

# Mutual dilution of infection by an introduced parasite in native and invasive stream fishes across Hawaii

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(Received 24 December 2015; revised 11 April 2016; accepted 1 May 2016)

## SUMMARY

The presence of introduced hosts can increase or decrease infections of co-introduced parasites in native species of conservation concern. In this study, we compared parasite abundance, intensity, and prevalence between native *Awaous stamineus* and introduced poeciliid fishes by a co-introduced nematode parasite (*Camallanus cotti*) in 42 watersheds across the Hawaiian Islands. We found that parasite abundance, intensity and prevalence were greater in native than introduced hosts. Parasite abundance, intensity and prevalence within *A. stamineus* varied between years, which largely reflected a transient spike in infection in three remote watersheds on Molokai. At each site we measured host factors (length, density of native host, density of introduced host) and environmental factors (per cent agricultural and urban land use, water chemistry, watershed area and precipitation) hypothesized to influence *C. cotti* abundance, intensity and prevalence. Factors associated with parasitism differed between native and introduced hosts. Notably, parasitism of native hosts was higher in streams with lower water quality, whereas parasitism of introduced hosts was lower in streams with lower water quality. We also found that parasite burdens were lower in both native and introduced hosts when coincident. Evidence of a mutual dilution effect indicates that introduced hosts can ameliorate parasitism of native fishes by co-introduced parasites, which raises questions about the value of remediation actions, such as the removal of introduced hosts, in stemming the rise of infectious disease in species of conservation concern.

Key words: Biological invasions, dilution effect, Hawai'i, poeciliid, *Awaous stamineus*, *Camallanus cotti*.

## INTRODUCTION

The co-introduction of exotic hosts and their parasites is contributing to the rise of infectious disease in native species of conservation concern (Daszak *et al.* 2000; Peeler *et al.* 2010; Peeler and Feist, 2011). Spillover, or 'enemy addition' (Gagne *et al.* 2015), can occur if introduced hosts serve as sources of co-introduced parasites that can infect native species (Prenter *et al.* 2004; Peeler and Feist, 2011; Britton, 2013; Sachman-Ruiz *et al.* 2015; Sheath *et al.* 2015). It remains unclear, however, whether parasitism of introduced hosts drives infection of native hosts following initial co-introduction. Parasitism of native species by co-introduced parasites has been attributed to the distribution and densities of introduced hosts (Gozlan *et al.* 2005; Peeler and Feist, 2011), but an emerging literature suggests that, with time, infection of native species by co-introduced parasites may have little or nothing to do with introduced hosts (Kirk, 2003; Haddaway *et al.* 2012, Gagne *et al.* 2015). For example, shifts in host preference can result in greater prevalence of

co-introduced parasites in novel host species (Frankel *et al.* 2015). Additionally, co-introduced parasites sometimes achieve higher intensities (Kirk, 2003) and increased virulence (Haddaway *et al.* 2012) in native species. Co-introduced parasites also can become established in native hosts with the capacity to complete their life cycle independent of the introduced host (Gagne *et al.* 2015).

Streams across the Hawaiian archipelago have long served as a natural laboratory for examining conditions that influence infection of native and introduced hosts by co-introduced parasites. As a consequence of extensive species introductions and naturally depauperate communities of hosts and parasites (Font and Tate, 1994; Font, 2003), the non-native intestinal nematode *Camallanus cotti* (Fujita, 1927) has become the most widespread and abundant parasite of Hawaiian stream fishes (Font, 2003; Gagne *et al.* 2015). *Camallanus cotti* is a generalist parasite with a complex life cycle; a range of fishes can serve as definitive hosts from which mature female parasites release first stage larvae into the freshwater environment, after which larvae are consumed by cyclopoid copepods that serve as an intermediate host (Font and Tate, 1994; Font, 2003). There is some experimental evidence that

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suggests *C. cotti* larvae can be directly transmitted, although this has never been documented in the wild (Levsen and Jakobsen, 2002). Although *C. cotti* is native to Asia, where it primarily infects cyprinid fishes (e.g. carps, true minnows, and relatives), poeciliids (e.g. guppies, swordtails) introduced from Texas (USA) are thought to have served as the primary vector of introduction to Hawai'i (Font and Tate, 1994; Gagne, 2015). Since being introduced, *C. cotti* has infected four of the five native stream fishes (*Awaous stamineus*, *Lentipes concolor*, *Eleotris sandwicensis* and *Stenogobius hawaiiensis*, but not *Sicyopterus stimpsoni*) (Font and Tate, 1994). The pathology of *C. cotti* in native fishes is not known, but *C. cotti* infection causes apathy, reduced libido, haemorrhage and erosion of the rectum mucosa in other species (Menezes *et al.* 2006). Thus it is considered a potential threat to native fishes in Hawaiian streams (Font, 2003).

Recent work indicates that parasitism of native Hawaiian stream fishes by *C. cotti* is no longer associated with introduced hosts (Gagne and Blum, 2015; Gagne *et al.* 2015). For example, parasitism of the endemic goby *A. stamineus* by *C. cotti* extends beyond the distribution of introduced hosts and appears to reflect a complex set of factors rather than poeciliid densities (Gagne *et al.* 2015). Although this suggests that native fishes are capable of sustaining *C. cotti* in Hawaiian streams, the extensive variation in parasitism across the archipelago indicates that native hosts may not provide stable transmission pathways (Gagne *et al.* 2015). In contrast, parasitism in poeciliids does not appear to differ significantly across years (Vincent and Font, 2003b), suggesting that coincident introduced hosts provide stable transmission pathways that increase transmission and sustain infections of *C. cotti* in native fishes. If so, then parasitism of native hosts might be amplified by, or covary with, parasitism of coincident introduced hosts. Conversely, introduced hosts could reduce parasitism in a native host via the dilution effect, where increased diversity lowers parasite intensity in a focal species due to uptake of parasites by other available hosts (Keesing *et al.* 2006).

In this study, we examined the abundance, intensity, and prevalence of *C. cotti* in populations of native and introduced hosts across the Hawaiian archipelago. We examined spatial variation to assess whether these parameters reflect host availability and other abiotic and biotic factors across watersheds. Specifically, we examined: (1) whether parasite abundance, intensity and prevalence differ between coincident and non-coincident native and introduced hosts, respectively; and (2) whether biotic and abiotic factors associated with parasitism are equivalent for native and introduced hosts. We also examined temporal variation because parasite abundance, intensity and prevalence can track

shifts in conditions over time, which can sometimes manifest as stochastic outbreaks or cyclical variation in parasitism (Schall and Marghoob, 1995; Glenn and Pugh, 2006; Violante-González *et al.* 2008; Lachish *et al.* 2011). Examining parasite abundance, intensity and prevalence, in space and time not only offered further perspective on the conditions that promote the success of co-introduced parasites, but it also provided a novel lens for understanding the potential value of remediation actions, such as the removal of introduced hosts, in stemming the rise of infectious disease in species of conservation concern like native Hawaiian stream fishes.

## METHODS

### *Sample collection and laboratory methods*

In 2009 and 2011, we sampled 42 watersheds on the five Hawaiian Islands containing perennial streams. Between two and four sites were sampled in each watershed. In accordance with permits reflecting conservation concerns, a total of 970 *A. stamineus* (2009,  $n = 562$ ; 2011  $n = 408$ ) and 929 poeciliids (2009,  $n = 630$ ; 2011,  $n = 323$ ; *Poecilia reticulata*,  $n = 744$ ; *Poecilia sphenops*,  $n = 123$ ; *Xiphophorus hellerii*,  $n = 62$ ) were collected from all watershed using hand nets (Gagne *et al.* 2015). All specimens were necropsied for intestinal macroparasites using a dissecting microscope following Hoffman (1999).

Parallel datasets on parasite abundance, intensity and prevalence as well as potential abiotic and biotic factors were developed for the 2009 and 2011 samples following Gagne *et al.* (2015). Biotic factors measured included the population density of both introduced and native hosts, as well as host length. Abiotic factors included watershed land use and water chemistry (soluble reactive phosphate, ammonium, nitrate, total nitrogen, total suspended solids, total dissolved solids, water temperature and conductivity). Characterization of watershed land use was only assessed once, corresponding to the 2001 National Land Cover Dataset (Homer *et al.* 2007). Estimates of genetic diversity were excluded from all analyses because genetic variation was not identified as an important indicator of *C. cotti* abundance, intensity or prevalence in *A. stamineus* and because genetic data were not available for introduced poeciliids (Gagne *et al.* 2015). Watershed area and mean annual rainfall were considered in addition to the other factors examined by Gagne *et al.* (2015) because both can influence surface flow, which in turn can influence *C. cotti* intensity and abundance in *A. stamineus* (Gagne and Blum, 2015). Data on watershed area were obtained from the Atlas of Hawaiian Watersheds and Their Aquatic Resources (Parham *et al.* 2008). Mean annual rainfall from 1987 to 2007 was calculated for each watershed using the Online Rainfall Atlas of Hawai'i (Giambelluca *et al.* 2013).

### Data analysis

Three measures of parasitism were calculated for each site and year following Bush *et al.* (1997): abundance (mean number of parasites per fish); intensity (mean number of parasites per infected fish); and prevalence (percentage of fish infected with *C. cotti*) for each site.

We first assessed spatial variation and temporal stability of parasite abundance, intensity and prevalence in *A. stamineus* and all poeciliids, respectively, in 2009 and 2011. We also examined interspecific variation by comparing each measure between *A. stamineus* and all poeciliids across sites and years. Because the majority of poeciliids collected were guppies (*P. reticulata*) we also analysed guppies separate from other poeciliid species. Although we found *C. cotti* in *A. stamineus* from 36 of 38 watersheds and *C. cotti* in poeciliids from 19 of 22 watersheds (Table 1), site level analyses only included sites where  $\geq 3$  individuals were scored for parasites (*A. stamineus* = 849 individuals, 35 watersheds, 73 sites; Poeciliids = 929 individuals, 39 watersheds, 44 sites). Finally, we performed comparisons between *A. stamineus* and poeciliids when co-occurring and when the other was not present. The reciprocal comparison also only included sites where  $\geq 3$  individuals were collected and that had corresponding snorkel survey data (*A. stamineus* without poeciliids = 378 individuals from 36 sites; *A. stamineus* with poeciliids = 314 individuals from 37 sites; Poeciliids without *A. stamineus* = 165 individuals from nine sites; Poeciliids with *A. stamineus* = 757 poeciliids from 36 sites). Brunner and Munzel test, which has been shown to be a robust non-parametric approach for comparing parasite abundance and intensity between groups (Brunner and Munzel, 2000; Neuhäuser and Poulin, 2004), was performed to compare parasite abundance and intensity between species and years. Chi-squared tests were performed to compare prevalence. Because poeciliid population densities were often much greater than those of *A. stamineus*, we also assessed the relative abundance of *C. cotti* in native and introduced hosts by multiplying mean abundance by host density, respectively. Thus relative abundance is a measure of the total number of parasites within a species (or categorical group of species) at a given site. We compared relative abundances between species using the Brunner and Munzel test.

Generalized linear mixed models (GLMM) were constructed using site level data to examine the influence of host body length (i.e. the length of individual fish), native (*A. stamineus*) and introduced (poeciliid) host population densities, watershed characteristics [area, % agricultural and urban land use (hereafter %ag-urb), mean annual rainfall] and water chemistry (PC1 and PC2 derived from a Principle Components Analysis of soluble reactive phosphorus, ammonium, nitrate, total nitrogen,

total suspended solids, water temperature, conductivity and total dissolved solids) on each metric of parasitism in *A. stamineus* and poeciliids, respectively. To recognize non-independence among sites and years, models included random effects of year, sites within watersheds and watersheds within islands. Values of %ag-urb were arc-sine transformed. Poisson error distributions were used for models examining the effects of site-level attributes on *C. cotti* intensity and prevalence, and a negative binomial distribution was used for models of parasite abundance. GLMM analyses only included sites where  $\geq 3$  *A. stamineus* or poeciliids were scored for parasites as well as where corresponding populations and ecological data was available. Consequently, parasite abundance and prevalence models for *A. stamineus* included 692 individuals from 29 watersheds containing 65 sites, and parasite intensity models (which consider only infected fish) included 59 sites. Models for poeciliids included 504 individuals from 15 watersheds and 26 sites. All analyses were conducted in R (R Core Team, 2012) using the glmmADMB package (GLMMs) (Skaug *et al.* 2006) and the lawstat package (Brunner and Munzel test) (Gastwirth *et al.* 2013).

## RESULTS

### Parasitism of *A. stamineus*

Taking both years into account, 43.8% of the sampled *A. stamineus* were infected by *C. cotti*, exhibiting an average abundance (i.e. worms/fish) of 2.77 and an intensity (i.e. worms/infected fish) of 6.33 (Table 1). Parasite abundance, intensity and prevalence were significantly higher in 2009 than in 2011 (abundance,  $P = 0.0048$ ; intensity,  $P < 0.0001$ ; prevalence,  $P < 0.0001$ ; Fig. 1, Table 1). Notably, evidence of parasitism was found in Hanakapiai in 2011, but not in 2009 or any prior surveys (Font and Tate, 1994; Font, 2003; Gagne *et al.* 2015). Alelele watershed on Maui was the only watershed with no infected *A. stamineus* throughout both years of the study ( $n = 29$  individuals; Table 1).

Although some shifts occurred, the watersheds with the highest abundance, intensity and prevalence of *C. cotti* infecting *A. stamineus* remained relatively consistent between sampling years (Fig. S1, Table 1). Notably, in 2009 three watersheds on Molokai (Pelekunu, Wailau, and Waikolu) registered abundance and intensity values that were three to five times greater than any other watershed sampled in either year (Fig. S1, Table 1). Despite large reductions in abundance and intensity, Pelekunu, Wailau and Waikolu watersheds remained among the five watersheds harbouring the most heavily parasitized individuals in 2011 (Fig. S1, Table 1). A similar, but less asymmetric, pattern was observed for estimates of prevalence (Fig. S1, Table 1).

Table 1. The abundance, intensity and prevalence of *Camallanus cotti* infecting *Awaous stamineus* (*Awaous*), poeciliids and *Poecilia reticulata* in 2009 and 2011 listed by watershed and grouped by island. The density of the host fish is also provided

Watershed	Awaous 2009					Awaous 2011					All Poeciliids					<i>Poecilia reticulata</i>			
	Density	<i>n</i>	Abundance	Intensity	Prevalence	Density	<i>n</i>	Abundance	Intensity	Prevalence	Density	<i>n</i>	Abundance	Intensity	Prevalence	<i>n</i>	Abundance	Intensity	Prevalence
<b>Molokai</b>																			
Honouli Wai	0.21	30	1.70	2.55	0.67	0.29	15	2.47	3.36	0.73	0.02	9	0.89	2.67	0.33	0			
Halawa	0.41	30	2.83	4.25	0.67	0.53	15	2.27	3.40	0.67	0.00	0			0				
Pelekunu	1.50	21	17.05	18.42	0.91	0.43	13	4.85	7.00	0.69	0.00	0			0				
Wailau	0.43	17	26.59	28.25	0.94	0.12	15	3.80	8.14	0.47	0.00	0			0				
Waikolu	0.13	19	21.89	29.71	0.74	0.10	15	3.27	6.13	0.53		0			0				
Kamalo Gulch	0.19	2	0.00	0.00	0.00		0				4.23	20	0.00	0.00	0.00	0			
<b>Kauai</b>																			
Lawai	0.45	24	3.92	3.92	1.00	1.51	15	0.87	2.17	0.40	0.67	94	0.29	1.05	0.21	71	0.30	1.31	0.23
Kapaa	0.19	20	2.10	5.00	0.42	0.28	14	4.43	6.89	0.64	0.31	47	2.15	3.16	0.68	46	2.15	3.26	0.67
Anahola		5	3.20	5.33	0.60		0				0.00	0			0				
Waimea	1.92	17	1.88	3.09	0.48	0.47	14	1.14	2.29	0.44	0.27	88	1.56	3.11	0.50	68	1.81	3.42	0.53
Hanalei		5	1.80	3.00	0.60		0				0.00	0			0				
Nawiliwili	0.02	0					0				0.68	21	0.93	2.70	0.34	21	0.93	2.70	0.34
Wainiha		14	6.21	7.25	0.86		4	1.00	2.00	0.50	0.00	0			0				
Moloaa	0.17	17	1.06	0.50	0.42	0.07	12	0.83	2.50	0.33	0.40	34	0.06	1.00	0.06	0			
Hanakapiai	0.23	15	0.00	0.00	0.00	0.43	14	1.50	4.20	0.36	0.00	0			0				
<b>Hawaii</b>																			
Hakalau		0				0.12	15	1.67	2.27	0.73	0.00	0			0				
Kaieie		9	0.78	1.50	0.50	0.01	15	2.47	4.63	0.53	0.09	36	1.03	2.18	0.47	36	1.03	2.18	0.47
Niulii	0.16	9	0.00	0.00	0.00	0.06	15	3.00	5.00	0.60	0.01	0			0				
Waipio	0.08	18	0.50	2.00	0.25	0.32	15	0.80	2.40	0.33	0.08	20	1.15	2.09	0.55	0			
Honolii	0.47	17	0.47	1.50	0.32	0.39	10	0.00	0.00	0.00	0.00	0			0				
Wailoa R	0.87	15	0.38	2.00	0.19		0				0.00	40	0.10	1.33	0.08	22	0.00	0.00	0.00
Wai'aha		0					0					16	0.00	0.00	0.00	0			
Nanue	0.04	4	2.00	2.50	0.80		0				0.01	0			0				
Waiulaula		0					0				0.00	35	3.49	4.88	0.71	35	3.49	4.88	0.71
<b>Oahu</b>																			
Waimea	0.02	18	1.33	6.00	0.22	0.06	5	0.00	0.00	0.00	0.33	0			0				
Alawai	0.02	21	0.29	3.00	0.10	0.01	2	0.00	0.00	0.00	1.07	41	0.51	2.63	0.20	41	0.51	2.63	0.20
Kahana	0.12	26	0.46	1.50	0.31	0.13	14	0.57	1.33	0.43	0.09	15	0.47	2.33	0.20	15	0.47	2.33	0.20
Wailupe		1	36.00	36.00	1.00		0				0.00	0			0				
Kaluanui		10	0.10	0.13	0.80	0.16	15	2.33	5.83	0.40	0.06	0			0				
Ki'iki'i		15	1.80	3.38	0.53		0					0			0				
Kahaluu		14	1.53	2.55	0.60	0.13	13	0.54	1.17	0.46	0.00	0			0				
Keaahala	0.30	19	1.47	3.11	0.47	0.39	9	2.00	3.60	0.56	1.01	161	0.23	1.38	0.16	159	0.23	1.44	0.16
Waiahole	0.13	15	0.36	3.00	0.14	0.18	13	0.77	2.00	0.38	0.33	40	0.28	1.38	0.20	28	0.36	1.43	0.25
Waikane	0.10	15	0.20	1.00	0.20	0.16	14	0.21	1.50	0.14	0.23	20	0.05	1.00	0.05	14	0.07	1.00	0.07
Waimanalo	0.10	20	1.00	2.25	0.44	0.00	7	0.43	1.50	0.29	0.93	32	0.00	0.00	0.00	29	0.00	0.00	0.00
<b>Maui</b>																			
Waihee	0.06	18	1.72	6.20	0.19	0.13	15	0.07	1.00	0.07	0.01	15	3.53	4.42	0.80	15	3.53	4.42	0.80
Iao	0.01	19	5.58	11.78	0.47	0.01	15	5.00	15.00	0.33	0.94	44	2.52	3.96	0.64	44	2.52	3.96	0.64
Alelele	0.23	14	0.00	0.00	0.00	0.65	15	0.00	0.00	0.00	0.00	0			0				
Honokohau	0.28	14	1.25	3.57	0.35	0.21	15	0.87	2.17	0.40	0.33	37	1.05	2.44	0.43	36	1.08	2.60	0.42
Kahakuloa		1	0.00	0.00	0.00	0.70	10	0.90	2.25	0.40	0.00	0			0				
Piinaau		14	1.00	2.00	0.50	0.21	15	1.07	1.78	0.60	0.00	0			0				
Ukumehame		0				0.21	15	0.60	2.25	0.27	0.00	64	0.50	1.78	0.28	64	0.50	1.78	0.28

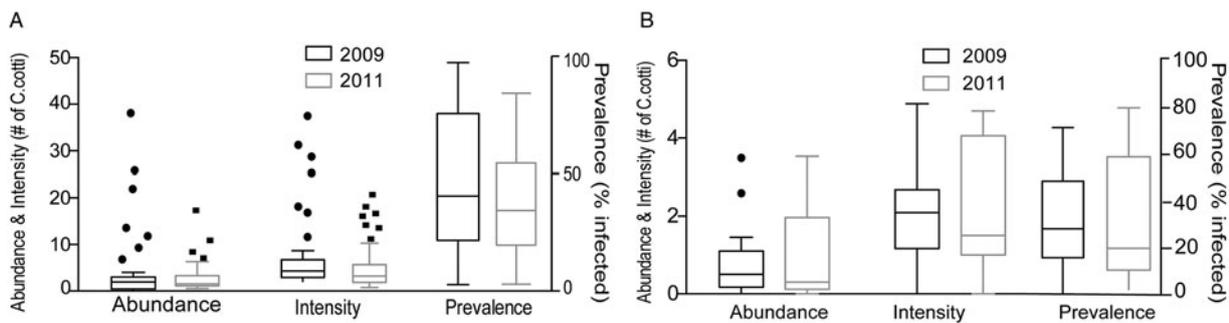


Fig. 1. (A) The abundance, intensity, and prevalence of co-introduced *Camallanus cotti* infecting native *Awaous stamineus* in 2009 and 2011. Whiskers are drawn following the Tukey method. (B) The abundance, intensity, and prevalence of co-introduced *Camallanus cotti* infecting introduced poeciliids in 2009 and 2011. Whiskers are drawn following the Tukey method.

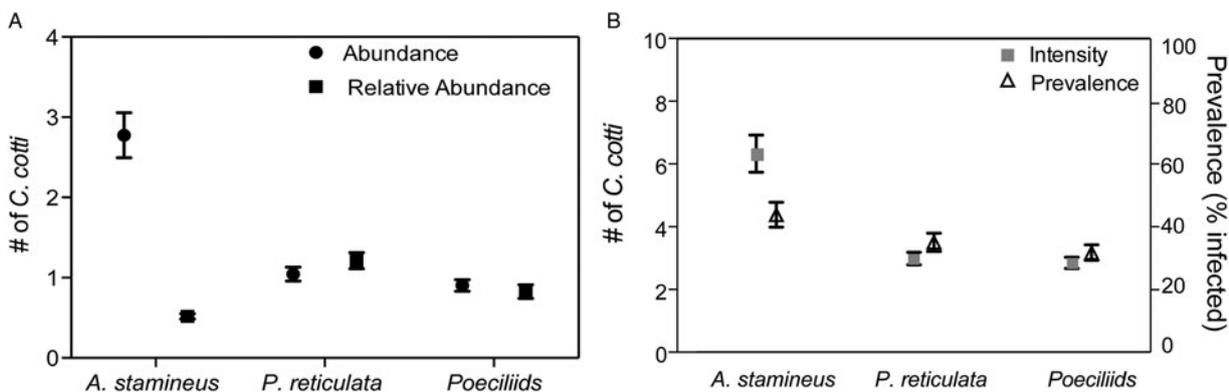


Fig. 2. (A) The abundance and relative abundance of co-introduced *Camallanus cotti* infecting native *Awaous stamineus*, all poeciliids and *Poecilia reticulata*, with error bars representing 95% confidence intervals (B) The intensity and prevalence of co-introduced *C. cotti* infecting native *A. stamineus*, all poeciliids, and *P. reticulata*, with error bars representing 95% confidence intervals.

### Parasitism of Poeciliids

Across all watersheds and years, 31.7% of poeciliids were infected by *C. cotti*, with an average abundance of 0.90 and an intensity of 2.85. The abundance and prevalence of *C. cotti* infecting poeciliids were significantly higher in 2009 than in 2011 ( $P < 0.001$ , Fig. 1, Table 1). The watersheds that contained poeciliids without *C. cotti* infections were (1) Kamalo Gulch on Molokai, where *C. cotti* also were not found in co-occurring *A. stamineus*; (2) Waimanalo on Oahu, where *A. stamineus* were infected; and (3) Waiaha on Hawai'i, where *A. stamineus* were absent. The highest abundance, intensity and prevalence of *C. cotti* occurred in poeciliids sampled from a diversion canal adjacent to Waihee stream on Maui (Table 1). Waiulaula, which sometimes exhibits discontinuous surface flow, had the highest abundance, intensity and prevalence of *C. cotti* in poeciliids sampled from a stream channel (Table 1). Consistent with previous studies (Vincent and Font, 2003a), we found that *P. reticulata* had the highest abundance, intensity and prevalence of *C. cotti* although the levels were generally representative of parasitism of the full complement of introduced poeciliids sampled in the study (Fig. 2).

### Comparison of parasitism between native and introduced hosts

Abundance, intensity and prevalence of *C. cotti* were all greater in *A. stamineus* than in co-occurring poeciliids (abundance and intensity,  $P < 0.001$ ; prevalence,  $P = 0.002$ ; Fig. 2). Parasite abundance, intensity and prevalence in *A. stamineus* were also significantly greater than in *P. reticulata*, the most common poeciliid species collected (abundance, intensity and prevalence,  $P < 0.001$ ; Fig. 2). Relative abundance, which accounts for the density of the host species, was significantly greater for *C. cotti* in poeciliids (relative abundance = 0.827) than *A. stamineus* (relative abundance = 0.518,  $P = 0.038$ , Fig. 2).

Parasite abundance and prevalence in *A. stamineus* was significantly greater when poeciliids were not present (abundance without poeciliids = 4.23, s.e. = 0.58, abundance with poeciliids = 1.82, s.e. = 0.24,  $P = 0.006$ ; prevalence without poeciliids = 50%, s.e. = 2.6%, prevalence with poeciliids = 41%, s.e. = 2.8%,  $P = 0.024$ , Fig. 3). Differences in parasite intensity approached significance (without poeciliids = 8.41, s.e. = 1.07; with poeciliids = 4.48, s.e. = 0.51,  $P = 0.059$ ). Parasite abundance, intensity and prevalence in poeciliids also were significantly greater

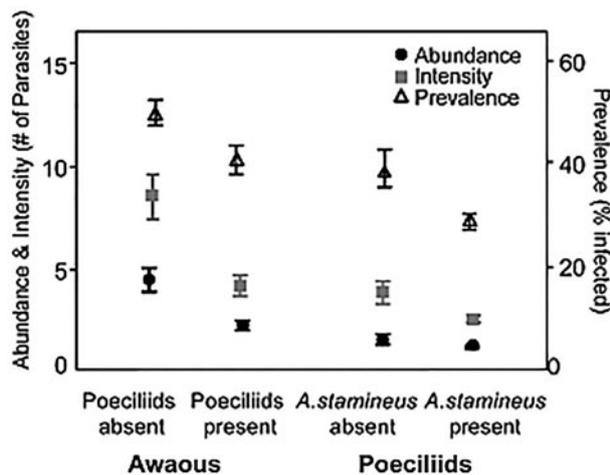


Fig. 3. Abundance, intensity and prevalence within poeciliids when coincident with *Awaous stamineus* and non-coincident with *A. stamineus*, and the abundance, intensity and prevalence within *A. stamineus* when coincident with poeciliids and non-coincident with poeciliids.

when *A. stamineus* were not present (abundance without *A. stamineus* = 1.43, S.E. = 0.25, abundance with *A. stamineus* = 0.73, S.E. = 0.07,  $P = 0.006$ ; intensity without *A. stamineus* = 3.69, S.E. = 0.54; intensity with *A. stamineus* = 2.65, S.E. = 0.18,  $P < 0.001$ ; prevalence without *A. stamineus* = 39%, S.E. = 3.8%, prevalence with *A. stamineus* = 29%, S.E. = 1.6%,  $P = 0.012$ , Fig. 3).

#### Predictors of parasitism

The intensity and abundance of *C. cotti* in *A. stamineus* were positively related to host length (Table 2). Additionally, parasite intensity in *A. stamineus* was negatively associated with poeciliid density and positively associated with water chemistry PC1 (Table 2), which explained 54.2% of the variation and contained strong loadings ( $>0.6$ ) for all three nitrogen forms, conductivity, total dissolved solids and total suspended solids (only soluble reactive phosphate loaded strongly on to water chemistry PC2). No variables were significantly related to parasite prevalence in *A. stamineus*. GLMM for parasite abundance and prevalence in poeciliids recovered positive relationships with land use (% ag-urb; Table 2) and models for parasite intensity recovered a negative relationship with water chemistry PC1.

#### DISCUSSION

Following initial spillover, infection of native species by co-introduced parasites may or may not be influenced by parasitism of introduced hosts. In prior work (Gagne *et al.* 2015), we showed that *A. stamineus* have become infected with the co-introduced parasitic nematode *C. cotti* in remote

watersheds that do not harbour introduced poeciliid hosts, suggesting that parasitism in native fish across the Hawaiian archipelago is no longer contingent on poeciliids serving as reservoirs. The current study provides further support for this inference. Not only did we find that *C. cotti* abundance, intensity and prevalence are greater in native than introduced hosts, we found that parasite intensity in native *A. stamineus* are inversely related to poeciliid density. Additionally, we found that infection levels in *A. stamineus* are largely consistent (i.e. sustained) in the absence of introduced poeciliids over time. Evidence that parasite abundance and intensity is lower in the presence of poeciliids also suggests that introduced poeciliids hosts reduce rather than elevate parasitism of native fish by *C. cotti*.

#### Parasitism of native vs introduced hosts

The higher abundance, intensity and prevalence of *C. cotti* in native *A. stamineus* relative to introduced poeciliid hosts possibly reflects differences in size and lifespan of host species. Native *A. stamineus* generally have longer lifespans (i.e. 3–4 years) and are larger (i.e. adults are  $\geq 100$  mm) than introduced poeciliid hosts (i.e.  $\leq 1.5$  years, adults are  $\leq 60$  mm) (Reznick *et al.* 2005; Hogan *et al.* 2014). A longer lifespan allows for the accumulation of higher parasite intensities as a result of greater exposure (Price and Clancy, 1983). In addition, food consumption by host fish generally increases with body size, thereby raising the odds of consuming an infected intermediate host (Lo *et al.* 1998). Contrasting feeding preferences may also exacerbate differences in infection risk over time as poeciliids have ontogenetic dietary variation, which has not been observed in *A. stamineus* (Hutchings *et al.* 2003; Vincent and Font, 2003a; Blum *et al.* 2014).

Abundance of *C. cotti* in *A. stamineus* is notably much higher than for hosts within the native range of *C. cotti*, which suggests that native Hawaiian stream fish are naïve hosts that lack defences against infection (Smith *et al.* 2009). Abundance is  $< 0.85$  in all host species in the native range of *C. cotti* (Wu *et al.* 2007) compared with 2.77 in *A. stamineus* and 0.9 in poeciliids in Hawaiian streams. Native Hawaiian stream fish, which are all endemic to the archipelago (Lindstrom *et al.* 2012), have likely evolved without the pressures imposed by a complex parasite assemblage. This may translate into comparatively weak defensive responses to parasite infection (Font, 2003). For example, naïve hosts may not show dietary adjustments or other behavioural responses to reduce risk of exposure (Hutchings *et al.* 2003), such as ontogenetic shifts in feeding preferences like those exhibited by guppies, which progressively feed less at stream margins where intermediate copepod hosts are most plentiful (Vincent and Font, 2003a). Thus, native Hawaiian stream fish may feed more heavily upon

Table 2. GLMM model outcomes for the abundance, intensity and prevalence of *Camallanus cotti* infecting native *Awaous stamineus* and poeciliids according to: host length (Length), agricultural and urban land use (% Ag-urb), density of *A. stamineus* (Density Awaous), the density of poeciliids (Density Poeciliids), water chemistry principle components one (PC1) and two (PC2), watershed area (WS-area) and average watershed rainfall (Rainfall). Models were run with a combined 2009 and 2011 dataset with year as a random factor. Sites and watersheds were also included as random factors

Species	Abundance			Intensity			Prevalence		
	Est	S.E.	P	Est	S.E.	P	Est	S.E.	P
<b><i>Awaous</i></b>									
Length	$1.41 \times 10^{-2}$	$2.18 \times 10^{-3}$	<0.001	$1.24 \times 10^{-2}$	$7.81 \times 10^{-4}$	<0.001	$1.84 \times 10^{-4}$	$1.57 \times 10^{-3}$	0.906
% Ag-urb	$5.21 \times 10^{-1}$	$6.85 \times 10^{-1}$	0.447	$3.91 \times 10^{-1}$	$8.21 \times 10^{-1}$	0.633	$9.74 \times 10^{-2}$	$4.30 \times 10^{-1}$	0.821
Density <i>Awaous</i>	$2.65 \times 10^{-1}$	$2.18 \times 10^{-1}$	0.223	$1.06 \times 10^{-1}$	$1.45 \times 10^{-1}$	0.466	$1.50 \times 10^{-1}$	$1.24 \times 10^{-1}$	0.226
Density Poeciliids	$-4.43 \times 10^{-2}$	$2.40 \times 10^{-2}$	0.065	$-3.93 \times 10^{-2}$	$1.49 \times 10^{-2}$	<b>0.008</b>	$-1.65 \times 10^{-2}$	$1.68 \times 10^{-2}$	0.327
PC1	$5.09 \times 10^{-3}$	$6.55 \times 10^{-2}$	0.938	$1.39 \times 10^{-1}$	$4.31 \times 10^{-2}$	0.001	$2.17 \times 10^{-3}$	$4.63 \times 10^{-2}$	0.963
PC2	$2.04 \times 10^{-1}$	$1.28 \times 10^{-1}$	0.110	$-6.24 \times 10^{-2}$	$5.77 \times 10^{-2}$	0.2792	$8.64 \times 10^{-2}$	$7.66 \times 10^{-2}$	0.259
WS-area	$3.97 \times 10^{-3}$	$4.45 \times 10^{-3}$	0.373	$2.81 \times 10^{-3}$	$5.19 \times 10^{-3}$	0.906	$2.51 \times 10^{-3}$	$2.51 \times 10^{-3}$	0.638
Rainfall	$2.21 \times 10^{-5}$	$1.74 \times 10^{-4}$	0.899	$2.44 \times 10^{-5}$	$2.07 \times 10^{-4}$	0.588	$-5.11 \times 10^{-5}$	$1.09 \times 10^{-4}$	0.317
<b>Poeciliids</b>									
Length	0.0123	0.008	0.150	0.008	0.006	0.221	$-4.26 \times 10^{-4}$	$1.16 \times 10^{-3}$	0.714
% Ag-urb	<b>5.1567</b>	<b>2.109</b>	<b>0.0145</b>	0.458	0.801	0.567	<b><math>3.51 \times 10^{-1}</math></b>	<b><math>1.08 \times 10^{-1}</math></b>	<b>0.001</b>
Density <i>Awaous</i>	0.9347	0.813	0.251	-0.275	0.279	0.324	$3.75 \times 10^{-1}$	$3.32 \times 10^{-1}$	0.258
Density Poeciliids	-0.0220	0.063	0.728	0.019	0.024	0.435	$-1.20 \times 10^{-2}$	$2.38 \times 10^{-2}$	0.613
PC1	-0.2116	0.146	0.148	<b>-0.082</b>	<b>0.041</b>	<b>0.048</b>	$-7.04 \times 10^{-1}$	$2.99 \times 10^{-1}$	0.018
PC2	-0.0028	0.517	0.996	0.086	0.041	0.612	$-8.23 \times 10^{-1}$	$6.43 \times 10^{-1}$	0.201
WS-area	0.0143	0.008	0.113	0.005	-0.001	0.678	$1.01 \times 10^{-2}$	$2.92 \times 10^{-3}$	0.127
Rainfall	0.0011	0.001	0.079	-0.001	0.005	0.110	$9.10 \times 10^{-3}$	$1.04 \times 10^{-2}$	0.381

Bold indicates significant at  $P < 0.05$ . Est, estimate + direction of effect; S.E., standard error; P, significance (P).

cyclopid copepods (the intermediate host of *C. cotti*) than introduced fishes. Native fish may also be more susceptible to infection due to lower immunocompetency, as has been found in other fishes with naturally low exposure to parasites (Scharsack *et al.* 2007).

We found striking evidence of a mutual dilution effect. Co-occurrence and higher densities of alternative hosts appear to reciprocally reduce infection of native and introduced hosts by *C. cotti*. As in our prior research (Gagne *et al.* 2015), we found an inverse relationship between parasite intensity in *A. stamineus* and introduced host densities. We also found that parasite intensity, abundance and prevalence in *A. stamineus* was reduced when poeciliids were present and that parasite intensity, abundance and prevalence in poeciliids was reduced when *A. stamineus* were present. This suggests that co-occurrence and greater densities of native and introduced fishes dilute parasitism by increasing the overall availability of definitive hosts. Increased host diversity has been shown to reduce infection via the dilution effect, whereby increased diversity reduces infection due to the presence of dead end hosts (Keesing *et al.* 2006). Our findings indicate that higher densities and diversity of viable definitive hosts also serve to reduce infection of each species present, with the dilution effect caused by a wider range and number of hosts able to take up parasites from a finite pool (Johnson and Thielges, 2010). Thus, the relationship of reduced parasite abundance and intensity in native Hawaiian stream fish with increasing densities of poeciliids could be a result of poeciliids harbouring parasites that might otherwise infect native hosts. This inference is also supported by our finding that poeciliids cumulatively host a comparably larger number of parasites relative to *A. stamineus*. If so, then following spillover from an initial introduction of poeciliids into a watershed, the influence of introduced hosts on parasitism of native hosts by co-introduced parasites might shift from negative to positive.

#### *Abiotic and biotic influences on parasitism*

Parasite burdens may vary if susceptibility to conditions that promote infection differ among hosts (Dunn and Dick, 1998; Plowright *et al.* 2008). Our results indicate that parasitism of native and introduced hosts by *C. cotti* reflects different biotic and abiotic factors known to influence susceptibility to infection. For example, a positive relationship between host length and parasite abundance and intensity were observed only in native *A. stamineus*, which likely reflects the accumulation of parasites over time in *A. stamineus* (Gagne *et al.* 2015) as opposed to poeciliids (Vincent and Font, 2003a). The positive association detected between agricultural and urban land use and parasite abundance and prevalence in poeciliids could be an outcome

of fewer co-occurring alternative hosts, because *A. stamineus* is less tolerant of stream conditions associated with intense watershed land use (Blum *et al.* 2014).

Our results also indicate that variation in parasitism of native and introduced hosts can be a consequence of different responses to the same factor. Water chemistry PC1 was found to be associated with infection of both native and introduced hosts by *C. cotti*; however, it was positively associated with parasite intensity in *A. stamineus* and negatively associated with parasite intensity in poeciliids. Water chemistry PC1 largely corresponded to nitrogen variability. In other host–parasite systems, nutrients drive parasitism of definitive hosts by influencing the prevalence of intermediate hosts (Johnson *et al.* 2008). In Hawai'i, nutrient-driven shifts in intermediate host populations would likely result in parallel rather than contrasting trends in parasitism of native and introduced definitive hosts (i.e. as the abundance of intermediate hosts increases, so does parasitism of all definitive hosts), which suggests that other outcomes of nitrogen variability are shaping parasitism of definitive fish hosts in Hawaiian streams. Stable isotope and gut content analyses indicate that the positive association between nutrients and parasite intensity in *A. stamineus* reflect changes in diet, where greater carnivory (i.e. consumption of copepods) in nutrient-enriched streams elevate risk of infection (Blum *et al.* 2014). Not much is known about dietary preferences of introduced poeciliids under different nutrient regimes, but it is possible that greater competition with native fish for invertebrate prey could reduce parasite intensity in poeciliids in nutrient-rich streams (Holitzki *et al.* 2013).

With some notable exceptions, the set of factors found to influence parasitism of *A. stamineus* by *C. cotti* were consistent with those identified in prior studies (Gagne and Blum, 2015; Gagne *et al.* 2015). Gagne *et al.* (2015), for example, found that host size and conditions associated with nutrient loading were positively associated with parasite abundance and intensity in *A. stamineus* based on a single year of data. Gagne *et al.* (2015) also found, however, that parasite intensity and prevalence tracked *A. stamineus* population density. We did not find the same relationship in the current study, which might be due to temporal shifts in parasite abundance, intensity, and prevalence in watersheds harbouring some of the highest densities of *A. stamineus* across the archipelago. A comparison across a natural rainfall gradient and a broader comparison among islands also found that parasite abundance, intensity and prevalence increase with declining precipitation (Gagne and Blum, 2015). Average rainfall was lower and parasitism was higher in 2009, but we did not detect a statistically significant relationship with rainfall, possibly because our rainfall estimates

were based on 30-year averages rather than annual measures, which are not readily available for each watershed (Giambelluca *et al.* 2013).

#### Temporal changes in parasitism

Although we found higher levels of parasite abundance, intensity and prevalence in *A. stamineus* in 2009 compared with 2011, *C. cotti* infections were relatively stable in the majority of watersheds sampled over the study period. This is consistent with evidence of stable levels of parasite abundance and prevalence in poeciliids in one watershed on Oahu over 5 years (Vincent and Font, 2003b). Nonetheless, we observed notable shifts in parasite abundance, intensity and prevalence in three watersheds on the remote north shore of Molokai. The transient spike on Molokai may have corresponded to an epizootic event, which can increase host mortality (Kennedy *et al.* 2001); periods of increased parasitism are often driven by environmental and host population dynamics and subside following a crash in host populations (Kennedy *et al.* 2001; Briggs *et al.* 2010; Heins *et al.* 2011). Consistent with this, we found that densities of *A. stamineus* declined in these watersheds between 2009 and 2011, but we cannot say whether the declines are attributable to parasite-induced mortality. Parasite abundance and prevalence within poeciliids also was greater in 2009 than 2011, suggesting that yearly differences in parasitism could be indicative of regional rather than local shifts in environmental conditions (e.g. rainfall and surface flow) over time. Further work to better characterize the magnitude and footprint of temporal variation in parasitism is warranted, as transient spikes could be detrimental to populations of *A. stamineus*, particularly if coupled with other stressors, such as habitat degradation.

In conclusion, the greater abundance, intensity, and prevalence of *C. cotti* in native *vs* introduced fishes, as well as higher parasite abundance, intensity, and prevalence in watersheds that lack introduced fishes, show that the incidence of parasitism in native species is no longer strictly determined by the distribution and densities of the original introduced hosts in the Hawaiian archipelago. Evidence of a dilution effect also indicates that the co-occurrence and prevalence of introduced hosts can ameliorate parasitism of native fish by co-introduced parasites, which runs counter to the prevailing notion that the presence of introduced species is always detrimental to native fish (Font, 2007; Holitzki *et al.* 2013). Indeed, our findings suggest that efforts to control (i.e. remove) introduced poeciliids could inadvertently increase parasitism in native fish. Importantly, other benefits of removing introduced poeciliids (e.g. reduced resource competition) could outweigh any increases in parasitism. Thus a better understanding of responses of native

fishes to introduced fish is warranted to support management decisions. A more thorough pathological assessment, for example, would help address this concern and related concerns, like the possibility of parasite-induced mortality from epizootic events or interactions with other stressors that threaten amphidromous Hawaiian stream fishes (Brasher, 2003; Walter *et al.* 2012).

#### SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at <http://dx.doi.org/10.1017/S0031182016001001>.

#### ACKNOWLEDGEMENTS

We thank E. Childress, J. Fenner, G. Glotzbecker, T. Haas, J.D. Hogan, B. Lamphere, D.P. Lindstrom, K. Moody, D. Oele, T. Rayner, J. Rossa and R.P. Walter for field assistance, as well as J. Thore, E. Hamann, A. Gulacheski and S. Piper for laboratory assistance. We also thank H. Bart, C. Criscione and W. Font for valuable guidance. All collections were approved and conducted in accordance with Special Activities Permits issued by the Hawaii Division of Aquatic Resources.

#### FINANCIAL SUPPORT

This study was funded by Tulane University (to M.B. and R. G.) and the US Department of Defense Strategic Environmental Research Development Program (SERDP) through project RC-1646 (to M. B., P. M. and J. G.).

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