

Parasites of invasive freshwater fishes and the factors affecting their richness

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Abstract: Freshwater environments are threatened by nonnative species introductions, often involving fishes. Parasites and pathogens introduced with fishes have the potential to infect native taxa and should be investigated. We examined 726 fishes representing 6 invasive species from 27 ponds in California to evaluate how parasite richness, infection prevalence, and parasite abundance varied with host body size, host species, pond identity, and environmental variables. We identified 14 parasitic taxa based on morphological and molecular techniques. Overall, 60% of fish were infected with ≥ 1 parasite taxa: 6 trematodes, 3 monogeneans, 3 nematodes, 1 larval cestode (unidentified), and 1 acanthocephalan. *Micropterus salmoides* had the highest average infection prevalence ($85\% \pm 0.08$ SE, $n = 17$ site-year combinations), the highest average infection abundance (292.51 ± 119.10 SE parasites/fish), and the 2nd-highest γ diversity (10 taxa; *Lepomis macrochirus* had 11 total parasite taxa). In contrast, *Gambusia affinis* consistently supported the lowest parasite abundance, infection prevalence, and richness. Across all fish species, larger body size was associated with a broader diversity of parasites, but host sex had no effect. Increased pond area, pH, and aquatic community diversity also were correlated positively with parasite richness in fish populations, consistent with potential influences of colonization opportunities and the ‘diversity begets diversity’ hypothesis. A shortage of historical information on the native ranges of observed parasites precluded an assessment of whether these infections were introduced with their fish hosts. Nonetheless, they have the potential to directly or indirectly affect interactions between introduced fishes and native fauna, particularly given the near-absence of native lentic fishes in this region and the fact that several infections are shared with sensitive endemic taxa, such as amphibians.

Key words: invasion biology, parasites, invasive fish, parasite biodiversity, aquatic conservation, food web, amphibian decline

Over the last 30 y, the estimated number of introduced species has nearly doubled, amplifying the problem of biotic homogenization and threatening global biodiversity (Charles and Dukes 2008, Gozlan 2008, 2009, Johnson et al. 2008, Leprieur et al. 2009). Freshwater environments, such as streams, lakes, and ponds, are among the most invaded and most threatened ecosystems worldwide (Cohen 2002, Saunders et al. 2002, Ricciardi 2003, Dudgeon et al. 2006). Fishes often are introduced for food, through the aquarium trade, or for sport (Moyle and Marchetti 2006) and have been recognized as significant contributors to the extinction of native taxa in freshwaters (Olden et al. 2010). Olden and colleagues (2010) showed that introduced fishes had effects across multiple levels of biological organization, including genetic, individual, population, community, and ecosystem effects. However, these estimates of the ecological effect of fish introductions vary considerably. In part, this variability probably is a result of variation in the indirect pathways, including parasitism and disease risk, through which introduced

species affect native taxa and habitats (Williamson 1999, Olden et al. 2011, Simberloff et al. 2013).

Theoretical and empirical research suggests that invasive species can affect host–parasite interactions via diverse pathways (Torchin et al. 2003, Prenter et al. 2004, Kelly et al. 2009). One mechanism is ‘pathogen pollution’, in which invasive species introduce new parasites that can infect and threaten native species (Dobson and Foufopoulos 2001, Prenter et al. 2004, Telfer et al. 2005, Kelly et al. 2009). For example, introduced Grass Carp, *Ctenopharyngodon idella*, to North America were accompanied by the pathogenic tapeworm, *Bothriocephalus acheilognathi*, which is now established in Gila Chubs (*Gila intermedia*) in Arizona and California (Choudhury et al. 2006). A 2nd mechanism is alteration of the spread of endemic infections among native hosts by invasive hosts (Reinhart et al. 2003, Telfer et al. 2005, Kelly et al. 2009, Paterson et al. 2011). Recent studies of invasive species have detected numerous instances in which nonnative taxa altered the abundance of parasites

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that infect native species (Ostfeld and Keesing 2012). In some cases, introduced species tend to enhance infections among native hosts, whereas in others they dampen it. In a meta-analytic comparison of the native and introduced ranges of 53 species (26 plant and 27 animal species), Parker et al. (2013) found a 96% likelihood of enhanced reproduction or higher abundance in their introduced ranges in the absence of their associated predators and parasites ('enemy release hypothesis'; see also Torchin et al. 2003). The multiple pathways by which parasites and invasive species can interact emphasize the importance of identifying infectious agents within introduced species.

The western USA generally and California in particular support large numbers of nonnative fishes (Dill and Cordone 1997, Marchetti et al. 2004). Approximately 68 species of fishes are native to California, of which >30% occur exclusively or primarily within the state (Moyle 2002, Moyle and Marchetti 2006). However, since the 1870s, >110 nonnative fish species have been introduced (Moyle 2002, Moyle and Marchetti 2006). Relatively few scientists conducting field studies have intensively examined what parasites occur in freshwater fish communities from California, either among native or introduced taxa (see Hoffman 1999 for a summary). Over a 3-y period, Haderlie (1953) surveyed 2010 fishes representing 36 species from lakes, streams, and rivers in northern California, from which 59 taxa of helminths (trematodes, cestodes, and nematodes), copepods, and leeches were reported. While working in freshwater lakes in the Sacramento–San Joaquin delta, Edwards and Nahhas (1968) examined 236 fishes representing 26 species and detected 6 trematode taxa, 6 cestodes, 1 acanthocephalan, and several unidentified taxa of nematodes. However, over the last 45 y, authors of comparatively few published studies have examined the parasite infections from invasive California freshwater fishes, even as the number of introductions and their geographic ranges has continued to increase (Moyle and Marchetti 2006).

We surveyed the parasites of introduced fishes from natural and artificial pond ecosystems in the East Bay region of California over a 3-y period. Specifically, we studied 6 species of invasive fishes distributed across 27 ponds that were sampled between 2013–2015. We define these fishes as invasive species because they fit both criteria set out in Presidential Executive Order 13112 as taxa: 1) that are nonnative to the ecosystem, and 2) whose introduction causes or is likely to cause environmental or economic harm. These systems often support nonnative fishes introduced for sport fishing (Moyle 1976, Gozlan et al. 2010), through aquarium releases (Bearden 1967, Moyle and Marchetti 2006), or for biological control (Gozlan et al. 2010). Our aims were to characterize the macroparasite communities of these invasive fish species and to explore how parasite prevalence, abundance, and richness varied in relation to host size, species identity, and pond characteristics. We evaluated how

host traits (species identity, body size, and sex) and pond-level features (surface area, amount of forested area, water chemistry, and aquatic biodiversity) were correlated with parasite richness and abundance.

METHODS

Collection of fish and abiotic factors

In May and June 2013–2015, we collected fishes from 27 freshwater ponds in the Bay Area of California distributed across Alameda, Contra Costa, and Santa Clara counties (Fig. 1). We used habitat-stratified dipnet sweeps (45.7-cm D-frame with 1.2-mm mesh), hauls with 1 of 2 seine nets (1.2 × 1.8 m or 4.5 × 1.8 m, each with 3-mm mesh), and rod and reel. These methods often sample different size classes.

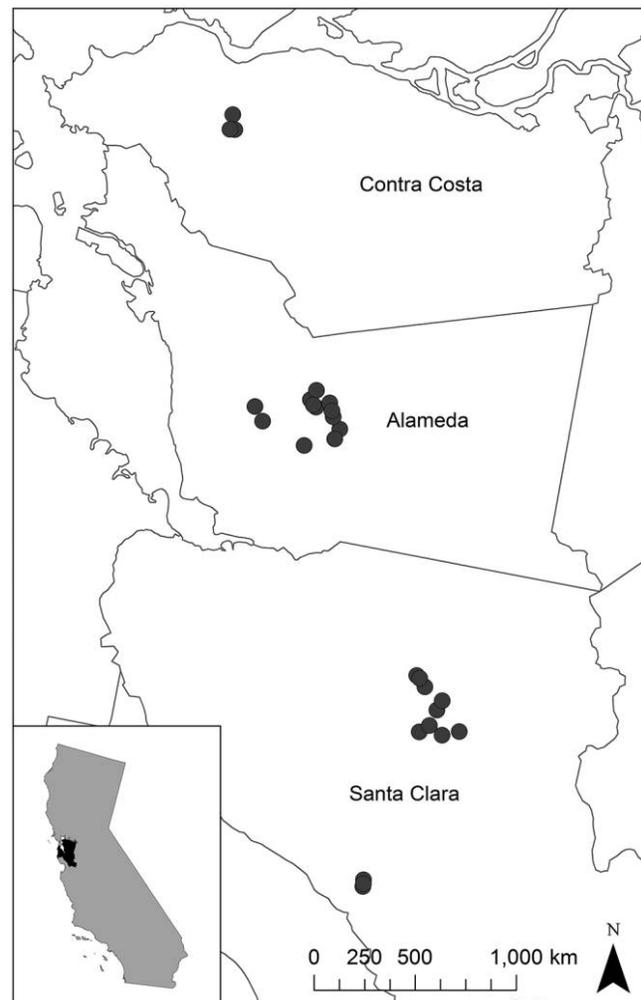


Figure 1. Map showing the locations of 27 ponds in the East Bay Region of California sampled between 2013 and 2015. Ponds with fishes were selected from a larger pool of sampled ponds that were distributed across publicly accessible and private conservation areas, regional parks, municipal utility districts, and ranches.

Rod and reel are the primary means of capture for adult *Lepomis* spp. and *Micropterus salmoides*, whereas seines and dip nets tend to capture juvenile fishes or *Gambusia affinis* and *Carassius auratus*. We euthanized fish humanely on site with MS-222 following guidelines approved by the University of Colorado Institutional Animal Care and Use Committee protocol (1302.01) and then froze them until necropsy. Host-level characteristics were recorded (host species, total length, sex) before examination for parasites (see below). We aimed to capture a minimum of 10 to 15 individuals of each fish species per pond per year. However, not all ponds were sampled for fishes in all years because of variation in fish abundance and hydroperiod; e.g., 7 sites went dry in 2014 and, therefore, supported no fishes thereafter. The intensifying drought beginning in 2012 was classified as among the most severe in the last 1200 y (Griffin and Anchukaitis 2014). Thus, we sampled 20 sites in 2013, 12 in 2014, and 11 in 2015, for a total of 43 site-year combinations.

Pond-level covariates (water pH, conductivity, total dissolved N, total dissolved P, and dissolved organic C, pond surface area, perimeter, and taxonomic richness of other aquatic organisms [e.g., amphibians, aquatic insects, and mollusks]) were measured at each pond during the fish collections (see Johnson et al. 2013, 2016 for additional details on data collection). In brief, we measured pH and conductivity with a handheld multiparameter (PCSTest™ 3; Oakton, Vermon Hills, Illinois). Water samples were filtered using a Millex® 5.00- μm micropore filter and analyzed at the Arikaree Environmental Laboratory (<https://instaar.colorado.edu/research/labs-groups/arikaree-environmental-lab/page/26755/>) for total dissolved N, organic C, and P. We estimated pond surface area by walking the perimeter with a handheld global positioning system (GPS) unit (GPSMAP 60; Garmin, Olathe, Kansas) while using the 'track' function. We also used data derived from the National Land Cover Database (NLCD) to estimate the percentage of land-cover types within a 1-km radius of each pond (Vogelmann et al. 2001, Johnson et al. 2013). Land-cover types were expected to influence visitation rates by potential definitive hosts (for complex life-cycle helminths) and colonization opportunities by fishes and intermediate hosts. We specifically estimated forested area as the sum of deciduous evergreen and mixed forest and grassland area as the sum of grassland, herbaceous, shrub, and scrub areas (Johnson et al. 2011). Last, we used all observed aquatic macroinvertebrates and vertebrates documented at the site through any collection method to calculate aquatic species richness (see Johnson et al. 2013, 2016 for details on sampling methods; California Department of Fish and Wildlife permit number: SC-3683). Multihost data often are lacking in parasitological field studies, but growing evidence suggests that free-living richness can be an important determinant of parasite richness and species composition (e.g., Hudson et al. 2006, Kamiya et al. 2014, Johnson et al. 2016).

Necropsy and parasite collection

After measuring the total length of each fish using digital calipers, we examined the eyes, nares, internal organs, and musculature for macroparasites (Trematoda, Nematoda, Cestoidea, Acanthocephala, and Monogenea) with the aid of an Olympus SZX10 stereodissection microscope. We also removed each gill raker, placed it in a sterile conical tube filled with 45 mL of deionized water, and shook the vial vigorously for 1 min to dislodge any parasites from the gill filaments. We then filtered the contents through a series of sieves and examined the material that passed through the final 50- μm filter on a gridded Petri dish to quantify groups, such as monogeneans. We also removed and examined each lateral-line scale to aid in detection of *Ribeiroia ondatrae* metacercariae. We identified all isolated parasites with the aid of taxonomic keys published by Schell (1985), Hoffman (1999), Jones et al. (2005), and Anderson et al. (2009). We stained voucher samples with aqueous Meyer's hematoxylin solution, dehydrated in graded ethanol series, cleared with methyl salicylate, and mounted in Canada balsam to further facilitate identification (Calhoun et al. 2013). Molecular vouchers of representative parasites containing a minimum of 5 metacercariae or 1 nematode, cestode, or acanthocephalan of each identified taxa from each location in 95% ethanol and stored at -20°C for genomic analysis.

For descriptive purposes, we defined α diversity as the per-host parasite taxonomic richness (i.e., the number of unique parasite taxa in a given individual fish) and γ diversity as the total number of parasite taxa recorded in a given fish species among all ponds and years of sampling. Infection prevalence was calculated at the collection level as the proportion of fish infected with a particular parasite for each species-pond-year combination. Similarly, infection abundance was the number of parasites/individual fish host. When calculating average values of prevalence and abundance for each pond-year-species combination, we excluded ponds at which a given parasite was not detected but included hosts with infection loads of 0, provided other conspecifics from that pond were infected (Bush et al. 1997).

Whenever possible, we confirmed taxonomic identifications using 28S ribosomal gene sequences via the Basic Local Alignment Search Tool (BLAST) in GenBank, for which a $\geq 95\%$ match was used as the threshold for molecular verification. We extracted genomic DNA from individual helminths with Zymo Quick DNA™ MiniPrep (Zymo Research, Irvine, California) following methods described by Calhoun et al. (2013) and the manufacturer's instructions. We amplified DNA fragments measuring $\geq \sim 2000$ base pairs (bp), including the 3' and 5' of the 28S gene, from the extracted DNA by polymerase chain reaction (PCR). Forward primer digl2 (5'-AAGCATATCACTAAGCGG-3' and reverse primer 1500R (5'-GCTATCCTGAGGGAACTTCG-5') were used in reactions (Olson et al. 2003, Olson and Tkach 2005, Calhoun et al. 2013). We purified PCR products

with Zymo DNA Clean and Concentrator™, and personnel at the Institute for Cellular and Molecular Biology at the University of Texas, Austin, sequenced our samples via capillary-based ABI 3730/3730XL DNA Analyzers (Applied Biosystems Inc., Foster City, California). Forward and reverse sequences were edited using Geneious version 7.1.7 (Kearse et al. 2012) and consensus sequences were constructed. The consensus sequences were screened against the GenBank® database using the BLAST algorithm and representative sequences are deposited in GenBank (MG544871, MG544872, and MG544873).

Statistical analysis

We restricted statistical analyses to the subset of samples for which we had complete sets of measured host- and site-level variables ($n = 597$ hosts from 43 pond–year combinations). This subset included 6 sites visited in all 3 y, 8 sites visited in 2 y, and 8 sites visited in a single year. We excluded 1 host that was missing a measurement of body size and 9 site–year combinations that were lacking data on water chemistry, turbidity, or free-living richness. We also excluded *C. auratus* because this species was detected at only 1 site. We used generalized linear mixed models (GLMMs) to test which factors affected parasite richness among host individuals. GLMMs allow inclusion of fixed effects (typically considered covariates or predictors) and random effects, which account for nonindependence among observations (e.g., because of expected correlations of hosts from the same site or repeated sampling of a specific site over time). This approach is superior to dividing complex data sets by site, date, year, or species because it takes advantage of all observations to offer improved estimates of parameters, especially for unbalanced designs typical of field surveys. GLMMs also allow specification of nonnormal error distributions, such as binomial, Poisson, gamma, or negative binomial, which helps to accommodate many types of ecological data in ways that may be preferable to transformation or nonparametric methods (e.g., Bolker et al. 2009).

To examine variation among individual hosts, we modeled parasite richness based on a Poisson distribution with a log-link function and included fixed effects related to the host (species identity, body size, and sex) and to the pond from which the host was collected. Poisson distributions are often appropriate for discrete counts of richness, which we verified by examining histograms of the data and comparing the Akaike Information Criterion (AIC) scores between models formulated with a Poisson error distribution vs Gaussian (normal) and negative binomial (overdispersed) distributions. We used a similar approach to evaluate what factors affected abundance of the most common parasite species, *Posthodiplostomum minimum*, but we modeled parasites/host as a negative binomial distribution. For pond characteristics, we included surface area (\log_{10} -transformed), ele-

vation, the amount of forested area within a 1-km radius of the pond, free-living taxonomic richness (i.e., the number of amphibian, snail, aquatic insect, and other macroinvertebrate taxa detected), and characteristics of the water, such as pH, water temperature, turbidity ($\log_{10}[x]$ -transformed), and total dissolved N ($\log_{10}[x]$ -transformed). With the exception of elevation, each of these variables was year-specific (i.e., measured within the specific sampling year that fish were collected, thereby allowing us to take interannual variation into account). We excluded variables that were highly collinear (Pearson $r > 0.6$) with these selected predictors, such as pond perimeter, dissolved organic C, total dissolved P, water conductivity, and grassland area.

We wanted to test for the effects of fish size independently from fish species identity, so we converted individual body sizes into z -scores by subtracting the mean body size for a given fish species (among all individuals of that species from all sites) and dividing by its standard deviation. This approach effectively allowed us to test for the general influence of host body size on infection responses as distinct from the relationship with host species, which was included as a categorical variable. We incorporated random intercept terms for pond identity and sampling year to account for the co-occurrence of multiple hosts and fish species in the same pond or year. After specifying the full model with all covariates, we removed nonsignificant terms and compared AIC changes (using maximum likelihood estimation) to identify the best-fitting model (Crawley 2002). We scaled all variables prior to inclusion to standardize the resulting coefficients. We examined the potential for spatial autocorrelation in the residuals of the final model by plotting the spline correlogram of correlations as a function of spatial distance (Rhodes et al. 2009). We did not include interaction terms between fish species identity and other covariates because of the large number of additional terms it would incur (with corresponding risk of overfitting) and because of a lack of a priori theoretical justification.

Based on our past observations that infection patterns in *G. affinis* vary in response to the presence of other fish species (DMC, unpublished data), we analyzed how parasite richness and total parasite abundance on individual *G. affinis* depended on co-occurrence of *Lepomis* spp. or *M. salmoides*. We included pond surface area and total free-living richness as covariates to address potential variation in the types of ponds supporting multiple fish taxa, and we selected among nested models based on likelihood ratio tests. Fish body size was included in all models. All analyses were conducted using R (version 3.3.2; R Project for Statistical Computing, Vienna, Austria). Models of parasite richness were analyzed with the *lme4* package (Bates et al. 2014), whereas those of parasite abundance were analyzed with the *glmmADMB* package (Bolker et al. 2012). We obtained p - and R^2 - values for GLMMs with *lmerTest* and *rsquaredglmm*, respectively.

RESULTS

Fishes

Over 3 y, we collected 726 fishes from 27 ponds, most of which were sampled in multiple years, to obtain 43 site-year combinations. This collection of fishes included 293 *G. affinis*, 187 *L. macrochirus*, 136 *M. salmoides*, 79 *L. cyanellus*, 24 *C. auratus*, and 7 *L. microlophus*. *Lepomis macrochirus* were the most commonly detected fish and occurred at 16 ponds, followed closely by *G. affinis* (14 ponds). *Carassius auratus* were found at only 1 pond. Most ponds harbored 1 to 2 species of fishes, and 1 site supported 4 (*G. affinis*, *L. cyanellus*, *L. macrochirus*, and *M. salmoides*). No native fishes were detected at any of the ponds sampled.

Parasites

We identified 14 taxa of parasites using a combination of morphological and molecular techniques (Tables 1, 2, S1). Three adult gill monogeneans (*Actinocleidus fergusonii*, *Cleidodiscus* spp., and *Dactylogyrus* spp.) were found in ~36, 8, and 2% of hosts, respectively. We recorded metacercariae representing 6 trematodes (*Bucephalus* spp., *Clinostomum* spp., *Echinostoma* spp., *P. minimum*, *Gorgoderina* spp., and *R. ondatrae*), with *P. minimum* as the most prevalent and most abundant parasite (overall prevalence = 36%, mean \pm SE abundance = 56.68 ± 8.59 parasites/dissected fish), often occurring in the liver, heart, kidneys, and body cavity of infected hosts. *Ribeiroia ondatrae*, which has rarely been recorded from fishes in California (Orlofske et al. 2012), was found in the lateral-line canal scales of 13% of sampled fishes, with an average intensity of 4.54 ± 0.84 parasites/infected fish. Only 2 individual fishes (*M. salmoides* and *L. cyanellus*) harbored larval cestodes (unidentified), whereas 6.9% of dissected fishes contained the nematode, *Contracaecum* sp. (mean \pm SE = 0.26 ± 0.06). Adult acanthocephalans (*Neoechinorhynchus cylindricus*) were detected in *M. salmoides*, *G. affinis*, *L. macrochirus*, *L. microlophus*, with an overall prevalence of 3%.

For 3 of the detected trematodes (*Clinostomum* spp., *R. ondatrae*, and *Echinostoma* spp.), we sequenced a partial fragment (>1500 bp) of the 28S ribosomal (r)DNA gene that aligned closely with previously identified genera within GenBank®. The $\geq 95\%$ bp match in conjunction with our morphological identifications adds confidence to the taxonomic identifications (we acknowledge the high potential for cryptic diversity within these taxa and treat our parasite richness estimates as conservative). Cestodes, monogeneans, and nematodes were identified based on morphological identification alone because of limited taxonomic resolution of the genera in GenBank.

Patterns of infection varied among fish species and among pond communities. Of the fishes examined ($n = 726$), 60% were infected with ≥ 1 parasite species with an average parasite richness of 1.21 ± 0.05 (Table 1). All but 1 pond sup-

ported ≥ 1 parasite species. Infection prevalence averaged $60 \pm 2.0\%$ SE ($n = 27$ site-year combinations). Five collections exhibited 100% infection prevalence (e.g., all fishes examined had ≥ 1 parasites). Despite being common at our ponds, *G. affinis* were the least infected. Only 26% of examined individuals were infected ($n = 293$), and average infection abundance was 1.51 ± 0.43 parasites/fish (range 0–31, $n = 27$ site-year combinations). *Micropterus salmoides* was the most heavily infected species, with an average infection prevalence of 85% ($n = 17$ site-year combinations in which bass were detected), and an average infection abundance of 292.51/host (Fig. 2A). One adult *M. salmoides* supported 2917 parasites (256 *A. fergusonii*, 1 *Clinostomum* spp., 2 larval *Contracaecum* sp., 2636 *P. minimum*, and 15 *R. ondatrae* [new host record]). *Lepomis* spp. ($n = 36$ site-year combinations pooled among 3 *Lepomis* hosts collected) had intermediate levels of infection prevalence and variable parasite abundance values (Fig. 2A). *Carassius auratus* were sampled from only 1 pond over 3 y, for which infection prevalence averaged 0.48 ± 0.11 , and parasite abundance averaged 2.87 ± 0.94 . *Micropterus salmoides* exhibited the greatest mean α diversity (3.82 ± 0.54 parasite taxa/site-year combination, $n = 17$; Fig. 2B). Among species in the genus *Lepomis*, *L. macrochirus* exhibited the highest average α diversity (2.79 ± 0.28 parasite taxa/site-year combination, $n = 24$). *Lepomis macrochirus* also supported the highest γ diversity (11 total parasite taxa), followed closely by *M. salmoides* (10 total parasite taxa; Fig. 2B). *Gambusia affinis* supported the lowest α diversity (Fig. 2B).

Ecological correlates of parasite richness and abundance

Based on the model-selection procedure, parasite taxonomic richness/fish host was positively associated with aquatic taxonomic richness, pH, and host body size (z -scored by fish species), with a negative relationship to the amount of nearby forested area (GLMM with Poisson distribution, standardized coefficients for predictors: total richness = 0.172 ± 0.07 , $p = 0.014$; z -score [pH] = 0.161 ± 0.071 , $p = 0.024$; z -score [host size] = 0.358 ± 0.047 , $p < 0.0001$; z -score [forest] = -0.232 ± 0.118 , $p = 0.049$; $n = 597$ hosts from 43 site-year combinations). Fish species identity also had a strong influence, and *G. affinis* consistently exhibited lower levels of parasite richness relative to other host species ($p < 0.0001$). *Micropterus salmoides* had the greatest diversity of parasites. The conditional R^2 for the best-fitting model was 0.63. Parasite richness was not significantly associated with water temperature, pond elevation, turbidity, or total N. Among the subset of adult hosts ($n = 586$), sex had no added influence on per-host patterns of parasite richness ($p > 0.3$). Including sample year as a random-intercept term tended to worsen model fit ($\Delta\text{AIC} > 2$).

For the abundance of *P. minimum*, which was the most common parasite, the best-supported model included pos-

Table 1. Total prevalence and average abundance of parasites detected across the 3-y study in freshwater ponds in California. Total number of invasive fishes infected with each detected parasite taxon. Number of ponds with infection is reported at the site-by-year combination. * designates a new species recorded, ** designates a new geographical record.

Parasite taxon	Number of infected fish	Fish host(s) with infection	Number of sites with infection	Mean total prevalence (%)	Mean abundance/ dissected fish
Monogenea					
<i>Actinocleidus fergusonii</i>	260	<i>Lepomis macrochirus</i> , <i>Micropterus salmoides</i> , <i>Gambusia affinis</i> *, <i>Lepomis cyanellus</i> , <i>Lepomis microlophus</i> , <i>Carassius auratus</i> *	22	35.81	19.14 ± 2.81
<i>Dactylogyrus</i> spp.	17	<i>Gambusia affinis</i> *, <i>Lepomis macrochirus</i> , <i>Carassius auratus</i> *	4	2.34	0.13 ± 0.04
<i>Cleidodiscus</i> spp.**	60	<i>Gambusia affinis</i> *, <i>Lepomis macrochirus</i> , <i>Micropterus salmoides</i> *	12	8.26	6.15 ± 1.42
Trematoda					
<i>Bucephalus</i> spp.**	7	<i>Lepomis macrochirus</i>	2	0.96	0.13 ± 0.06
<i>Clinostomum</i> spp.	73	<i>Micropterus salmoides</i> *, <i>Lepomis macrochirus</i> , <i>Gambusia affinis</i> *, <i>Lepomis microlophus</i> , <i>Lepomis cyanellus</i>	8	10.06	0.80 ± 0.24
<i>Echinostoma</i> spp.**	22	<i>Micropterus salmoides</i> *, <i>Lepomis macrochirus</i> *, <i>Lepomis cyanellus</i>	7	3.03	0.47 ± 0.17
<i>Posthodiplostomum minimum</i>	262	<i>Lepomis macrochirus</i> , <i>Gambusia affinis</i> , <i>Lepomis cyanellus</i> , <i>Micropterus salmoides</i> , <i>Lepomis microlophus</i>	17	36.09	56.68 ± 8.59
<i>Gorgoderina</i> sp.**	2	<i>Lepomis macrochirus</i> *, <i>Micropterus salmoides</i> *	2	0.28	0.01 ± 0.004
<i>Ribeiroia ondatrae</i> **	96	<i>Lepomis macrochirus</i> , <i>Micropterus salmoides</i> *, <i>Lepomis cyanellus</i> *, <i>Carassius auratus</i> *	10	13.22	4.54 ± 0.84
Nematoda					
<i>Contracaecum</i> sp.	50	<i>Lepomis macrochirus</i> , <i>Micropterus salmoides</i> , <i>Gambusia affinis</i>	9	6.89	0.26 ± 0.06
<i>Eustrongylides</i> sp.	1	<i>Gambusia affinis</i>	1	>0.001	0.001 ± 0.001
Unidentified adult nematode	1	<i>Lepomis cyanellus</i>	1	>0.001	0.001 ± 0.001
Cestoidea					
Unknown larval cestode	2	<i>Micropterus salmoides</i> , <i>Lepomis cyanellus</i>	2	0.28	6.15 ± 1.42
Acanthocephala					
<i>Neoechinorhynchus cylindratus</i>	25	<i>Gambusia affinis</i> *, <i>Micropterus salmoides</i> , <i>Lepomis macrochirus</i> , <i>Lepomis microlophus</i>	4	3.44	0.65 ± 0.31

itive effects for pH, free-living richness, water turbidity, N concentration, and host size (GLMM with negative binomial distribution, total richness = 0.951 ± 0.282 , $p = 0.0007$; pH = 0.806 ± 0.254 , $p = 0.0015$; z-score [host size] = 1.433 ± 0.125 , $p < 0.00001$; turbidity [$\log_{10}(x)$ -transformed] = 0.357 ± 0.162 , $p = 0.027$, total N [$\log_{10}(x)$ -transformed] = 0.873 ± 0.285 , $p = 0.0022$; 421 hosts from 17 sites). *Gambusia affinis* supported significantly lower infection loads than all other fish species ($p < 0.0001$), whereas *M. salmoides* and *L. macrochirus* tended to have higher infection abundances

($p < 0.0005$). For this analysis, inclusion of a random effect for sample year (as a factor) improved model fit ($\Delta AIC \approx 30$).

When *G. affinis* individuals co-occurred with other fish species, they tended to support a higher infection prevalence and a greater infection load than when they occurred in isolation. This effect was strongest with *M. salmoides*, the presence of which was associated with a $2.2\times$ increase in parasite richness within co-occurring *G. affinis* (GLMM with a Poisson distribution, *M. salmoides* presence = 1.63 ± 0.67 , $p = 0.015$; z-score (fish size) = 0.046 ± 0.016 , $p = 0.006$), a

Table 2. Total prevalence and mean (\pm SE) and range of abundance of parasite taxa detected in invasive fish species across the 3-y study of freshwater ponds in California.

Monogenea	<i>Lepomis macrochirus</i> (n = 187)			<i>Lepomis cyanellus</i> (n = 79)			<i>Lepomis microlophus</i> (n = 7)			<i>Micropterus salmoides</i> (n = 136)			<i>Gambusia affinis</i> (n = 293)			<i>Carassius auratus</i> (n = 24)		
	Prevalence	Abundance	Range	Prevalence	Abundance	Range	Prevalence	Abundance	Range	Prevalence	Abundance	Range	Prevalence	Abundance	Range	Prevalence	Abundance	Range
<i>Actinocleidus fergusoni</i>	54.01	24.77 \pm 3.37	0–260	43.04	4.92 \pm 0.10	0–43	71.43	52.71 \pm 24.32	0–179	64.71	61.50 \pm 13.43	0–1,326	9.55	0.28 \pm 0.07	0–9	20.83	1.65 \pm 0.88	0–15
<i>Dactylogyrus</i> spp.	0.53	0.01 \pm 0.01	0–1	—	—	—	—	—	—	—	—	—	3.41	0.22 \pm 0.09	0–17	25.00	1.17 \pm 0.49	0–8
<i>Cleidodiscus</i> spp.	16.04	19.81 \pm 4.86	0–391	7.60	0.22 \pm 0.09	0–4	—	—	—	8.82	4.83 \pm 0.36	0–303	4.10	0.28 \pm 0.11	0–23	—	—	—
Trematoda																		
<i>Bucephalus</i> spp.	3.74	0.51 \pm 0.21	0–23	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Clinostomum</i> spp.	10.70	1.34 \pm 0.86	0–145	18.99	1.56 \pm 0.47	0–19	14.29	1.57 \pm 1.57	0–11	22.80	1.39 \pm 0.36	0–30	2.05	0.02 \pm 0.01	0–2	—	—	—
<i>Echinostoma</i> spp.	1.60	0.02 \pm 0.10	0–2	2.53	0.24 \pm 0.23	0–18	—	—	—	12.5	2.36 \pm 0.87	0–90	—	—	—	—	—	—
<i>Potheadiplostomum minimum</i>	62.03	121.82 \pm 25.05	0–3,876	58.23	28.94 \pm 7.71	0–500	14.29	0.57 \pm 0.57	0–4	67.65	117.59 \pm 27.37	0–2,636	2.39	0.10 \pm 0.06	0–17	—	—	—
<i>Gorgoderina</i> sp.	0.53	0.02 \pm 0.20	0–3	—	—	—	—	—	—	0.74	0.01 \pm 0.01	0–1	—	—	—	—	—	—
<i>Ribeiroia ondatrae</i>	17.11	2.41 \pm 0.87	0–140	8.86	0.76 \pm 0.36	0–20	—	—	—	41.18	20.43 \pm 4.50	0–452	—	—	—	4.17	0.04 \pm 0.04	0–1
Nematoda																		
<i>Contracaecum</i> sp.	2.14	0.15 \pm 0.12	0–23	—	—	—	—	—	—	30.89	1.15 \pm 0.28	0–32	1.02	0.02 \pm 0.01	0–3	—	—	—
<i>Eustrongylides</i> sp.	—	—	—	—	—	—	—	—	—	—	—	—	0.34	0.003 \pm 0.003	0–1	—	—	—
Unidentified adult nematode	—	—	—	1.27	0.01 \pm 0.01	0–1	—	—	—	—	—	—	—	—	—	—	—	—
Cestodea																		
Unknown larval cestode	—	—	—	1.27	0.08 \pm 0.08	0–6	—	—	—	0.74	0.62 \pm 0.62	0–84	—	—	—	—	—	—
Acanthocephala																		
<i>Neochinorhynchus cylindricus</i>	0.53	0.14 \pm 0.14	0–26	—	—	—	14.29	0.86 \pm 0.86	0–6	3.68	2.55 \pm 1.63	0–210	6.14	0.31 \pm 0.12	0–31	—	—	—

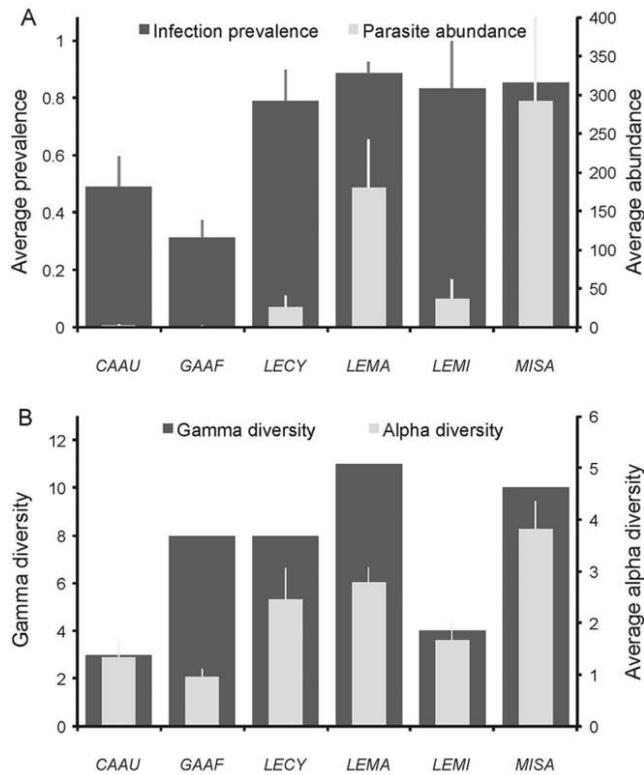


Figure 2. Mean (+SE) infection prevalence and average parasite abundance/host (A) and parasite taxonomic richness (α) by site-year and the total diversity (γ) of all parasite taxa detected in that host among ponds and years (B). Hosts are nonnative fish species in 27 ponds in California (2013–2015). The number of site-years sampled for each host species were: *Carassius auratus* (CAAU): $n = 3$, *Gambusia affinis* (GAAF): $n = 27$, *Lepomis cyanellus* (LECY): $n = 9$, *Lepomis macrochirus* (LEMA): $n = 24$, *Lepomis microlophus* (LEMI): $n = 3$, and *Micropterus salmoides* (MISA): $n = 17$.

1.9 \times increase in infection prevalence (GLMM with a binomial distribution, *M. salmoides* presence = 3.07 ± 1.29 , $p = 0.017$; z-score [fish size] = 0.716 ± 0.239 , $p = 0.0027$), and a 2.3 \times increase in total infection load summed among all parasite taxa (GLMM with a negative binomial distribution, *M. salmoides* presence = 2.62 ± 1.24 , $p = 0.035$; z-score [fish size] = 0.085 ± 0.023 , $p = 0.0003$). Neither pond size nor free-living richness significantly influenced these relationships, and these terms were dropped from the final models based on Δ AIC and a likelihood ratio test. When *G. affinis* occurred at ponds with *M. salmoides* and *L. macrochirus*, their parasite fauna included *N. cylindratus* and *P. minimum*, neither of which was detected when they occurred without these fish species.

DISCUSSION

We reported on the parasite fauna from 6 introduced fish species in California ponds. Among the 726 individual

fishes examined, we detected 14 unique taxa of parasites representing 6 digenetic trematodes, 3 monogeneans, 2 larval nematodes, 1 unidentified adult nematode, 1 unidentified larval cestode, and 1 acanthocephalan. These included 16 new host records and 5 new geographic records (Table 1). Most of the identified parasites ($n = 11$) were relative generalists, with few obvious patterns of host specialization. However, the trematode *Bucephalus* spp. was found only in *L. macrochirus*, despite previous studies reporting this parasite from a diversity of freshwater and marine fishes (Gibson et al. 2002). Among the fish species sampled, the patterns for parasite richness, infection prevalence, and infection abundance exhibited broadly similar trends. *Micropterus salmoides* supported the highest prevalence, intensity, and richness of parasites, whereas *G. affinis* were among the least infected, with a low prevalence and infection load (see Table 2). *Lepomis* spp. had intermediate levels of parasite richness and abundance, but some differences emerged. For instance, these fishes reside within the same genus, but their average parasite abundance varied considerably (*L. macrochirus*: 170.98 ± 27.74 ; *L. microlophus*: 55.71 ± 23.73 ; *L. cyanellus*: 36.72 ± 7.85 ; Fig. 2A).

Few parasitological surveys of nonnative freshwater fishes have been conducted in California, but our findings offer an interesting comparison with the last such major undertaking from 1953. Five of the 14 parasite taxa we identified (or close congeners) were observed in a survey of introduced fishes from the Merced River in San Joaquin Valley to Copco Lake on the Oregon–California border (*Actinocleidus* spp., *Clinostomum* spp., *P. minimum*, *N. cylindratus*, and *Contracaecum* sp.; Haderlie 1953). Of the fish host species processed in both surveys, *M. salmoides* and *L. macrochirus* were among the most consistently infected species (~80–93% infected in both surveys), whereas *G. affinis* were among the least (<30%). Both surveys also cited digenetic trematodes as the most frequently encountered parasite group. For instance, *P. minimum* was the most common parasite taxon, occurring in 5 of 6 host species sampled here and 12 of 36 from Haderlie (1953). In contrast, we detected more ectoparasitic monogeneans, which could reflect differences in water quality (e.g., Thulin 1991, Lafferty 2008, Gilbert and Avenant-Oldewage 2016) or methods. We used extensive filtering of material from gills to remove parasites that can otherwise be difficult to isolate, especially in the presence of mucus. One notable parasite not previously recorded in California fishes is the trematode *R. ondatrae*, which can cause dermal pathology in the lateral-line canal in some fishes (Calhoun et al. 2015) and limb malformations in amphibians (Johnson et al. 2002, 2004, Johnson and Sutherland 2003). Our study provided 2 new host records for *R. ondatrae* (*L. cyanellus* and *M. salmoides*) that add to the previously reported 9 species of fishes known to support this parasite in North and Central America (Johnson et al. 2004, Calhoun et al. 2015). However, because of its tendency

to infect scales of the lateral-line canal (Beaver 1939, Nassi 1978, Calhoun et al. 2015), metacercariae of this parasite could have been overlooked in previous surveys.

Our results highlight the importance of both pond- and host-level variables in affecting parasite richness within nonnative hosts. Parasite richness was associated with fish species identity and body size, consistent with previous research (Crofton 1971, Gregory et al. 1996, Poulin and Rohde 1997). *Micropterus salmoides* and *Lepomis* spp. supported a higher average parasite richness than did *G. affinis*, whereas larger hosts tended to have more parasites regardless of the host species under consideration. Sex did not affect parasite richness. Lo et al. (1998) reported that parasite richness increased with fish host size, and Bell and Burt (1991) found that host size and diet explained 40% of the variation in helminth diversity among Canadian fishes. The recurrent link between body size and infection across parasites with multiple forms of transmission could stem from the positive influence of body size on parasite encounter rate, increased exposure to infections with age, or shifts in total food consumption and the form of prey with ontogeny. For instance, *M. salmoides*, which supported 2.25× more parasites (average richness) than *G. affinis*, grow to a maximum size of 97 cm and can live up to 23 y (Froese and Pauly 2016), probably increasing their exposure to a wide range of parasites relative to smaller hosts with shorter lifespans. *Gambusia* spp. typically live 3 y and rarely are >7 cm long (Froese and Pauly 2016). *Micropterus salmoides* also tend to consume a large variety of vertebrate and invertebrate prey items (e.g., small fishes, crayfish, amphibians, crustaceans, and insects) that could increase the diversity of trophically transmitted parasites encountered (Haderlie 1953, Combes 2001, Hechinger and Lafferty 2005, Dunne et al. 2013).

Attributes of the pond, including surface area, free-living taxonomic richness, and water quality, also were correlated with parasite richness/host. Links between habitat size and species richness are well documented (Williams 1964, Thornton et al. 1993, Kruess and Tschardt 2000), and pond area probably influences establishment of parasites with complex life cycles by increasing visitation by definitive hosts, such as birds and mammals, and by facilitating the occurrence of necessary intermediate hosts (e.g., through increases in the richness of aquatic host taxa; Kennedy and Burrough 1978, Marcogliese and Cone 1991). Marcogliese and Cone (1991) found that parasite richness in salmonid fishes from Newfoundland increased from small ponds to medium- and larger-sized lakes. Larger bodies of water also may support bigger and more stable fish populations, which could reduce the likelihood of stochastic extinction events. Increases in pH of the pond also were associated with higher parasite richness, whereas high amounts of forest near the ponds were associated with lower parasite richness. Higher acidity, which is sometimes associated with inputs of dissolved organic C from nearby forests, can negatively affect snail populations

(Glass and Darby 2009), potentially reducing survival of parasites, such as trematodes, which depend on snail intermediate hosts (Gumble et al. 1957, Koprivnikar et al. 2010, Richgels et al. 2013) and other free-living parasitic stages that move through water (Blum and Fridovich 1983, Sures 2004, Kotze et al. 2005).

Why *G. affinis* supported so few parasites is unclear. Relative to other hosts species, *G. affinis* were infected with 42–78% fewer parasite taxonomic groups, on average, and supported 97% fewer total parasites/fish, results that parallel other surveys of this species in its introduced range (e.g., Haderlie 1953, Pyke 2008). In contrast, authors of parasitological surveys of *G. affinis* in its native range have reported higher parasite diversity. Davis and Huffman (1977) found 16 parasite taxa and an overall infection prevalence of 68% from *G. affinis* populations in Texas, which is thought to be where the California population originally derived. However, when *G. affinis* co-occurred with other fish species, and particularly with *M. salmoides*, they supported significantly higher parasite richness, increased prevalence, and greater average abundance than when they occurred in isolation. Such patterns could stem from overall differences in the characteristics of ponds in which fish co-occur or could be the result of spillover, particularly if *G. affinis* are ill-suited to support the infection in the absence of another reservoir host.

Most of the parasites documented in our survey are generalist species with limited historical data on their native geographic ranges in the USA, so determining whether these parasites existed in California prior to the introduction of nonnative fishes or were introduced alongside their hosts is difficult. *Posthodiplostomum minimum*, a trematode parasite with a complex life cycle that uses birds as definitive hosts, was reported in 5 California freshwater fishes (Haderlie 1953, Colley and Olsen 1963, Edwards and Nahhas 1968). Some of the parasites reported here have been observed extensively within the native range of these fishes (Wilson et al. 1996, Hoffman 1999), suggesting that introduced species might have brought nonnative parasites or genetic strains to California (i.e., pathogen pollution). For example, *N. cylindratus*, an adult acanthocephalan of fishes, frogs, and turtles, has been found in all examined *Lepomis* species and *Gambusia* species in their native range (Hoffman 1999). However, *Clinostomum* spp. was not previously documented in any of our examined species in their native ranges (Hoffman 1999), suggesting these introduced fishes might have become infected from the native fishes or amphibians within this region functioning as either infection sinks (dilution hosts) or sources (reservoir or amplification hosts).

Interactions between invasive species and parasites have been recognized as an important contributing factor in understanding and predicting the net effects of nonnative taxa in ecological communities (Torchin et al. 2003, Prenter et al.

2004, Hatcher et al. 2006). These interactions can include the role of introduced hosts in bringing novel infections along with them (Kennedy 1993, Gozlan et al. 2005, Thieltges et al. 2009) and the potential for nonnative species to alter interactions between native hosts and endemic infections (Torchin et al. 2003, Thieltges et al. 2009). Assessing the fraction of parasites observed in our study that are endemic to California ponds or are more recent arrivals is difficult, but the potential for the introduced fish taxa in our study to influence parasite transmission in native hosts warrants further attention. Native fish are uncommon in these ponds, but sensitive species, such as Sacramento Perch (*Archoplites interruptus*) and Sticklebacks (*Gasterosteus aculeatus*), both of which are currently classified as of 'critical concern' by the California Department of Fish and Wildlife (Moyle et al. 2015), could experience shifts in infection in habitats where introduced fish co-occur. In addition, 3 of the parasites (*R. ondatrae*, *Clinostomum* spp., and *Echinostoma* spp.) detected in fishes in our study also infect larval amphibians, sometimes with adverse effects (Belden 2006, Johnson and McKenzie 2009). Invasive fishes are a major contributing factor in amphibian population declines in the western USA because of effects such as direct predation (Lawler et al. 1999, Vredenburg 2004, Joseph et al. 2016). However, if pathogenic infections become amplified in fishes and subsequently spillover into amphibians, their net effects could be exacerbated. For instance, if the trematode *R. ondatrae* spreads from invasive fishes to native amphibians, it could contribute to increased mortality or development of severe limb malformations (e.g., Johnson et al. 2013). Such spillover events could be relevant for declining amphibians within this region, such as California red-legged frogs, California tiger salamanders, and western toads (Fisher and Shaffer 1996, Raffel and Anderson 2009).

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