

Spread of an introduced parasite across the Hawaiian archipelago independent of its introduced host

RODERICK B. GAGNE*, J. DEREK HOGAN*^{†,‡}, BRENDA M. PRACHEIL^{‡,§}, PETER B. MCINTYRE[‡], ERNIE F. HAIN[¶], JAMES F. GILLIAM[¶] AND MICHAEL J. BLUM*

*Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, U.S.A.

[†]Department of Life Sciences, Texas A&M- Corpus Christi, Corpus Christi, TX, U.S.A.

[‡]Center for Limnology, University of Wisconsin, Madison, WI, U.S.A.

[§]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, U.S.A.

[¶]Department of Biology, North Carolina State University, Raleigh, NC, U.S.A

SUMMARY

1. Co-introductions of non-native parasites with non-native hosts can be a major driver of disease emergence in native species, but the conditions that promote the establishment and spread of non-native parasites remain poorly understood. Here, we characterise the infection of a native host species by a non-native parasite relative to the distribution and density of the original non-native host species and a suite of organismal and environmental factors that have been associated with parasitism, but not commonly considered within a single system.
2. We examined the native Hawaiian goby *Awaous stamineus* across 23 catchments on five islands for infection by the non-native nematode parasite *Camallanus cotti*. We used model selection to test whether parasite infection was associated with the genetic diversity, size and population density of native hosts, the distribution and density of non-native hosts, land use and water quality.
3. We found that the distribution of non-native *C. cotti* parasites has become decoupled from the non-native hosts that were primary vectors of introduction to the Hawaiian Islands. Although no single intrinsic or extrinsic factor was identified that best explains parasitism of *A. stamineus* by *C. cotti*, native host size, population density and water quality were consistently identified as influencing parasite intensity and prevalence.
4. The introduction of non-native species can indirectly influence native species through infection of co-introduced parasites. Here, we show that the effects of ‘enemy addition’ can extend beyond the range of non-native hosts through the independent spread of non-native parasites. This suggests that control of non-native hosts is not sufficient to halt the spread of introduced parasites. Designing importation regulations to prevent host–parasite co-introductions can promote native species conservation, even in remote areas that may not seem susceptible to human influence.

Keywords: *Awaous stamineus*, biological invasions, enemy addition, parasite, *Camallanus cotti*

Introduction

The spread of non-native species is a widely recognised threat to biological diversity worldwide, particularly in aquatic ecosystems (Sala *et al.*, 2000; LePrieur *et al.*, 2008). Species introductions can lead to loss of native species through direct interactions, such as predation and competition and indirect effects such as those arising from co-introduced pathogens. The process of ‘enemy addition’, whereby non-native parasites or

pathogens transfer from introduced to native host species, can be a major driver of disease emergence in species of concern (Prenter *et al.*, 2004; Peeler *et al.*, 2011). For instance, *Myxobolus cerebralis*, the etiological agent of whirling disease, decimated populations of native cutthroat trout (*Oncorhynchus clarki*) in North America following the introduction of infected rainbow trout (*O. mykiss*) (Koel *et al.*, 2006). Enemy addition can also have non-lethal consequences that diminish fitness of native species, such as reducing body condition and

fecundity, shifting predator–prey interactions and altering responses to environmental stress (Heins *et al.*, 2004; Prenter *et al.*, 2004; Szuroczki & Richardson, 2009). Despite a growing appreciation of the potential impacts of non-native species on native species and ecosystems, the conditions that promote establishment and spread of introduced parasites remain poorly understood (LePrieur *et al.*, 2008; Vignon & Sasal, 2010).

A variety of factors, both extrinsic (e.g. land use, chemical pollution) and intrinsic to the host (e.g. genetic diversity, body size), have been identified as potential drivers of parasite intensity and prevalence. Increased nutrients or contaminants generated by land-use practices, for example, can alter parasite carrying capacity of host populations by influencing host density (Johnson *et al.*, 2010), condition (Brown, Loosli & Schmid-Hempel, 2000) or immune response (Rohr *et al.*, 2008). Parasitism, however, can also be reduced as a result of environmental impairment as free-living larvae and intermediate hosts are often susceptible to environmental contaminants (Lafferty & Kuris, 1999; MacKenzie, 1999; McIntyre *et al.*, 2005). Factors intrinsic to host populations also can be important predictors of the likelihood and degree of parasitism; older hosts, for example, have more time to accumulate parasites (Clers, 1991; Poulin, 2000), and greater genetic diversity of host populations has been linked to lower parasite transmission rates as well as increased probability of parasite extirpation (Hughes & Boomsma, 2005; Altermatt & Ebert, 2008).

Oceanic island streams offer exceptional opportunities to evaluate factors that control the spread, intensity and prevalence of introduced parasites (Font, 2003). Insular stream ecosystems exhibit a broad range of biotic and abiotic conditions, yet their geographic isolation produces depauperate native host and parasite communities compared with the diversity of introduced species that serve as vectors and hosts of non-native parasites (Font, 2003). For example, in many streams across the Hawaiian archipelago, particularly on O'ahu, the diversity of non-native fishes can be an order of magnitude higher than that of native fishes (Eldredge, 2000; Yamamoto & Tagawa, 2000). Non-native poeciliid fishes (e.g. guppies, swordtails) also have become widespread across the entire Hawaiian archipelago and have resulted in the introduction of non-native parasites that infect four of the five species of native stream fish (Font & Tate, 1994; Font, 2003). Non-native parasites introduced with poeciliids have become the most abundant and widespread members of the freshwater parasite community, far exceeding all parasites that have colonised the archipelago via natural mechanisms (Font, 2003). To

date, however, introduced parasites have only been found in streams that harbour introduced poeciliids, and it is believed that variation in parasitism within native hosts reflects differences in poeciliid densities among catchments (Font, 2003, 2007).

In this study, we evaluated whether the distribution and abundance of introduced hosts act as primary constraints on the spread and abundance of the non-native intestinal nematode *Camallanus cotti* infecting the native amphidromous goby *Awaous stamineus* (Eyedoux & Souleyet, 1850, Lindstrom *et al.*, 2012) across the Hawaiian archipelago. To test for host control of parasitism rates, we characterised the population density of both introduced and native hosts, as well as the body size, condition and genetic diversity of the native host. We also examined probable alternatives to host control of parasitism, based on evidence that the spread and pathogenicity of non-native parasites can depend on land use (McKenzie, 2007), aquatic habitat condition (McIntyre *et al.*, 2005; Rohr *et al.*, 2008) and native host distribution (van Riper *et al.*, 1986). This involved surveying sites spanning a broad gradient of stream impairment (relatively pristine to heavily degraded sites) to evaluate whether parasitism reflects water quality or land-use conditions. Combined, these parallel comparisons of intrinsic and extrinsic factors provided a powerful test of the controls on the intensity and prevalence of introduced parasites within a native host species across the archipelago.

Methods

Parasite life history

The intestinal nematode *Camallanus cotti* is the most widespread of the introduced parasites that infect native Hawaiian stream fishes (Font & Tate, 1994; Font, 2003). It has a global distribution and an indirect life cycle (Dove, 1999; Font, 2007). Cyclopoid copepods serve as the intermediate host and fishes serve as the definitive host, with fish becoming infected by ingesting infected copepods (Font, 2007). *Camallanus cotti* infects a wide range of host species and can cause severe damage to the intestine that may result in host mortality (Kim, Hayward & Heo, 2002; Menezes *et al.*, 2006). *Camallanus cotti* is considered a potential threat to the health of host fish (Kim *et al.*, 2002; Font, 2003; Menezes *et al.*, 2006), but the severity of disease it causes in native Hawaiian fishes is not known. Infection incidence in Hawaiian gobies peaks in summer months although parasite abundance and prevalence remain relatively stable across years, suggesting an absence of annual epizootic cycles (Vincent & Font, 2003).

Host life history

Poeciliids that occur throughout the Hawaiian Islands are freshwater obligate species that have been introduced and further translocated among catchments by humans for mosquito control. Poeciliids are believed to have been the original vector by which *C. cotti* was spread across Hawai'i (Font, 2003). Streams across the archipelago are also inhabited by five endemic amphidromous fish species of conservation concern (McDowall, 2003; Lindstrom *et al.*, 2012; Walter *et al.*, 2012). Adults of obligatory amphidromous species live and spawn in freshwater streams, but newly hatched larvae quickly drift to the sea where they develop for up to 6 months prior to recruiting back into fresh water (Radtke, Kinzie & Folsom, 1988). In facultative amphidromous species, the majority of larvae may remain in fresh water or nearshore environments (e.g. estuary, river plume) (Hogan *et al.*, 2014). We focused on the facultative amphidromous goby *Awaous stamineus* (Lindstrom *et al.*, 2012; Hogan *et al.*, 2014) because this species exhibits broad in-stream distributions, high population densities and resilience to anthropogenic stream degradation compared with other native stream fishes (Walter *et al.*, 2012). *Awaous stamineus* therefore offered opportunities to: (i) examine biological response to conditions across environmental gradients; (ii) sample individuals from multiple reaches within a catchment and (iii) collect native fishes without risk of detrimental effects on local populations.

Field data and sample collection

From June to October 2009, 23 catchments were sampled across the five Hawaiian Islands with perennial streams (Hawai'i, Maui, Moloka'i, O'ahu and Kaua'i; Fig. 1). Survey sites encompassed a broad gradient of stream impairment from relatively pristine to heavily degraded, allowing us to assess the influence of water quality and land use on parasitism. At each site, snorkelers surveyed the densities of native and non-native fishes within thirty 1 m² quadrats distributed randomly along a 140-m-long reach (following Higashi & Nishimoto, 2007). These standardised surveys were supplemented with the presence-absence records of poeciliids compiled by the Hawai'i Division of Aquatic Resources (DAR) from a variety of survey methods implemented from 1956 to 2008 (Atlas of Hawaiian Watersheds & Their Aquatic Resources, 2008). Following the quantitative fish surveys, adult and juvenile *A. stamineus* were collected with hand nets. A total of 919 specimens were collected across 48 sites in the study catchments. A small tissue clip from

the second dorsal fin of each individual was stored in 95% ethanol for genetic analysis. Census of parasites in *A. stamineus* requires euthanasia, and strict regulations limited the number of fish that we could sacrifice per catchment. Therefore, a subset of individuals ($n = 421$) were euthanised for parasite examination and other purposes. Whole *A. stamineus* were necropsied for all intestinal macroparasites with a dissecting microscope following Hoffman (1999).

Host attributes: physical condition and genetic analyses

Phenotypic and genotypic traits of *A. stamineus* were characterised to investigate whether infection by *C. cotti* varied according to host attributes. Total length and wet mass of each fish were measured prior to necropsy. The body condition of *A. stamineus* was quantified as the residual deviation in mass from a regression of log₁₀ of wet mass against log₁₀ of total length for all individuals (Anderson & Neumann, 1996). Population-level genetic diversity was assessed by genotyping *A. stamineus* individuals at 10 microsatellite loci. Nine of the loci were previously published in Hogan, Blum & Walter (2010): Agua A4, Agua B1, Agua B2, Agua C4, Agua D3, Agua D103, Agua D110, Agua D135 and Agua D136. The remaining locus, Agua D117, exhibits a repeat motif of (TATC)₉ with an optimal annealing temperature of 48 °C. Primer sequences for amplification of Agua D117 are (F) GCCAAACTCATACAAGAGGT and (R) GGTCTGCTTCTGAAATCTC. Genomic DNA was extracted from fin clips using a DNeasy Blood and Tissue extraction kit (Qiagen, Inc., Valencia, CA, USA). Polymerase chain reactions (PCRs) were performed in 15 µL volumes using MBS Satellite 0.2G thermo cyclers (Thermo Electron Corporation Waltham, MA, USA) with conditions following Hogan *et al.* (2010). HEX, 6-FAM or NED fluorescently dye-labelled forward primers were used to generate labelled PCR amplicons for sizing the loci against a 500 ROX™ size standard (ABI) on an ABI 3100 DNA analyzer. Electropherograms were scored using GeneMarker v1.85 (SoftGenetics LLC, State College, PA, USA). Observed heterozygosity was used as a measure of genetic diversity (Isomursu *et al.*, 2012) across hierarchical levels (i.e. site, catchment and island) with MSA v4.05 (Dieringer & Schlötterer, 2003).

Environmental conditions: water chemistry and land use

Water chemistry was measured at every site at the time fish were collected. Water samples were collected from the deepest point in the stream channel (the thalweg),

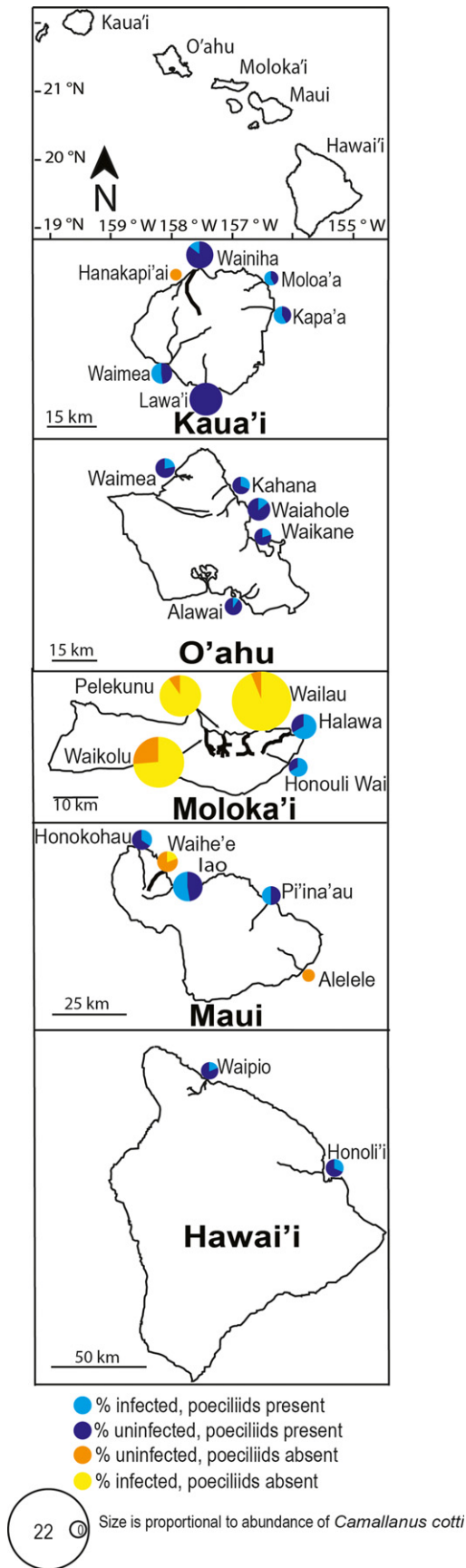


Fig. 1 Map of the Hawaiian Islands. Study streams are identified on each island with reference to the presence of *Camallanus cotti*, the abundance of *C. cotti* and the presence of poeciliids.

filtered immediately (Whatman GFX, 0.45 μm pore size) and stored frozen until analysis for soluble reactive phosphate (SRP), ammonium, nitrate and total nitrogen by standard colorimetric methods. Total suspended solids were quantified by filtering 1–3 L of stream water onto pre-combusted, pre-weighed filters (Reweight[®]) that were kept frozen until oven drying at 60 °C for 24 h. Field measurements of water temperature, conductivity and total dissolved solids were taken using hand-held metres (YSI 550a and ECTestr 12). For this study, only broad patterns of differences in water chemistry were of interest; therefore, principal component analysis (PCA) was used to distill the water chemistry data into the first (PC1) and second (PC2) principal axes of variation for further analyses.

We estimated agricultural and urban land use (%ag-urb) as the per cent of landscape classified as either developed (open space, low, medium or high intensity), pasture/hay or cultivated crops based on the 2001 National Land Cover Dataset (Homer *et al.*, 2007). The % ag-urb metric was determined at the site level using catchment boundaries derived from the National Hydrography Dataset Plus (NHD+), reflecting the land surface that drains directly into a NHD+ flow line without first flowing into an upstream flow line (McKay, Bondelid & Dewald, 2012).

Data analysis

Two measures of parasitism, mean intensity (mean number of *C. cotti* per infected *A. stamineus*) and prevalence (percentage of *A. stamineus* infected with at least one *C. cotti*), were calculated for each site following Bush *et al.* (1997). Comparison of parasite presence/absence with poeciliid presence/absence was conducted at the catchment level based on at least 3 snorkel surveys by our team in addition to historical surveys (Atlas of Hawaiian Watersheds & Their Aquatic Resources, 2008). Spearman's rank correlations were run between the two metrics of parasitism. We also examined pairwise correlations of parasite measures with poeciliid densities to test the prevailing hypothesis that parasitism of native hosts depends on the prevalence of introduced poeciliids. We tested for parasite effects on hosts by examining body condition of infected and uninfected *A. stamineus* using a Student's *t*-test as well as by inspecting the correlation between host body condition and the number of parasites per gram.

Generalised linear models were constructed to examine the influence of site-averaged host attributes (length, heterozygosity), native and non-native host population densities (*A. stamineus* density, poeciliid density), land use (%ag-urb) and water chemistry (PC1 and PC2) on both measures of parasitism. Sites where fewer than three *A. stamineus* could be examined for parasites were excluded from analysis, resulting in 48 sites with prevalence data and 27 sites for intensity (which only considers infected fish). In preliminary data exploration (Walters *et al.*, 2008; Freckleton, 2011; Blum *et al.*, 2012), collinearity ($r > 0.6$) was first assessed by examining pairwise correlations among sets of variables within each of the extrinsic and intrinsic categories described above. For both measures of parasitism, a best-subsets regression approach for all possible models was used whenever the number of variables was less than one-tenth the numbers of observations. To address heteroscedasticity in residuals and to identify the error distribution that best fits our data, the fit of error distributions was compared to data using Vuong's closeness test, a likelihood ratio-based approach that evaluates competing, non-nested models using Kullback-Leibler information criterion (Vuong, 1989). Poisson error distributions were used for models examining effects of site-level attributes on *C. cotti* intensity (Proc Countreg) and prevalence (Proc Genmod, SAS ver. 9.3, SAS Institute, Cary, NC, USA). Models were ranked using Akaike's information criteria (AIC) where best models were defined as those having a $\Delta\text{AIC} \leq 2$. The frequency of each predictor variable in the best models was used to assess its general contribution to each metric of parasitism. Recognising that sampling across islands and multiple sites within catchments adds a potential geographic constraint to our data, we included both island and catchment as a class variable in all models.

Results

Host distribution and density

Awaous stamineus were found in all 23 catchments sampled (Fig. 1). Streams on Hawai'i, Moloka'i and Kaua'i had the highest average densities followed by Maui and O'ahu (Table 1). At the catchment level, highest densities were found in the Waimea catchment on Kaua'i, the Waipi'o catchment on Hawai'i and the Pelekunu catchment on Moloka'i (Table 1). The lowest densities were in the Waimea and Alawai catchments on O'ahu, and the Iao catchment on Maui (Table 1). Poeciliid fishes were observed in 17 of 23 catchments. The most abun-

dant poeciliid species were the guppy, *Poecilia reticulata*, the short fin molly, *P. latipinna*, the green sword tail, *Xiphophorus helleri*, and the mosquito fish, *Gambusia affinis*. The highest average densities of poeciliids occurred on Hawai'i and O'ahu and the lowest densities on Moloka'i (Table 1). We did not observe poeciliids in the main channel of Waihe'e in Maui, but we did note their presence in water diversion canals adjacent to and downstream of the stream survey sites. No introduced fishes of any kind were found in streams or side pools in three catchments on Moloka'i. These results are consistent with previous surveys that found no evidence of poeciliids or other non-native fishes in these catchments (Pelekunu – 17 surveys between 1956 and 2005; Wailau – 9 surveys between 1977 and 1999; Waikolu – 22 surveys between 1979 and 2002).

Parasite density and distribution

The intensity and prevalence of *C. cotti* varied across the Hawaiian archipelago. Across all catchments, an average of 45% of individuals had infections and the mean intensity was 5.8 parasites. There was no overall difference in body condition between parasitised and unparasitised *A. stamineus* ($t = -0.507$, d.f. = 562, $P = 0.61$) nor was there a correlation between body condition and number of parasites per gram ($r = 0.072$, $P = 0.082$). Other internal macroparasites had comparatively low prevalence; the next most prevalent parasite was the introduced *Bothriocephalus acheilognathi*, which had less than 4% prevalence. As a result, parasites other than *C. cotti* were not considered in subsequent analyses.

Although measures of parasitism were moderately to strongly correlated with one another ($r = 0.47$, $P < 0.001$), the intensity and prevalence of *C. cotti* nonetheless varied widely across the Hawaiian archipelago. Intensity was highest in the Wailau, Pelekunu and Waikolu catchments on the remote windward coast of Moloka'i (Table 1). Prevalence was highest in the Lawai catchment on Kaua'i and the Wailau and Pelekunu catchments on Moloka'i (Table 1).

The introduced parasite *C. cotti* was found in native *A. stamineus* from 21 of the 23 sampled catchments. Alelele catchment on Maui and Hanakapi'ai catchment on Kaua'i were the only two sampled catchments where no *C. cotti* infections were found. Parasites were present in *A. stamineus* from four of the six catchments where poeciliids were not observed during snorkel surveys, with three of the six having no historical record of poeciliids. Our surveys in Alelele and Hanakapi'ai catchments did not detect poeciliids, but they have been previously

Table 1 The number of *Awaous stamineus* examined for parasites (*n*), along with the mean intensity (Inten) and prevalence (Prev) of *Camallanus cotti* parasites in *A. stamineus* in each sampled catchment relative to the average length of host fish in millimetres (Len), average weight of *A. stamineus* host in grams (Mass), average condition of *A. stamineus* host (Cond), density of poeciliids (POEC), density of *A. stamineus* (Den AS), number of *A. stamineus* genotyped (Gen n), observed heterozygosity (Ho), water chemistry principal component one (PC1; primary loadings from all three nitrogen forms, conductivity, total dissolved solids and total suspended solids) and principle component two (PC2, primary loading from SRP), per cent agricultural and urban land use (%ag-urb). N/A = not applicable

Stream	<i>n</i>	Parasitism		<i>A. stamineus</i> condition			Host densities		<i>A. stamineus</i> genetics		Water chemistry		Land use %ag-urb	
		Inten	Prev	Len	Mass	Cond	POEC	Den AS	Gen n	Ho	PC 1	PC 2		
Kaua'i	ISLAND	97	3.29	52.95	105.8	15.05	-0.004	2.01	0.54	183	0.552	N/A	N/A	28.5
	Hanakapi'ai	14	0.00	0.00	112.5	18.63	-0.012	0.00	0.23	11	0.491	-0.658	-0.256	0.0
	Wainiha	18	7.25	85.70	104.9	15.39	0.018	0.00	0.22	37	0.535	-1.417	-0.042	16.5
	Moloa'a	17	0.50	42.10	99.8	9.42	-0.034	2.60	0.21	10	0.529	-0.534	-0.922	35.9
	Kapa'a	19	5.00	42.10	129.0	29.95	0.021	1.99	0.19	25	0.601	0.304	-0.604	57.7
	Lawa'i	25	3.92	100.00	96.6	8.32	-0.011	5.28	0.45	44	0.591	4.024	1.126	47.5
O'ahu	Waimea	18	3.09	47.80	92.4	8.57	-0.004	2.20	1.92	56	0.566	0.585	-1.942	13.4
	ISLAND	94	2.90	19.36	120.1	21.44	0.004	2.89	0.10	138	0.526	N/A	N/A	16.9
	Kahana	26	1.50	30.80	103.8	13.16	0.022	0.71	0.12	67	0.559	-0.618	-0.228	3.5
	Waikane	15	1.00	20.00	110.2	16.23	0.014	1.72	0.16	19	0.473	0.309	-0.255	3.2
	Waiahole	14	3.00	14.30	108.9	14.81	0.011	1.29	0.20	31	0.519	-0.933	3.235	5.0
	Alawai	21	3.00	9.50	121.0	25.32	0.014	8.12	0.02	6	0.413	1.512	-1.064	62.3
Moloka'i	Waimea	18	6.00	22.20	156.5	37.68	-0.042	2.59	0.02	15	0.667	-0.434	-1.108	10.7
	ISLAND	117	16.64	78.34	138.2	31.55	0.008	0.03	0.54	189	0.585	N/A	N/A	0.0
	Waikolu	19	29.71	73.70	154.6	47.92	0.023	0.00	0.13	14	0.760	-0.429	1.602	0.0
	Pelekunu	21	18.42	90.50	166.9	52.09	-0.012	0.00	1.50	49	0.493	-0.423	0.849	0.0
	Wailau	17	28.25	94.10	158.3	31.32	0.061	0.00	0.43	49	0.539	-1.047	-0.911	0.0
	Halawa	30	4.25	66.70	106.8	13.31	-0.017	0.00	0.40	45	0.521	-1.526	-0.255	0.0
Maui	Honouli Wai	30	2.55	66.70	104.5	13.13	-0.012	0.14	0.21	32	0.612	-0.931	0.334	0.2
	ISLAND	88	4.71	30.32	128.7	34.82	0.012	2.30	0.23	223	0.527	N/A	N/A	18.3
	Honokohau	20	3.57	35.00	143.4	37.12	0.016	3.84	0.41	61	0.560	-1.021	1.142	0.2
	Waihe'e	18	6.20	19.20	123.9	32.37	-0.003	0.00	0.09	25	0.484	-1.251	0.559	21.1
	Iao	19	11.78	47.40	194.4	84.45	0.040	7.63	0.01	23	0.499	-1.045	-0.224	66.1
	Pi'ina'au	14	2.00	50.00	95.5	11.19	-0.009	0.02	0.42	49	0.541	-0.385	-1.348	3.7
Hawai'i	Alelele	17	0.00	0.00	86.1	8.99	0.016	0.00	0.23	65	0.551	-1.506	-0.405	0.2
	ISLAND	39	1.75	28.30	89.8	8.96	0.002	4.62	0.20	69	0.542	N/A	N/A	46.7
	Waipi'o	20	2.00	25.00	89.7	8.90	0.006	0.60	0.08	29	0.531	0.872	2.993	20.4
Honoli'i	19	1.50	31.60	89.9	9.01	-0.002	8.63	0.32	40	0.553	-0.704	-0.718	73.0	

recorded in these catchments (Atlas of Hawaiian Watersheds & Their Aquatic Resources, 2008).

Predictors of parasitism

Values obtained for host length, body condition, observed heterozygosity, %ag-urb, water chemistry PC1 and PC2 are presented in Table 1. PC1 explained 54.2% of the variation in water chemistry among catchments with strong loadings (>0.6) from all three nitrogen forms, conductivity, total dissolved solids and total suspended solids. PC2 explained 15.3% of the variation in water chemistry and had a strong loading only from SRP.

Model selection results did not support the prevailing hypothesis that parasitism is driven by the prevalence of non-native poeciliid hosts. The density of poeciliids

alone was not significantly correlated with intensity ($r = -0.137$, $P = 0.35$) or prevalence ($r = -0.062$, $P = 0.68$) of *C. cotti*. Although the density of poeciliids was included in 33% of the top models for intensity as well as the single best model for prevalence (Table 2 and Appendix S1), model selection identified all candidate predictors in at least one top model except for water chemistry PC1 and per cent agricultural and urban land use (Table 2 and Appendix S1). When models were run with all predictor variables, we found significant relationships between all variables and intensity. We also found significant relationships between prevalence and density of *A. stamineus* and water chemistry PC2 (Table 3). Additionally, host length was the most frequently identified predictor of parasitism of *A. stamineus* (Table 2 and Appendix S1; Fig. 2). In models including all factors, the density of *A. stamineus* was positively

Table 2 Frequency of appearance of variables in models with $\Delta AIC < 2$. Direction of association indicates whether each variable had a + or - β value. Factors included host length, density of *Awaous stamineus*, density of poeciliids, % agricultural and urban land use, water chemistry principal components one (primary loadings from all three nitrogen forms, conductivity, total dissolved solids and total suspended solids) and two (primary loading from SRP) (PC1, PC2) and observed host heterozygosity

Variable	Direction of association	Top model frequency
Intensity ($N = 27$ sites)		
Length	+	3
PC2	+	1
Density of poeciliids	-	1
Observed heterozygosity	+	1
Prevalence ($N = 48$ sites)		
Length	+	1
PC2	+	1
Density of poeciliids	-	1
Density of <i>A. stamineus</i>	+	1

associated with prevalence and intensity, whereas the density of poeciliids was negatively associated with intensity (Table 3).

Discussion

Previous studies have suggested that *Camallanus cotti* only infects native hosts in streams where non-native poeciliid hosts have been introduced (Font & Tate, 1994; Font, 2003), but our surveys revealed that the distribution of *C. cotti* has become decoupled from that of their introduced hosts. Some of the highest densities of *C. cotti* occurred in streams with no recorded observations of poeciliids, including the Pelekunu, Wailau and Waikolu catchments on the windward coast of Moloka'i. Although we cannot rule out the possibility that poeciliids are present but were not detected in these catchments during our work and previous surveys, the physical isolation of the catchments minimises the likelihood of species introductions, including guppies and other poeciliids that have been introduced via aquarium releases or intentionally released into streams for mosquito control (Font, 2003, 2007).

Evidence of introduced parasites spreading beyond the range of introduced hosts represents an important counterpart to more celebrated cases of invasions being enhanced by 'enemy release' where non-native species have a competitive advantage over native species by carrying relatively lower parasite loads in areas of introduction compared with historic ranges (Torchin, Lafferty & Kuris, 2001; Torchin *et al.*, 2003; Liu &

Table 3 P -values and direction of effect for the model that included all variables, host length, density of *Awaous stamineus*, density of poeciliids, % agricultural and urban land use (% ag-urb), water chemistry principal component one (primary loadings from all three nitrogen forms, conductivity, total dissolved solids and total suspended solids) and two (primary loading from SRP) (PC1, PC2) and observed host heterozygosity

	Intensity		Prevalence	
	Direction	P	Direction	P
Length	+	<0.001	+	0.98
%ag-urb	-	<0.001	+	0.4009
Density of <i>A. stamineus</i>	+	<0.001	+	<0.001
Density of poeciliids	-	0.002	-	0.254
Observed heterozygosity	+	0.013	-	0.448
PC1	+	<0.001	-	0.603
PC2	+	0.01	+	0.014

Stiling, 2004). Like enemy release, enemy addition can promote the spread of the original, non-native host species at the expense of native species (Prenter *et al.*, 2004; Rushton *et al.*, 2006). For example, the spread of the North American signal crayfish, *Pacifastacus leniusculus*, across Europe has been aided by the microsporidian parasite, *Thelohania contejeani*, which reduces feeding behaviour in the native white clawed crayfish, *Austropotamobius pallipes*, but is benign in the introduced crayfish (Haddaway *et al.*, 2012). Our results further demonstrate that the introduction of non-native host species can result in secondary invasions of co-introduced parasites. We also show that secondary invasions of non-native parasites can proceed on pathways independent of the original non-native host, which reinforces concerns that a single species introduction can have multiplicative and unanticipated outcomes (Prenter *et al.*, 2004; Ehrenfeld, 2010).

The spread of non-native parasites independent of their original hosts is especially intriguing in this study system as the ocean is the only connection between catchments in the Hawaiian Islands. Larvae of native Hawaiian amphidromous fishes, including *A. stamineus*, are not infected with parasites prior to entering the ocean and thus do not function as a conduit for parasite dispersal (Font, 2003). The most likely means of colonising new streams (i.e. where introduced fishes are absent) are via euryhaline fish hosts that routinely move between marine and freshwater environments, such as flagtails (*Kuhlia xenura*). *Camallanus cotti* infections have been recorded in *Kuhlia xenura* in Hawaiian estuaries as well as in *Kuhlia marginata* in New Caledonia (Moravec & Justine, 2006; Font 2007). Another possible dispersal pathway is the transport of infected copepods by water

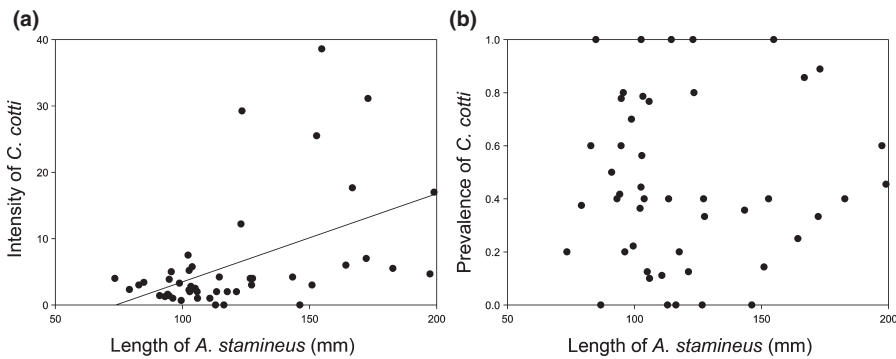


Fig. 2 (a) Intensity of *Camallanus cotti* infections relative to the average length of *Awaous stamineus* at each site ($r^2 = 0.234$ $P = 0.001$). (b) Prevalence of *C. cotti* relative to the average length of *A. stamineus* at each site ($r^2 = 0.002$ $P = 0.766$).

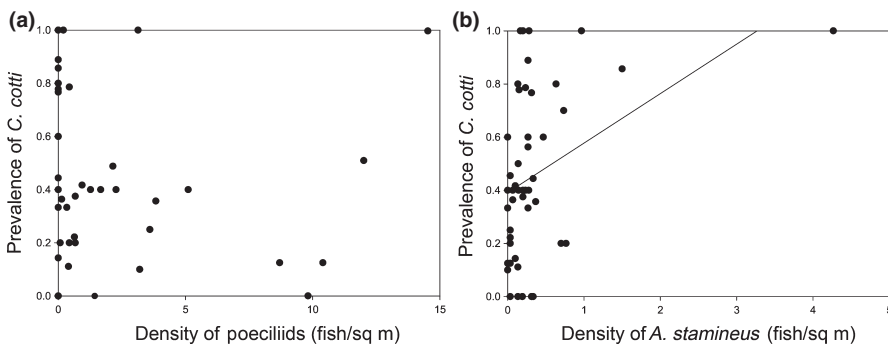


Fig. 3 (a) Prevalence of non-native *Camallanus cotti* parasites in native *Awaous stamineus* hosts across sites with respect to the density of non-native poeciliid hosts ($r^2 = 0.003$, $P = 0.68$). (b) Prevalence of non-native *C. cotti* parasites in native *A. stamineus* hosts across sites with respect to the density of *A. stamineus* ($r^2 = 0.094$, $P = 0.031$).

birds moving between catchments (Frisch, Green & Figuerola, 2007). Regardless of the mechanism of dispersal, the spread of introduced parasites independently of their original host greatly expands the potential for parasitism of native stream fishes across the archipelago.

Our statistical model results suggest that parasitism of *A. stamineus* by the introduced nematode *C. cotti* is influenced by a portfolio of factors rather than being solely attributable to the presence or density of non-native poeciliids. Our top models included a suite of variables rather than a single or small number of strong predictors. These results are consistent with other studies that suggest complex controls on parasitism rates (Paterson *et al.*, 2012; Pérez-Rodríguez *et al.*, 2013). For example, Pérez-Rodríguez *et al.* (2013) identified top models encompassing multiple types of predictors (e.g. landscape features, climate) and showed that predictors of parasitism may not be consistent even among closely related host species. Paterson *et al.* (2012) similarly found that parasitism of introduced fish by native parasites is not attributable to a few quantifiable predictors and suggested that ecological, immunological and physical characteristics may all factor into parasite acquisition. Our results are indicative of the complex nature of parasitism and highlight the potential pitfalls of characterising parasitism by focusing on a single or small number of *a priori* selected environmental and biological vari-

ables. Some caution must also be taken, however, when selecting multiple factors for analysis as there can be a risk of overfitting models (Ginzberg & Jensen 2004). As we have done here, careful consideration should be given to selecting plausible factors that are known to influence parasitism in other systems.

Although no single factor explains parasitism of native Hawaiian goby hosts by introduced parasites, our analyses suggest that parasitism is most often attributable to host size, native host densities and soluble reactive phosphate (PC2). It is probable that these factors individually and interactively influence parasitism. For example, greater parasitism of larger *A. stamineus*, which reflects a commonly observed pattern in studies of parasitism, is probably a result of accumulation of larger parasite loads over longer exposure times as length corresponds closely with age (Price & Clancy, 1983; E.F. Hain & B.A. Lamphere unpublished data). Although populations of parasites with indirect life cycles are typically not regulated by the densities of definitive hosts (Arneberg, 2001), direct transmission of *C. cotti* during spawning or other close interactions could contribute to the observed positive effect of *A. stamineus* density on the prevalence and intensity of *C. cotti* infections. Direct transmission of *C. cotti* has never been recorded in the field, but laboratory studies have demonstrated that *C. cotti* can be directly transmitted during close contact

among fishes (Levsen & Jakobsen, 2002). The observed trends also may partly reflect prevailing environmental conditions (Rohr *et al.*, 2008) in catchments that enable native hosts to reach greater size and densities. Some of the highest levels of parasitism were observed in remote, forest-dominated catchments on Kaua'i and Moloka'i where *A. stamineus* achieves the largest average body lengths and the highest densities. The association of parasitism with larger sized, high density populations of native hosts in more pristine catchments that also lack poeciliids is probably driving the negative relationship between parasitism of native hosts and non-native host density. This relationship raises the possibility, however, that the presence of non-native hosts may result in a situation similar to the dilution effect. Higher densities of alternative hosts could serve to reduce infection of native hosts by taking up parasites that otherwise would be available to infect native hosts (Johnson & Thielges, 2010). The influence of alternative hosts may be greater if there is a finite pool of parasites or if there is preferential infection of these species over native species. It is also possible that parasitism is higher in more pristine catchments, with higher soluble reactive phosphate, because intermediate hosts or free-living life stages of the parasite are susceptible to environmental degradation but are often positively influenced by increased nutrients such as phosphate (Lafferty & Kuris, 1999; McIntyre *et al.*, 2005; McKenzie, 2007; Schotthoefer *et al.*, 2011).

Relatively small sample sizes per site, as well as lack of data on intermediate hosts and other native definitive hosts, may have constrained our ability to fully resolve the factors driving parasitism of *A. stamineus* by *C. cotti*. Concerns over the status and viability of local populations of native fishes limited the number of individuals that we were permitted to remove from local populations. We compensated for this limitation by weighting our analysis to reflect small sample sizes and by considering patterns across a large number of sites. Nonetheless, larger sample sizes could have augmented our capacity to identify factors governing parasitism. In future work, characterising the abundance of the intermediate copepod host might also be helpful for reconstructing pathways of interactions between environmental degradation and infection of native hosts by introduced parasites (Rohr *et al.*, 2008). For example, our finding that parasitism generally increases with increasing soluble reactive phosphate could be attributable to responses of copepods to increased nutrients, but the ecology and abundance of intermediate host(s) of *C. cotti* in Hawaiian streams are not well understood (Font & Tate, 1994; Font, 2003).

The spread of introduced parasites independent of their original host greatly expands the potential influence of species introductions on native species. Although non-native parasites may be introduced to a naïve ecosystem via a non-native host, our findings indicate that host and parasite distributions can become decoupled such that the reach of introduced parasites can extend well beyond the distribution of the non-native host species. As a result, control of non-native hosts alone will not be an adequate step to prevent the spread of introduced parasites. Our results affirm that eradication of existing poeciliid populations would not prevent *C. cotti* infections of native fishes.

Prevention is probably the most efficient way to combat the expansion of species invasions (Vander Zanden *et al.*, 2010), and our findings suggest a need for a new dimension of prevention: screening or routine prophylaxis of imported fishes against parasites. Implementation of more extensive risk assessment might also prove useful (Williams, Britton & Turnbull, 2013), although the absence of key predictors of parasite acquisition may constrain assessment outcomes (Paterson *et al.*, 2012; Pérez-Rodríguez *et al.*, 2013). Similarly, remediation of nematode infections in native Hawaiian stream fishes is likely to be difficult because parasite loads in native hosts are influenced by multiple environmental factors and because non-native parasites appear capable of spreading through novel dispersal pathways that could enable them to rapidly re-establish following treatment.

Finally, our findings raise questions about what sites should be considered as reference sites for conservation and restoration efforts in the Hawaiian archipelago. The relatively pristine sites where we found the highest loads of introduced parasites are generally treated as the standard against which all other catchments are judged. The fact that introduced parasites are thriving in these catchments suggests a more nuanced view may be needed to guide conservation and restoration decisions. Even though our results suggest that *C. cotti* have limited pathogenicity in *A. stamineus* (i.e. host body condition does not differ between infected and non-infected individuals), the presence of *C. cotti* in these catchments nonetheless represents a potential risk to native fishes. It is possible, for example, that compensatory factors are masking the influence of *C. cotti* on *A. stamineus*; greater resource availability could be enhancing the capacity of *A. stamineus* to survive or support more severe infections (Brown *et al.*, 2000; Johnson *et al.*, 2010). Parasitism might detrimentally affect *A. stamineus* if conditions become less favourable with shifts in biotic or abiotic conditions (e.g. an epizootic event). It is also possible

that *C. cotti* have greater pathogenicity in other native fish hosts (Kim *et al.*, 2002). Improved understanding of pathogenicity, as well as temporal variation in parasitism (i.e. the possibility of epizootic events), would not only further clarify the potential effect of *C. cotti* on native hosts, but also help parameterise reference conditions for conservation of native stream fishes across the Hawaiian archipelago.

Acknowledgments

We thank C. Criscione for laboratory training assistance, E. Childress, J. Fenner, G. Glotzbecker, T. Haas, B. Lamphere, D.P. Lindstrom, K. Moody, D. Oele, T. Rayner, J. Rossa, R.P. Walter for field assistance, J. Thore and E. Hamann for laboratory analyses, N. Keutler and P. Crump for assistance with statistical analysis and two anonymous reviewers that provided useful comments that greatly improved this manuscript. This study was funded by the US Department of Defense Strategic Environmental Research Development Program (SERDP) through project RC-1646.

References

- Altermatt E. & Ebert D. (2008) Genetic diversity of *Daphnia magna* populations enhances resistance to parasites. *Ecology Letters*, **11**, 918–928.
- Anderson R.O. & Neumann R.M. (1996) Length, weight, and associated structural indices. In *Fisheries Techniques*, 2nd edn. (Eds B.R. Murphy & D.W. Willis), pp. 447–482. American Fisheries Society, Bethesda, MD.
- Arneberg P. (2001) An ecological law and its macroecological consequences as revealed by study of relationships between host densities and parasite prevalence. *Ecography*, **24**, 352–358.
- Atlas of Hawaiian Watersheds and Their Aquatic Resources. (2008) A joint project between the Hawai'i Division of Aquatic Resources and Bishop Museum.
- Blum M.J., Bagley M.J., Walters D.M., Jackson S., Daniel B., Chaloud D. *et al.* (2012) Genetic diversity and species diversity of stream fishes covary across a land use gradient. *Oecologia*, **168**, 83–95.
- Brown M.J.F., Loosli R. & Schmid-Hempel P. (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos*, **91**, 421–427.
- Bush A.O., Lafferty K.D., Lotz J.M. & Shostak A.W. (1997) Parasitology meets ecology on its own term: margolis *et al.* revisited. *Journal of Parasitology*, **83**, 575–583.
- Clers S.D. (1991) Functional relationship between sealworm (*Pseudoterranova decipiens*, Nematoda, Ascaridoidea) burden and host size in Atlantic cod (*Gadus morhua*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **245**, 85–89.
- Dieringer D. & Schlötterer C. (2003) Microsatellite Anlyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, **3**, 167–169.
- Dove A.D.M. (1999) Richness patterns in parasites communities of exotic poeciliid fishes. *Parasitology*, **120**, 609–623.
- Ehrenfeld J.G. (2010) Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, **41**, 59–80.
- Eldredge L.G. (2000) Non-indigenous freshwater fishes, amphibians, and crustaceans of the Pacific and Hawaiian islands. In: *Invasive Species in the Pacific: A Technical Review and Draft Regional Strategy* (Ed. G. Sherley), pp. 173–190. South Pacific Regional Environment Program, Apia, Samoa.
- Font W.F. (2003) The global spread of parasites: what do Hawaiian streams tell us? *BioScience*, **53**, 1061–1067.
- Font W.F. (2007) Parasites of Hawaiian stream fishes: sourced and impacts. *Bishop Museum Bulletin in Cultural and Environmental Studies*, **3**, 157–169.
- Font W.F. & Tate D.C. (1994) Helminth parasites of native Hawaiian freshwater fishes: an example of extreme ecological isolation. *The Journal of Parasitology*, **80**, 682–688.
- Freckleton R. (2011) Dealing with collinearity in behavioral and ecological data: model averaging and the problems of measurement error. *Behavioral Ecology Sociobiology*, **65**, 91–101.
- Frisch D., Green A.J. & Figuerola J. (2007) High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquatic Sciences*, **69**, 568–574.
- Ginzburg L.R. & Jensen C.X. (2004) Rules of thumb for judging ecological theories. *Trends in Ecology & Evolution*, **19**, 121–126.
- Haddaway N.R., Wilcox R.H., Heptonstall R.E.A., Griffiths H.M., Mortimer R.J.G., Christmas M.J. *et al.* (2012) Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. *PLoS ONE*, **7**, e32229.
- Heins D.C., Ulinski B., Johnson J. & Baker J.A. (2004) Effect of the cestode macroparasite *Schistocephalus pungitii* on the reproductive success of the nine spine stickleback *Pungitius pungitius*. *Canadian Journal of Zoology*, **82**, 1731–1737.
- Higashi G.R. & Nishimoto R.T. (2007) The point quadrat method: a rapid assessment of Hawaiian streams. In: *Biology of Hawaiian Streams and Estuaries* (Eds N.L. Evenhuis & J.M. Fitzsimons), pp. 305–313. Bishop Museum Bulletin in Cultural and Environmental Studies, **3**, The Bishop Museum, Honolulu, HI.
- Hoffman G.K. (1999) *Parasites of North American Freshwater Fishes*. Comstock Publishing Associates, Ithaca, NY.
- Hogan J.D., Blum M.J. & Walter R.P. (2010) Characterization of ten novel microsatellite markers in *Atwous guamen-*

- sis with comments on cross amplification in congeners and other amphidromous fish native to Hawai'i. *Conservation Genetic Resources*, **3**, 275–277.
- Hogan J.D., McIntyre P.B., Blum M.J., Gilliam J.F. & Bickford N. (2014) Consequences of alternative dispersal strategies in a putatively amphidromous fish. *Ecology*, **95**, 2397–2408.
- Homer C., Dewitz J., Fry J., Coan M., Hossain N., Larson C. *et al.* (2007) Completion of the 2001 National Land Cover Database for the conterminous United States. *Photogrammetric Engineering and Remote Sensing*, **73**, 337–341.
- Hughes W.O.H. & Boomsma J.J. (2005) Does genetic diversity hinder parasite evolution in social insect colonies? *Journal of Evolutionary Biology*, **19**, 132–143.
- Isomursu M., Ratti O., Liukkonen T. & Helle P. (2012) Susceptibility to intestinal parasites and juvenile survival are correlated with multilocus microsatellite heterozygosity in the Capercaillie (*Tetrao urogallus*). *Ornis Fennica*, **89**, 1–10.
- Johnson P.T. & Thielges D.W. (2010) Diversity, decoys and the dilution effect: how ecological communities affect disease risk. *The Journal of Experimental Biology*, **213**, 961–970.
- Johnson P.T., Townsend A.R., Cleveland C.C., Gilbert P.M., Howarth R.W., McKenzie V.J. *et al.* (2010) Linking environmental nutrient enrichment in disease emergence in humans and wildlife. *Ecological Applications*, **20**, 16–29.
- Kim J.H., Hayward C.J. & Heo G.J. (2002) Nematode worm infections (*Camallanus cotti*, Camallanidae) in guppies (*Poecilia reticulata*) imported to Korea. *Aquaculture*, **205**, 231–235.
- Koel T.M., Mahony D.L., Kinnan K.L., Hudson C.J., Murcia S. & Kerans B.L. (2006) *Myxobolus cerebralis* in native cutthroat trout in Yellowstone Lake ecosystem. *Journal of Aquatic Animal Health*, **18**, 157–175.
- Lafferty K.D. & Kuris A.M. (1999) How environmental stress affects the impacts of parasites. *Limnology Oceanography*, **44**, 925–931.
- LePrieur F., Beauchard O., Blanchet S., Oberdorff T. & Brosse S. (2008) Fish invasions in the world's river systems: when natural processes are blurred by human activities. *PLoS Biology*, **6**, 404–410.
- Levsen A. & Jakobsen P.J. (2002) Selection pressure towards monoxeny in *Camallanus cotti* (Nematoda: Camallanidae) facing an intermediate host bottleneck situation. *Parasitology*, **124**, 625–629.
- Lindstrom D.P., Blum M.J., Walter R.P., Gagne R.B. & Gilliam J.F. (2012) Molecular and morphological evidence of distinct evolutionary lineages of *Awaous guamensis* in Hawai'i and Guam. *Copeia*, **2012**, 293–300.
- Liu H. & Stiling P. (2004) Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions*, **8**, 1535–1545.
- MacKenzie K. (1999) Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin*, **38**, 955–959.
- McDowall R.M. (2003) Hawaiian biogeography and the islands' freshwater fish fauna. *Journal Biogeography*, **30**, 703–710.
- McIntyre P.B., Michel E., France K., Rivers A., Hakizimana P. & Cohen A. (2005) Individual and assemblage-level effects of anthropogenic sedimentation on snails in Lake Tanganyika. *Conservation Biology*, **19**, 171–181.
- McKay L., Bondelid T. & Dewald T. (2012) "NHD Plus Version2: User Guide" (ftp://ftp.horizonsystems.com/NHD-Plus/NHDPlusV21/Documentation/NHD-PlusV2_User_Guide.pdf)
- McKenzie V.J. (2007) Human land use and patterns of parasitism in tropical amphibian hosts. *Biological Conservation*, **137**, 102–116.
- Menezes R.C., Tortelly-Neto R., Noronha D. & Pinto R.M. (2006) *Camallanus cotti* Fujita, 1927 (Nematoda, Camallanidae) in ornamental aquarium fishes: pathology and morphology. *Memórias do Instituto Oswaldo Cruz*, **101**, 683–687.
- Moravec F. & Justine J.L. (2006) *Camallanus cotti* (Nematoda: Camallanidae) an introduced parasite of fishes in New Caledonia. *Folia Parasitologica*, **53**, 287–296.
- Paterson R.A., Townsend C.R., Tompkins D.M. & Poulin R. (2012) Ecological determinants of parasite acquisition by exotic fish species. *Oikos*, **121**, 1889–1895.
- Peeler E., Oidtmann B., Midtlyng P., Miossec L. & Gozlan R. (2011) Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions*, **13**, 1291–1303.
- Pérez-Rodríguez A., Fernández-González S., de la Hera I. & Pérez-Tris J. (2013) Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Global Change Biology*, **19**, 1365–2486.
- Poulin R. (2000) Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. *Journal of Fish Biology*, **56**, 123–137.
- Prenter J., MacNeil C., Dick J.T.A. & Dunn A.M. (2004) Role of parasites in animal invasion. *Trends in Ecology and Evolution*, **19**, 385–390.
- Price P.W. & Clancy K.M. (1983) Patterns in number of helminth parasite species in freshwater fishes. *The Journal of Parasitology*, **69**, 449–454.
- Radtke R.L., Kinzie R.A. III & Folsom S.D. (1988) Age at recruitment of Hawaiian freshwater gobies. *Environmental Biology of Fishes*, **23**, 205–213.
- van Riper C., van Riper S.G., Goff M.L. & Laird M. (1986) The epizootology and ecological significance of malaria in Hawaiian land birds. *Ecological Monographs*, **56**, 327–344.
- Rohr J.R., Schotthoefer A.M., Raffle T.R., Carrick H.J., Halstead N., Hoverman J.T. *et al.* (2008) Agrochemicals increase trematode infections in declining amphibian species. *Nature*, **455**, 1235–1240.
- Rushton S.P., Lurz P.W.W., Gurnell J., Nettleton P., Bruemmer C., Shirley M.D.F. *et al.* (2006) Disease threats posed

- by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. *Epidemiology and Infection*, **134**, 521–533.
- Sala E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R. *et al.* (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.
- Schotthoefer A.M., Rohr J.R., Cole R.A., Koehler A.V., Johnson C.M., Johnson L.B. *et al.* (2011) Effects of wetland vs. landscape variables on parasite communities of *Rana pipiens*: links to anthropogenic factors. *Ecological Applications*, **21**, 1257–1271.
- Szuroczki D. & Richardson J.M.L. (2009) The role of trematode parasites in Larval Anuran Communities: an aquatic ecologist's guide to the major players. *Oecologia*, **161**, 371–385.
- Torchin M.E., Lafferty K.D., Dobson A.P., McKenzie V.J. & Kuris A.M. (2003) Introduced species and their missing parasites. *Nature*, **421**, 628–630.
- Torchin M.E., Lafferty K.D. & Kuris A.M. (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions*, **3**, 333–345.
- Vander Zanden M.J., Hansen G.J.A., Higgins S.N. & Kornis M.S. (2010) A pound of prevention, plus a pound of cure: early detection and eradication of invasive species in the Laurentian Great Lakes. *Journal of Great Lakes Resources*, **36**, 199–205.
- Vignon M. & Sasal P. (2010) Fish introduction and parasites in marine ecosystems: a need for information. *Environmental Biology of Fishes*, **87**, 1–8.
- Vincent A.G. & Font W.F. (2003) Seasonal and yearly population dynamics of two exotic helminthes, *Camallanus cotti* (Nematode) and *Bothriocephalus acheilognathi* (Cestode), parasitizing exotic fishes in Waianu stream, O'ahu, Hawai'i. *Journal of Parasitology*, **89**, 756–760.
- Vuong Q.H. (1989) Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica*, **57**, 307–333.
- Walter R.P., Hogan J.D., Blum M.J., Gagne R.B., Hain E.F., Gilliam J.F. *et al.* (2012) Climate change and conservation of amphidromous endemic to Hawaiian streams. *Endangered Species Research*, **16**, 261–272.
- Walters D.M., Blum M.J., Rashleigh B., Freeman B.J., Porter B.A. & Burkhead N.M. (2008) Red shiner invasion and hybridization with blacktail shiner in the upper Coosa River, USA. *Biological Invasions*, **10**, 1229–1242.
- Williams C.F., Britton J.R. & Turnbill J.F. (2013) A risk assessment for managing non-native parasites. *Biological Invasions*, **15**, 1273–1286.
- Yamamoto M.N. & Tagawa A.W. (2000) *Hawai'i's Native and Exotic Freshwater Animals*. Mutual Publishing, Honolulu, HI, 200 pp.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Best models (those having a $\Delta AIC < 2$) for site-level mean intensity and prevalence showing model variables, AIC value, ΔAIC from top-ranked model, number of parameters (k) and model rank.

(Manuscript accepted 1 October 2014)