Host preference of an introduced 'generalist' parasite for a non-native host

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ABSTRACT

Parasites can invade new ecosystems if they are introduced with their native hosts or if they successfully infect and colonise new hosts upon arrival. Here, we ask to what extent an introduced parasite demonstrates specialisation among novel host species. Infection surveys across three field sites in Gatun Lake, Panama, revealed that the invasive peacock bass, *Cichla monoculus*, was more commonly infected by the introduced trematode parasite *Centrocestus formosanus* than were three other common cichlid fishes. Laboratory infection experiments were conducted to determine whether parasitism might be driven by differential encounter/exposure to parasites or by differential infection susceptibility/preference across different host species. These experiments were performed by controlling for parasite exposure in single host (compatibility) experiments and in mixed host (preference) experiments. In all cases, the peacock bass exhibited higher infection rates with viable metacercariae relative to the other potential fish hosts. Our experiments thus support that an introduced generalist parasite shows apparent specialisation on a specific novel host. Further studies are needed to determine whether these patterns of specialisation are the result of local adaptation following invasion by the parasite.

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1. Introduction

The widespread introduction of non-native species is responsible for major environmental and economic impacts worldwide (Vitousek et al., 1997). Of particular concern are introduced parasites and pathogens, which can lead to emerging diseases of humans (Daszak et al., 2000), commercially valuable species (Cleaveland et al., 2001) and wildlife (Dobson and Foufopoulos, 2001). When parasites and pathogens are introduced into regions where their original "native" hosts are already established, a ready-made biotic environment exists for the parasite/pathogen to persist and spread (Woolhouse and Gowtage-Sequeria, 2005). However, some introduced parasites/pathogens establish and spread in new communities lacking hosts from their native range, which they accomplish by infecting novel hosts (Woolhouse et al., 2001). Yet to establish, persist and spread without their native hosts, introduced parasites must either be generalist species that can infect a broad variety of new hosts with some degree of success or they must specialise on new hosts in a novel community (Combes, 1981; Font, 2003). Here we ask to what extent does an

introduced generalist parasite specialise on specific novel hosts in a new environment?

The relative contribution of host species to parasite reproduction and transmission, R_0, (Anderson and May, 1981), will depend on their encounter rates and infection compatibility with different hosts, and can also be affected by host preference exhibited by the parasite (Combes, 1991). These properties (e.g., encounter, compatibility and preference), jointly determine a parasite's host range, also expressed as the extent to which a parasite specialises on a given set of potential host species. Parasite–host range is invariably shaped by the biogeographical and evolutionary history of both parasites and hosts, and is an important determinant of the extent to which introduced parasites can infect host species in novel ecological communities and persist in new ranges. Yet while host range is a central feature of the ecology and evolutionary history of host–parasite interactions (Combes, 2001; Poulin and Keeney, 2008), little is known about the factors underpinning a parasite's host range in nature (Perlman and Jaenike, 2003). This is because it is typically impossible to discern the relative contributions of differences in encounter rates and compatibility across different potential host species under natural conditions (Kuris et al., 2007). Thus, in order to investigate drivers of host specialisation, laboratory experiments that manipulate host–parasite encounter rates are needed to disentangle the likely drivers of parasite–host

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specificity (Bush and Clayton 2006; Kuris et al. 2007). Here, we evaluate the extent to which generalist species can demonstrate increased specialisation across potential host species, and attempt to discern the proximate causes (e.g., encounter, compatibility or preference), eliciting natural patterns of infection with laboratory experiments.

To this end, our study is unique in two ways. First, tests that attempt to elucidate ecological and evolutionary drivers of host specificity typically focus on native species and long-established host–parasite associations (Little et al., 2006; Simková et al., 2006; Sears et al., 2012). By contrast, our use of non-native parasites and novel hosts is more informative for inferring interactions during the early stages of host–parasite community assembly. Second, laboratory experiments testing for patterns of host specificity typically focus on parasites thought to be highly host-specific, and often document infection compatibility with an unexpectedly broad range of novel host species typically not encountered in nature (Perlman and Jaenike, 2003; King and Cable, 2007; Poulin and Keeney, 2008). Conversely, our experiments consider whether a parasite putatively considered a generalist (from its native range) shows unexpectedly high host-specificity in its introduced range among a set of host species with which the parasite does not share a common evolutionary history. Overall, our combined survey-experimental approach for introduced parasites on novel hosts should provide theoretical and practical insights concerning the ecological and evolutionary processes underpinning host–parasite associations in expanded geographical ranges.

The global spread of the trematode, *Centrocestus formosanus* is of concern due to its ability to infect and cause disease in wild and endangered fishes (Mitchell et al., 2000), and commercially valuable species (Vélez-Hernández et al., 1998). This parasite has spread to freshwater habitats around the world following the invasion of its first intermediate snail host (Salgado-Maldonado et al., 1995), the Asian red-rimmed melania, *Melanoides (Thiara) tuberculata*, which was first reported in Panama in 2003 (Garcés and Garcia, 2004). *Centrocestus formosanus* has a complex life cycle; free-swimming larval cercariae emerge from snail hosts and encyst as metacercariae in the gills of second intermediate fish hosts (Scholz and Salgado-Maldonado, 2000). The parasite is then trophically transmitted to a piscivorous avian or mammalian definitive host (where the parasite sexually reproduces) when the infected fish is eaten, thereby completing its life cycle (Chen, 1942). The parasite, while it is highly specialised to its first intermediate snail host, has been reported to infect dozens of fish species across different families throughout its broad geographic range (Scholz and Salgado-Maldonado, 2000).

While this parasite is reported to have a broad host range throughout its global distribution, its potential to specialise on particular fish host species in its native or introduced ranges has not been examined. In order to measure specialisation in nature, a robust comparison of parasite prevalences and abundances across multiple co-occurring host species is necessary (Poulin, 2011). In order to gain some insight into the possibility of *C. formosanus* to specialise on a particular host species in a novel habitat, we evaluated natural patterns of parasitism across four potential cichlid fishes common in Gatun Lake, Panama, the native cichlid "vieja" (*Vieja maculicauda*) and three introduced cichlids, peacock bass (*Cichla monoculus*), oscar (*Astronotus ocellatus*), and Nile tilapia (*Oreochromis niloticus*). These four species are the most common cichlids in the Gatun Lake (Gonzalez-Gutierrez, 2000) and, importantly, none of these species shares an evolutionary/biogeographical history with *C. formosanus*, which is native to southeastern Asia (Scholz and Salgado-Maldonado, 2000).

We first compared prevalences and abundances of *C. formosanus* infection across the four fish species to evaluate natural patterns of parasitism of this introduced parasite. This is the first known report of *C. formosanus* in Panama and unlike reports from elsewhere, our field comparison suggests that the parasite is not broadly distributed across the fish hosts but rather appears to be specialised on the peacock bass, a novel fish host with which the parasite does not a share a long-term co-evolutionary history. We hypothesise that the patterns of infection in the field could be driven by differences in encounter/exposure rates, susceptibility of the fishes to the parasite or the host preference of *C. formosanus*. To distinguish these hypotheses, we used laboratory experiments that (i) held encounter rates constant to test for differences in infection compatibility in single-species trials and (ii) tested host preference in mixed-species experiments. Our laboratory experiments corroborate field comparisons, suggesting that higher infection rates in the peacock bass are due to both greater host preference for peacock bass by *C. formosanus* and increased compatibility with that host compared with the other fishes. The potential specialisation by this introduced parasite on a common introduced host could provide further insight into how introduced parasites establish, integrate and potentially evolve in novel assemblages of hosts in recently expanded geographic ranges. However, additional research is needed to discern whether this potential specialisation is the result of local adaptation of the parasite to a common introduced host.

2. Materials and methods

2.1. Field comparisons

Gatun Lake, part of the Panama Canal, was formed when the Chagres River was dammed early in the 20th century. The lake now has a biotic community of native and introduced species with broad biogeographic origins at all trophic levels, including aquatic plants, invertebrates such as snails and clams, and vertebrates such as fishes and reptiles (Zaret and Paine, 1973; Gonzalez-Gutierrez, 2000). The Asian red-rimmed melania snail, *M. tuberculata*, was first reported in Panama in 2003 as one of the two most abundant introduced mollusks in Gatun Lake (Garcés and Garcia, 2004), yet the pathway and chronology of the introduction and invasion (and that of its trematode parasite, *C. formosanus*) in Panama is unknown.

In February and March of 2010, we quantified prevalences, abundances and intensities (Bush et al., 1997) of *C. formosanus* in four common and co-occurring cichlid fish species (*C. monoculus*, *A. ocellatus*, *O. niloticus* and *V. maculicauda*) across four sites within Gatun Lake: Gamboa (09°09′22″ N, 79°51′22″ W), Barro Colorado Island (09°10′02″ N, 79°50′07″ W), Rio Gatun (09°15′21″ N, 79°46′46″ W) and Rio Chagres (09°12′23″ N, 79°38′07″ W). All of the fish were sampled near the shore where they were likely exposed to *C. formosanus* cercariae shed from *M. tuberculata* in the littoral zone. Fish were caught using a 30 m monofilament gill net (divided into three panels with 1.5 cm, 3 cm and 4.5 cm wide filaments). The net was set three to four times each morning at each site over a 60 day period to reach a comparable sample size ($n = 15$) for each species at each site. Approximately 1 h after the nets were set, live fish were removed and transported in oxygenated 189.27 L coolers to the Smithsonian Tropical Research Institute (STRI) Naos Marine Laboratory in Panama City where they were euthanised by spinal incision and dissected for parasites. We removed and examined gill arches immediately after the fish were euthanised using stereomicroscopes. Encysted *C. formosanus* metacercariae were identified using a compound microscope that allowed visual inspection of diagnostic features of this parasite (Yanohara and Kagei, 1983).

2.2. Experimental design

To test for infection susceptibility and host preference, we used two experiments that exposed the four focal fish species to *C. formosanus* in aquaria. All experimental fish were collected from a location near Barro Colorado Island, Panama, where *C. formosanus* is absent in first intermediate snail hosts (*n* = 1200) (VM Frankel, personal observations) and native and introduced second intermediate cichlid fish hosts (Roche et al., 2010) including peacock bass (*n* = 42) (VM Frankel, personal observations). Fish were caught and transported to Naos Marine Laboratory in Panama City as described above.

The first laboratory experiment examined infection susceptibility across the four cichlid fish species using each fish species as a separate treatment (hereafter referred to as single species experiments). For this experiment, we placed four individuals of each species of comparable size (standard length, mean 22.67 cm, S.D, 3.71) into the same aquarium using eight to 10 replicate aquaria per species. We then exposed the fishes to approximately 500 larval trematode cercariae shed from *M. tuberculata* (Lo and Lee, 1996). The second experiment tested whether *C. formosanus* cercariae preferentially infected particular fish species by simultaneously exposing the four target species to *C. formosanus* cercariae (hereafter referred to as mixed species trials). Here, one individual of each species of similar size (mean 21.74 cm, S.D. 2.44) was placed into the same aquarium, replicated 10 times. Fishes were exposed to *C. formosanus* cercariae as above.

2.3. Experimental details

Prior to the experiment, fish were kept for 2 weeks in 1 m² 189.27 L fibre-glass tanks filled with filtered tap water and de-chlorinated by running an air pump for 24 h before the fish were introduced. The tanks were maintained under ambient conditions (23.5–26.0 °C, dissolved oxygen 8.1–8.5 mg/l, pH 6.7–7.2). All fish were fed once each week with comparable amounts of live fish (1 g for peacock bass and oscar) or fresh fruit (1 g for Nile tilapia and vieja). At the beginning of the holding period, each tank was treated with 5 ml of API STRESS COAT® and API STRESS ZYME® (Aquarium Pharmaceuticals, USA) to treat possible tissue damage from netting and transport. After 24 h of this treatment, a 25% water change was conducted each day for five consecutive days to clear these chemicals from the water. One week after the last water change, the fish were transferred to the experimental aquaria (see below) where they were housed for the rest of the experiment.

We used 1 m² 189.27 L fibre-glass aquaria, each divided into four 0.5 m² quadrants, each quadrant containing a single fish. The quadrants were divided using plastic screening (1 cm mesh size) that allowed free passage of trematode cercariae, but not fish. The fish were acclimated for 24 h before introducing 500 cercariae to the centre of a tank. After a 24 h period we changed 25% of the water daily for 2 weeks, after which the fish were dissected for parasites as above. We allowed 2 weeks for *C. formosanus* to develop into metacercariae in the experimentally exposed fishes. We then haphazardly sampled *C. fomrosanus* metacercariae in each of the experimentally infected fish to confirm viability of trematodes inside the cysts. Specifically, we visually observed living worms in the cysts and measured metacercariae, all which were within the size range (0.145 and 0.200 mm diameter) of *C. formosanus* metacercariae described across various fish species (Mitchell et al., 2002; Salgado-Maldonado et al., 1995; Scholz and Salgado-Maldonado, 2000).

2.4. Statistical analysis

Comparisons of *C. formosanus* prevalences and abundances were conducted using generalised linear mixed models (GLMMs) with a log-linear link function and binomial-distributed (prevalence) or Poisson-distributed (abundance) errors. Field and laboratory experiments were analysed separately. All models were fitted using maximum likelihood (Bates and Maechler, 2010) and Poisson models incorporated an observation-level random effect to account for over-dispersion (Elston et al., 2001). Confidence intervals around model coefficients were obtained through *n* = 1000 model simulation runs (Gelman and Hill, 2006). The significance level for all models was set to = 0.05.

All experiments were analysed with GLMMs that incorporated fish species as a fixed effect and body size as a covariate. We set peacock bass as the reference group (intercept) with the species prediction, such that all other species were evaluated by comparison. For the field data, GLMMs also incorporated a species-by-site interaction in the random structure to allow site-specific inferences of species differences. For the single species trials, GLMMs incorporated a species-within-tank random effect, as well as an individual level random effect. GLMMs for the mixed species trials incorporated a tank-level random effect as well as an individual-level random effect. Significance of random structures was estimated based on likelihood ratio tests that compared the full model with a reduced model without the random structure in question (Gelman and Hill, 2006). For single and mixed species experiments, we accounted for infection levels in individual fish as

Table 1
Summary statistics of field surveys investigating parasitism by *Centrocestus formosanus* in four cichlid fish species (peacock bass (*Cichla monoculus*), oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*)) at three field sites in Gatun Lake, Panama. Prevalence (%) is the proportion of all sampled hosts infected by at least one parasite. Abundance is the mean number of parasites per individual host, calculated for each fish species at each of three sites, which includes 0 values for uninfected individuals. Intensity, similar to parasite load, is the mean number of parasites per individual host, but excludes uninfected individuals. Trammel nets were set daily over a 60 day period to sample all four cichlid fish species at sites where they all co-occurred with *Melanoides tuberculata* snails infected with *C. formosanus*.

Site	Host species	Prevalence (%)	Abundance (Mean/S.D.)	Intensity (Mean/S.D.)	Body size (cm)/S.D.
Gamboa	*Cichla*	100	133.27/190.77	133.27/190.77	27.8/5.48
	Astronotus	33.3	2.07/3.81	6.2/4.32	26.93/2.52
	Oreochromis	0.0	0.0	0.0	30.31/2.55
	Vieja	0.0	0.0	0.0	21.85/3.11
Chagres	*Cichla*	66.7	18.53/40.31	27.8/47.36	28.26/7.03
	Astronotus	0.0	0.0	0.0	25.72/3.86
	Oreochromis	0.0	0.0	0.0	28.46/4.67
	Vieja	0.0	0.0	0.0	19.98/3.23
Gatun	*Cichla*	66.7	16.87/31.36	25.3/35.96	26.63/7.30
	Astronotus	0.0	0.0	0.0	24.55/2.68
	Oreochromis	0.0	0.0	0.0	28.17/2.77
	Vieja	0.0	0.0	0.0	22.65/2.87

replicates. As individual fish were grouped into groups of four in infection tanks for both single species and mixed species trials, we accounted for dependence by treating species within tanks as a random effect, as well as by incorporating an individual-level random effect.

3. Results

3.1. Field comparisons

At all four sites (Gamboa, Gatun, Chagres and Barro Colorado Island), peacock bass was by far the most commonly caught fish, being four to five times more frequently caught per unit effort (CPUE) (mean = 2.36 CPUE, S.D. 1.61) than the second most abundant fish, oscar (mean = 0.53 CPUE, S.D. 0.31), followed by Nile tilapia (mean = 0.38 CPUE, S.D. 0.24). The native vieja was the least frequently caught fish (mean = 0.29 CPUE, S.D. 0.47). The prevalences, abundances and intensities of *C. formosanus* were highest in the peacock bass at each of the three sites where the parasite occurred (see Table 1). We did not recover *C. formosanus* in any of the fishes from Barro Colorado Island, nor did we find *C. formosanus* in Nile tilapia or vieja at any of the sites. We only found *C. formosanus* infecting oscar at one site, Gamboa, at low prevalence and intensity (see Fig. 1, Table 1). Fish species (fixed effect) had a significant effect on both infection prevalence (χ^2 = 13.679, P = .003) and mean abundance (χ^2 = 50.180, P < .001). Host body size (covariate) did not significantly affect prevalence (χ^2 = 2.553, P = .110) but did have a significant effect on abundance (χ^2 = 6.167, P = .013) and parasites were more abundant on larger fish. Site (random effect) significantly improved the model (ΔAIC = 31.01, χ^2 = 51.01, $P \leqslant$ 0.001), so we report site-specific estimates for species differences in parasite abundance (see insert plots, Fig. 1). Overall, peacock bass were more heavily infected compared with the other fish species across the sites (Fig. 1, Tables 1 and 2).

3.2. Laboratory experiments

Laboratory experiments testing infection susceptibility and host preference corroborated the patterns observed in field surveys (Table 2). In the laboratory experiments, all four fish species were infected by *C. formosanus* but peacock bass were infected twice as frequently in single-species experiments and more than eight times as frequently in mixed-species experiments compared with the next most heavily infected fish species, oscar (Figs. 2 and 3, Table 3). Peacock bass were, on average, infected by approximately 70% of the 500 introduced cercariae in the single-species experiments compared with approximately 25% for oscar. Over 50% of the 500 *C. formosanus* cercariae in the mixed-species experiments infected peacock bass compared with only 10% infecting all other species combined (Table 3). Fish size did not affect infection rates in the experiments and aquarium (random effect) did not significantly improve the model (single-species trials: ΔAIC = 2, χ^2 = 0, P = 0.99, ΔAIC = 2, χ^2 = 0, P = 0.99; mixed-species trials: ΔAIC = 0.36, χ^2 = 1.64, P = 0.21).

4. Discussion

The degree of a parasite's host specificity, or host range, is a primary determinant of its ability to spread to novel host species and expand into new biogeographical ranges (Cleaveland et al., 2001). For this reason, generalist parasites are typically considered to be more successful in invading and establishing in new ranges compared with specialist species, yet parasites that specialise on locally common hosts species can benefit from associations with those
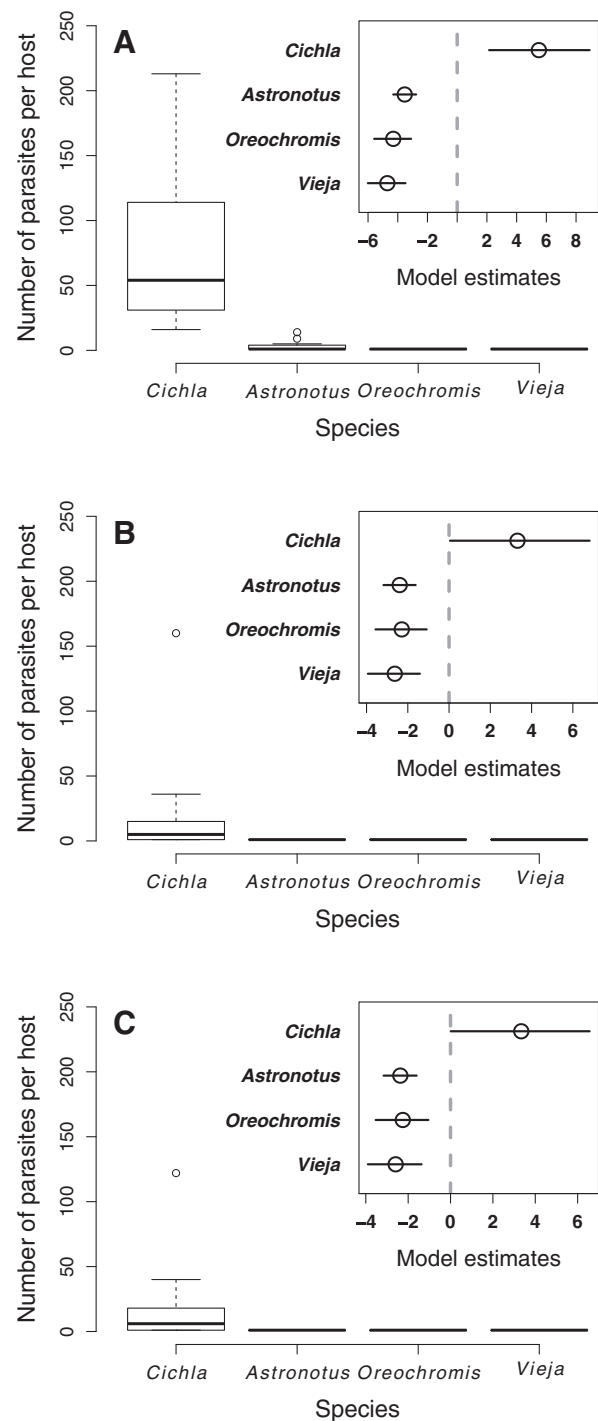

Fig. 1. Field survey of parasitism by *Centrocestus formosanus* across four cichlids at three sites in Gatun Lake, Panama: (A) Gamboa, (B) Chagres, (C) Gatun. Box plots for three sites depicting the number of parasites per host demonstrate significant differences in parasitism, calculated as the mean infection abundance (*n* = 15 fish per species) for each of four species (two outlier data points were omitted from the Gamboa plot for visual purposes). Our data indicate that parasitism in nature was significantly higher in the peacock bass (*Cichla monoculus*) compared with three other species consistently sampled at all three sites: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*). Insert plots depict model estimates from generalised linear mixed models with 95% confidence boundaries.

species that become widespread in new geographic ranges (Font, 2003). The introduced trematode *C. formosanus* is considered to be a generalist parasite with an extensive global distribution and broad host range in second intermediate fish species

Table 2
Poisson generalised linear mixed model estimates for infection intensity among four cichlid fish species, peacock bass (*Cichla monoculus*), oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*), in the field survey of Gatun Lake, Panama and in two laboratory experiments. The first laboratory experiment (single species) examined infection susceptibility to *Centrocestus formosanus* for each of the four cichlid species separately. For this, four individuals from the same species were exposed to approximately 500 larval trematode cercariae in the same aquarium. The second experiment (mixed species) tested whether *C. formosanus* cercariae preferentially infected particular fish species by simultaneously exposing one of each of the four cichlid species to approximately 500 cercariae in the same aquarium. In all trials, the peacock bass (*C. monoculus*) had significantly higher infection rates than all other three cichlid species in this study.

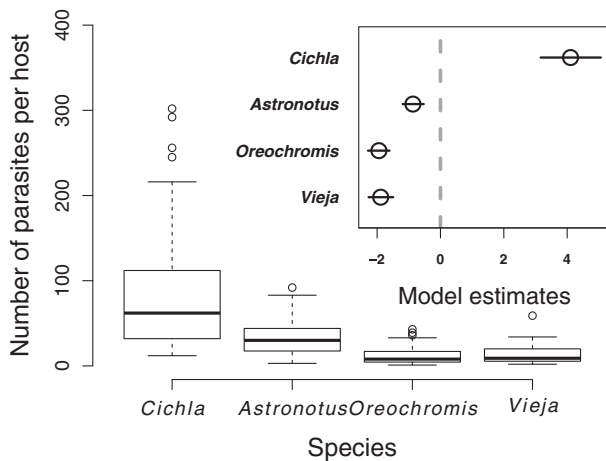| Host species	Field experiments			Laboratory experiments (single)			Laboratory experiments (mixed)		
	Coef.	z	P	Coef.	z	P	Coef.	z	P
Cichla	3.96	5.05	<0.001	4.09	8.58	<0.001	5.43	8.26	<0.001
Astronotus	−2.72	−6.83	<0.001	−0.87	−4.96	<0.001	−2.19	−15.69	<0.001
Oreochromis	−2.87	−4.59	<0.001	−1.94	−10.77	<0.001	−2.98	−19.44	<0.001
Vieja	−3.20	−4.94	<0.001	−1.87	−9.08	<0.001	−3.66	−21.19	<0.001

Coef., model coefficient.

Fig. 2. Experimental infection of four cichlids in single-species trials. Box plots demonstrate that all four species can be infected by *Centrocestus formosanus* but the invasive peacock bass, *Cichla monoculus*, is the more susceptible host for infection than three other species included in this study: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*). Insert plot depicts model estimates from generalised linear mixed models with 95% confidence boundaries.
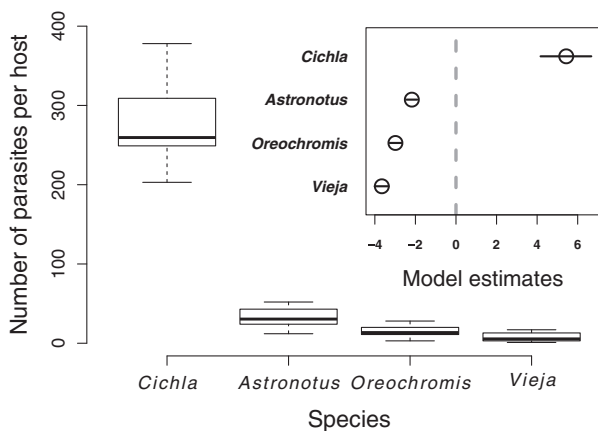

Fig. 3. Experimental infection of four cichlids in mixed-species trials. Box plots demonstrate significant differences in infection preference for the peacock bass over three other species tested: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*). Insert plot depicts model estimates from generalised linear mixed models with 95% confidence boundaries.

(Salgado-Maldonado et al., 1995; Scholz and Salgado-Maldonado, 2000; Mitchell et al., 2002). By contrast, our field survey suggested that, where it was introduced in Panama, *C. formosanus* could specialise on peacock bass, a particularly common species that was

also introduced, but with which *C. formosanus* does not share a long evolutionary history. In particular, *C. formosanus* prevalence in Gatun Lake was seven times greater, and its intensity 30 times higher, in peacock bass, than in three other common cichlid fish species (Table 1). Patterns of parasitism in two laboratory experiments that controlled encounter rates were consistent with the field surveys. These experiments yielded infection rates in peacock bass that were more than twice as high (relative to the other potential fish species) in single host species experiments and more than eight times as high in mixed host species experiments. In short, an introduced parasite that is normally considered a generalist can actually specialise on a particular novel host. This outcome can arise owing to a combination of differential encounter rates, host compatibility and infection preference, effects that we now consider in turn.

Encounter rates are an ecological "filter" for host–parasite interactions because, all else being equal, parasites should be more abundant on hosts they encounter more frequently (Combes, 2001). Encounter rates are certainly likely to vary among potential fish hosts given that peacock bass are the most abundant cichlid in near shore environments in Gatun Lake (Sharpe et al., unpublished data) and were captured in our nets more than five times more frequently than the other cichlids as described above (V.M. Frankel,

Table 3
Summary statistics for the experiments testing infection compatibility and infection preference of *Centrocestus formosanus* cercariae across four cichlid fish species, peacock bass (*Cichla monoculus*), oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*) and vieja (*Vieja maculicauda*). The first laboratory experiment (single species) examined infection susceptibility to *C. formosanus* for each of the four cichlid species separately. For this, four individuals from the same species were exposed to approximately 500 larval trematode cercariae in the same aquarium. The second experiment (mixed species) tested whether *C. formosanus* cercariae preferentially infected particular fish species by simultaneously exposing one of each of the four cichlid species to approximately 500 cercariae in the same aquarium. Parasite abundance is the mean number of parasites successfully established in each fish. Infection success is the proportion of parasites successfully establishing as metacercariae in each of four fish species relative to the total number of cercariae introduced into each tank. Each tank of four fish was considered one replicate ($n = 10$ for all experiments except where $n = 8$ for single species compatibility experiments on *Vieja*).

Host species	Parasite abundance (mean/S.D.)	Infection success (%)	n (trials)
Single species compatibility experiment			
Cichla	89.5/79.78	71.60	10
Astronotus	32.4/21.33	25.12	10
Oreochromis	11.35/10.60	9.48	10
Vieja	12.34/12.44	9.86	8
Mixed species preference experiment			
Cichla	276.5/49.23	55.30	10
Astronotus	31.6/12.87	6.32	10
Oreochromis	13.7/7.01	2.74	10
Vieja	6.99/6.44	1.40	10

unpublished data). Differential encounter rates are unlikely to be the sole reason for differential parasitism rates in nature because our laboratory experiments controlled exposure and yet yielded qualitatively similar patterns, as we will discuss below. However, we suggest that encounter rates might be an important contributor as the differential infection rates were more extreme in nature (30 times greater in peacock bass) than in the laboratory experiments (two times greater in peacock bass in infection compatibility experiments and eight times greater in host preference experiments).

Host compatibility is an intrinsic physiological "filter" because, when all else is equal, parasites should be more abundant on susceptible hosts in which their likelihood of successful establishment and transmission is higher (Combes, 2001). In our experiments, *C. formosanus* was found to infect and develop into viable metacercariae in all four exposed cichlid species. However, peacock bass were more susceptible to infection, with infection rates twice as high as for oscar, the next most heavily infected fish. One reason for these differences in susceptibility across the four cichlid species could be variation in immunological responses to parasite infection (Mitchell et al., 2002). However, infected fish did not display obvious visible reactions to the parasites (such as gill cartilage filament distortion or epithelial hyperplasia) or immune responses around the metacercarial cysts, as have been observed in other species infected by *C. formosanus* (Mitchell et al., 2002). Thus, the differences in infection rates might instead be the result of parasite preference.

Parasite preference for particular host species can also underpin patterns of host specialisation (Combes, 1991; Esbérard et al., 2005) and should be evolutionarily shaped by both encounter and compatibility (Combes, 2001). In our experiments, more than half of the 500 cercariae in the mixed-species experiments infected peacock bass, whereas we would expect no more than 25% if parasites showed no preference across the four potential host species. This difference in host preference patterns could be achieved through host recognition (Ohhashi et al., 2007) and chemotaxis (Gerardo et al., 2006) of free-swimming larval cercariae. Indeed, other species of trematode cercariae have been shown to actively swim toward the most susceptible host species when given multiple alternatives (Sears et al., 2012). Alternatively, the pattern could be explained by differential attachment of cercariae to host gills. Additional behavioural experiments could discriminate among these possibilities.

Using the conceptual framework of encounter and compatibility filters for host–parasite interactions (Euzet and Combes 1980; Combes 2001), we combined a series of field observations and laboratory experiments to begin to disentangle the apparent specialisation of the introduced trematode, *C. formosanus*, on a non-native host, the peacock bass. A particularly intriguing possibility is that *C. formosanus* has become locally adapted to infect peacock bass in Gatun Lake but further experiments are needed to formally test this possibility. Such experiments should include testing host susceptibility and parasite preferences across different host and parasite populations from different locations and evolutionary histories. If it turns out that *C. formosanus* have indeed become locally adapted to peacock bass since their introduction, the logical next question is what factors have favoured this adaptation. One possibility is variation in evolutionary time (Hawkins and Cornell, 1994; Torchin and Mitchell, 2004) but it seems unlikely in this case given that the obligate snail host for *C. formosanus* (*M. tuberculata*) was first recorded (2003) long after all of the fish species were well established in the lake: vieja (native), peacock bass (in 1967; Zaret and Paine, 1973), tilapia (in 1976; Gonzalez-Gutierrez, 2000; Roche et al., 2010) and (in 1991; Gonzalez-Gutierrez, 2000). Another possibility is that density mediated transmission rates are driving selection to the peacock bass through differential encounter rates with this highly invasive fish. Indeed, as noted earlier, the peacock bass is the most abundant cichlid in Gatun Lake (Sharpe et al., unpublished data; V.M. Frankel, unpublished data). A third possibility is that frequency mediated transmission rates are driving selection that drives local adaptation to the peacock bass. In particular, the peacock bass is the fish most frequently caught by fishermen and after processing the day's catch, fish remains, including infected gill tissues, are commonly consumed by birds (V.M. Frankel, unpublished data) which are their final hosts (Scholz and Salgado-Maldonado, 2000). Of course, more work will be needed to discriminate among these possibilities.

Host specificity is a central facet of a parasite's natural history that can determine the ability of a parasite to spread to new habitats and invade novel communities of hosts in new geographic ranges (Holt, 2003; Holt et al., 2003). Yet the extent to which introduced parasites interact with a given set of novel host species in an expanded geographic range is often unknown, as evaluations of host range in natural communities require the collection of robust parasitological data of multiple host species that co-occur and potentially interact with a given parasite species (Poulin, 2011). Clearly, research investigating the introduction of parasite species to new habitats provides both practical insights on the species that are most affected by the geographic expansion of parasite species and the transmission pathway of emerging infectious diseases in novel habitats (Ruiz-Gonzalez and Brown 2006; Olstad et al., 2007). In addition to these practical considerations, research investigating a parasite's host range in an expanded geographic range can also provide important natural experiments with which to test patterns of host ranges of parasite species putatively considered specialists or generalists, and can allow tests of theoretical predictions concerning the ecological and evolutionary drivers of host–parasite associations, and the success of introduced species in expanded geographic ranges.

Biological invasions are ultimately an irreversible component of human-induced environmental change, a process that continues to reshuffle species distributions, re-shapes biotic communities, and thus facilitates novel biotic interactions (Vitousek et al., 1996), including host–parasite associations, in ecosystems around the world. Yet basic epidemiological data on emerging host–parasite associations in nature, accompanied by simple laboratory experiments, can provide important insights concerning ecological and evolutionary processes underpinning host–parasite interactions, the dominant life-style on Earth, in an increasingly globalised and rapidly changing world.

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