

Differences in ecological structure, function, and native species abundance between native and invaded Hawaiian streams

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Abstract. Poeciliids, one of the most invasive species worldwide, are found on almost every continent and have been identified as an “invasive species of concern” in the United States, New Zealand, and Australia. Despite their global prevalence, few studies have quantified their impacts on tropical stream ecosystem structure, function, and biodiversity. Utilizing Hawaiian streams as model ecosystems, we documented how ecological structure, function, and native species abundance differed between poeciliid-free and poeciliid-invaded tropical streams. Stream nutrient yields, benthic biofilm biomass, densities of macroinvertebrates and fish, and community structures of benthic algae, macroinvertebrates, and fish were compared between streams with and without established poeciliid populations on the island of Hawai'i, Hawaii, USA. Sum nitrate ($\Sigma\text{NO}_3^- = \text{NO}_3^- + \text{NO}_2^-$), total nitrogen, and total organic carbon yields were eight times, six times, and five times higher, respectively, in poeciliid streams than in poeciliid-free streams. Benthic biofilm ash-free dry mass was 1.5× higher in poeciliid streams than in poeciliid-free streams. Percentage contributions of chironomids and hydroptilid caddisflies to macroinvertebrate densities were lower in poeciliid streams compared to poeciliid-free streams, while percentage contributions of *Cheumatopsyche analis* caddisflies, *Dugesia* sp. flatworms, and oligochaetes were higher. Additionally, mean densities of native gobies were two times lower in poeciliid streams than in poeciliid-free ones, with poeciliid densities being approximately eight times higher than native fish densities. Our results, coupled with the wide distribution of invasive poeciliids across Hawaii and elsewhere in the tropics, suggest that poeciliids may negatively impact the ecosystem structure, function, and native species abundance of tropical streams they invade. This underscores the need for increased public awareness to prevent future introductions and for developing and implementing effective eradication and restoration strategies.

Key words: benthic biofilm; biodiversity; community structure; Gobiidae; Hawaii; macroinvertebrates; nutrient yields; Poeciliidae; stream function.

INTRODUCTION

Invasion of native ecosystems by exotic species is occurring throughout the world at unprecedented rates, which is largely due to the breakdown of biogeographic barriers from increased global commerce, as well as the intentional release of exotic species into novel ecosystems (Vitousek et al. 1997, Lowe et al. 2000, Mooney and Cleland 2001). The establishment of exotic species in novel ecosystems can significantly alter community structure (Levine et al. 2003, Asner et al. 2008), ecosystem function (Vitousek 1990, Chapin et al. 1997), and native biodiversity (Simberloff and Von Holle 1999, Mooney and Cleland 2001, Clavero and Garcia-Berthou 2005) at both local and global scales. Furthermore, the invasion of native ecosystems can have

economic consequences through the loss of ecosystem services, as well as through the allocation of resources for exotic species eradication, control, and prevention (Chapin et al. 2000, Pimentel et al. 2001, 2005).

Loss of biodiversity from invasion of native ecosystems is perhaps the greatest cause for concern for many conservation biologists and is most commonly attributed to direct impacts exotic species have on natives. Direct impacts include predation on native species or competition for limiting resources such as space, light, or nutrients. Invasive species can also have indirect impacts on native species, and thus biodiversity, by engineering or altering native habitat quality or structure. Exotic species that have the capability to alter their environment through indirect impacts are thought to pose the greatest threat to native ecosystem function and biodiversity (Vitousek et al. 1997, Crooks 2002). For example, increased nitrogen loading and decreased light inputs that occur when native forests are invaded by fast-growing, canopy-forming, exotic nitrogen (N)-fixing trees (Compton et al. 2003, Volk et al. 2003,

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Hughes and Denslow 2005), alter ecosystem function (e.g., leaf litter breakdown, food web structure [Wipfli and Musslewhite 2004, Hughes and Uowolo 2006]) and create conditions more suitable for other invasive species (Asner and Vitousek 2005, Asner et al. 2008). Freshwater exotic species, especially fish, are also expected to have indirect impacts on native species by altering the native environments they invade; yet few studies have examined this. For example, while direct impacts of exotic fish on food webs have been well documented (e.g., consumption of native species or their resources [Vander Zanden et al. 1999, Angeler et al. 2002, Maezono et al. 2005, Kovalenko et al. 2010]), exotic fish can also influence food web structure by altering the behavior of native species (Capps et al. 2009) or their prey (Flecker and Townsend 1994). The introduction of exotic fish can also alter their invaded habitat by exposing native species to new parasites (Font 2003), impacting water quality through increased turbidity or increased nutrient loading that results from foraging or feeding on novel food resources (Angeler et al. 2002, Rowe 2007, MacKenzie and Bruland 2012), or increasing algal growth (Flecker and Townsend 1994).

Members of the topminnow family (Poeciliidae) are excellent model organisms to further our understanding of how exotic fish may alter the native ecosystems that they invade. Information on the direct and indirect impacts of poeciliid fish is also relevant at the global scale as these fish have successfully invaded aquatic ecosystems on every continent except Antarctica (Pyke 2005), have been identified as one of the 100 most invasive species in the world (Lowe et al. 2000, Alemadi and Jenkins 2007), and have established populations in 32 countries including Australia (Rowley et al. 2005), Mexico (Valero et al. 2008), New Zealand (Rowe et al. 2007), and even in the warm water effluent from a power plant in Canada (Hass et al. 2003). In the United States, poeciliids have been described as one of the most destructive introduced species in the western United States (Ayala et al. 2007), as well as an "invasive species of concern" in the states of Hawaii, Washington, Oregon, and New Mexico (HDAR 2003, OISC 2005, WDFW 2008). While the impacts of invasive poeciliids on native species have been somewhat documented, most of these studies have focused specifically on the direct consumption of native species by *Gambusia affinis* (mosquitofish) (Baird and Gerard 1853) in temperate ecosystems (Leyse et al. 2004). Few studies have documented poeciliid impacts in tropical stream ecosystems (Holitzki 2010), let alone how poeciliids are altering their invaded habitat via indirect impacts in temperate or tropical regions.

Hawaiian streams are ideal model ecosystems to examine impacts of poeciliids on tropical streams. The remote nature of Hawaiian streams has resulted in depauperate, relatively simple, stream communities that lack many functional groups (e.g., leaf shredders) commonly found in continental streams (Resh and

deSzalay 1995, Larned 2000). Furthermore, native fish (i.e., *Sicyopterus stimpsoni* (Gill 1860)), shrimp (i.e., *Atyoida bisulcata* (Randall 1840)), and snails (i.e., *Neritina granosa* (G. B. Sowerby I 1825)) have evolved to fill specialized niches in the absence of competition with exotic generalist species (Ford and Kinzie 1982). Thus, direct impacts of poeciliids on these isolated streams are expected to be more apparent and easier to measure than elsewhere.

To date, at least seven species of poeciliids have been introduced to Hawaiian streams. These include mosquitofish (*Gambusia affinis*), guppy (*Poecilia reticulata* [Peters, 1859]), sailfin molly (*Poecilia latipinna* [Lesueur, 1821]), Mexican molly (*Poecilia* sp. hybrid complex), Cuban topminnow (*Limia vittata* [Guichenot, 1853]), green swordtail (*Xiphophorus helleri* [Heckel, 1848]), and moonfish (*Xiphophorus maculatus* [Gunther, 1866]) (Yamamoto and Tagawa 2000). *Gambusia affinis*, *P. reticulata*, and *X. helleri* were the focus of this study, as they are the most prevalent in our study systems (Holitzki 2010). *Gambusia affinis*, whose native range extends from the eastern coast of the United States to central Mexico (Hoese and Moore 1977), was first introduced to Hawaii in the early 1900s. *P. reticulata*, native to northern South America (Rodriguez 1997), and *X. helleri*, native to North and Central America (Wischnath 1993), were introduced in 1922. While the introduction of all three species is commonly attributed to mosquito control (Van Dine 1907, Englund 1999), *X. helleri* was likely introduced as unwanted aquarium stock (Myers 1940, Brock 1952). All three species have since established populations in streams throughout the Hawaiian Islands with densities often 10–30× higher than native fish (Brasher et al. 2006). Potential impacts of poeciliids on Hawaiian stream fauna include reduced fitness and reproductive output of native gobies infested with exotic parasites that arrived with poeciliids (Font and Tate 1994). Poeciliids may also feed directly on larval native fish, larval shrimp, and aquatic insects (Englund 1999; R. Nishimoto, *personal communication*), as well as native species food resources (e.g., algae, invertebrates) (Pyke 2005; R. A. MacKenzie, T. Holitzki, and K. J. McDermid, *unpublished manuscript*). Poeciliids may also impact spawning and feeding behavior of native fish species. For example, native fauna may escape predation from or competition with poeciliids by occupying another stream habitat or by shifting foraging behavior to different times of the day (Mills et al. 2004, Ayala et al. 2007, Capps et al. 2009), ultimately altering their reproductive or feeding patterns. Finally, high densities of poeciliids may impact nutrient dynamics in streams by feeding on and releasing nutrients from previously unavailable forms (e.g., algae, detritus) into more readily available dissolved forms via excretion (Vanni 2002). Increased nutrient inputs from fish excretion can not only impact stream benthic algal growth and community structure (Knoll et al. 2009,

Kohler 2010), but may also change stream food web structure and ecological functions.

The goal of our study was to determine if there were differences in the ecological structure, function, and native species abundance between tropical streams with and without exotic poeciliids. Various ecological parameters were compared between five streams with and four streams without established populations of poeciliids along the Hāmākua coast of the island of Hawai'i, Hawaii, USA. Ecological parameters included (1) stream nutrient yields, (2) benthic biofilm biomass, (3) densities of macroinvertebrates and fish, and (4) community structures of benthic algae, macroinvertebrates, and fish. It was hypothesized that, compared to poeciliid-free streams, poeciliid streams would exhibit increased stream nutrient yields, increased biomass of benthic biofilm, decreased densities of macroinvertebrates, and decreased densities of native fish.

METHODS

Study location and design

We initially surveyed over two dozen stream reaches along the Hāmākua coast of the island of Hawai'i to find streams suitable for our study (Fig. 1). We chose streams that were relatively easy to access, and that were located at similar elevations (~100–150 m above sea level) to ensure similar community composition of native fish, shrimp, and snails, as interspecific differences in climbing abilities result in species distributions across elevational gradients (Yamamoto and Tagawa 2000). Stream reaches had similar substrate (i.e., cobble), stream width, riparian canopy cover (i.e., *Syzygium jambos*, *Psidium cattleianum*, *Ficus* sp., *Hibiscus* sp.), and land use. Snorkel surveys were conducted to determine the presence or absence of poeciliids before streams were selected. All streams that met these criteria were selected for this study, which resulted in a total of five stream reaches with and four stream reaches without established populations of exotic poeciliids. Poeciliid streams drained watersheds that were $63.5\% \pm 10.2\%$ (mean \pm SE) forested, $27.1\% \pm 3.5\%$ grasslands, and $1.2\% \pm 0.7\%$ agriculture (excluding one poeciliid stream, in which agriculture was 8.8% of the watershed). Poeciliid-free streams were $65.6\% \pm 12.3\%$ forested, $29.5\% \pm 8.9\%$ grassland, and $1.8\% \pm 1.0\%$ agriculture land. Human population densities varied across watersheds, but were generally less than 10 people/km². The exceptions were one poeciliid-free stream (12.2 people/km²) and one poeciliid stream (58.7 people/km²) (HDAR 2008).

In each stream, five cross-sections were then established every 10 m along a 50 m long stream reach. Streams were sampled three times during the wet season (November–April), when this region of Hawai'i receives 2400–4000 mm of precipitation, and three times in the dry season (May–October), when this region receives 850–2300 mm of precipitation (Giambelluca et al. 1986).

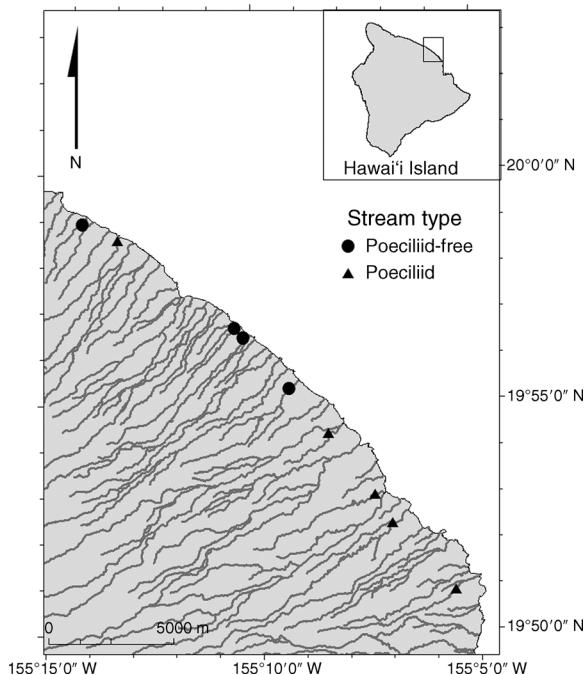


FIG. 1. Stream study sites (poeciliid-free and poeciliid) on the island of Hawai'i along the Hāmākua coast, Hawaii, USA.

Physicochemical parameters

Photosynthetically active radiation (PAR) was measured at each stream reach by placing a PAR quantum sensor (Li-Cor Biosciences, Lincoln, Nebraska, USA) above the stream surface and recording PAR via a handheld Li-Cor 250a data logger. Percentage light transmittance to the stream surface was determined by dividing stream PAR measurements by those taken simultaneously in adjacent clearings using a Li-Cor 1400 data logger. Specific conductivity, temperature, and dissolved oxygen concentrations were measured at the surface of the water column using a YSI Model 85 multi-parameter probe (YSI, Yellow Springs, Ohio, USA); pH was measured using a Symphony SP70P pH meter (VWR, West Chester, Pennsylvania, USA). All measurements were taken at cross-sections 0, 20, and 40 m in each stream reach. Stream water velocities were measured monthly at the same cross-section of each stream using a Rickly Pygmy flow meter (Rickly Hydrological Company, Columbus, Ohio, USA). Discharge was calculated as the product of stream water velocity and cross-sectional stream area (Gore 2007), which was calculated from stream widths and depths.

Nutrient dynamics

Water samples were collected each sampling trip from the 0-m cross-section of each stream using acid-washed, 1-L amber high-density polyethylene bottles. A sample of unfiltered water was immediately placed on ice, while another water sample was filtered through pre-combusted (6 h at 500°C) GF/F filters (Whatman International,

Piscataway, New Jersey, USA), and then placed on ice. Samples were stored frozen until analysis. Sum nitrate ($\Sigma\text{NO}_3^- = \text{NO}_3^- + \text{NO}_2^-$, detection limit [DL] 0.1 $\mu\text{mol/L}$; USEPA method 353.2 [O'Dell 1993a]), soluble reactive phosphorus (SRP, referred to hereon as phosphate $[\text{PO}_4^{3-}]$, DL 0.1 $\mu\text{mol/L}$; USEPA method 365.1 [O'Dell 1993b]), total dissolved phosphorus (TDP, DL 0.1 $\mu\text{mol/L}$; USGS method 1-4650-03 [Fishman 1993]), and ammonium (NH_4^+ , DL 1 $\mu\text{mol/L}$, USGS method I-2525-89 [Fishman 1993]) concentrations were measured from filtered water samples using a Technicon four-channel autoanalyzer (Pulse Instrumentation, Saskatoon, Saskatchewan, Canada). Total organic carbon (TOC, DL 10.0 $\mu\text{mol/L}$; USEPA method 415.1 [USEPA 1999]) and total nitrogen (TN, DL 5.0 $\mu\text{mol/L}$; ASTM D5176 [ASTM 2008]) were measured on unfiltered water samples on a Shimadzu TOC-V, TNM-1 (Shimadzu, Columbia, Maryland, USA).

Stream nutrient yields ($\text{mol}\cdot\text{d}^{-1}\cdot\text{km}^{-2}$) were calculated to normalize data for statistical comparisons because watershed areas and channel lengths were different sizes and had different discharges. Nutrient yields were calculated by dividing the nutrient flux by the drainage area of the watershed; drainage areas were obtained from the Hawaii Division of Aquatic Resources (HDAR 2008). Nutrient fluxes ($\mu\text{mol/s}$) were calculated as the product of stream discharge (L/s) and nutrient concentration ($\mu\text{mol/L}$).

Benthic biofilm

Chlorophyll *a* concentration (chl *a*) was used as a biomass proxy for the photosynthetic component of benthic biofilm, while ash free dry mass (AFDM) was equal to the combined biomass of the photosynthetic component, the non-photosynthetic benthic organic matter (e.g., bacteria, nematodes, protozoans, slime), and the non-living organic material within the biofilm of the streambed (Feminella and Hawkins 1995, Lowe and Pan 1996). Five rocks were haphazardly collected from the 0, 20, and 40-m cross-sections in each study stream only once during the dry season at the end of August 2009. Biofilm was scrubbed from rocks using a soft bristled toothbrush, washed into acid-washed polyethylene amber bottles, placed on ice, returned to the laboratory, and brought to a known volume. Two slurry subsamples of known volumes were filtered through GF/F filters, and analyzed for chl *a* (Arar and Collins 1997) and AFDM (Steinman and Lamberti 1996). Chl *a* was extracted from filters in 90% acetone for 18 h, read at 202 and 295 nm on a Turner Designs 10-AU Fluorometer (Turner Designs, Sunnyvale, California, USA), and converted to algal biomass as described by Arar and Collins (1997). AFDM was determined from loss of organic matter after combustion at 500°C for 4 h.

Surface areas of rocks were determined by wrapping sampled areas of rocks with aluminum foil. The foil was then weighed to the nearest 0.1 mg. Aluminum foil squares cut into known areas were also weighed and the

resulting area-weight regression was used to determine rock area. Chl *a* and AFDM were then standardized by dividing by the sampled rock area and average values were calculated for each reach.

Metabolic status of benthic biofilm was also calculated using an autotrophic index (AI). The AI for each stream was determined by dividing AFDM (mg/m^2) by chl *a* concentrations (mg/m^2). Autotrophic indices quantify the relative importance of autotrophs vs. heterotrophs in a community. Values greater than 200 indicate heterotrophy, while values lower than 200 indicate autotrophy (APHA 1995).

Benthic invertebrate community

Benthic macroinvertebrate samples were collected from the 0, 20, and 40-m cross sections from each of the nine streams using a surber sampler with an area of 0.25 m^2 (Rickly Hydrological Company, Columbus, Ohio, USA). Three surber samples were collected from each stream every month. Substrate type within the sampler was recorded, and the area within the sampler was scrubbed by hand for 1 minute. Organisms were preserved in 70% ethanol, identified to the lowest practical taxonomical unit using Merritt et al. (1984) and Flint and Englund (2003), counted, lengths measured to the nearest millimeter, and weighed to the nearest milligram. Densities were calculated by dividing the number of organisms by the area sampled; total biomass was calculated from the product of densities and invertebrate weights. In order to normalize invertebrate data, percentage contributions of each taxa to total macroinvertebrate densities were calculated by dividing the density of a specific taxa by the total density of macroinvertebrates. Comparisons between stream types were then made with taxa that represented more than 5% of the total invertebrate community.

Fish community structure

Visual surveys for fish and large invertebrates were conducted using snorkel gear within each of the five cross-sections from each study reach. Snorkel surveys were conducted instead of electrofishing surveys as the latter is illegal in Hawaii. Surveys began at the most downstream-most station and continued upstream to minimize disturbance. A random point was selected along each cross-section surveyed, fauna were identified to species and counted, and the area of the visual survey was recorded. Area was determined by selecting landmarks on the stream bed and counting all organisms within the selected area. Densities ($\text{individuals}/\text{m}^2$) were calculated by dividing counts by the stream area sampled.

Poeciliid nutrient excretion rates

Nutrient excretion rates of poeciliids were determined from three of the five poeciliid-invaded streams in the winter of 2010 ($n = 15$ samples) and from one of the five poeciliid-invaded streams in the winter of 2011 ($n = 5$

samples). Nutrient excretion rates of native gobies were also determined from two of the four poeciliid-free streams in the winter of 2011 ($n = 10$ samples). Stream water was collected within an acid-washed plastic 1-L amber bottle from which 250 mL was filtered through a pre-combusted GF/F filter, and placed into one of five acid-washed plastic bags. An individual exotic *P. reticulata* or native *Lentipes concolor* (Gill 1860) (goby) was then placed into each bag. Fish were randomly selected from the stream using hand nets to ensure individuals represented populations in streams. Three acid-washed bags without fish were filled with 250 mL of filtered stream water and used as controls. After 60 minutes, fish were removed from each bag, weighed, and 50 mL of water from the bag was filtered through a pre-combusted GF/F filter and immediately placed on ice. Water samples were stored frozen until analysis. Water was analyzed for ΣNO_3^- , PO_4^{3-} , NH_4^+ , TDP, and total dissolved nitrogen (TDN, same analytical method as TN) using methods described earlier. Nutrient concentrations in control bags were assumed to represent initial nutrient concentrations and were subtracted from the nutrient concentrations in fish bags. These corrected values were then used to calculate fish excretion rates ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) as described in Schauss et al. (1997). Contributions of fish excretion to streams ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) were determined by

$$\text{fish}_{\text{contribution}} = \text{fish}_{\text{excretion rate}} \times \text{fish density} \times \text{fish mass}. \quad (1)$$

Total nitrogen standing stock

Standing stocks of N in benthic biofilm, invertebrates, and fish were compared between two poeciliid-free and three poeciliid streams. Samples were collected, sorted, and rinsed clean in deionized water, and stored at 4°C until processed. Samples were then dried at 60°C to a constant mass, homogenized using a Wig-L-Bug grinding mill (Reflex Analytical Corporation, Ridgewood, New Jersey, USA), weighed, and packed into tin cups for percentage N analysis using a Costech Elemental Analyzer Conflo III (Costech Instrumentation, Valencia, California, USA). N standing stocks from poeciliid and poeciliid-free streams were the product of biomass and percentage N data measured from AFDM, invertebrates, and native and exotic fish.

Statistical analyses

Pearson's product moment correlations were used to examine associations between poeciliid densities and stream nutrient concentrations, fluxes, yields, percentage contribution of individual invertebrate groups (e.g., chironomids, flatworms), and total macroinvertebrate densities in both poeciliid-free and poeciliid streams.

Because Pearson's correlation cannot detect differences between seasons, data were also compared between streams and seasons using two-way analysis

of variance (ANOVA) for physicochemical parameters, stream nutrient yields, macroinvertebrate densities, percentage contribution of individual taxa to total invertebrate densities in each stream, as well as native and invasive fish densities. Fixed effects included stream (poeciliid vs. poeciliid-free) and season (wet vs. dry), as well as all two-way interactions. If data did not meet assumptions of normality, they were either $\log(x + 1)$ or square-root transformed. NH_4^+ data were rank transformed (Potvin and Roff 1993). Two-sample t tests were used to compare N standing stock, fish excretion rates, chl *a* concentrations, AFDM, and AI values between stream types (poeciliid and poeciliid-free).

Community structure of algae, macroinvertebrates, and fish (natives and invasives) were compared between stream types and seasons by creating Bray-Curtis similarity matrices using densities from microscope slides, surber samples, or visual surveys, respectively. Data were then arranged into a two-dimensional ordination space using a nonmetric multidimensional scaling (NMDS) test. NMDS analysis allows data to be visually explored for patterns of similarity and examines the factors responsible for any differences (e.g., presence or absence of poeciliids). NMDS tests were run on square-root-transformed data.

A one-way analysis of similarity (ANOSIM) was used to compare community structure of algae between stream types because algae were collected only once. A two-way ANOSIM was also used to compare community structure of macroinvertebrates and fish between stream types and between seasons. ANOSIM estimates community similarity using community composition and taxa abundance in a nonparametric permutation procedure applied to a Bray-Curtis similarity matrix (Clarke 1993). Macroinvertebrate and algal densities were square-root transformed, while fish densities were $\log(x + 1)$ -transformed prior to analysis. If significant differences were determined from ANOSIM, species responsible for those differences were identified using a similarity of percentage (SIMPER), which measures dissimilarity by making pairwise comparisons of abundances between stream type and season. Species were then ranked in order of their percentage contribution to dissimilarity.

All Pearson's product moment correlations, ANOVAs, and t tests were conducted using SYSTAT 11 (SPSS, Chicago, Illinois, USA). NMDS, ANOSIM, and SIMPER analyses were conducted using Primer 6 (Primer-E, Lutton, UK) (Clarke and Warwick 2001).

RESULTS

Physicochemical parameters

Percentage light, temperature, specific conductivity, dissolved oxygen, pH, and wetted stream widths were similar between poeciliid and poeciliid-free streams, as well as between seasons (wet and dry). Discharge was two times higher in the wet season than the dry season ($df = 1,6$, $F = 17.135$, $P < 0.05$; Table 1). To determine if

TABLE 1. Values of stream parameters in poeciliid-free (PF) and poeciliid (P) streams along the Hāmākua coast of the island of Hawai‘i, Hawaii, USA.

Parameter	Wet season		Dry season	
	PF	P	PF	P
pH	7.9 ± 0.1	7.9 ± 0.3	8.0 ± 0.0	8.0 ± 0.1
DO (mg/L)	8.8 ± 0.3	8.8 ± 0.1	8.5 ± 0.3	8.3 ± 0.1
DO (%)	98.7 ± 2.8	101.0 ± 1.0	98.4 ± 2.6	97.3 ± 1.2
Temperature (°C)	21.1 ± 0.5	21.9 ± 0.5	22.7 ± 0.3	23.3 ± 0.2
Sp. Cond. (µs/cm)	97.1 ± 10.0	103.6 ± 5.6	99.7 ± 11.4	98.5 ± 8.9
Light (%)	17.1 ± 5.7	11.2 ± 5.2	12.9 ± 5.2	12.4 ± 13.8
Discharge (m ³ /s)	1.03 ± 0.3	1.04 ± 0.4	0.41 ± 0.1	0.42 ± 0.1
Wetted widths (m)	4.7 ± 0.4	4.1 ± 0.4	4.2 ± 0.5	3.2 ± 0.2

Notes: Values are means ± SE. Abbreviations are: DO, dissolved oxygen concentration; Sp. Cond., specific conductivity, and light, percentage light transmission (a ratio of light in the stream to light outside of the stream). Boldface type highlights significant differences between seasons ($P < 0.05$).

streams were different without the two sites with higher human population densities, the two-way ANOVA’s were run without these streams. The results did not change from the original analysis with these streams removed.

Nutrient dynamics

Correlations between nutrient concentration and fish densities were variable. ΣNO_3^- , NH_4^+ , and TN concentrations were significantly and positively correlated to poeciliid fish density. Correlations between PO_4^{3-} and TOC concentrations with invasive fish densities were not significant.

ΣNO_3^- yields were eight times higher in poeciliid streams than in poeciliid-free streams ($df = 1,8, F = 17.39, P < 0.01$), as well as between the wet and dry season ($df = 1,8, F = 14.76, P < 0.05$; Table 2). TN yields were six times higher in poeciliid streams than in poeciliid-free streams ($df = 1,8, F = 6.671, P < 0.05$), and five times higher in the wet season than in the dry season ($df = 1,8, F = 5.417, P < 0.05$). TOC yields were five times higher in poeciliid streams than in poeciliid-free streams ($df = 1,8, F = 12.14, P < 0.01$), but were similar between seasons. NH_4^+ and PO_4^{3-} yields were similar between stream types and seasons (Table 2).

Benthic biofilm

Chl *a* concentrations were 1.3× higher in poeciliid streams ($16.7 \pm 4.44 \text{ mg/m}^2$) than in poeciliid-free

streams ($12.5 \pm 6.56 \text{ mg/m}^2$), but differences were not significant. AFDM was three orders of magnitude greater than chl *a* concentrations and significantly higher in poeciliid streams ($37.7 \pm 3.67 \text{ g/m}^2$) than in poeciliid-free streams ($28.1 \pm 5.99 \text{ g/m}^2; P < 0.05, t = -2.72$; Fig. 2). Autotrophic index (AI) values were indicative of heterotrophic conditions, but were not significantly different between poeciliid-free (3270 ± 865) and poeciliid streams (2990 ± 740).

Benthic invertebrate community

Total invertebrate densities were higher in poeciliid-free streams than poeciliid streams in the wet (913.4 ± 294.3 vs. 734.1 ± 356.2 individuals/ m^2) and dry season (3300.7 ± 1131.6 vs. 2761.9 ± 925.8 individuals/ m^2), although this pattern was not significant. Chironomids were the most abundant macroinvertebrate collected, followed by the exotic trichopterans *Cheumatopsyche analis* and *Hydroptila* sp., the flatworm *Dugesia* sp., and then oligochaetes and ostracods (Table 3). All of these species were present throughout the sampling period, except *Hydroptila* sp., which were only present during the last wet season sampling event and throughout the dry season. Macroinvertebrates that represented less than 5% of total macroinvertebrate densities included: coleopterans, collembolans, hemipterans, lepidopterans, amphipods, polychaetes, arachnids, and formicids.

Percentage contributions of chironomids to total invertebrate densities were significantly and negatively

TABLE 2. Nutrient yields ($\text{mol}\cdot\text{d}^{-1}\cdot\text{km}^{-2}$) in poeciliid-free (PF) and poeciliid (P) streams along the Hāmākua coast of Hawai‘i.

Yields	Wet season		Dry season	
	PF	P	PF	P
ΣNO_3^-	2.5 ± 0.8	20.9 ± 0.5	0.6 ± 0.2	2.5 ± 0.5**
NH_4^+	3.7 ± 3.1	3.9 ± 3.0	0.2 ± 0.2	0.4 ± 0.2
TN	7.0 ± 1.3	12.3 ± 2.2	9.5 ± 2.4	9.5 ± 2.0*
TOC	69.1 ± 6.2	117.2 ± 25.9	215.0 ± 48.3	238 ± 37.0**
PO_4^{3-}	0.4 ± 0.2	0.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.1

Notes: Values are means ± SE. Boldface type highlights significant differences between seasons ($P < 0.05$), while asterisks represents significant differences between stream type. * $P < 0.05$; ** $P < 0.01$.

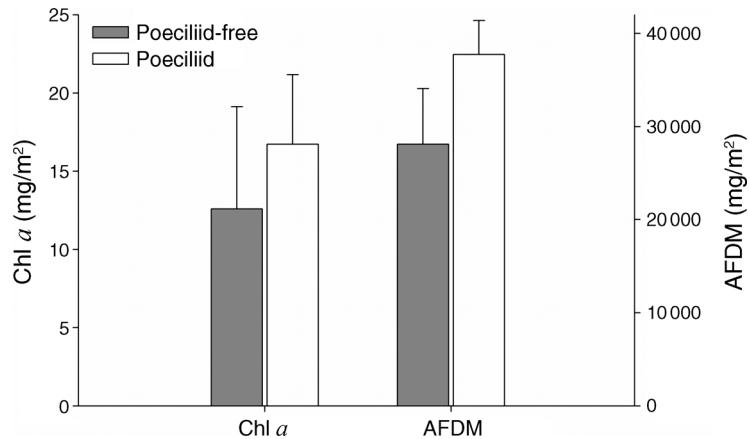


FIG. 2. Benthic biofilm chlorophyll *a* (chl *a*) and ash-free dry mass (AFDM) values (mean \pm SE) in poeciliid-free and poeciliid streams along the Hāmākua Coast of Hawai‘i.

correlated to poeciliid densities ($r = -0.47$, $P < 0.01$), while percentage contributions of *Dugesia* sp. were significantly and positively correlated to poeciliid densities ($r = 0.55$, $P < 0.001$). Percentage contributions of oligochaetes were significantly and negatively correlated to percentage contributions of native fish ($r = -0.331$, $P < 0.05$).

Comparisons of macroinvertebrate data between stream types revealed that average percentage contributions of chironomids were 1.6 \times lower in poeciliid streams ($31.4\% \pm 5.1\%$) than in poeciliid-free streams ($51.2\% \pm 5.9\%$; $df = 1,8$, $F = 8.94$, $P < 0.05$). Average percentage contributions of *Hydroptila* sp. were also 1.6 \times lower in poeciliid streams ($7.7\% \pm 2.4\%$) than in poeciliid-free streams ($12.0\% \pm 5.4\%$; $df = 1,8$, $F = 9.02$, $P < 0.05$). In contrast, average densities and percentage contributions of *C. analis*, *Dugesia* sp., oligochaetes, and ostracods were all higher in poeciliid streams compared to poeciliid-free streams (Fig. 3), although none of these patterns were significant.

Total invertebrate biomass was higher in poeciliid streams (264.2 ± 81.3 mg/m³) than in poeciliid-free streams (159.3 ± 36.8 mg/m³). Average biomass of *C. analis* and *Dugesia* sp. was higher in poeciliid streams compared to poeciliid-free streams in the dry season

(Table 4), although these patterns were not significant. Average biomass of *Hydroptila* sp. and chironomids was two times higher in poeciliid-free streams than poeciliid streams, although these patterns were not significant.

Visual exploration of macroinvertebrate densities using NMDS plots revealed some separation between macroinvertebrate communities from poeciliid and poeciliid-free streams (Fig. 4). ANOSIM results revealed significant, but weak differences between stream types ($R = 0.1$, $P < 0.05$) and between seasons ($R = 0.13$, $P < 0.05$). These weak correlations were attributed to differences in densities of chironomids, *C. analis*, and *Hydroptila* sp., each of which contributed to 22%, 18%, and 14% of dissimilarity, respectively.

Fish community structure

Native fish communities were dominated by *L. concolor*, which represented 49–56% of the native fish observed in poeciliid and poeciliid-free streams, respectively. *Awaous guamensis* (Valenciennes 1837) (goby) represented 30–51% of the native fish in poeciliid-free and poeciliid streams, respectively. *Sicyopterus stimpsoni* were present only in poeciliid-free streams and represented 14% of native fish observed. Invasive fish

TABLE 3. Macroinvertebrate densities (individuals/m²) in poeciliid-free (PF) and poeciliid (P) streams collected with a surber sampler along the Hāmākua coast of Hawai‘i.

Macroinvertebrate groups	Wet season		Dry season	
	PF	P	PF	P
<i>Cheumatopsyche analis</i>	87.5 \pm 32.8	127.0 \pm 9.8	905.0 \pm 245.8	1166.2 \pm 651.7
<i>Hydroptila</i> sp.	34.2 \pm 18.2	72.6 \pm 72.0	630.2 \pm 213.2	350.0 \pm 125.7
Chironomidae	666.2 \pm 207.7	339.3 \pm 207.8	1383.6 \pm 465.1	799.3 \pm 88.6
Ostracoda	0.4 \pm 0.2	5.9 \pm 2.8	0.9 \pm 0.5	1.5 \pm 0.9
<i>Dugesia</i> sp.	34.7 \pm 13.2	95.2 \pm 43.1	131.1 \pm 80.8	191.1 \pm 21.7
Oligochaete	25.8 \pm 11.2	42.9 \pm 4.2	42.2 \pm 36.4	114.7 \pm 23.0
Other	64.6 \pm 11.0	51.2 \pm 16.5	207.7 \pm 89.8	139.1 \pm 14.2

Notes: Values are means \pm SE. The group “other” includes invertebrates that represented less than 5% of the total invertebrate densities. These are coleopterans, collembolans, hemipterans, lepidopterans, amphipods, polychaetes, arachnids, and formicids.

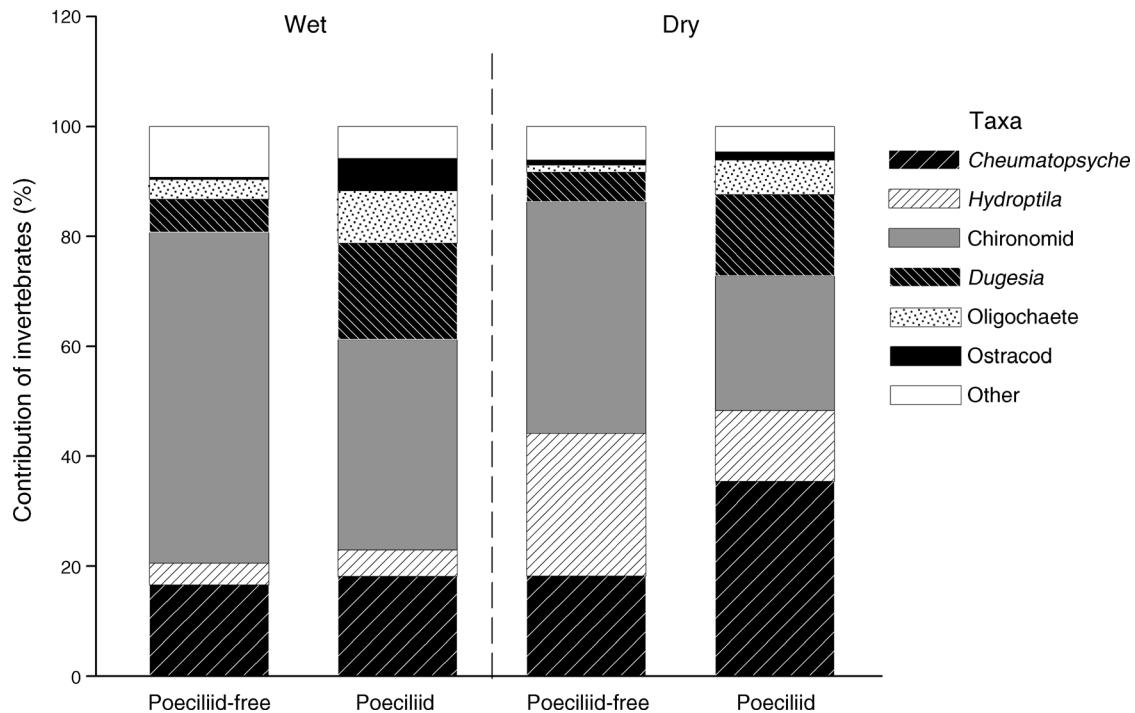


FIG. 3. Contributions of various taxa to total macroinvertebrates in poeciliid-free and poeciliid streams, as well as season (wet and dry), on the Hāmākua Coast of Hawai‘i. Values are means. The group “other” includes any macroinvertebrate taxon that contributed <5% to total macroinvertebrate densities. These are coleopterans, collembolans, hemipterans, lepidopterans, amphipods, polychaetes, arachnids, and formicids.

communities in poeciliid streams were dominated by *X. helleri* and *P. reticulata*. Although these two species were present throughout this study, *X. helleri* densities were 57% higher than *P. reticulata* densities in the wet season, while *P. reticulata* densities were 55% higher than *X. helleri* in the dry season, although neither pattern was significant (Table 5).

Native fish densities, averaged over species and season, were nearly two times lower in streams with poeciliids (0.86 ± 0.2 fish/m²) than in streams without poeciliids (1.60 ± 0.3 fish/m²; $df = 1,44$, $F = 8.23$, $P < 0.01$). Native fish densities averaged across poeciliid and poeciliid-free streams were not significantly different between dry (1.30 ± 0.3 fish/m²) and wet seasons (1.02

± 0.3 fish/m²). In contrast, poeciliid densities from poeciliid streams were 2.5× lower in the wet (10.0 ± 3.0 fish/m²) than the dry season (24.4 ± 3.7 fish/m²). NMDS and ANOSIM analyses revealed no significant differences in fish community structure between stream types or seasons.

TABLE 4. Macroinvertebrate biomass (mg/m³) in poeciliid-free (PF) and poeciliid (P) streams collected with a surber sampler along the Hamakua coast of Hawai‘i, for a concurrent study in the dry season.

Macroinvertebrate groups	Biomass	
	PF	P
<i>C. analis</i>	90.6 ± 41.1	204.9 ± 88.4
<i>Hydroptila</i> sp.	12.8 ± 3.1	6.8 ± 2.9
Chironomidae	34.6 ± 12.5	16.7 ± 8.9
<i>Dugesia</i> sp.	21.3 ± 13.1	35.8 ± 16.4
Total biomass	159.3 ± 36.8	264.2 ± 81.3

Notes: Values are means ± SE. There were no significant differences observed.

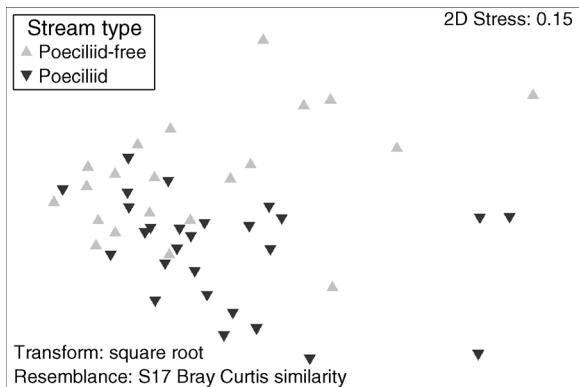


FIG. 4. Nonmetric multidimensional scaling (NMDS) plots of macroinvertebrate community structure from poeciliid-free and poeciliid streams on the Hāmākua coast of Hawai‘i, determined from individual species densities (individuals/m²) using Bray-Curtis similarity index and poeciliid-free and poeciliid as grouping factors. Data were square-root transformed.

TABLE 5. Fish densities (individuals/m²) in poeciliid-free (PF) and poeciliid (P) streams along the Hāmākua coast of Hawai'i.

Species	Wet season		Dry season	
	PF	P	PF	P
<i>Lentipes concolor</i>	1.8 ± 0.3	1.1 ± 0.2	2.5 ± 0.7	2.0 ± 0.5
<i>Awaous guamensis</i>	1.1 ± 0.3	1.5 ± 0.3	0	2.0 ± 0.4
<i>Sicyopterus stimpsoni</i>	0.5 ± 0	0	0	0
<i>Poecilia reticulata</i>	0	8.0 ± 4.8	0	17.1 ± 3.0
<i>Xiphophorus helleri</i>	0	10.5 ± 3.0	0	14.0 ± 7.8

Notes: Values are means ± SE. Fish species in bold type are native species, while the remaining two are invasive species.

Poeciliid excretion experiment

In the fish excretion experiment, native *L. concolor* mass averaged 1.71 ± 0.39 g, while *P. reticulata* fish mass averaged 0.44 ± 0.04 g. Concentrations of N and P from fish excretion experiment bags were higher than the control bags. Concentrations of NH₄⁺ and ΣNO₃⁻ increased with the addition of fish (Table 6). Production rates of ΣNO₃⁻ (18.1 ± 3.3 μmol·m⁻²·h⁻¹; *P* < 0.05, *t* = -4.22), NH₄⁺ (18.1 ± 3.1 μmol·m⁻²·h⁻¹; *P* < 0.05, *t* = -4.52), and TDN (47.2 ± 4.1 μmol·m⁻²·h⁻¹; *P* < 0.05, *t* = -6.427) by *P. reticulata* were significantly higher than those of native *L. concolor* (2.54 ± 1.58, 6.76 ± 0.79, 14.8 ± 2.80 μmol·m⁻²·h⁻¹, respectively). *Poecilia reticulata* populations also produced more PO₄³⁻ (1.10 ± 0.01 μmol·m⁻²·h⁻¹) and TDP (2.62 ± 1.28 μmol·m⁻²·h⁻¹) than *L. concolor* (0.44 ± 0.10, 0.70 ± 0.04 μmol·m⁻²·h⁻¹, respectively), but these differences were not significant.

Total nitrogen standing stocks

Standing N stocks in benthic biofilm, *C. analis*, and *Dugesia* were higher in poeciliid streams (987.42 ± 123.92, 15.94 ± 6.83, and 2.84 ± 1.07 mg/m², respectively) than in poeciliid-free streams (701.91 ± 182.89, 5.68 ± 3.19, and 2.13 ± 1.37 mg/m², respectively). Standing N stocks in *Hydroptila* sp., chironomids, and *L. concolor* were lower in poeciliid streams (0.53 ± 0.23, 1.34 ± 0.72, and 0.36 mg/m² respectively) than in poeciliid-free streams (1.00 ± 0.26, 2.08 ± 0.71, and 0.52 ± 0.09 mg/m² respectively). None of these differences were significant. Standing N stocks in exotic *P. reticulata* (0.74 ± 0.06 mg/m²) were 2× greater than native *L. concolor* (Table 7).

DISCUSSION

Using Hawaiian streams as model systems, we documented differences in the ecological structure, function, and species abundance of poeciliid vs. poeciliid-free streams. Comparisons of nutrient yields between poeciliid and poeciliid-free streams partially supported our first hypothesis that nutrient yields would be higher in poeciliid streams. Significantly higher ΣNO₃⁻, TN, and TOC yields were found in poeciliid streams, while other nutrient yield comparisons showed no trends. Higher biomass of benthic biofilm (AFDM) in poeciliid streams partially supported our second hypothesis, that algal biomass would be greater in poeciliid streams, although chl *a* was not significantly higher. Macroinvertebrate densities were generally higher in poeciliid-free streams than poeciliid streams, but comparisons of individual macroinvertebrate densities and percentage contributions to total macroinvertebrate densities produced mixed results and only partially supported our third hypothesis, that macroinvertebrate densities would be lower in poeciliid streams. Percentage contributions of chironomids and *Hydroptila* sp. were significantly lower in poeciliid streams; all other dominant taxa had higher percentage contributions. Finally, the twofold reduction in native fish densities in poeciliid streams supported our fourth hypothesis, that native fish densities would be lower in poeciliid streams. Our data suggest that invasion of streams by poeciliids may be altering the ecological structure, function, and species abundance of those streams. While differences could have also been influenced by conditions that existed prior to poeciliid invasion (e.g., abandoned cesspools or pasturelands), we lacked the before and after data to

TABLE 6. Fish excretion rates and nutrient contributions to streams by fish from native *Lentipes concolor* in poeciliid-free and invasive *Poecilia reticulata* in poeciliid streams.

Nutrient	Fish excretion rates (μmol·g ⁻¹ ·h ⁻²)		Fish nutrient contribution (μmol·m ⁻² ·h ⁻¹)	
	<i>L. concolor</i>	<i>P. reticulata</i>	<i>L. concolor</i>	<i>P. reticulata</i>
ΣNO ₃ ⁻	0.78 ± 0.25	3.35 ± 1.32	2.54 ± 1.58	18.10 ± 3.30
NH ₄ ⁺	1.94 ± 0.47	2.67 ± 0.59	6.76 ± 0.79	18.10 ± 3.09
TDN	5.11 ± 0.93	7.67 ± 1.24	14.80 ± 2.80	47.20 ± 4.08
PO ₄ ³⁻	0.21 ± 0.04	0.18 ± 0.03	0.44 ± 0.08	1.10 ± 0.01
TDP	0.22 ± 0.04	0.32 ± 0.09	0.70 ± 0.04	2.62 ± 1.28

Note: Values are means ± SE.

TABLE 7. N standing stock (mg/m²) in poeciliid-free and poeciliid streams.

Species	N standing stock	
	PF	P
<i>L. concolor</i>	0.52 ± 0.09	0.36
<i>P. reticulata</i>	NA	0.74 ± 0.06
<i>Hydroptila</i>	1.00 ± 0.26	0.53 ± 0.23
<i>Dugesia</i>	2.13 ± 1.37	2.84 ± 1.07
<i>C. analis</i>	5.68 ± 3.19	15.9 ± 6.83
Chironomid	2.08 ± 0.71	1.34 ± 0.72
Benthic biofilm	701.91 ± 182.89	987.42 ± 182.89

Notes: Values are means ± SE. Fish were not present in PF streams; therefore NA represents not available. Only one native fish was found in P streams; therefore, no SE is shown.

address this. Future studies using controlled mesocosms would validate that the environmental changes reported here are a direct result of poeciliid invasion.

Impacts of exotic poeciliids on stream nutrient dynamics

Fish are an important component of nutrient cycling in aquatic ecosystems (Tátrai and Istvánovics 1986, Vanni 2002, Vanni et al. 2006, McIntyre et al. 2008). While the importance of nutrient inputs from fish in meeting the nutrient demands of temperate streams has recently been questioned, it appears that they may be more important in the nutrient cycling of tropical streams (Wilson and Xenopoulos 2011). The ingestion of particulate nutrients (e.g., detritus, phytoplankton, animal tissue) by fish, and the ultimate excretion of more readily available, dissolved nutrients is an important nutrient source that supports aquatic microbial and primary production (Vanni 2002). Thus, the establishment of high densities of exotic omnivorous fish such as poeciliids was expected to increase concentrations of dissolved nutrients in invaded streams. This was evident from the significantly higher nutrient yields reported from poeciliid-invaded streams, as well as significantly higher nutrient excretion rates from poeciliids compared to native gobies. While the primary form of N excreted by fish is NH₄⁺ (McIntyre et al. 2008), ΣNO₃⁻ also proved to be a large component of dissolved inorganic nitrogen that accumulated in our excretion experiments, and sometimes its accumulation was even greater than NH₄⁺. Similar results were observed in poeciliid excretion experiments conducted in Hawaiian wetlands, where ΣNO₃⁻ increased over time in fish excretion bags and represented up to 3% of the dissolved inorganic nitrogen pool (MacKenzie and Bruland 2012; R. MacKenzie, unpublished data). Increased ΣNO₃⁻ may have been due to bacterial nitrification of NH₄⁺ excreted from fish within the excretion bags, as bacteria were likely present in the water used in the excretion experiments since it was only filtered through a 0.7-μm filter. Nitrifying bacteria may have also been present in the stomachs or mucus layer of the fish used in our excretion experiments. This suggests that excretion studies that ignore ΣNO₃⁻ contributions may signifi-

cantly underestimate NH₄⁺ inputs from aquatic organisms. Additional studies are needed to verify if a portion of NH₄⁺ excreted by fish is indeed converted to ΣNO₃⁻ over the duration of excretion experiments. Results from our excretion experiments also suggest that high densities of poeciliids contributed to the increased ΣNO₃⁻ and TN yields in poeciliid-invaded streams. An alternative explanation for high ΣNO₃⁻ yields in poeciliid streams could be differences in land cover or human population, as N in streams draining Mauna Kea and Kohala watersheds on Hawai'i Island have been positively correlated with these factors (Michaud and Wiegner 2011). However, land cover was similar for all streams sampled in this study, except for one. Additionally, human population densities varied across watersheds and, with the exception of one poeciliid stream, were generally lower than 10 people/km².

NH₄⁺ yields were expected and found to be higher in poeciliid streams than poeciliid-free ones, but this trend was not significant. The lack of significance is probably a result of both nitrification, as discussed above, and NH₄⁺ uptake by greater levels of benthic biofilm in poeciliid streams. Regardless, it appears that high densities of poeciliids are influencing dissolved N dynamics in invaded streams.

N standing stocks (e.g., benthic biofilm, invertebrates, fish) were compared between poeciliid and poeciliid-free streams in an attempt to identify potential sources for increased N yields observed in poeciliid-invaded streams. Combined N standing stocks of *P. reticulata* and *L. concolor* populations in poeciliid-invaded streams were greater than *L. concolor* in poeciliid-free streams, which was due to increased levels of fish biomass from high densities of *P. reticulata*. The higher N from the larger poeciliid biomass in the invaded streams was likely met by consumption of *Hydroptila* sp. and chironomids by poeciliids (R. A. MacKenzie, T. Holitzki, and K. J. McDermid, unpublished manuscript), as contributions of *Hydroptila* sp. and chironomids to invertebrate densities and their lower N standing stocks were lower in these streams. In contrast, the N standing stocks of benthic biofilm and *C. analis* were also much higher in poeciliid streams. Mass balance suggests that these increases in N standing stocks were more than enough to offset N lost from *Hydroptila* sp. and chironomids. This suggests that a potential poeciliid food resource was not sampled: leaf litter. A recent study conducted in this region using stable isotopes estimated that leaf litter can contribute up to 20% of poeciliid diets (R. A. MacKenzie, T. Holitzki, and K. J. McDermid, unpublished manuscript). Standing stocks of leaf litter from another stream in this region that was of similar size and elevation to the streams sampled in our study ranged from 23.3 to 70.5 g/m² (Larned 2000). Using an average %N value of 1 for mixed leaf litter (Hughes and Denslow 2005), the N standing stock in leaf litter would be equivalent to 2000–7000 mg/m². As there were no differences in riparian forest structure or

canopy cover among our streams, we assumed leaf litter inputs would be similar across our streams, and thus consumption of that litter by *P. reticulata* may have contributed to additional N inputs in poeciliid streams.

TOC yields were also higher in poeciliid streams. This was attributed to the higher levels of benthic algal and biofilm biomass that occurred in poeciliid streams. As algal cells and other organisms within the benthic biofilm photosynthesize or decompose organic matter, TOC is released into the stream water (Stauss and Lamberti 2002); this pattern has been observed downstream from large benthic algal patches in other systems (Kaplan and Bott 1989, MacKenzie 2008). Higher leaf litter decomposition within the stream may have also contributed to higher levels of TOC in poeciliid streams, especially as increased N levels, that also occurred in poeciliid streams, can stimulate microbial activity and significantly increase leaf decomposition rates (Gergel et al. 1999, Mulkins et al. 2002, MacKenzie 2008). Increased levels of TOC in poeciliid streams may have also been due to sloppy feeding by high densities of poeciliids (Richardson et al. 2009). Differences in riparian forest composition can also influence C inputs to streams and thus C dynamics (Mulkins et al. 2002). However, riparian forest structure was similar across our streams.

Impacts of poeciliids on stream algal communities

Benthic biofilms in tropical streams are influenced by bottom-up (i.e., nutrient additions, increased sunlight [Mosisch et al. 2001, Flecker et al. 2002, MacKenzie 2008]) and top-down (i.e., grazing [Pringle 1996, Flecker et al. 2002, March et al. 2002]) controls. Higher levels of benthic biofilm biomass in poeciliid streams were attributed to both controls. High N yields in poeciliid streams may have increased benthic biofilm biomass through release from nutrient limitation, as tropical streams are typically N limited (Larned and Santos 2000, Mosisch et al. 2001). Exotic *P. reticulata* and *X. helleri* have been shown to feed heavily on aquatic macroinvertebrates that commonly feed on benthic biofilm (Arthington 1989, Englund 1999, Atwood et al. 2010). Thus, lower densities of chironomid and *Hydroptila* sp. in poeciliid streams, which were attributed to increased predation from high densities of both poeciliid species, may have released biofilm from grazing pressure, and thus increased biofilm biomass, as several species of chironomids and hydroptilids graze on periphyton (Merritt and Cummins 1996). Significant increases in AFDM and chl *a* from New Zealand streams invaded by brown trout (*Salmo trutta* Linn. 1758) were attributed to the combination of invertebrate predation by trout, as well as decreased grazing that occurred via predator avoidance behavior of grazing invertebrates (Flecker and Townsend 1994, McIntosh and Townsend 1996).

High levels of benthic biofilm AFDM reported here, as well as from other tropical streams (MacKenzie 2008;

T. Moulton, *personal communication*), also suggest that Pacific Island streams are heterotrophic ecosystems. This is supported by the fact that the autotrophic index (AI) values for both poeciliid-free and poeciliid stream types were much higher (2990 to 67 300) than the 200 value that delineates heterotrophic from autotrophic ecosystems in temperate streams (Uehlinger and Brock 2005, Feio et al. 2010). Previous studies have suggested that Pacific Island streams are heterotrophic (LaPerriere 1995, MacKenzie 2008), which may be largely influenced by light limitation (Larned and Santos 2000, MacKenzie 2008), and possible high leaf litter inputs that occur year-round (Larned 2000). Higher levels of benthic biofilm AFDM in poeciliid streams compared to poeciliid-free ones, coupled with higher AI values in poeciliid streams and the lack of difference in chl *a* values, suggests that the invasion of streams by poeciliids may alter benthic biofilms by increasing the abundance of heterotrophic organisms. Additional studies are needed to identify how and the mechanisms by which benthic biofilms change with invasion by exotic organisms.

Impacts of poeciliids on stream macroinvertebrate communities

Fish can also influence macroinvertebrate communities in streams, although the magnitude and direction of that influence varies across systems (see Wallace and Webster 1996). High densities of exotic fish can significantly lower invertebrate densities in streams (Flecker and Townsend 1994, Kido et al. 1999, Baxter et al. 2004) and other aquatic ecosystems (Schindler and Parker 2002, Capps et al. 2009). However, it is not clear if reduced invertebrate densities are due to direct predation or from predator avoidance (Nyström et al. 2003, Capps et al. 2009). Closer examination of community structure from our study revealed that the decrease in total invertebrate densities was largely due to lower chironomid densities in poeciliid streams. The microcaddisfly, *Hydroptila* sp., also had lower densities in poeciliid streams, but only in the dry season. The lack of differences in the wet season was due to the general absence of *Hydroptila* sp. from all stream samples. *Hydroptila* sp. were likely present, but in their earlier instars that can easily pass through the 250- μ m mesh of our surber samplers (McIntosh et al. 2003). In contrast, densities of *C. analis*, ostracods, and oligochaetes were all higher in poeciliid streams. Decreased densities of chironomids and hydroptilids in poeciliid streams were likely due to predation, especially as chironomids have been shown to be a dominant component in poeciliid diets (Arthington 1989, Bassar et al. 2010) and are small enough (chironomid mean length 3.3 ± 0.32 mm), to be eaten by *P. reticulata* (mean gape 1.45 ± 0.07 mm) and *X. helleri* (mean gape 2.11 ± 0.18 mm). Higher densities of *C. analis* and oligochaetes may have been due to their larger size (mean length 7.3 ± 1.5 and 10.3 ± 2.0 mm, respectively) relative to the mouth size of either species

of poeciliid. However, native fish, with a gape size nearly four to five times larger than that of the invasive poeciliids, *A. guamensis* (mean gape 4.17 ± 0.27) and *L. concolor* (mean gape 5.12 ± 0.97 mm; Maie et al. 2009), would have effortlessly fed on these larger invertebrates. Yet, the aggressive territorial behavior of the poeciliids may have minimized foraging time by native gobies. Higher densities of *C. analis* may have also been due to increased benthic biofilm in poeciliid streams. While *C. analis* are net spinning caddisflies whose diet is largely composed (~90%) of detritus (Parker and Voshell 1983, Sanchez and Hendricks 1997), the gut contents of exotic *C. analis* from a Moloka'i stream contained nearly 50% of diatoms and algae (Kondratieff et al. 1997). Stable isotopes have also demonstrated that algae is an important food source for *C. analis* (Larned et al. 2008, Atwood et al. 2010). This suggests that, on Hawai'i, *C. analis* may be grazing off of substrate surfaces more than their nets (Kondratieff et al. 1997) or that algae are growing on *C. analis* nets.

The impacts of established poeciliid populations on native gobies

Poeciliid densities were on average 85% higher than native goby densities in poeciliid streams. This may be partially due to the rapid reproductive cycles of poeciliids (Thibault and Schultz 1978), where the lack of an egg stage results in the immediate release of large numbers of live young a few weeks after insemination (Thibault and Schultz 1978) that can occur several times a year (McGuire 2007). In contrast, native gobies have longer, more complicated life cycles that involve migrations to and from the ocean over a three to six-month period thought to occur only once a year (McDowall 1997, 2007, Radtke et al. 2001). Migration also involves moving through lower stream reaches and estuaries where juveniles can be preyed upon by piscivores (e.g., *Eleotris sandwicensis*, *Kuhlia* spp.) typically absent from stream reaches above waterfalls (Fitzsimons et al. 2007, Schoenfuss and Blob 2007). Migration also often involves climbing waterfalls that can be hundreds of meters tall (McDowall 2003, Blob et al. 2006, Fitzsimons et al. 2007). This amphidromous life cycle exposes multiple stages of young gobies to risks not encountered by poeciliids, and may partially explain the lower abundances of gobies compared to poeciliids in invaded streams.

Despite the fact that poeciliid densities were consistently higher than gobies in the invaded streams examined, there were distinctive seasonal patterns in poeciliid densities. During the wet season, poeciliid densities were lower than they were in the dry season. This was likely due to higher water discharge in the wet season that results from greater levels of precipitation (Oki 2003, 2004). Unlike native gobies, poeciliids have not evolved mechanisms to colonize streams during these high flood events. Gobies, however, have evolved several morphological features to help maintain their

position in stream channels during high flow events. These include suction-cup-like pelvic fins and fusiform body shapes (Maie 2007, Schoenfuss and Blob 2007). Alternatively, higher densities of poeciliids in the dry season could have been due to concentrated populations of fish that result from reduced habitat from decreased stream discharge (Dudgeon 1993). However, Dudgeon (1993) reported a 75% decrease in stream discharge while stream discharge in our study was only reduced by 40%. Stream widths were also not significantly different between wet and dry seasons in our study. While we did not measure stream habitat, the lack of difference in stream width suggests there was not a marked difference in stream habitat. Furthermore, increased stream flow had a greater impact on poeciliid densities than change in habitat availability in an intermittent tropical stream in Costa Rica (Chapman and Kramer 1991).

Lower *L. concolor* densities may have also resulted from resource competition with poeciliids, as there were significantly lower densities of chironomids and hydroptilids in poeciliid streams, and these two invertebrates are important food sources for this goby (Kido 1996, Fitzsimons et al. 2007). Preliminary stable isotope evidence from a concurrent study supports this supposition, as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values show a decreased importance of chironomids in the diets of gobies in poeciliid streams, as well as an increased importance of *C. analis* (R. A. MacKenzie, T. Holitzki, and K. J. McDermid, *unpublished manuscript*). It is unclear how this shift in food resources impacts native goby populations, but it could result in decreased fitness, reproductive output, and ability to resist parasitic infections (Kido 1997).

The presence of poeciliids may also alter the behavior of native gobies. In Utah, native least chub shifted their natural habitat from warmer shallow marshes used for spawning to cooler, deeper springheads (Ayala et al. 2007). While this allowed the least chub to escape attacks by poeciliids, the longer time spent foraging in cooler, deeper waters resulted in decreased growth and fecundity rates of least chub (Ayala et al. 2007). In Michigan, the aggressive behavior of the invasive round goby (*Neogobius melanostomus*) forced native mottled sculpins (*Cottus bairdi*) to seek refuge in deeper habitats, making them more susceptible to predation by larger fish (Dub and Corkum 1996). In Hawaii, native shrimp in poeciliid-invaded anchialine pools feed only at night, while native shrimp in non-invaded pools feed throughout the day (Capps et al. 2009). This change in behavior has significantly increased the algal biomass within these pools, as grazing pressure by shrimp has been partially released (Capps et al. 2009).

Finally, exotic poeciliids may be directly feeding on native gobies. Significantly lower densities of native fish and amphibians are commonly reported from mesocosm experiments and are attributed to direct predation by poeciliids on eggs, juveniles, or adults, competition for limited resources, or a combination of many of these

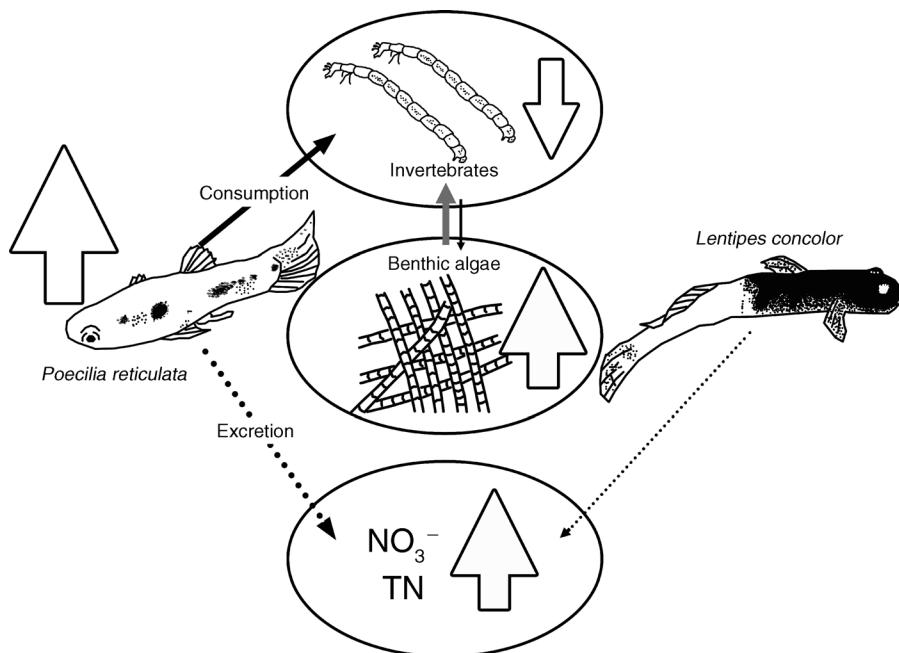


FIG. 5. Food web structure in a Hawaiian stream invaded by poeciliids. Solid black arrows represent top-down impacts via consumption. Gray arrows represent bottom-up impacts. Dotted arrows represent excretion. Solid white arrows represent the response of each standing stock. The size of the arrow indicates the magnitude of the effect. High densities of poeciliids are decreasing macroinvertebrates (chironomids and *Hydroptila*) through consumption and increasing nutrient yields through excretion, which is stimulating benthic biofilm growth. Lower consumption of invertebrates by native gobies coupled with lower densities of invertebrates is thought to result in decreased densities of gobies.

factors (Lawler et al. 1999, Hamer et al. 2002, Mills et al. 2004, Rogowski and Stockwell 2006, Reynolds 2009). While direct predation on native fish has not yet been documented in Hawaii, cannibalism by poeciliids has been observed in invaded coastal wetlands (R. MacKenzie, unpublished data). Thus, decreased densities of native gobies reported in our study could have also been due to predation by exotic poeciliids, especially during the migration of small goby juveniles from the ocean (Eldridge 2000).

Bottom-up and top-down effects of poeciliids

Poeciliids are having both bottom-up and top-down effects on Hawaiian stream structure and function. High densities of poeciliids appear to have increased biofilm biomass through increased nutrient yields in Hawaiian streams. This bottom-up effect was expected to increase invertebrate densities. However, increased biofilm biomass only increased densities of certain invertebrates, specifically, the exotic caddisfly, *C. analis*. Lower densities of chironomids and *Hydroptila* sp. in poeciliid streams were attributed to consumption by exotic poeciliids in high densities. This top-down effect would have also led to increased biofilm biomass, as these invertebrates feed on algae (Merritt and Cummins 1996). While the decreased densities of invertebrates also resulted in decreased food resources for native gobies, increased *C. analis* densities, assumed to result from increased biofilm biomass, may have decreased the

magnitude of the top-down effects of poeciliids on goby populations by providing an alternative food source for gobies (Fig. 5).

Implications

Results from this study revealed Hawaiian streams invaded by exotic poeciliids had higher nutrient levels and biofilm biomass, decreased densities of certain macroinvertebrates, and decreased densities of native gobies. Together, these factors suggest that invasion by poeciliids are impacting the ecological structure, function, and native biodiversity of Hawaiian streams through alterations of essential ecosystem processes such as nutrient cycling, primary production, predator-prey relationships, and food web structure, although additional studies are needed to verify causation. The magnitude of these results may be greater than those predicted for continental streams due to the disharmonic biota of Hawaiian streams that have resulted from the extreme isolation of the island chain. While similar impacts may not occur in every tropical aquatic ecosystem invaded by poeciliids, we expect similar impacts to occur in other tropical island streams in the Pacific and elsewhere (i.e., Puerto Rico), as these ecosystems also support depauperate community assemblages comprised of endemic flora and fauna. Furthermore, the aquatic invaders predicted to have the greatest impact on an aquatic ecosystem are those that belong to genera not already present in the system (Ricciardi and Atkinson 2004). Thus, the impacts reported in our study

are likely to occur in other tropical aquatic ecosystems that lack similar poeciliid genera or poeciliids altogether. The negative impacts from poeciliid invasion reported here underscore the need for local resource managers to develop effective eradication campaigns, especially in island streams and wetlands that support unique assemblages of flora and fauna (Resh and deSzalay 1995, March et al. 2003, MacKenzie and Bruland 2012). The use of piscicides, such as rotenone or antimycin, have successfully removed poeciliids from Hawaiian anchialine pools (A. Mokiao-Lee, *personal communication*). While these chemicals would also eradicate native species, their amphidromous life cycle would provide a natural mechanism to repopulate treated streams. Furthermore, results presented here suggest that the application of piscicides immediately after the wet season may be most effective, as this is when poeciliid populations are lowest. Before applying piscicides, a monitoring system should be set up to observe whether poeciliids increase in density in poeciliid streams and whether poeciliid-free streams remain free of invasion. However, the most effective management strategy is the prevention of future introductions of poeciliids into intact native streams. This could be accomplished through local media outlets or community meetings to facilitate public education concerning the consequences of these seemingly harmless releases. Pet storeowners should also be educated as they could provide a warning to purchasers of these highly invasive fish. Finally, resource managers should monitor new aquatic species arrivals when first reported to ensure that they are not introduced or spread to other streams. A combination of all of these efforts is needed to protect the many ecosystem services of island streams that are vital for the native assemblages of flora and fauna, clean water, and the ultimate well-being of human populations residing on islands.

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