

Effects of invasion at two trophic levels on diet, body condition, and population size structure of Hawaiian red shrimp

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Abstract. We examined the degree to which invasion by non-native, nitrogen (N)-fixing riparian trees and poeciliid fish alters diet of a common grazer/detritivore in brackish ponds on the dry coast of Hawai'i Island. Because this shrimp, *Halocaridina rubra* ('opae'ula), displays a preference for autotrophic components of epilithon, we hypothesized that tree canopy would reduce their body condition and abundance, but that this would be moderated by nutrient quality of leaf litter (high-quality, non-native, nitrogen-fixing tree vs. low-quality, endemic non-nitrogen-fixing tree). We hypothesized that poeciliid invasion would reduce population size and body condition of *H. rubra* by direct predation and by inducing reductions in feeding rate. Analysis of stable isotopes (δD , $\delta^{13}C$, and $\delta^{15}N$) showed that direct consumption of leaf litter detritus supplemented *H. rubra* diet in ponds with canopy cover. In addition, epilithon in ponds with canopy cover was isotopically more similar to leaf litter than epilithon in ponds without canopy cover, and our evidence suggests this may be due to contributions of allochthonous nutrient sources to epilithon assemblages. However, canopy cover, whether native or invasive, did not decrease epilithon growth rates and had little effect on shrimp body condition or population size. In contrast, poeciliid invasion reduced daylight grazing on the benthos, and caused a reduction in body condition among larger shrimp. Our results indicate that primary succession by invasive trees may shift food web linkages in these ponds from autotrophic to heterotrophic support, without negative impacts on native grazer/detritivore communities. We suggest that poeciliid invasions have sub-lethal effects on *H. rubra* as well as their direct predation effects, with potential long-term implications for the ecosystem function of anchialine ponds and other coastal systems.

Key words: anchialine pond; canopy; carbon isotopes; estuarine; grazing; *Halocaridina rubra*; Hawai'i; hydrogen isotopes; invasion; nitrogen isotopes; poeciliid; trophic.

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INTRODUCTION

Introductions of invasive species are increasingly altering energy and nutrient flows to the base of food webs (Strayer 2010). For grazers,

invasion may cause bottom-up change to the quality (e.g., N content) and quantity of basal food resources, and top-down alterations to food availability through competitive or trait-mediated effects (Kennedy et al. 2005, Capps et al. 2009,

Holitzki et al. 2013, MacKenzie et al. 2013). Trait-mediated effects arise from a predator's influence on prey traits (e.g., where access to food increases susceptibility to predation [McIntosh and Townsend 1996, Anholt and Werner 1998, Holitzki et al. 2013]). Alterations to grazer food sources may have significant implications for the resilience of ecosystems, and dictate management strategies for their protection.

Allochthonous primary producers provide considerable sources of energy and nutrients to freshwater (Cole et al. 2011, Cole and Solomon 2012) and estuarine ecosystems (Sobczak et al. 2002). In particular, litterfall inputs from invasive nitrogen-fixing trees can increase N availability in aquatic systems (Atwood et al. 2010, Mineau et al. 2011, Wiegner et al. 2013). Where endemic and invasive leaf litter samples differ in nutrient content, differences in species composition of riparian tree communities could alter bottom-up effects on autochthonous primary producers. Leaf litter inputs may directly provide an alternate nutrient source for invertebrate primary consumers through consumption of leaf litter or indirectly by supporting the heterotrophic components of epilithon via litter contributions to dissolved organic carbon (DOC) (Wiegner et al. 2005, Kaplan et al. 2008). While increases in nutrient enrichment may benefit aquatic consumer communities in the short term (Cross et al. 2006), longer-term consequences to benthic food webs from invasion by N-fixing trees are poorly understood, given that higher-quality litter may also decrease food availability due to increased litter breakdown rates (MacKenzie et al. 2013). In addition, reduction in preferred algal food resources by canopy succession may negatively impact primary consumer populations (Schiesari 2006) by reducing available light below the canopy and the productivity, diversity, and abundance of aquatic photoautotrophs (Bunn et al. 1999, Skelly et al. 2002).

Invasions at higher trophic levels (TLs) can cause both bottom-up effects (e.g., changes to nutrient cycling) that alter the quality of available food sources, and top-down effects that alter the ability of endemic primary consumers to access food (Byrnes et al. 2007). In pond systems, invasive fish have shown to cause trophic shifts and negatively affect production and growth rates of sympatric fishes (Britton et al. 2010), and invasive

poeciliids have been suggested to elevate nutrient concentrations, change native fish and macroinvertebrate assemblages, and alter overall ecosystem function in tropical streams (Holitzki et al. 2013) and wetlands (MacKenzie and Bruland 2012).

In Hawai'i, anchialine ponds provide a useful model system for comparison of invasion effects on invertebrate primary consumers in coastal ecosystems. Anchialine ponds contain diverse assemblages of many endemic species, but the benthic palaemonid shrimp *Halocaridina rubra* is the dominant primary consumer; counts of this species commonly exceed 95% of visible fauna in anchialine ponds (Wong 1975, Maciolek 1983, Bailey-Brock and Brock 1993, Sakihara et al. 2015). Anchialine ponds are also influenced by many non-native species. They are often surrounded by introduced vegetation, particularly introduced nitrogen-fixing trees and shrubs that have proved particularly successful in colonizing these N-deficient, P-rich, bare or sparsely vegetated young lava flows (Vitousek 1995, Hughes and Denslow 2005). In these ponds, *H. rubra* shows a tendency to prefer the autotrophic component of epilithon; therefore, alterations to the presence or grazing behavior of *H. rubra* can have marked effects on benthic primary production and epilithon biomass (Dalton et al. 2012, Sakihara et al. 2015). In addition, many Hawaiian anchialine ponds also contain populations of poeciliids—guppies (*Poecilia reticulata*), mollies (*Poecilia* sp.), and/or mosquitofish (*Gambusia affinis*; MacKenzie and Bruland 2012). Capps et al. (2009) observed that presence of invasive fish in anchialine ponds generated cryptic behavior during the day by *H. rubra*, a reduction in daytime grazing, and a resulting increase in epilithon biomass. Both invasion by poeciliids and canopy cover may also alter abundance of the native *Metabetaeus lohena*, an alpheid shrimp scavenger that shares the habitat and distribution of *H. rubra*, and may prey on it (Holthuis 1973, Sakihara 2012, Seidel et al. 2015). Hence, invasion of anchialine ponds by the N-fixing *Prosopis pallida* and poeciliids may affect *H. rubra* populations by altering (1) the quantity and quality of allochthonous inputs, (2) the quantity and quality of autochthonous primary production, and (3) the abundance and foraging behavior of individuals. The absence of native fish from the

endemic composition of most anchialine pond communities, and N-fixing species from Hawaiian coastal dryland tree assemblages suggest a potential susceptibility of anchialine pond ecosystems to invasion impacts by these species (Ricciardi and Atkinson 2004, Scheffer et al. 2006).

Natural abundance stable isotope techniques are potentially powerful tools to quantify dietary and biogeochemical changes in ecosystems. Within coastal mixing zones however, spatial and temporal variations in abiotic factors (e.g., in nutrient availability, light, and temperature) have been shown to cause variation in carbon and nitrogen isotope ratios of primary producer tissue (Cloern et al. 2002, Cornelisen et al. 2007, Dudley et al. 2010). Increasing the number of isotope tracers that can be used in food web studies has the potential to improve reliability of mixing models (Fry 2006, 2013, Soto et al. 2013). Stable hydrogen isotope ratios (δD) appear particularly suited to provide extra resolution to estimates of terrestrial vs. aquatic energy sources for freshwater consumers, because while δD values of leaf tissue in higher plants may be variably affected by fractionation during transpiration, the net depletion between source water and non-exchangeable H in higher plant tissue appears generally less than for algal primary production (Smith and Ziegler 1990, Finlay et al. 2010). This results in differences in δD values between allochthonous and autochthonous energy sources to aquatic systems that can be $>100\%$ (Doucett et al. 2007). Furthermore, δD values of benthic algae appear not to vary greatly over gradients of turbulence, light, or temperature (Macko et al. 1983, Finlay et al. 2010). Recent analytical developments have solved many of the obstacles to the use of hydrogen isotopes in aquatic food web studies (Wassenaar and Hobson 2000, 2003, Solomon et al. 2009, Soto et al. 2013); however, published instances of δD use in brackish ecosystem studies are rare.

Here, using two controls (no canopy cover and canopy cover of endemic, non-nitrogen-fixing milo, *Thespesia populnea*), we examine the effect of the invasive N-fixing tree, *P. pallida*, canopy presence on (1) availability and quality of potential food sources (leaf litter and epilithon) to *H. rubra* and (2) differences in diet, body condition, densities, and size structure of populations of *H. rubra*. In ponds without canopy cover, we

examined the effects of populations of non-native poeciliid fish on (1) growth rate and quality of epilithon, (2) diurnal migration of *H. rubra* to avoid poeciliids, and (3) differences in diet, body condition, densities, and structure of populations of *H. rubra*.

We predicted firstly that presence of canopy cover would result in decreased epilithon growth, and a shift in *H. rubra* diet from algae to lower-quality leaf litter. Secondly, we predicted that this reduction in food quality would result in reduced individual body condition of *H. rubra*. Where canopy cover around anchialine ponds was dominated by N-fixing *P. pallida*, we predicted that the higher-quality leaf litter deposited into ponds would enhance the body condition of *H. rubra* relative to ponds dominated by *T. populnea*. Finally, we predicted that fish presence would cause cryptic behavior (known to reduce grazing), and reduced diet quality (due to competition for preferred food sources), reducing both population size and individual body condition of the shrimp. We aimed to quantify the dietary and body condition response of *H. rubra* to these two invasions, advance our understanding of interactions between invasive and native species in lentic ecosystems, and aid biodiversity conservation efforts for anchialine ponds and similar systems.

MATERIALS AND METHODS

Study system

The Hawaiian archipelago is home to 600 or more anchialine ponds, around 70% of which are located along the leeward coast of the youngest island, Hawai'i (Brock et al. 1987). These ponds exhibit dampened tidal fluctuations, have no surface connection to the sea or terrestrial water bodies, and are most commonly mesohaline to oligohaline (Holthuis 1973, Ridgley and Chai 1990). Hawaiian anchialine ponds are particularly common in areas where terrestrial patterns of succession are periodically reset by lava flows, and range from having no surrounding vegetation to having tree canopy cover on older flows. However, these patterns of succession in Hawai'i have changed greatly since the first European settlement in the late 18th and 19th centuries. On the dry, leeward side of the islands, native coastal dry shrublands previously subject to grazing by cattle have largely been replaced by invasive grasses

and succeeded by non-native nitrogen-fixing tree species, particularly *Leucaena leucocephala* and *P. pallida* (Wagner et al. 1999). Where groundwater is shallow, *P. pallida* tends to dominate, although *T. populnea* and Naupaka (*Scaevola* spp.) form stands in some areas. *Prosopis pallida* shrubland now covers ~3.55% of the total land area of the Hawaiian Islands (around 59,000 ha), almost exclusively in lowland parts of the leeward coasts (Gallaher and Merlin 2010), and in many areas forms monospecific stands around anchialine ponds. Water chemistry in the ponds strongly reflects groundwater flows, with residence times ranging from 1.7 to 5.5 h; pond shows similar total dissolved nitrogen, salinity, pH, and temperature across pond types (Dudley et al. 2014). Groundwater nutrient concentrations in this region are high, and groundwater supplies N and P across these ponds at concentrations in excess of periphyton growth requirements (Sakihara et al. 2015). However, ponds with heavy canopy cover tend to have lower dissolved oxygen concentrations and a higher proportion of total dissolved N in organic and ammoniacal forms.

Study design

We examined the effect of the invasive *P. pallida* tree and poeciliid fish on quantity and quality of available food, diet, and individual body condition of the dominant herbivore (*H. rubra*), and the structure of native shrimp communities in Hawaiian anchialine ponds. Ponds with no surrounding vegetation or fish (open; $n = 4$) were compared with ponds surrounded by the invasive *P. pallida* (invasive canopy; $n = 4$), those surrounded by a non-N-fixing endemic tree—*T. populnea* (endemic canopy; $n = 4$), and open ponds with resident populations of poeciliid fish (open with poeciliids; $n = 4$) along a ~45-km stretch of Hawai'i's leeward coast (Fig. 1; Table 1).

Abiotic pond characteristics

Pond surface area and depth were measured to the nearest cm within 1 h of high tide. Pond depth was measured using a meter stick, and surface area was estimated from approximate pond shape at high tide (e.g., circular, rectangular, scalene triangle) and dimensions (see Appendix S1: Table S1 for pond dimensions and locations). Photosynthetically active radiation (PAR) was measured three times on three dates

between June 2012 and October 2013 at the surface of ponds using LI-COR quantum sensors coupled to LI-COR LI-250 light meters (LI-COR Environmental, Lincoln, Nebraska, USA). Each pond surface PAR measurement coincided with a simultaneous reading made in a nearby open area with an unobstructed view of the sky within 38° of the zenith. The percentage of incident light outside the canopy that reached the pond surface was calculated as $\text{PAR}_{\text{pond}}/\text{PAR}_{\text{open}} \times 100$.

Potential food sources

Net accumulation of epilithon (a potential food source for *H. rubra*) was compared between ponds using grazer-excluded settlement plates. Terracotta plates with a surface area of 53.5 cm² were covered in a layer of 500- μm Nitex bolting cloth raised above the plate surface with a plastic frame to exclude grazers. One plate was placed on a level area of the benthic surface near the center of each pond for each time period. At collection, we removed the mesh and scrubbed clean the surface of each plate with a denture brush for 1 min. We then rinsed the brush and plate with microfiltered (Whatman 0.7 μm GF/F filters) pond water to make a slurry of known volume. We stored slurry samples on ice in the dark until they were returned to the laboratory for processing. We then mixed the slurry sample by shaking and stirring, and vacuum-filtered subsamples through precombusted and preweighed 0.7- μm GF/F filters. Filters were analyzed for chlorophyll *a* (Chl *a*) content according to Pringle and Triska (2006). Plates were first deployed on 14–16 December 2011 and processed/exchanged for fresh plates sequentially on 14–16 February, 16–18 April, 14–15 June 2012, and 30–31 August. Plates were removed on 25–26 October. Epilithon dry mass, ash-free dry mass (AFDM), and Chl *a* data were divided by the number of days of deployment to give accumulation rates comparable between pond types, although we note that true daily accumulation rates are likely to be non-linear. We calculated an autotrophic index ($\text{AI} = \text{AFDM}/\text{Chl } a$) to assess relative shifts toward autotrophy or heterotrophy in epilithic communities. A higher AI indicated relative heterotrophy, whereas a lower AI indicated relative autotrophy (Steinman et al. 2006). Water samples were also collected from the surface of each pond using an acid-washed grab

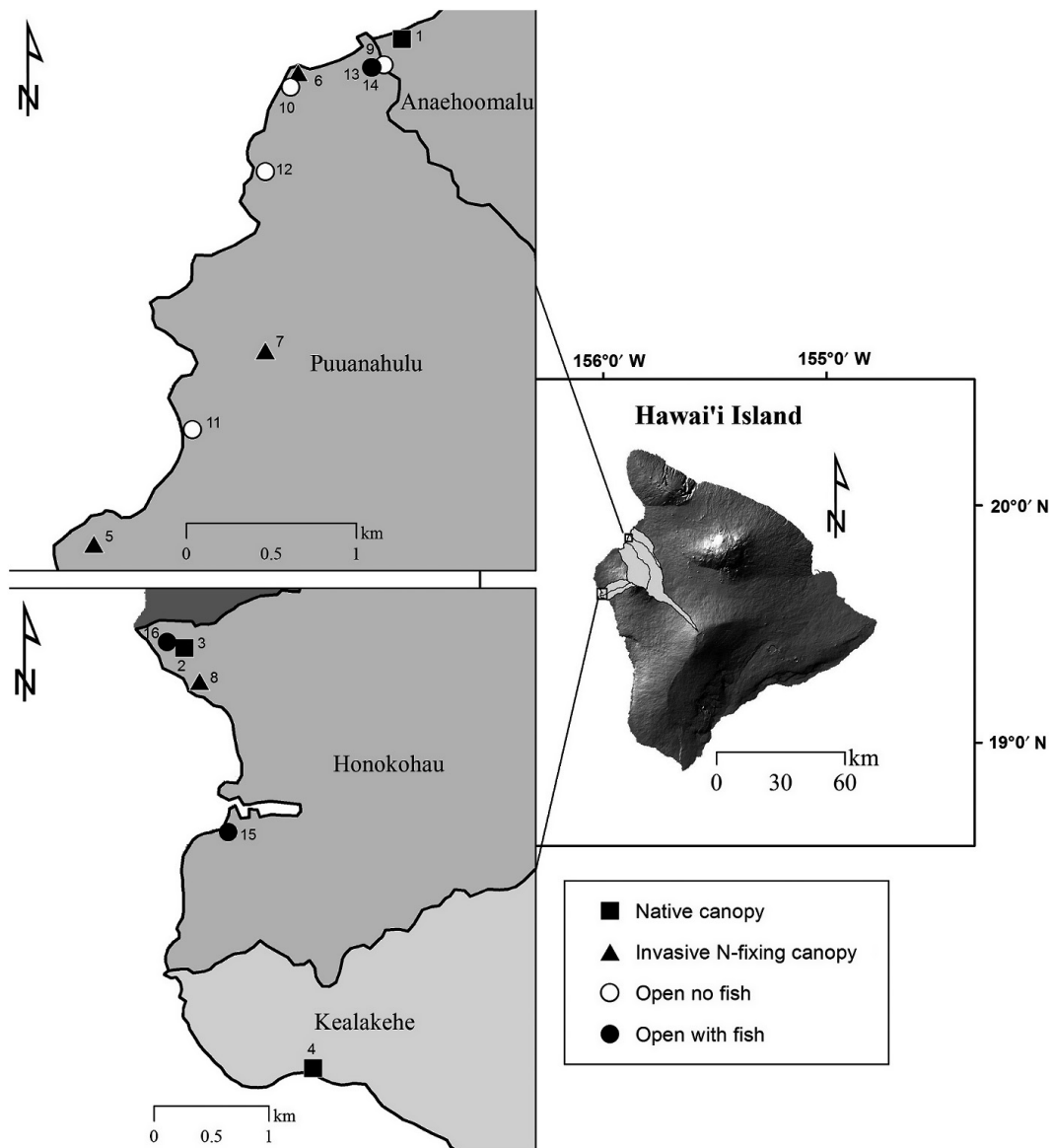


Fig. 1. Pond locations. Gray partitions show watershed delineations. Numbers in panels are pond identification numbers.

sampler. An unfiltered water sample was immediately placed on ice, while a second water sample was filtered through a 0.7- μm Nitex nylon filter and also placed on ice. Samples were returned to the laboratory, frozen, and later analyzed for total organic carbon (TOC, unfiltered samples) and DOC (filtered samples) (TOC/DOC, DL 10.0 $\mu\text{mol/L}$; USEPA method 415.1 [USEPA1999]) on a Shimadzu TOC-V, TNM-1 (Shimadzu, Columbia, Maryland, USA).

Diet

Samples of potential food resources for *H. rubra* were collected from all ponds between February and June 2012 at the same dates as the removal of epilithon growth plates. Samples of leaf litter were collected haphazardly from the top 10 mm of the benthic substrate of ponds on each date. The samples from each pond were composited to form a single leaf litter sample for each pond on each sampling date. Epilithon for

Table 1. Physical properties and quality and quantity of potential food sources for *Halocaridina rubra* across all ponds.

Pond	Pond class	PAR (%)		Litter inputs†, ‡		Litter C:N		Epi.§ dry weight¶		Epi. C:N		Epi. AFDM¶		Epi. Chl a#		Epi. AI		DOC		TOC	
		n = 3	n = 3	n = 3	n = 3	n = 5	n = 3	n = 5	n = 3	n = 5	n = 5	n = 5	n = 5	n = 5	n = 4	n = 4					
1	Endemic canopy	1.17	0.19	3.46	35.80	8.73	2.44	0.57	9.52	0.02	0.21	0.06	0.061	0.014	4029	1034	1379	936	1417	536	
2	Endemic canopy	13.07	11.40	3.05	36.58	3.70	1.15	0.53	8.82	0.26	0.19	0.11	0.157	0.0123	4714	1393	199	90	790	676	
3	Endemic canopy	27.85	15.5	0.80	22.13	2.91	0.81	0.15	7.85	0.74	0.41	0.08	1.562	0.360	258	19	536	453	240	150	
4	Endemic canopy	2.75	0.36	3.41	33.38	2.69	1.08	0.52	9.08	0.07	0.20	0.11	0.024	0.009	5534	1780	874	634	7206	6343	
5	Invasive canopy	15.65	11.44	1.21	17.51	0.77	1.53	0.61	10.22	0.12	0.34	0.14	0.400	0.268	1171	555	526	255	1188	1049	
6	Invasive canopy	5.30	2.43	1.76	25.7	4.16	0.23	0.073	11.01	1.77	0.11	0.04	0.717	0.206	119	23	104	23	894	803	
7	Invasive canopy	14.03	6.98	1.27	27.47	2.57	0.13	0.07	9.22	0.92	0.05	0.03	0.247	0.108	107	36	138	35	90.3	11.2	
8	Invasive canopy	45.62	16.80	0.39	28.52	1.32	0.47	0.16	8.47	0.65	0.20	0.07	0.497	0.154	487	192	255	123	296	174	
9	Open	100	0	–	–	–	0.65	0.09	6.70	0.27	0.33	0.05	0.455	0.143	653	70	64.0	7.2	63.1	7.1	
10	Open	100	0	–	–	–	0.37	0.10	11.38	2.49	0.18	0.05	0.296	0.069	590	49.3	67.7	19.6	54.9	4.3	
11	Open	100	0	–	–	–	0.2	0.01	8.54	0.17	0.08	0.05	0.264	0.117	257	55	102	35	49.9	3.9	
12	Open	100	0	–	–	–	0.19	0.06	6.82	0.17	0.06	0.02	0.298	0.043	242	97	369	284	200	144	
13	Open with poeciliid	100	0	–	–	–	0.77	0.04	9.56	0.16	0.48	0.02	0.344	0.057	1414	191	61.3	17.6	72.3	22.5	
14	Open with poeciliid	100	0	–	–	–	0.81	0.16	8.21	0.45	0.48	0.09	0.512	0.130	1051	133	169	110	162	86	
15	Open with poeciliid	100	0	–	–	–	1.07	0.22	6.22	0.32	0.56	0.11	1.083	0.269	558	64	222	35	735	528	
16	Open with poeciliid	100	0	–	–	–	0.70	0.05	6.57 (n = 1)	0.41	0.04	0.04	0.47	0.07	885	3.5	84.7	13.0	67.2	10.8	

Notes: PAR values given are % transmittance through the canopy to the pond surface. AFDM, ash-free dry mass; AI, autotrophic index; DOC, dissolved organic carbon; PAR, photosynthetically active radiation; TOC, total organic carbon.

† From Nelson-Kaula et al., *in press*.

‡ Litterfall in $\text{g m}^{-2} \text{d}^{-1}$.

§ Epilithon.

¶ Accumulation in $\text{g m}^{-2} \text{d}^{-1}$.

Accumulation in $\text{mg m}^{-2} \text{d}^{-1}$.

|| Concentration in pond water in $\mu\text{mol/L}$.

isotope analysis was collected by removing rocks from the pond benthos, rinsing them gently with pond water, and scrubbing them with a denture brush. Brush and rocks were rinsed into a steel tray with microfiltered pond water to form an algal slurry. Litter samples and slurry samples were stored in whirlpacks, kept on ice during transport to the laboratory, refrigerated at 4°C, and processed within 48 h.

Halocaridina rubra were collected for isotope analysis at the time of the final food source collection in June 2012. Twenty to 30 individuals were collected from each pond during daylight hours by lifting rocks and immediately sweeping the benthos beneath the rock with a hand net. Shrimp were kept cool during transport to the laboratory and held in filtered pond water without food for 24 h to expel their gut contents. All food source and shrimp samples were dried at 60°C to a constant weight, and pulverized using a Wig-L-Bug sample grinder. Dried shrimp were not acidified prior to isotope analysis; carbon derived from non-organic sources appears to form only a small proportion of total tissue carbon in shrimp (Bosley and Wainright 1999) and

acidification may alter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of other body tissues (Jacob et al. 2005).

Stable isotope analysis

Dried and ground samples were analyzed for %C, %N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ at the EPSCoR analytical laboratory at the University of Hawai'i at Hilo using a Costech 4010 elemental combustion system interfaced to a Thermo DeltaV advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Duplicate samples of 2.5 mg of powder were loaded into tin capsules for the analysis of C and N isotopes and percent C and N composition. All $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were normalized to PeeDee Belemnite and air standards, respectively, using USGS samples 40 and 41. The standard analytical error between duplicate analyses was calculated using NIST standard 1547 and was lower than 0.3‰ for nitrogen and 0.1‰ for carbon. Samples were also analyzed for hydrogen stable isotopes (δD values) and %H at the Colorado Plateau Stable Isotope Laboratory (CPSIL) at Northern Arizona University using a Thermo-Chemical Elemental Analyser interfaced to a

Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Organic δD values were calculated according to Doucett et al. (2007) and Finlay et al. (2010). All δD values are expressed in per mil (‰) notation relative to Vienna Standard Mean Ocean Water (VSMOW). Analytical error on several internal organic standards was always lower than 3.0‰ (1 SD). We estimated potential effects of differences in lipid content on $\delta^{13}C$ values using C:N values according to Post et al. (2007). To assess the effect of sample lipid content on H isotope values in this system, a subset of leaf, epilithon, and shrimp samples were lipid-extracted and reanalyzed for δD at CPSIL. Standards used were KHS—Kudu Horn Keratin (54‰ 2‰), Keratin—SC Lot SJ (122‰ 2‰), and CBS—Caribou hoof keratin (197‰ 2‰). We reanalyzed 10 samples each of leaf litter (five endemic canopy and five invasive canopy), epilithon, and shrimp from 10 ponds in order to compare results with and without lipid extraction. For this reanalysis, lipids were extracted from sample tissue according to Soto et al. (2013). See Appendix S1: Fig. S2 for relationships between lipid-extracted and non-lipid-extracted data.

Because an aim of this study was to determine which primary producers were fueling the base of each food web, $\delta^{13}C$ and $\delta^{15}N$ signatures of potential food sources were corrected for isotopic trophic shifts using Eq. 1,

$$\delta^{13}C_{\text{food}} \text{ or } \delta^{15}N_{\text{food}} = \delta^{13}C_i \text{ or } \delta^{15}N_i - (\alpha \text{ TL}) \quad (1)$$

where $\delta^{13}C_{\text{food}}$ or $\delta^{15}N_{\text{food}}$ is the corrected food source value, $\delta^{13}C_i$ or $\delta^{15}N_i$ is the initial stable C or N isotopic signature of the food source, α is the mean trophic fractionation factor (TF), and TL is the trophic level of the consumer being analyzed (in this case 2). TF values for *H. rubra* were calculated from mean differences between epilithon and *H. rubra* tissue in ponds without canopy cover where we had replicated epilithon samples, that is, ponds 9–14. In these ponds, *H. rubra* grazes epilithon assemblages nearly completely—epilithon mats remaining in these ponds were difficult to see with the naked eye—and there are no other apparent food sources. Differences between epilithon and *H. rubra*

tissues in these ponds were 0.2–6.6 (SD) for δD , 3.5–1.07 SD for $\delta^{13}C$, and 4.2‰–1.1‰ (SD) for $\delta^{15}N$.

Because there was no precedent for calculating trophic shifts in δD in mixohaline waters, we compared these field-derived values for δD with values calculated for each pond using the equation of Solomon et al. (2009).

$$\delta D_{\text{consumer}} = (\omega \times (\delta D_{\text{water}} + \epsilon_{\text{water}})) + ((1 - \omega) \times (\delta D_{\text{food}} + \epsilon_{\text{food}})) \quad (2)$$

This was rearranged to give the average δD value of food sources to *H. rubra*:

$$\delta D_{\text{food}} = ((\delta D_{\text{consumer}} - \omega) \times (\delta D_{\text{water}} + \epsilon_{\text{water}})) / (1 - \omega) - \epsilon_{\text{food}} \quad (3)$$

where ω is the contribution of environmental water to consumer tissue, δD_{water} is the δD of pond water, δD_{food} is the δD of the consumer diet, and ϵ_{water} and ϵ_{food} are trophic enrichment terms for the incorporation of water and food into bulk consumer tissue. Previous laboratory experiments that manipulated the δD of water and food for cladoceran zooplankton, insects, and fishes provided strong evidence that fractionation of δD between TLs was negligible, and so we assumed ϵ_{water} and ϵ_{food} to be zero (Solomon et al. 2009). We estimated ω to be 0.2, based on laboratory study results for invertebrate zooplankton, and the standard deviation around this value at 0.12, based on variation across several invertebrate consumer groups (Solomon et al. 2009). To estimate the average δD values of water from each pond, samples of surface ocean water (salinity >35 ppt) were taken from ~500 m from this coast during December 2013. δD values of water from each pond were sampled twice in December 2013. Samples were split for later analysis of salinity and δD values. Isotope samples were crimp-sealed in vials without headspace on site. We then regressed the salinity of ocean water and pond water samples against their δD values, and used this relationship to calculate average pond water δD values from long-term average salinities at each pond (Dudley et al. 2014). This method assumes that alterations in δD values of pond water due to evaporation from the pond surface are minor, an assumption

likely to be met given the very low residence times of water in these ponds (<2–6 h; Dudley et al. 2014). A further assumption is that of temporal consistency in the δD value of the endmembers, groundwater, and seawater. We assumed variation in seawater δD to be negligible, and assessed consistency in groundwater δD using samples taken quarterly from two lava tubes within the Pu'u Wa'awa'a watershed from December 2010 to September 2012.

SIAR calculations

We examined shifts in food sources in ponds with differing canopy cover by estimating the range in percent contribution of epilithon and leaf litter in *H. rubra* diet. These contributions of organic C, N, and H were calculated using the SIAR version 4.2 package of R statistical software (Parnell and Jackson 2011). Because a single pooled *H. rubra* sample was used in the mixing model calculation for each pond, we used the “siarsolomcmcv4” function in SIAR, which does not require a consumer error term. Percent contributions of food sources to consumer diet are reported as the 95% Bayesian credibility intervals. Stable isotopic values of leaf litter and epilithon were averaged over the three collections and used in dietary analysis of consumers collected from each pond. We compared mixing model results calculated using TF values derived from open ponds, with results calculated using TF values derived from the literature. Literature TF values and dietary water effects for δD were calculated as above, and for C and N, we used values for invertebrate consumers (0.2‰–0.21‰ for C and 2.2‰–0.30‰ for N) from McCutchan et al. (2003).

Fauna densities

Densities of *H. rubra* were estimated by visual observation counts using three to six 0.25-m² quadrats placed across the longitudinal axis of each pool. We measured *H. rubra* densities three times at night and three times in the day in each sampled pool, within 2 h of high tide. We also measured densities of *M. lohena*, to examine potential interactions between this species, *H. rubra*, and the two invasive species. Day sampling was conducted between 11:00 hours and 16:00 hours on 25–26 June 2012, 22 April 2014, and 9 June 2014. We conducted night sampling between 20:00 hours and

midnight on 25–26 June 2012, 5 February 2013, and 18 June 2014.

Condition

Twenty individuals of *H. rubra* were collected from each pool using hand nets at the same time as collections for isotope analysis. Samples were placed in 80% ethanol until analysis. Lengths of individual shrimp were measured for postorbital length (POL), the distance between the posterior margin of the postorbital cavity and the distal edge of cephalothorax, to the nearest 0.1 mm (Kuris et al. 1987). Individuals were dried at 60°C to a constant weight (dry weight), then combusted at 450°C, and reweighed to calculate AFDM. Condition was calculated according to LeCren (1951) using the formula:

$$BCI = AFDM/POL^3 \quad (4)$$

where BCI is body condition index, AFDM is ash-free dry mass in mg, and POL is postorbital length in mm.

Data analysis

We first tested differences in canopy cover species on PAR reduction (difference between PAR above the canopy and PAR at the pond surface) and litter quality (C:N values) using Welch's *t*-tests. We tested the influences of pond canopy cover and fish presence on aspects of potential food supply—epilithon growth rates (benthic AFDM), epilithon AI, epilithon Chl *a*, epilithon C:N, epilithon isotope values (δD , $\delta^{13}C$ and $\delta^{15}N$), and DOC concentrations in pond water with one-way analysis of variance (ANOVA). Because in this tropical system we did not anticipate large seasonal differences in these factors, we averaged data collected on repeated occasions at each pond prior to analysis. We assessed the effects of the categorical independent variable “pond type,” using Tukey–Kramer post-hoc tests to examine differences between individual pond types “open,” “invasive canopy,” “endemic canopy,” or “open with poeciliids.” Because we saw large differences in light reduction between ponds across both canopy species, and little difference in litter quality or quantity between species, we used the continuous predictor “PAR reduction” as a measure of canopy density effects in a separate analysis. This was performed on the same set of independent variables while excluding ponds with fish

from the analysis. The assumptions of ANOVA were checked using the `gvlma` function of R statistical software (Pena and Slate 2006), and variables were transformed prior to analysis where necessary to meet assumptions.

We tested effects of pond type and canopy density (again approximated by PAR reduction) on daytime and nighttime counts of *H. rubra* with separate generalized linear mixed models (GLMM) using a Poisson distribution with a log-link function to best represent count data. Pond type and PAR were the fixed effects for these models, and pond was a random factor. We modeled observations as random effects when data were overdispersed relative to a Poisson distribution: residual deviance > residual df (Harrison 2014). Effects on *H. rubra* grazing behavior were first assessed by examining the extent of diel migration between epigeal (surface-exposed) to hypogeal (subterranean) habitat, calculated as the difference between *H. rubra* densities (per square meter) in daylight hours and those on the next night sampling, divided by the greater density (Dalton et al. 2012). Proportions were then assigned a positive (net daytime migration from epigeal to hypogeal habitat) or negative (net daytime migration from hypogeal to epigeal habitat), and inverse-log-transformed prior to analysis. We tested for effects of pond type and canopy density (PAR reduction) on diel migration using GLMM with a binomial distribution and log-link function, with values for each pond weighted by average density in day and night sampling (Crawley 2005). Pond type and PAR were the fixed effects for these models, and pond was a random factor. To use this method to examine pond type effects on *M. lohena* migration, we removed the pond type “open with fish” from the analysis; *M. lohena* was not present in all but one pond in which poeciliids were present, and only on one occasion, causing missing values for this pond type. For all GLMM analyses, we calculated *P*-values for differences between pond categories using Tukey’s range test. *Z* statistics and *P*-values for the continuous variable “PAR” are from Wald tests. Effects of pond type and canopy density on body condition and length of individual *H. rubra* were analyzed with linear mixed models with a nested design; the fixed effects were pond type and PAR, and pond was the random effect. We used the Satterthwaite

approximation for degrees of freedom for each of these models and used *F*-tests and Tukey’s range test to examine effects of pond type and canopy cover density. Statistical tests and transformations were conducted with the base, `gvlma`, `boot` (Canty and Ripley 2016), `lme4` (Bates et al. 2011), `lmerTest` (Kuznetsova et al. 2013), and `multcomp` (Hothorn et al. 2008) packages of the statistical software “R” (R Development Core Team 2011). We interpreted *P*-values < 0.05 as significant and 0.05–0.10 as marginally significant.

RESULTS

Reduction in PAR to the pond surface varied by a factor of 40 across ponds with canopy cover (Table 1). Surprisingly, differences in light reaching the pond surface did not appear to cause a reduction in the mass of epilithon that grew on plates placed in the ponds. There was a marginally significant effect of pond type on epilithon AFDM over all four treatments, and post-hoc Tukey’s analysis suggested that this was largely due to greater epilithon biomass in ponds with endemic canopy than open ponds and those with invasive cover (Table 2). Analysis of PAR as a driver of epilithon AFDM showed marginally higher epilithon growth where reduction in PAR was greater and thus canopy cover was denser (Table 2B). Chl *a* concentrations on plates were variable within and between pond categories and did not show significant differences between pond type, or a relationship to PAR. Relative autotrophy, however, was significantly lower (higher AI) in ponds with endemic canopy than in those with invasive or no canopy (with and without poeciliids). Analysis of PAR as a predictor of AI showed a marginally significant trend toward decreased autotrophy in well-shaded ponds. There was little indication that either canopy cover or the presence of invasive fish altered the quality of epilithon available for consumption by *H. rubra*; epilithon C:N was relatively consistent across pond categories and did not show a significant dependence on PAR. Similarly, leaf litter quality present at the benthic surface did not differ significantly between ponds with invasive or endemic canopy ($t = 1.5295$, $df = 5.57$, $P = 0.18$), but both leaf litter types had a substantially lower quality (higher C:N) than epilithon (Table 1). Both dissolved and TOC showed differences between pond types,

Table 2. ANOVA and Tukey's HSD results for linear models examining invasion effects on potential food sources for *Halocaridina rubra*.

Dependent variables	ANOVA <i>F</i> -tests			Tukey's pairwise comparisons. Pond types with differing letters are separated at an α of 0.05			
	df	<i>F</i>	<i>P</i>	Endemic canopy	Invasive canopy	Open	Open with poeciliids
(A) Effects of pond type							
Epilithon AFDM	3,12	7.53	0.066	a	b	ab	ab
Log(epilithon chlorophyll <i>a</i>)	3,12	0.90	0.247	a	a	a	a
SQRT (epilithon AI)	3,12	3.88	0.017*	a	b	b	ab
Epilithon C:N	3,12	0.90	0.468	a	a	a	a
δ D epilithon	3,12	11.7	0.009**	a	ab	bc	c
δ^{13} C epilithon	3,12	3.46	0.051	a	ab	ab	b
Log(δ^{15} N epilithon)	3,12	0.65	0.600	a	a	a	a
Water DOC	3,12	4.20	0.030*	a	ab	b	b
Log(water TOC)	3,12	4.50	0.024*	a	ab	b	ab
(B) Effects of canopy							
PAR transmittance							
Epilithon AFDM	1,10	0.09	0.067				
Log(epilithon chlorophyll <i>a</i>)	1,10	0.213	0.381				
Epilithon AI	1,10	4.137	0.087				
Epilithon C:N	1,10	1.61	0.233				
δ D epilithon	1,10	7.15	0.023*				
δ^{13} C epilithon	1,10	0.26	0.623				
δ^{15} N epilithon	1,10	9.10	0.013*				
Log(water DOC)	1,10	5.00	0.049*				
Log(water TOC)	1,10	15.3	0.002**				

Notes: Panel A shows results with "pond type" as a categorical predictor. Panel B shows results where ponds with fish were omitted from the dataset and PAR reduction to the benthos by riparian canopy was used as a continuous predictor. AFDM, ash-free dry mass; AI, autotrophic index; DOC, dissolved organic carbon; PAR, photosynthetically active radiation; TOC, total organic carbon.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

being markedly higher in ponds with endemic canopy. Analysis with PAR reduction as a predictor of DOC and TOC suggested that thicker canopy resulted in higher concentrations of organic carbon in pond water.

Stable isotope analysis

Use of salinity as a proxy for long-term mean pond water δ D values was supported by low temporal variability in groundwater δ D values. Groundwater δ D values at the central coast well ranged from 48.6‰ to 45.8‰ between December 2010 and September 2012 (mean \pm 1 SD = 46.9‰ \pm 0.93‰, $n = 8$). Pond water δ D values ranged from 53.3‰ to 12.6‰ (Table 3), tending to be more enriched where ponds had higher salinity, and at southern coastal sites. Comparison of literature-derived TF values with those measured in open ponds showed that compensating for environmental water in lipid-corrected samples considerably overestimated trophic enrichment in 2 H; the increase in δ D calculated from the

literature was 13.6‰ \pm 4.6‰ (SD), while the increase measured in open ponds for δ D was 0.2 \pm 6.6 (SD). TF values for C and N from McCutchan et al. (2003) (0.2‰ \pm 0.21‰ for C and 2.2‰ \pm 0.30‰ for N) were substantially lower than those measured in open ponds (3.5‰ \pm 1.07‰ SD for C and 4.2‰ \pm 1.1‰ SD for N). We report mixing model results calculated using mean TF values derived from open ponds; however, we note that mixing model results using literature-derived TF values indicated shrimp diet dominated by epilithon in all but one pond.

Epilithon isotope values were not consistent across ponds; both epilithon δ D and δ^{15} N showed a tendency to be enriched in ponds with denser canopy cover. Furthermore, epilithon δ D values showed surprisingly little dependence on δ D of pond water (Table 3). Epilithon δ^{13} C values were particularly enriched in ponds containing populations of invasive fish, but showed no relationship to canopy density. When compared to leaf litter from the same ponds, epilithon had

Table 3. Stable isotope values for pond water, potential food sources for *Halocaridina rubra*, *H. rubra* tissue, and SIAR Bayesian mixing model estimates for epilithon and litter contribution to *H. rubra* diet across all ponds.

Pond	δD Pond water†	δD litter $n = 3$		$\delta^{13}C$ litter $n = 3$		$\delta^{15}N$ litter $n = 3$		δD epi.‡ $n = 3$		$\delta^{13}C$ epi. $n = 3$		$\delta^{15}N$ epi. $n = 3$		δD <i>H. rubra</i> $n = 1$ (20)	$\delta^{13}C$ <i>H. rubra</i> $n = 1$ (20)	$\delta^{15}N$ <i>H. rubra</i> $n = 1$ (20)	Epi. in diet (%)	Litter in diet (%)
1	46.36	111.0	2.3	27.2	0.2	5.5	0.6	110.7	3.0	24.0	0.7	4.0	1.1	94.0	23.5	9.1	2–25	75–98
2	43.18	101.6	1.2	27.4	0.5	3.7	0.5	111.7	3.1	23.6	1.3	3.4	0.3	96.2	24.6	7.4	1–16	84–99
3	20.04	77.7	2.8	25.8	1.9	4.2	2.2	120.8	7.3	19.4	1.3	0.4	2.2	115.9	16.7	5.8	85–97	3–15
4	14.32	95.1	8.6	28.2	0.7	8.0	1.1	107.2	5.7	24.7	0.6	8.7	0.4	80.0	24.0	12.6	19–42	58–81
5	48.97	102.9	1.5	26.7	0.2	0.5	0.3	121.7	6.6	21.7	3.3	3.1	0.6	85.5	21.0	7.1	63–92	8–37
6	50.21	97.4	4.3	28.3	0.7	0.1	0.3	124.7	0.5	20.2	4.3	2.8	0.3	90.4	21.6	5.9	22–36	64–78
7	51.72	102.0	2.1	27.7	0.1	1.2	0.3	126.2	7.6	24.9	0.5	1.8	0.4	97.2	23.9	6.9	2–23	77–98
8	14.30	81.0	2.4	26.7	0.6	1.3	1.0	129.3	8.6	16.5	1.2	4.4	2.7	120.9	20.4	8.5	55–68	32–45
9	50.03	–	–	–	–	–	–	125.1	0.7	22.5	0.9	0.6	2.7	133.7	16.8	4.1	–	–
10	50.61	–	–	–	–	–	–	127.8	5.8	19.6	1.7	1.7	1.9	126.2	16.6	4.6	–	–
11	53.30	–	–	–	–	–	–	138.6	6.6	22.1	2.9	0.3	2.2	131.0	20.3	3.6	–	–
12	52.77	–	–	–	–	–	–	127.7	4.0	24.3	0.9	2.0	3.5	135.3	20.0	4.0	–	–
13	46.31	–	–	–	–	–	–	151.5	5.1	12.5	2.3	1.6	0.8	146.7	10.4	5.8	–	–
14	49.53	–	–	–	–	–	–	139.3	6.6	15.9	3.5	1.2	2.0	138.1	11.8	5.1	–	–
15	12.63	–	–	–	–	–	–	141.1	7.15	14.4	1.9	23.1	2.7	–	–	–	–	–
16	17.14	–	–	–	–	–	–	132.9 ($n = 1$)	–	22.5 ($n = 1$)	–	0.4 ($n = 1$)	–	121.2	13.5	5.4	–	–

Note: Percent contribution ranges given are 95% highest density regions.

† Estimated long-term value based on long-term salinity data and measured values. See Appendix S1: Table S3 for relationship between measured and estimated data.

‡ Epilithon.

generally more enriched carbon isotope values and more depleted hydrogen isotope values. Nitrogen isotope values of epilithon were generally more enriched than in litter, although this was most consistent in ponds with invasive canopy cover where litter showed $\delta^{15}N$ values near zero, characteristic of N-fixing vegetation.

Isotope values of the primary consumer, *H. rubra*, were well separated between ponds with and without canopy cover, most notably due to differences in δD values. *Halocaridina rubra* showed considerable spread in $\delta^{13}C$, largely reflecting epilithon $\delta^{13}C$ differences (Fig. 2). There were unlikely to have been differences in $\delta^{13}C$ resulting from lipid content differences in *H. rubra* populations; mean C:N of *H. rubra* tissue samples was very similar, ranging from 5.15 to 5.21 across pond categories. A distinct pattern in these results was that isotope values of epilithon in ponds with canopy cover tended to shift toward those of leaf litter, relative to values of epilithon in ponds without canopy cover; that is, epilithon from open ponds was isotopically very distinct from leaf litter, while leaf litter and epilithon from ponds with canopy cover were more similar (Fig. 2B, D, F). This reduced the confidence with which we could determine contributions of epilithon and litter to consumer diet in

ponds with tree canopy. However, SIAR analysis showed increasing contributions of litter to the diet of *H. rubra* in ponds with greater litterfall rates (Tables 2 and 3, ANOVA; $F_{1,6} = 5.4066$, $P = 0.05903$). These contributions appeared consistent between canopy types.

Population size and migration

Neither canopy cover nor the presence of poeciliids had a discernible effect on nighttime counts of *H. rubra*; however, resident populations of poeciliids caused cryptic behavior by *H. rubra* during daylight hours (Table 4). In ponds without poeciliids, ponds with higher-density canopy cover tended to have higher daytime populations of *H. rubra*, and this was not dependent on canopy species. Populations of the alpheid shrimp *M. lohena* were greatly altered both by fish presence and by canopy cover. Fish almost eliminated *M. lohena*; only one was observed over six sampling trips to four ponds with poeciliids, and *M. lohena* were much more likely to be cryptic during daylight hours in ponds with low or no canopy cover, regardless of canopy tree species.

Individual *Halocaridina rubra* size and condition

Open ponds (without poeciliids) had *H. rubra* with shorter POL, and ponds with denser

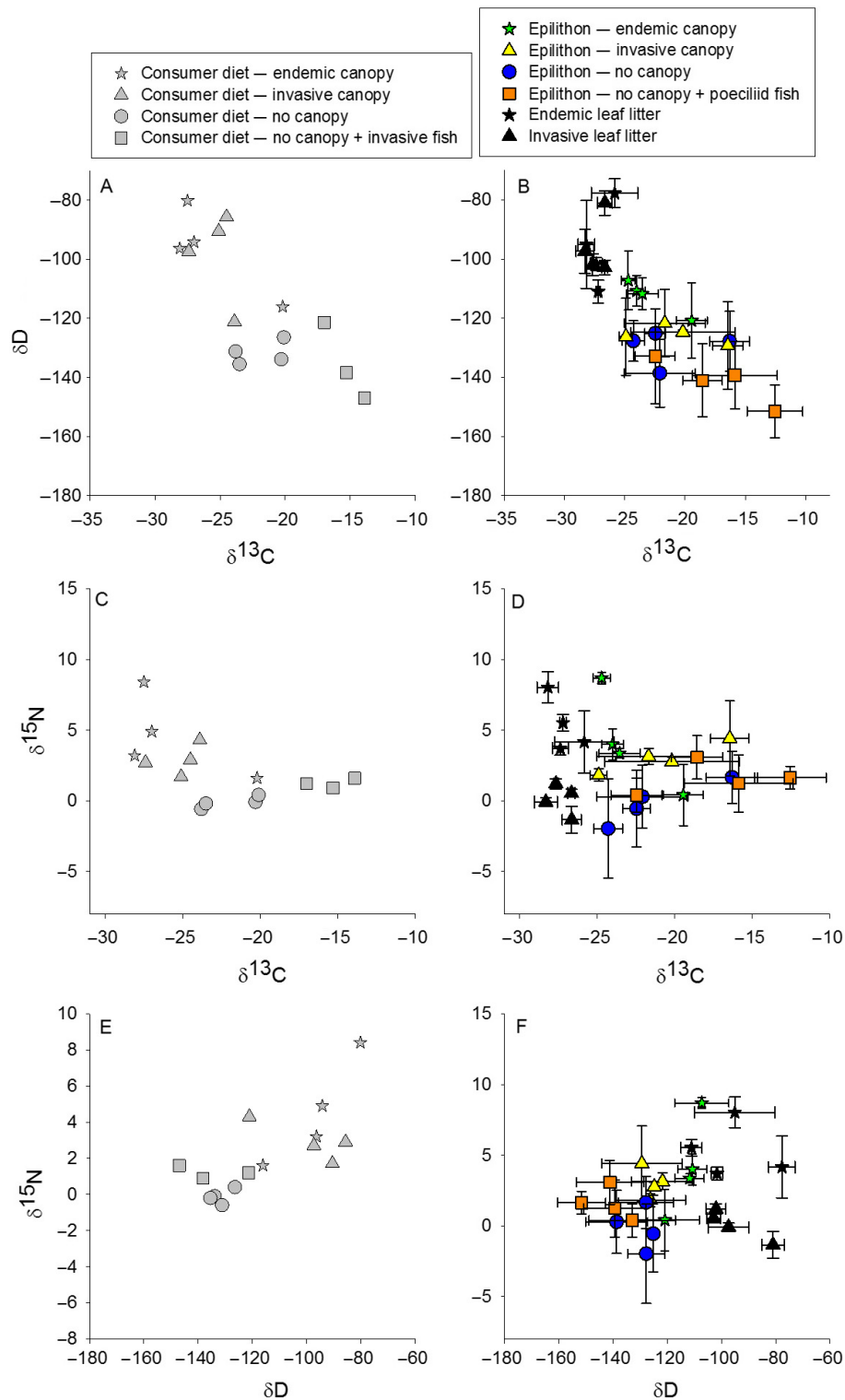


Fig. 2. Isotope values of food sources for consumers (*Halocaridina rubra*) calculated from composite samples of 20 individuals (plots A, C, and E), and average values (± 1 SD) for potential food sources; litter (black symbols in plots B, D, and F) and epilithon (colored symbols in plots B, D, and F).

Table 4. Results from generalized linear mixed models analyses examining invasion effects on invertebrate consumer populations.

(A) Effects of pond type		Tukey's pairwise comparisons. Pond types with differing letters are separated at an α of 0.05			
Dependent variables	Total obs.	Endemic canopy	Invasive canopy	Open	Open with poeciliids
<i>Halocaridina rubra</i> day counts	151	a	a	a	b
<i>H. rubra</i> night counts	176	a	a	a	a
<i>Metabetaeus lohena</i> day counts	151	a	a	b	b
<i>M. lohena</i> night counts	176	a	a	a	b
<i>H. rubra</i> migration	48	a	a	ab	b
<i>M. lohena</i> migration	36	a	ab	b	–

(B) Effects of canopy density		Wald tests		
Dependent variables	Total obs.	z	P	
<i>H. rubra</i> day counts	113	2.107	0.035*	
<i>H. rubra</i> night counts	128	0.093	0.926	
<i>M. lohena</i> day counts	113	4.60	<0.001***	
<i>M. lohena</i> night counts	128	0.820	0.412	
<i>H. rubra</i> migration	36	1.323	0.186	
<i>M. lohena</i> migration	36	2.69	0.007**	

Notes: Panel A shows results of Tukey's HSD tests with "pond type" as a categorical predictor. Panel B shows results of Wald z-tests where ponds with poeciliid populations were omitted from the dataset and photosynthetically active radiation reduction to the benthos by riparian canopy was used as a continuous predictor.

$P < 0.05$, $P < 0.01$, $P < 0.001$.

canopy cover tended to support populations of *H. rubra* with longer POL (Fig. 3, Table 5). Shorter *H. rubra* in open ponds did not appear to indicate poor body condition; there was no effect of canopy density on BCI, and among the smaller size class (1–2 mm), there were no significant effects of pond class. Among larger shrimp (>2 mm), pond category had a marginally significant effect on *H. rubra* BCI, driven by differences between ponds with poeciliid fish and those with endemic canopy (Tukey's honestly significant difference [HSD], $P = 0.048$) and invasive canopy (Tukey's HSD, $P = 0.054$).

DISCUSSION

We have shown effects from two concomitant invasions, an N-fixing tree and opportunistic poeciliids, on a tropical brackish pond food web. In contrast to our first hypothesis, tree canopy cover and litterfall, regardless of canopy species and light availability, were not primary drivers of epilithon growth. However, epilithon composition and standing biomass were significantly more heterotrophic and greater, respectively, under canopy cover. Higher levels of heterotrophy were likely supported by the large amounts of DOC measured in these ponds that is released

during leaf litter breakdown (Wiegner et al. 2005). This was especially true for ponds with endemic canopy cover. Riparian litter inputs featured increasingly in *H. rubra* diet as canopy cover increased, but this did not appear to affect body condition, regardless of canopy species. In contrast, and as we hypothesized, poeciliids significantly altered the behavior of *H. rubra* and therefore affected grazing rates and body condition of larger individuals. These invasive fishes, however, did not affect epilithon food quality as we would have expected.

Potential food sources

We predicted that increasing canopy cover would reduce autotrophic epilithon growth rates and thus availability of this high-quality food source for invertebrate primary consumers. Surprisingly, while relative autotrophy was reduced in ponds with endemic canopy cover, epilithon growth rates were highest in these ponds and generally higher in ponds with heavier canopy. It should also be noted that the "AI" is likely to overestimate the contribution of autotrophs to epilithon in shaded ponds, given that algae in lower light tend to increase chlorophyll stores relative to biomass (Dudley et al. 2010). This suggests increased contributions of the heterotrophic

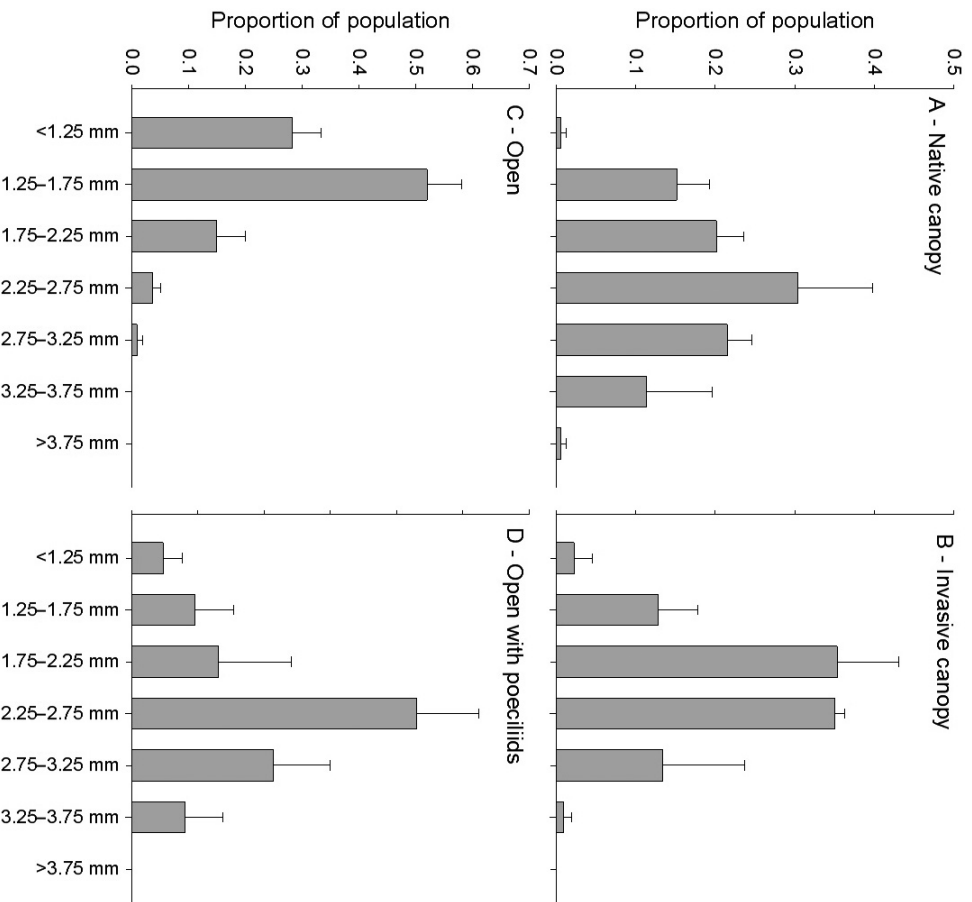


Fig. 3. Distribution of average (± 1 SE) of postorbital carapace lengths of *Halocaridina rubra* by pond category. Small size classes were disproportionally represented in “open” ponds without canopy cover or invasive poeciliids.

Table 5. Results from LMM analyses examining invasion effects on *Halocaridina rubra* body length and condition.

Dependent variables	F-tests			Tukey's pairwise comparisons. Pond types with differing letters are separated at an α of 0.05			
	df	F	P	Endemic canopy	Invasive canopy	Open	Open with poeciliids
(A) Effects of pond type							
<i>H. rubra</i> length	3, 9.8	8.85	0.003	ac	b	c	ab
<i>H. rubra</i> BCI (1–2 mm)	3, 13.5	1.35	0.298	a	a	a	a
<i>H. rubra</i> BCI (>2 mm)	3, 12.5	3.11	0.065	a	ab	ab	b
(B) Effects of canopy density							
<i>H. rubra</i> length	1, 9.8	15.26	0.003				
<i>H. rubra</i> BCI (1–2 mm)	1, 8.5	0.904	0.368				
<i>H. rubra</i> BCI (>2 mm)	1, 21.3	0.762	0.392				

Notes: Panel A shows results of F-test and Tukey's HSD tests with “pond type” as a categorical predictor. Panel B shows results of F-tests where ponds with poeciliid populations were omitted from the dataset and photosynthetically active radiation reduction to the benthos by riparian canopy was used as a continuous predictor. BCI, body condition index. $P < 0.05$, $P < 0.01$, $P < 0.001$.

component of epilithon in ponds with endemic canopy. The quality of epilithon as a food source, at least in terms of C:N, was similar in ponds with canopy cover and those without. There was also little difference in litter quality between tree species; while the C:N values of *P. pallida* litter were expected to be higher than those of *T. populnea* based on the N-fixing tendencies of the former species, C:N of decomposing litter collected from the benthic surface was similarly high for both canopy types and much higher than that of epilithon. This result was surprising, but supported by analysis of litterfall from around the same ponds over the duration of the study, which showed little difference between nitrogen contents of the two species (Nelson-Kaula et al. 2016).

Much of the variation in δD , $\delta^{13}C$, and $\delta^{15}N$ values of *H. rubra* living in ponds with canopy cover could be accounted for by a shift in diet from epilithon to litter as canopy cover increased. This occurred regardless of whether this canopy was endemic or invasive. However, we also observed enriched δD and $\delta^{15}N$ values of epilithon itself in ponds with heavier canopy cover. This could be caused by (1) contamination from leaf litter detritus in epilithon mats, (2) differences in isotopic discrimination during nutrient uptake by epilithon, (3) differences in nutrient sources to epilithon, or a combination of these three factors. We address these possible explanations below:

1. C:N values of leaf litter were considerably greater than those of epilithon, but there was little increase in epilithon C:N values in ponds with canopy cover that would indicate contamination from leaf litter detritus in epilithon mats.
2. Differences in isotopic discrimination could be possible if changes in the composition of epilithon communities, or abiotic effects (e.g., reduced light or water temperature), caused changes to isotope discrimination. Generally, discrimination against the heavier isotope is reduced when uptake rates of inorganic C and N are low relative to photosynthetic demand (Farquhar et al. 1989, Needoba and Harrison 2004). While differences in light environments between ponds may have influenced photosynthetic demand, this does

not explain the heavier δD values with increasing canopy cover; discrimination during assimilation of H by aquatic primary producers is not greatly altered by light or temperature (Macko et al. 1983, Finlay et al. 2010).

3. A possible mechanism for a shift in nutrient sources to epilithon is the observed increase in heterotrophs in epilithon in ponds with canopy cover. This would also result in a change in the nutrient source of epilithon from inorganic H, C, and N utilized by autotrophs to organic material from allochthonous sources, utilized by heterotrophs. This assumes that $\delta^{15}N$ and δD signatures of dissolved organic material are enriched relative to inorganic sources—but this is likely given the enriched isotope values of leaf litter itself relative to epilithon in open ponds. Water column DOC and TOC increased with increasing canopy cover, and uptake of DOC by heterotrophic epilithon has been shown to contribute considerably to freshwater microbial food webs (Datry et al. 2005, Wiegner et al. 2005, Augspurger et al. 2008).

Hence, while abiotic effects associated with canopy cover could explain some of the variation in epilithon $\delta^{13}C$ seen, highly enriched $\delta^{15}N$ and δD values imply altered nutrient sources to or TL of epilithon communities beneath canopy cover. This would present an additional pathway by which the base of anchialine food webs could be fueled by allochthonous primary production.

Comparison of literature-derived and field-derived trophic enrichment factors

For *H. rubra*, our calculated values for dietary water enrichment of 2H were considerably higher than those values (near zero) that we measured in the field. Furthermore, our measured trophic enrichment values for C and N isotopes differed substantially from mean values derived from published literature on similar species. Use of literature-derived values for all three isotopes would have caused us to draw quite different conclusions regarding the effects of canopy on diet. We note that, for example, our measured trophic shift for C was within the range of observations, but well separated from the mean

observed by Post (2002). Hence in similar studies on only one or few consumers, we would recommend experimental designs that enabled measurement rather than calculation or assumption of trophic enrichment.

Impacts of invasion on Halocaridina rubra population density, size, and condition

While canopy cover altered sources of energy to *H. rubra*, it did not appear to negatively impact population size or body condition of this species. The size of individuals in ponds with canopy cover was generally greater than in open ponds. In ponds with poeciliid populations, substantial shrimp migration during daylight hours from the epigeal (surface-exposed) to hypogeal (subterranean) habitats—and in one pond almost complete loss of *H. rubra* populations—suggests reduced grazing rates where fish were present, relative to ponds where they were absent. Given the similarity of epilithon quality across all pond types, reduced grazing rates are a likely cause of reduced *H. rubra* body condition in ponds with poeciliids; this supports the contention by Dalton et al. (2012) that poeciliids can negatively influence *H. rubra* populations through trait-mediated effects (i.e., inducing cryptic behavior during daylight hours). One explanation for the larger size of individuals in ponds with poeciliids is predation on smaller shrimp; experiments in tanks have shown that poeciliids will preferentially consume smaller individuals of *H. rubra* (Capps et al. 2009). However, this mechanism does not explain relatively larger shrimp in ponds with canopy cover. There are considerable differences in ages of the lava flows on this coast, and high levels of genetic differentiation between populations of *H. rubra* in this study; it is also possible that these population size structures are driven by genetic rather than environmental differences between pond types (Wolfe and Morris 1996, Santos 2006).

Ecological overview of invasion impacts

Through much of leeward Hawai'i, *P. pallida* is the first tree species to colonize young lava flows (Gallaher and Merlin 2010), forming monospecific stands around many anchialine ponds. It is reassuring therefore that increasing density of canopy cover had no discernible negative effects on *H. rubra* diet, population size, or condition.

We attribute this result to a shift for this species to litter-derived energy and nutrients, through direct consumption of litter, and via epilithon communities.

Poeciliid fish did not appear to reduce the quality of epilithon available for consumption, and when fish and grazers were excluded, epilithon growth rates were similar between ponds with and without fish. Hence, it is likely that the observed reductions in abundance of *H. rubra* and body condition of larger individuals are a result of trait-mediated effects rather than competition for preferred resources. This conclusion is supported by the almost complete absence of *M. lohena* in ponds with poeciliid populations; that is, top-down effects on *H. rubra* could only be attributed to poeciliids. Individual *H. rubra* populations have been reported to live for several years in captivity under minimal husbandry (Maciolek 1983)—conditions likely less suitable than their natural habitat—suggesting that they have at least equivalent life spans in anchialine pools. Poeciliid introductions to Hawaiian anchialine ponds have been occurring from >40 yr ago (Maciolek and Brock 1974) and are an ongoing invasion (Capps et al. 2009, Carey et al. 2010, Havird et al. 2013, Marrack 2016). For example, the tsunami generated by the East Japan earthquake of 2011 spread poeciliids into several ponds already selected for the study based on an absence of poeciliids (B. Dudley, *personal observation*). It is therefore likely that current pressures on *H. rubra* populations and anchialine food webs will not have their full effect for some years.

Here, we have described delicate shifts in basal food resource characteristics in isolated brackish ecosystems affected by invasion. We have also highlighted the resilience and adaptive traits of an endemic shrimp to both bottom-up and top-down stressors in a system that is, in theory, highly susceptible to disturbances. Although these invasions have altered natural habitat conditions and food resources in Hawaiian anchialine ponds, these dynamic systems naturally change, physically and biologically, as plant communities develop around ponds formed by new lava flows. It may not be surprising, therefore, that invertebrate consumers in these habitats possess a resilience to altered food web linkages caused by exotic species. However, we cannot infer that the resilience of these habitat specialists to relatively recent

invasions by fishes and invasive plants will be indefinite. One likely future impact of these two invasions is multitrophic-level interactions within a given system. While invasive species may or may not have deleterious effects on native communities independently, multitrophic-level invasions may cause interactions in recipient communities (Nyström et al. 2001, Rodriguez 2006). At the time and location of this study, we found few instances of dominant invasive tree canopy and the invasive fish occurring in the same ponds. However, some of the poeciliid invaders are detritivorous (Seidel et al. 2015), raising the possibility of multitrophic-level interactions between fish, tree canopy species, and native shrimp communities as these invasions spread.

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