hosts, the gregarine, *Hoplorhyncus* sp. parasite occurred at 62.9% (22/35) of sites and had a mean infection prevalence of $8.6 \pm 1.9\%$ SE across all sites. The digenetic trematode *Haematoloechus* sp., was the second most common parasite in our system which occurred at 45.7% (16/35) of the sampled sites with an average infection prevalence of $10.84 \pm 0.32\%$ SE. *Halipegus* sp. was relatively rare, occurring at only 11.4% (4/35) of sites and with a mean infection prevalence of $0.32 \pm 0.01\%$. An unidentified larval nematode occurred at 14.3% (5/35) of the sites and had a mean infection prevalence of $0.4 \pm 0.01\%$ SE. Mite infection occurred at 28.6% (10/35) of the sites with a mean infection prevalence of $1.55 \pm 0.21\%$.

Infection prevalence of Haematoloechus sp. – The first two scores of the PCA had eigenvalues of above one (1.92 and 1.27) and were thus retained for the analysis. The first PCA score had positive loadings for conductivity, salinity and turbidity with negative loadings for total dissolved solids and pH. The second PCA score had positive loadings for all variables except pH, which had a negative loading (See Appendix F for complete list of loadings and eigenvalues). Based on the intraclass correlation coefficient analysis, the majority of observed variation among odonate infection prevalence with

Haematoloechus sp. was grouped at the site level, with an intraclass correlation coefficient score of 0.75; variation grouped within the host genus scored an intraclass correlation coefficient score of 0.25. Of the models considered to help explain patterns of infection prevalence, the best-fitting model included two predictors associated with host identity (host body length and the host suborder) and five site-level factors (fish presence, host density, the two water chemistry PCA axes, and the interaction between fish status and host suborder). Body length positively predicted infection probability (scaled coefficient: 0.60 ± 0.11 , $p < 0.001$). Hosts in the suborder Zygoptera and those at sites with fishes were both associated with lower probabilities of infection (fish presence: estimated coefficient: -3.11 ± 1.45 , $p = 0.03$, Zygoptera estimated coefficient: -3.10 ± 0.83 , $p < 0.001$). However, there was strong, positive interaction between fish presence of fish and the suborder Zygoptera (interaction estimated coefficient: 4.93 ± 0.59 , $p < 0.001$) (Figure 2) such as in fishless sites Zygopterans had a lower infection prevalence compared to Anisopertan yet in sites with fish, Zygopterans had the higher infection prevalence. At the site level, both host density and the water chemistry PCA scores associated positively with infection (scaled coefficient PCA score 1: 0.39 ± 0.17 , $p = 0.02$, scaled coefficient PCA score 2: 0.37 ± 0.18 , p value = 0.04, host density scaled coefficient: 0.52 ± 0.24 , p value = 0.03). After the model fit, we recalculated the intraclass correlation coefficient for the remaining variation, which indicated that the site-level had high grouping with a score of 0.92, whereas the host taxa identity had a score of 0.08. Based on the pseudo- R^2 , the marginal R^2 value was 0.16 (fixed effects only) while the conditional R^2 was 0.79 (fixed and random effects).

Infection load by Haematoloechus sp. – Similar to infection prevalence, the site-level grouping for parasite infection load had the highest intraclass correlation score (0.74), with host-level identity exhibiting an ICC score of 0.26. The best-fitting model to explain infection load was also similar to that for prevalence, with two host-level covariates (body length and suborder), a single habitat-level covariate (fish presence), and the interaction between fish and suborder. An increase in body length was associated with an increase in a taxa's parasite load (scaled coefficient: 0.14 ± 0.02 , $p < 0.001$). Although taxa in the dragonfly suborder tended to have higher infection loads compared to damselflies (Zygoptera estimated coefficient: -2.77 ± 0.91 , $p = 0.002$), the presence of fish diminished this effect (Zygoptera-by-fish interaction estimated coefficient: 5.02 ± 0.43 , $p < 0.001$) (Figure 3) such as in sites with fish resulting in higher infection load in Zygoptera compared to sites without fish where Anisoptera had higher loads.

Discussion

Macroinvertebrates in pond ecosystems have historically played an important role in the study of community ecology (McPeck 1997; Benke and Huryn 2010; Chase 2010). However, the parasites of these organisms as well as the factors that influence infection risk have rarely been considered, despite evidence that such parasites can be both abundant and have strong effects on the community (Vance and Peckarsky 1996; Kohler and Wiley 1997). We surveyed replicate ponds across the landscape and examined dominant macroinvertebrate taxa for parasite infection, which revealed six unique taxa, including metacercariae of two digenetic trematodes, a gregarine parasite, a larval nematode, an acanthocephalan, and an ectoparasitic mite. The majority of our sites supported at least one taxa of parasite, illustrating the potential importance of parasites

within these communities, although patterns of parasite richness and load varied widely among examined host taxa. It should be noted that our estimates of parasite richness and prevalence are likely underestimates based on the subsampling employed, the challenges in detecting cryptic microparasite infections, and the shortage of morphological traits to definitively separate these parasites groups, particularly for larval stages.

Among sites with at least one parasite taxon detected, infection prevalence ranged from 5% to 75%. The majority of observed infections and parasitic taxa occurred within the arthropod order Odonata, which includes the dragon- and damselflies and is among the most abundant members in these small ponds (Preston et al. 2013).

Based on further examination of the drivers of *Haematoloechus* sp. infection prevalence and load within the odonates, our analyses indicated that patterns of infection associated positively with variables at both the site- and host-level. The majority of observed variation was grouped with the site level, as indicated by the intraclass correlation analysis. For both infection prevalence and load, site-level groupings accounted for 74 to 75% of the variation, relative to 25 to 26% for the host-level grouping. In the more detailed analyses of specific covariates, we found significant effects of both host- and site-level predictors. Host body length, host density, water chemistry, host suborder, and fish presence were all positively associated with infection prevalence and parasites per host. There is strong previous literature foundation supporting the trend of higher infection with larger body size (Kuris 1990; Poulin et al. 2003). For cercariae swimming through the water column, larger hosts result in larger targets, thus potentially increasing the likelihood of the parasite encountering the host (Arneberg 2002). Alternatively, size is often correlated with age, for which there is often

a positive relationship between probability of infection and age (Calhoun et al. 2013). Similarly, for many parasites, an increase in host density allows more opportunities for encountering a host and successfully infecting it, which is a pattern well-supported in multiple systems (Arneberg et al. 1998; Altizer et al. 2003). Habitat water chemistry has been shown to influence the survival of the free-living cercariae stage of the trematodes (Koprivnikar et al. 2010), with different trematode species responding uniquely to a variety of water conditions. Thus any change in any of our water chemistry metrics could result in a change in survival of the cercariae resulting in a corresponding change in infection prevalence.

Intriguingly, we also found consistent evidence for an interaction between the presence of introduced fishes and the odonate suborder (Zygoptera vs. Anisoptera) in determining infection prevalence and load. Thus, in sites with fishes, host taxa in the suborder Zygoptera (damselflies) were more likely to be infected than in sites without fish. For dragonflies (Anisoptera), the presence of fishes had a negative association on both the probability of infection or the number of parasites per host. A possible theory could suggest that the presence of fish could alter the odonate composition so that only the most susceptible Zygopterans are present in the fish sites resulting in the interaction. Yet the majority of the odonate taxa were present across both fish and fishless sites and we saw no difference between family level host density among fish and fishless sites. We suggest this interaction may stem from the effects of fish on host behavior and micro-habitat usage by odonates (Robinson and Wellborn 1987; Johnson 1991; Stoks and McPeck 2003). For hosts in the suborder Zygoptera, *Haematoloechus* sp. cercariae actively attach to the lateral caudal lamella and migrate to the posterior end of the host

before penetrating the tissue (Bolek and Janovy 2007). For dragonflies, in contrast, cercariae use a more passive transmission pathway, in which they are sucked into the host's abdomen during gas exchange and subsequently encyst on the brachial basket (Snyder and Janovy 1996). Previous research suggests that fish cause odonates to become less active and move closer to near shore habitats to avoid consumption (Johnson 1991). Thus, the presence of fish might lead to *reduced* infection of dragonfly larvae if the associated reduction in host activity lowers their movement and gas exchange (both of which offer opportunities for cercariae to enter the host) whereas fish might either *increase* or have no effect on infection in damselflies, for which a decrease in movement could enhance the ability of the *Haematoloechus* sp. cercariae to attach and penetrate the host.

In the absence of experimental data, we suggest these potential mechanisms for the interaction effect be approached with caution. Predators can have multiple and counteracting effects on patterns of infection within host populations. For instance, predators that selectively foraging on infected individuals (Johnson et al. 2006; Duffy et al. 2011) can cause a reduction in infection prevalence, potentially reducing transmission, whereas other predators can induce trait-mediated indirect effects resulting in a variety of changes in the infection prevalence (Orlofske et al. 2012; Bertram et al. 2013). Our current understanding of the roles predators play on infection is incomplete, and manipulative experiments are needed to test potential mechanisms and the influence of host behavior on overall infection prevalence and load (Orlofske et al. 2012; Rohr et al. 2015).

While research on the parasites of aquatic macroinvertebrates in ponds remains limited (Kohler 2008), our results are broadly consistent with previous studies on invertebrate hosts in freshwater ecosystems (Kohler and Wiley 1992; Vance and Peckarsky 1997; Mariluan et al. 2012). Compared to other taxa present in pond ecosystems, the macroinvertebrates studied here harbored a relatively low diversity of parasites. Similar studies done on amphibians, for instance, have found anywhere from 12 to 23 macroparasites using frogs as hosts, whereas in our study we identified six macroparasites and one microparsite, the gregarine *Hoplorhyncus* sp. (Muzzall et al. 2001; Pulis et al. 2011). Despite the large diversity of macroinvertebrates in our system, the majority of infections and parasite taxa occurred among the order Odonata, with most other host groups harboring one or no parasite taxa. This includes the order Ephemeroptera (mayflies), which has previously been found to be an important host for both mermithid nematodes and digenetic trematodes in stream ecosystems (Esch et al .1986; Vance and Peckarsky 1997). Similarly, aquatic Coleoptera (beetles) showed a surprisingly low level of infection prevalence and harbored only a single gregarine parasite species, despite their ubiquity as hosts across the landscape. The majority of the parasite taxa seemed to high host specificity at the order level, with the two trematodes infecting only Odonata and the gregarine infection only Odonata and Coleoptera. Yet at the genus level the two trematodes had less host specificity infecting the majority the odonate genera.

Infections within odonates have the potential to affect both community structure within aquatic ecosystems and influence nutrient flow into the surrounding terrestrial ecosystem. Because many odonates are key predators during their aquatic phase,

infections that affect their population abundance or dynamics could have broad-ranging effects on other members of the aquatic community, including in some cases vertebrates such as amphibian larvae. Moreover, macroinvertebrates and specifically Odonata are a key link for the aquatic – terrestrial communities where emergence of adult insects can number up to 20,000 per m⁻² per year⁻¹ (Baxter et al. 2005). These massive annual emergence events can provide a variety of terrestrial predators such as bats, birds, and small mammals with valuable prey resources (Murakami and Nankano 2002). Some studies suggest that aquatic invertebrate adults make up 26% of the annual energy budgets for terrestrial predators (Nankano and Murakami 2001) and up to 40% of given predator's annual budget (Baxter et al. 2005). Ignoring parasites of aquatic macroinvertebrates could therefore obscure an important contributing force for understanding what drives macroinvertebrate population and community dynamics as well as their influence on surrounding terrestrial ecosystems.

Tables

Table Captions

Table 1.

List of parasite taxa identified in our survey with the number of host utilize and infection prevalence \pm standard error.

Table 2

List of host order with corresponding number of parasite taxa utilizing host and mean infection prevalence \pm standard error.

Table 3

Infection prevalence \pm standard error of four parasite taxa infecting Odonata host. Host are broken down by genus with proportion of sites occupied by each genus and total sample size (n).

Table 1.

	Order	Parasite Taxa	n	Infection
1	Odonata	5	1612	0.27 ± 0.44
2	Ephemeroptera	0	15	0.00 ± 0.00
3	Hemiptera	0	75	0.00 ± 0.00
4	Coleoptera	1	43	0.07 ± 0.26
5	Malacostraca	1	69	0.04 ± 0.29

Table 2

	Parasite	Host Taxa	Number of Sites
1	<i>Haematoloechus</i> sp.	8	13
2	<i>Halipegus</i> sp.	5	6
3	<i>Hoplorhyncus</i> sp.	5	16
4	Nematode	3	6
5	Mite	4	7
6	Acanthocephalan	1	2

Table 3

Host Taxa					Mean Infection \pm	Standard Error		
Family	Genus	% Sites	n	Total infection	<i>Haematoloechus</i> sp.	<i>Halipegus</i> sp.	<i>Hoplorhyncus</i> sp.	Nematode
1 <i>Aeshnidae</i>	<i>Aeshna</i>	0.46	64	0.19 \pm 0.08	0.11 \pm 0.06	0.01 \pm 0.01	0.01 \pm 0.01	0.06 \pm 0.06
2 <i>Aeshnidae</i>	<i>Anax</i>	0.80	267	0.10 \pm 0.04	0.08 \pm 0.04	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
3 <i>Coenagrionidae</i>	<i>Coenagrion</i>	0.31	53	0.30 \pm 0.12	0.11 \pm 0.09	0.00 \pm 0.00	0.16 \pm 0.09	0.00 \pm 0.00
4 <i>Coenagrionidae</i>	<i>Ischnura</i>	1.00	408	0.39 \pm 0.06	0.16 \pm 0.05	0.00 \pm 0.00	0.21 \pm 0.04	0.01 \pm 0.00
5 <i>Coenagrionidae</i>	<i>Telebasis</i>	0.09	6	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
6 <i>Lestidae</i>	<i>Archilestes</i>	0.06	3	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
7 <i>Lestidae</i>	<i>Lestes</i>	0.40	100	0.07 \pm 0.07	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
8 <i>Libellulidae</i>	<i>Erythemis</i>	0.43	125	0.18 \pm 0.06	0.12 \pm 0.06	0.04 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00
9 <i>Libellulidae</i>	<i>Erythrodiplax</i>	0.06	10	0.06 \pm 0.06	0.06 \pm 0.06	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
10 <i>Libellulidae</i>	<i>Pachydiplax</i>	0.57	132	0.38 \pm 0.08	0.26 \pm 0.08	0.02 \pm 0.02	0.09 \pm 0.06	0.00 \pm 0.00
11 <i>Libellulidae</i>	<i>Planthemis</i>	0.46	78	0.03 \pm 0.02	0.00 \pm 0.00	0.00 \pm 0.00	0.01 \pm 0.01	0.00 \pm 0.00
12 <i>Libellulidae</i>	<i>Sympetrum</i>	0.77	261	0.13 \pm 0.04	0.10 \pm 0.03	0.01 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01
13 <i>Libellulidae</i>	<i>Tramea</i>	0.29	105	0.23 \pm 0.09	0.21 \pm 0.09	0.00 \pm 0.00	0.01 \pm 0.01	0.00 \pm 0.00

Figure Captions

Figure 1: Bipartite graph with parasite taxa on left and host taxa on right side. Lines indicate host utilized by each parasite taxa

Figure 2: The effect of the presence of fish on mean infection prevalence of *Haematoloechus* sp. for the two suborders of Odonata, Anisoptera (Dragonflies) and Zygoptera (Damselflies). Height of bars represents mean prevalence \pm standard error (From GLMM : Zygoptera ; fish interaction estimated coefficient: 4.93 ± 0.59 , p value < 0.001).

Figure 3: The effect of the presence of fish on mean infection load of *Haematoloechus* sp. for the two suborders of Odonata, Anisoptera (Dragonflies) and Zygoptera (Damselflies). Height of bars represents mean *Haematoloechus* sp. load \pm standard error (From GLMM: Zygoptera: fish interaction- estimated coefficient: 5.02 ± 0.43 , p value < 0.001).

Figure 1

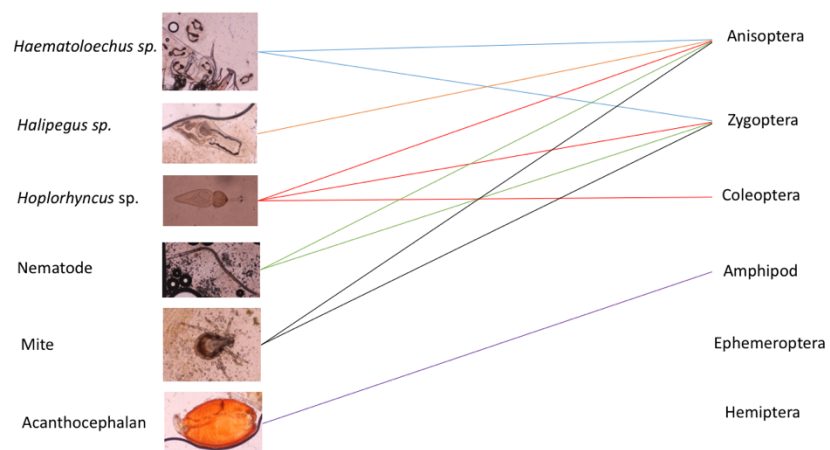


Figure 2

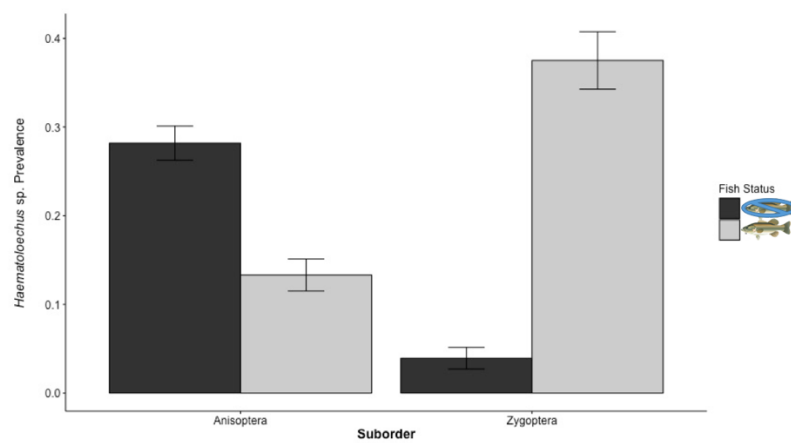
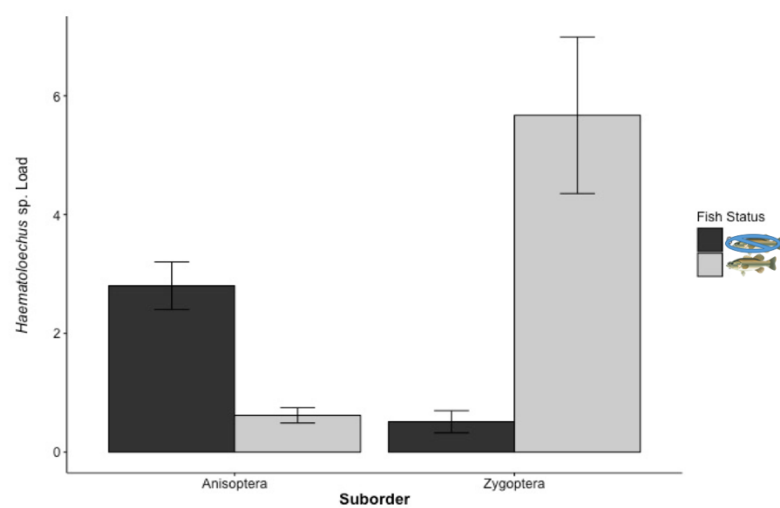


Figure 3



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Appendix

Appendix A

We conducted a principal components analysis on a suite of water chemistry and nutrient data using the PCA function in the R statistical software package **FactoMineR**. We retained the first two principal component scores, which accounted for 39.8% and 27.8% of the explained variation respectively for cumulative variation explain of 67.7%. (Table 1A). All the variables had positive loadings on the first principal components score, on the second principal component score total dissolved solids, salinity were strongly positively loadings (> 0.90) with total phosphorus having a small positive loadings (< 0.001), pH turbidity dissolved organic carbon and total nitrogen had negative loading between -0.1 to -0.31. (Table 2A)

Table 1A

	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	2.80	40.03	40.03
comp 2	1.97	28.14	68.17
comp 3	1.01	14.39	82.56
comp 4	0.77	10.98	93.54
comp 5	0.36	5.12	98.65
comp 6	0.08	1.13	99.78
comp 7	0.02	0.22	100.00

Eigenvalue and percent of variation explained for the factors in the principal component analysis

Table 2A

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
TDS	0.39	0.91	0.09	0.06	0.08
Salinity	0.39	0.91	0.09	0.02	0.08
pH	0.08	-0.16	0.95	-0.26	-0.03
TURBID	0.49	-0.31	0.24	0.77	0.07
DOC	0.86	-0.29	-0.14	-0.28	0.19
TOTN	0.88	-0.35	-0.08	-0.14	0.20
TOTP	0.85	0.01	-0.09	-0.03	-0.51

Factor loadings for the principal components scores, DIM = principal component dimensions Order. TDS= Total Dissolved Solids, DOC= Dissolved Organic Carbon, TOTN= Total Nitrogen, TOTP= Total Phosphorous

AppendixB

Table 1B

	Class	Order	Family	Genus
1	Archnida	Acari	Hydrachnidae	Hydracha
2	Branchopoda	Cyclestherida	Spinicaudata	Cyzicus
3	Clitellata	Hirudinea	Erpobdellidae	Erpobdella
4	Clitellata	Hirudinea	Glossiphoniida	Glossiphonia
5	Gastropoda	Planorbidae	Physidae	Physa
6	Gastropoda	Planorbidae	Planorbidae	Gyraulus
7	Gastropoda	Planorbidae	Planorbidae	Helisoma
8	Insecta	Coleoptera	Curculionidae	Bagous
9	Insecta	Coleoptera	Dytiscidae	Agabus
10	Insecta	Coleoptera	Dytiscidae	Celina
11	Insecta	Coleoptera	Dytiscidae	Cybister
12	Insecta	Coleoptera	Dytiscidae	Hydroporus
13	Insecta	Coleoptera	Dytiscidae	Laccophilus
14	Insecta	Coleoptera	Dytiscidae	Stictotarsus
15	Insecta	Coleoptera	Dytiscidae	Uvarus
16	Insecta	Coleoptera	Haliplidae	Halipus
17	Insecta	Coleoptera	Haliplidae	Peltodytes
18	Insecta	Coleoptera	Hydrophilidae	Berosus
19	Insecta	Coleoptera	Hydrophilidae	Enochrus
20	Insecta	Coleoptera	Hydrophilidae	Hydrophilus
21	Insecta	Coleoptera	Hydrophilidae	Laccobius
22	Insecta	Coleoptera	Hydrophilidae	Tropisternus
23	Insecta	Diptera	Chironomidae	N/A
24	Insecta	Diptera	Culicidae	N/A
25	Insecta	Diptera	Stratiomyidae	Hermetia
26	Insecta	Diptera	Tabanidae	Chrysops
27	Insecta	Diptera	Tipulidae	Tipula
28	Insecta	Ephemeroptera	Baetidae	Callibaetis
29	Insecta	Hemipterian	Belostomatidae	Belostoma
30	Insecta	Hemipterian	Belostomatidae	Lethocerus
31	Insecta	Hemipterian	Nepidae	Ranatra
32	Insecta	Hemipterian	Notonectidae	Martarega
33	Insecta	Hemipterian	Notonectidae	Notonecta
34	Insecta	Odonata	Aeshnidae	Aeshna
35	Insecta	Odonata	Aeshnidae	Anax
36	Insecta	Odonata	Coenagrionidae	Argia
37	Insecta	Odonata	Coenagrionidae	Coenagrion/Enallagma
38	Insecta	Odonata	Coenagrionidae	Ischnura
39	Insecta	Odonata	Coenagrionidae	Telebasis
40	Insecta	Odonata	Lestidae	Archilestes
41	Insecta	Odonata	Lestidae	Lestes
42	Insecta	Odonata	Libellulidae	Erythemis
43	Insecta	Odonata	Libellulidae	Libellula
44	Insecta	Odonata	Libellulidae	Pachydiplax
45	Insecta	Odonata	Libellulidae	Pantala
46	Insecta	Odonata	Libellulidae	Plathemis
47	Insecta	Odonata	Libellulidae	Sympetrum
48	Insecta	Odonata	Libellulidae	Tramea
49	Malacostraca	Amphipoda	Gammaridae	Gammarus

List of macroinvertebrates taxa found during the course of the sampling period. Samples taken from 36 ponds across the San Francisco Bay Area of California over a period of 4 years (2011–2014).

Appendix C

Figure 1C

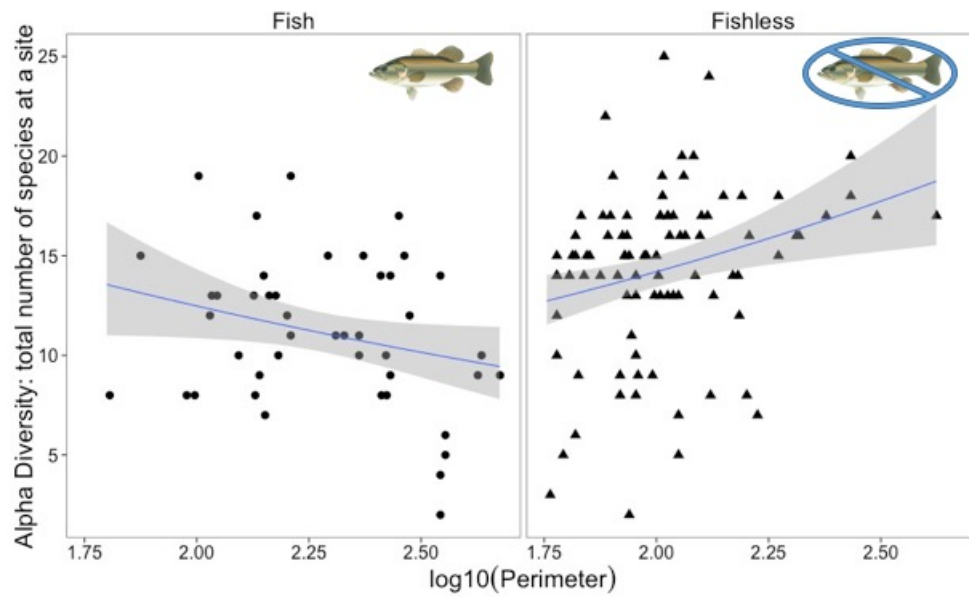


Figure 1C

The effect of perimeter in meters (log 10 transformed) on site level alpha diversity

interaction coefficient 0.35 ± 0.14 . A) Fishless sites coefficient B) Fish sites coefficient

Appendix D

In order to verify that we adequately captured the landscape level diversity we performed rarefactions curves across all sites, (Fig. 1D) fish sites, (Fig 2D) and fishless sites (Fig 3D). We used the `vegan` package in the R statistical software.

Figure 1D

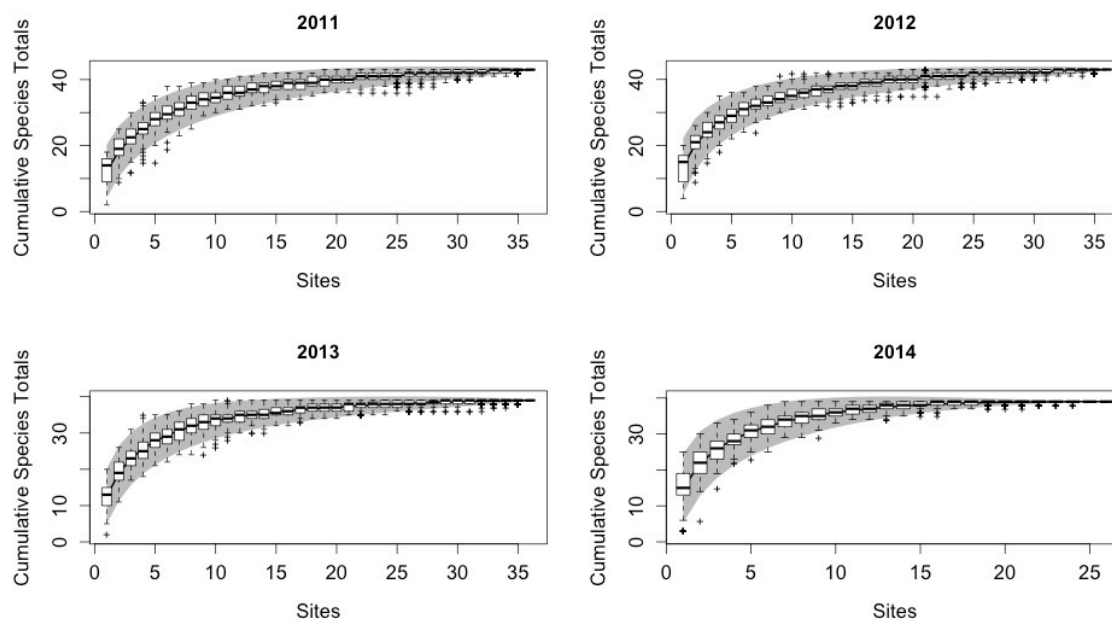


Figure 1D

Rarefaction curve among all sampled sites sampled for each sample year.

Figure 2D

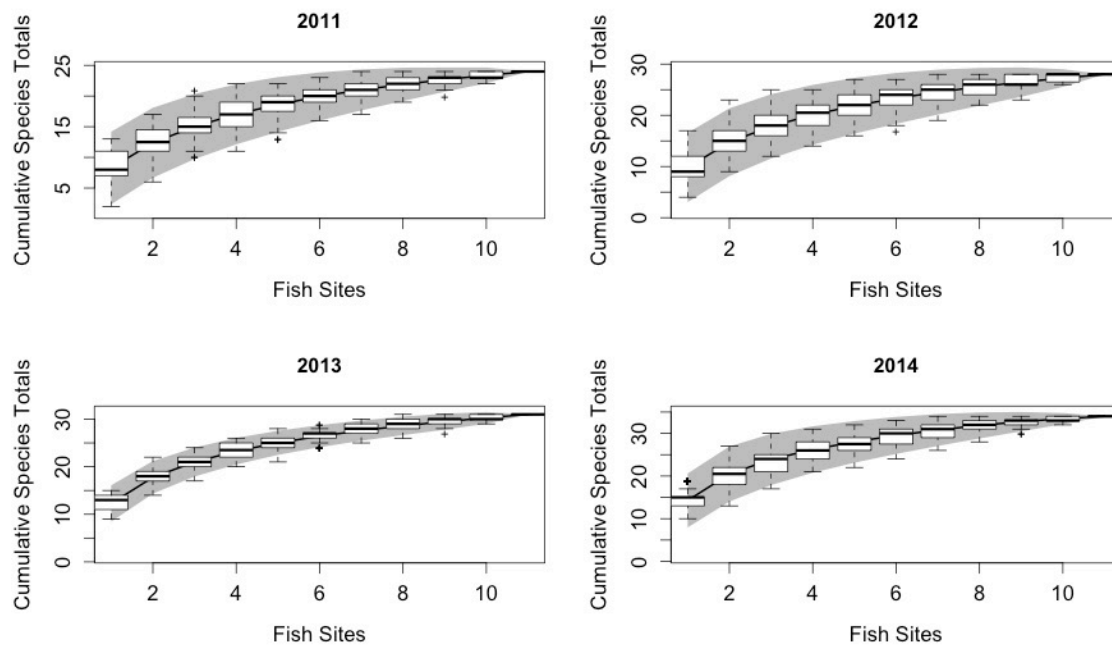


Figure 2D

Rarefaction curves among all fish present sites sampled for each sample year

Figure 3D

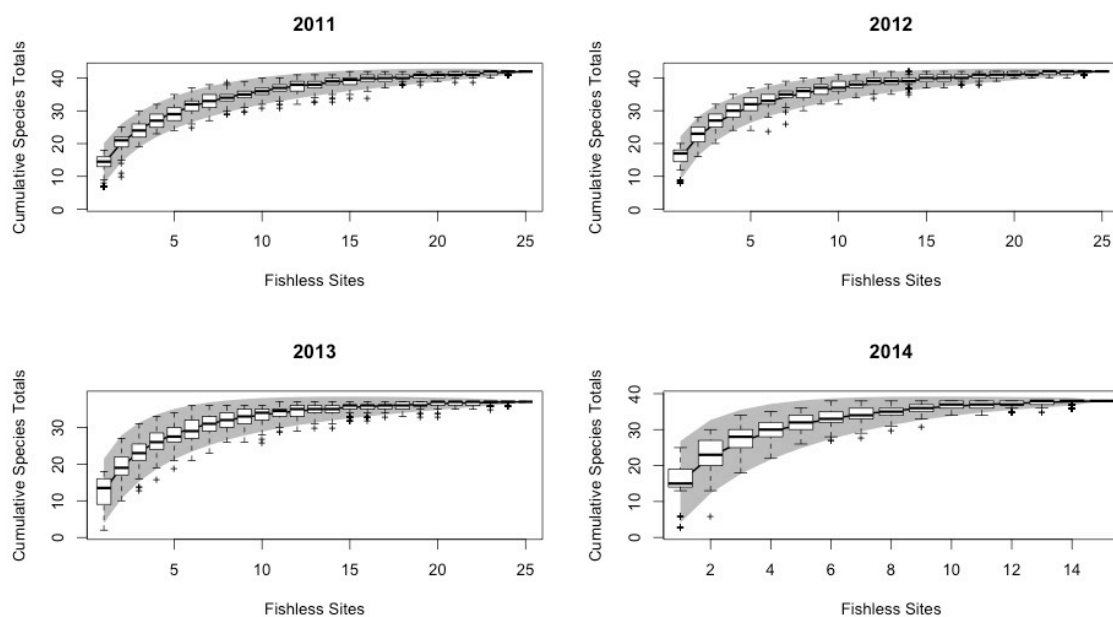


Figure 3D

Rarefaction curves among all fishless sites sampled for each sample year.

Appendix E

Table 1E

	Class	Order	Family	Genus
1	Malacostraca	Amphipoda	Gammaridae	<i>Gammarus</i>
2	Insecta	Odonata	Aeshnidae	<i>Anax</i>
3	Insecta	Odonata	Aeshnidae	<i>Aeshnidae</i>
4	Insecta	Odonata	Libellulidae	<i>Eythemis</i>
5	Insecta	Odonata	Libellulidae	<i>Pachydiplax</i>
6	Insecta	Odonata	Libellulidae	<i>Planthemis</i>
7	Insecta	Odonata	Libellulidae	<i>Sympetrum</i>
8	Insecta	Odonata	Libellulidae	<i>Tramea</i>
9	Insecta	Odonata	Coenagrionidae	<i>Ishnura</i>
10	Insecta	Odonata	Coenagrionidae	<i>Coenagrion</i>
11	Insecta	Odonata	Coenagrionidae	<i>Telebasis</i>
12	Insecta	Odonata	Lestidae	<i>Archilestes</i>
13	Insecta	Odonata	Lestidae	<i>Lestes</i>
14	Insecta	Hemipterian	Belostomatidae	<i>Belostoma</i>
15	Insecta	Hemipterian	Belostomatidae	<i>Lethocerus</i>
16	Insecta	Hemipterian	Notonectidae	<i>Notonecta</i>
17	Insecta	Ephemeroptera	Baetidae	<i>Callibaetis</i>
18	Insecta	Coleoptera	Halipidae	<i>Halipus</i>
19	Insecta	Coleoptera	Dytiscidae	<i>Laccophilus</i>
20	Insecta	Coleoptera	Dytiscidae	<i>Cybister</i>
21	Insecta	Coleoptera	Hydrophilidae	<i>Hydrophilus</i>
22	Insecta	Coleoptera	Hydrophilidae	<i>Tropisternus</i>

Table 1E

List of all macroinvertebrates species surveyed for parasite infection

Appendix F

We conducted a principal components analysis on a suite of water chemistry and nutrient data using the PCA function in the R statistical software package **FactoMineR**. We retained the first two principal component scores, which accounted for 38.5% and 25.3% of the explained variation respectively for cumulative variation explain of 63.8%. (Table 1F). Three of the five variables (conductivity, salinity, and turbidity) had positive loadings on the first principal components score, with two have negative loadings (total dissolved solids, pH). The second principal component score three of the five had positive loadings (conductivity, total dissolved solids, and salinity) with two had negative loading (pH and turbidity). For complete loading scores on the first two scores see table 2F

Table 1F

	eigenvalue	percentage of variance	cumulative percentage of variance
comp 1	1.92	38.48	38.48
comp 2	1.27	25.32	63.80
comp 3	0.97	19.50	83.30
comp 4	0.67	13.36	96.65
comp 5	0.17	3.35	100.00

Table 1F

Eigenvalue and percent of variation explained for the factors in the principal component analysis

Table 2F

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
Conductivity	0.59	0.76	0.09	-0.00	-0.24
Total Dissolved Solids	-0.51	0.78	-0.10	0.28	0.21
Salinity	0.87	0.12	0.23	-0.32	0.25
pH	-0.27	-0.04	0.95	0.15	-0.02
Turbidity	0.68	-0.25	-0.07	0.68	0.04

Table 2F

Loading scores for the variables among the dimensions in the principal component analysis