

Aquatic Insect Taxa as Indicators of Aquatic Species Richness, Habitat Disturbance, and Invasive Species Impacts in Hawaiian Streams¹

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Abstract

In this study we provide a synthesis of numerous stream assessments in the Hawaiian Islands that began in the early 1990s and have continued to the present. Data from numerous sites within the five major high Hawaiian Islands with flowing streams (excluding Lāna'i, which lacks flowing waters) were used to assess native and introduced aquatic insect communities, the impacts of various invasive freshwater species and the threats from habitat disturbance. The primary objective of this study was to provide the first comprehensive analysis of aquatic insect populations in various urbanized and virtually pristine stream reaches on the five major Hawaiian Islands, and to assess if various suites of introduced aquatic species may be impacting aquatic insect populations.

We were also interested in assessing the suitability of native aquatic insects as key indicator, flagship, or umbrella species regarding the overall health of Hawaiian aquatic ecosystems. If key indicator species can be found, then aquatic habitats with high native biodiversity can be identified and management efforts can be made to ensure this high level of biodiversity persists. These indicator species could also be used for monitoring future rehabilitation programs on disturbed streams.

Introduction

Detailed distribution and abundance data for invertebrates such as aquatic insects are lacking for most tropical regions, and this lack of basic knowledge hinders the development of conservation planning efforts. The Hawaiian Islands are an exception to this rule because of a long history of entomological collections starting in the 1800s, and the infrastructure of a major museum and large university in close proximity to a wide range of aquatic habitats. Because of its extreme isolation, Hawai'i has the greatest percentage of unique fauna in the world with an estimated 98% endemism rate for the 5,368 described insect species (Eldredge & Evenhuis, 2003). Most research efforts in the Hawaiian Islands have been focused on the amazing adaptive radiations and ecological adaptations found within the terrestrial insect fauna, with far fewer resources devoted to studying insects found within freshwater habitats. In aquatic systems, the insect group historically receiving the greatest attention has been the Odonata (damselflies and dragonflies), with other taxa such as aquatic flies (Diptera) or true bugs (Heteroptera) being assessed at various levels of intensity. While most

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of the early research involved taxonomic descriptions of new species, some early pioneers such as F.X. Williams conducted life history and basic ecological studies on the Hawaiian aquatic insect fauna (Williams, 1936).

Although life history and limited ecological studies have been conducted on a small number of Hawaiian aquatic insect species, this study is the first to examine broad scale patterns of entire communities found within individual watersheds, islands, or different islands. While various authors have demonstrated the impacts of specific introduced aquatic species on native Hawaiian freshwater species (Englund & Polhemus, 2001; Englund, 1999; Font, 1998; Font & Tate, 1994), a quantitative examination of the potential suitability of different aquatic insect taxa as indicator species representing the ecological health of a particular Hawaiian aquatic ecosystem has not previously been attempted. For the purposes of this study we define ecological health as an intact Hawaiian watershed containing greater numbers of native species than an urbanized and highly disturbed watershed.

In this study we provide a synthesis of numerous stream assessments in the Hawaiian Islands that started in the early 1990s and continue to the present. Data from numerous sites within 5 of the major high Hawaiian Islands with flowing streams (excluding Lānaʻi) were used to assess native and introduced aquatic insect communities, the impacts of various invasive freshwater species and the threats from habitat disturbance (see Figs. 1–5 for site maps).

The primary objective of this study was to provide the first comprehensive analysis of aquatic insect populations in various urbanized and virtually pristine stream reaches on the five major Hawaiian Islands, and to assess how various suites of introduced aquatic species may be impacting these aquatic insect populations. Additionally, given that one of the major goals for conservation biologists is maintaining biodiversity in highly endemic areas such as in Hawaii, we were also interested in assessing the suitability of native aquatic insects as key indicator, flagship, or umbrella species regarding the overall health of Hawaiian aquatic ecosystems. If key indicator species can be found, then aquatic habitats with high native biodiversity can be more readily identified and management efforts can be undertaken to ensure this high level of biodiversity persists. These indicator species can also be used for monitoring future rehabilitation programs on disturbed streams.

Many species of native Hawaiian aquatic insects are now threatened with extinction because of reduced ranges resulting from habitat loss and invasive species (Liebherr & Polhemus, 1997; Englund, 1999, 2001, 2002). Preserves for threatened and endangered species are often designed to protect habitats that permit the maximum number of species to be conserved, often by using surrogate species that are believed to represent the needs of other threatened species using the same habitat (Simberloff, 1998; Andelman & Fagan, 2000; Rubinoff, 2001). Three classes of surrogate species have been identified and include: (1) flagship species, or charismatic species attracting public support, (2) umbrella species, or species requiring large areas of habitat needing protection thereby also providing protection for other species, and (3) biodiversity indicators, or species whose presence indicates areas with high species richness (Andelman & Fagan, 2000).

In the present study we make the first attempt to assess the sensitivity of both native fish and aquatic insect species to introduced species and to other major watershed perturbations such as diversions or concrete channelization. This was done by collecting from a wide variety of aquatic insect habitats ranging from heavily urbanized and channelized streams, to pristine sections of watersheds accessible only by helicopter. A holistic evaluation of Hawaiian streams requires not only the assessment of the five native species of freshwater fish and several large species of easily observed invertebrates (i.e., crustaceans), but also the 300–400 estimated species of native Hawaiian aquatic insects. Unlike aquatic vertebrates, many aquatic insects have narrow habitat tolerances meaning they can only live in certain flowing water microhabitats, for example seeps or cascade splash-zones. These narrow habitat preferences also increase the vulnerability of aquatic insects to stream disturbances such as stream channelization, dewatering, sedimentation, and alien species introductions. Because native Hawaiian aquatic insects are much less flexible in their habitat requirements than aquatic vertebrates, it then follows that insects may provide a better monitoring and stream assessment tool than vertebrates. Stream macrofauna such as the native fish, crustaceans, and neritid snails are migratory and are not necessarily co-evolved to a specific stream system, unlike many Hawaiian

aquatic insects. This study therefore makes a first attempt at integrating the various factors that appear to be presently limiting the distributions of native aquatic insects in Hawaii, or factors that make habitats suitable for the survival of endemic species.

Materials and Methods

Streams on Kauai, O'ahu, Moloka'i, Maui, and Hawai'i islands (Table 1, Figs. 1–5) were surveyed for both native and introduced species in a wide range of aquatic habitats, ranging from coastal lowlands at sea level to high elevation reaches only accessible by helicopter, thus covering the entire gradient of habitats available in the islands. The highest elevation sampled in a particular stream reach was recorded and determined with a combination of USGS topographic maps and handheld altimeters. Efforts were made to standardize insect collections at each sample site as similar habitats and collecting techniques were used at each station.

Aquatic Insects

Collections of both immature and adult specimens were made with yellow pan traps, aerial sweep nets, aquatic dip nets, kick-netting, and Surber (benthic) samplers around all aquatic habitats at each study site. Visual observations of aquatic insects were also conducted above and around the stream. Sampling of damselflies and dragonflies (Odonata) was also emphasized, because six Hawaiian species are currently considered Candidate Species by the U.S. Fish & Wildlife Service.

Benthic sampling centered on kick-netting and involved vigorously disturbing the substrate upstream of a fine meshed aquatic net to displace any aquatic invertebrates inhabiting the stream substrate. The use of frequent kick-netting allowed for a greater sample size and resulted in increased effort for invertebrate collections. Benthic sampling also included collecting individual variously sized rocks and then using a toothbrush or forceps to remove immature insects. Above and below water visual observations for aquatic insects were also conducted as we hiked between sampling stations. Sampling effort was focused on all suitable aquatic habitats such as splash zones around riffles and cascades, wet rock faces associated with springs and seeps, waterfalls, nearby wetland areas associated with the streams, and variously-sized stream substrates. All aquatic habitats were sampled. All insect specimens were stored in 95% ethanol for curation and identification and voucher specimens are currently housed in the Bishop Museum and Smithsonian Institution collections.

Freshwater Fish, Introduced Crustaceans, and Amphibians

One of the primary objectives of this study was to assess where specific suites of aquatic organisms have been introduced into a particular Hawaiian watershed. Thus, observations and limited collections of freshwater fish, crustaceans, and amphibians were undertaken to verify species identities. Fish and introduced crustaceans and amphibians were either collected with nets and hand seines, or identified underwater while snorkeling. Many of these aquatic insect surveys were jointly conducted with biologists from the Hawaii Division of Aquatic Resources (HDAR) assessing native and introduced fish populations, thus we have integrated the results of their findings with our aquatic insect findings. HDAR fish collection data was accessed from their stream survey website at: [http://www.hawaii.gov/dlnr/dar/streams/stream_data.htm].

Statistical Analysis

Multiple-species data are notoriously difficult to analyze in a clear and meaningful manner. Multivariate statistical analysis of community data offer a means of detecting patterns in similarity of species composition of sample sites, and a means of identifying species associated with specific environmental conditions. Canonical correspondence analysis is an analytical method that can be used to unravel patterns in complex ecological data sets (Leps & Smilauer, 2003).

Presence/absence data for the insect species was subjected to canonical correspondence analysis (CCA), a direct gradient analysis method, which summarizes relationships between response variables (in this case, insect species assemblages in 39 study sites) and environmental variables

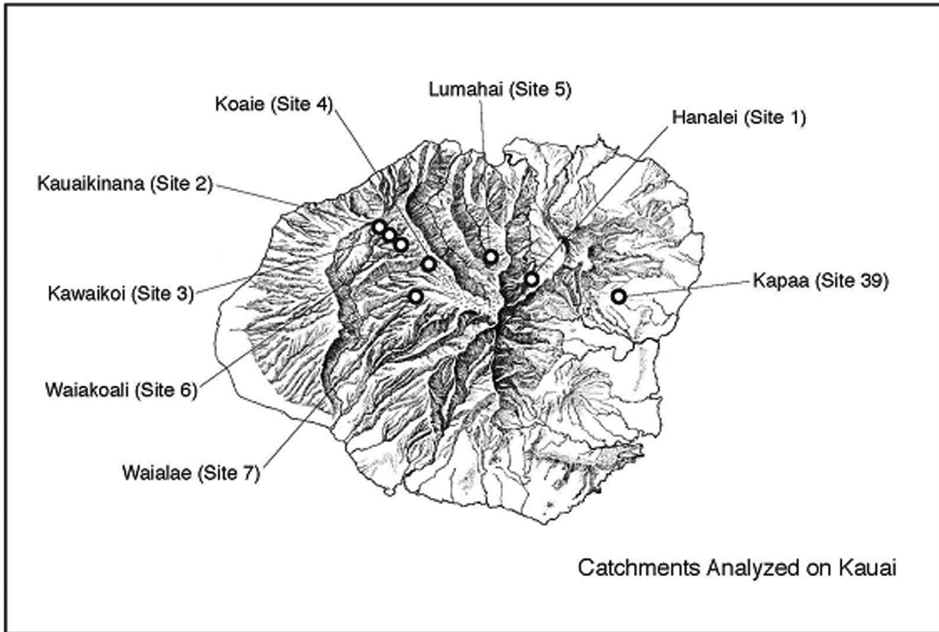


Figure 1. Streams sampled for aquatic biota during this study on the island of Kauai.

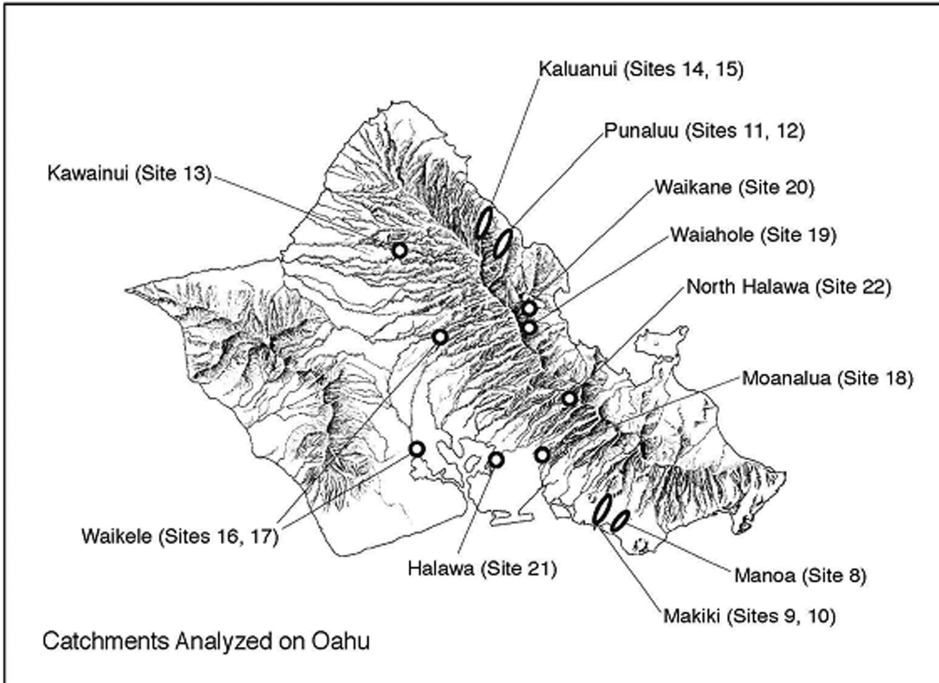


Figure 2. Streams sampled for aquatic biota during this study on the island of Oahu.

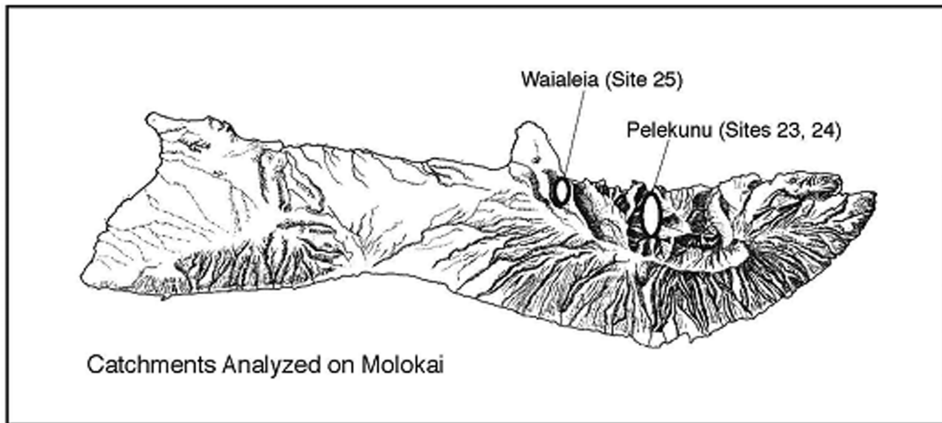


Figure 3. Streams sampled for aquatic biota during this study on the island of Molokai.

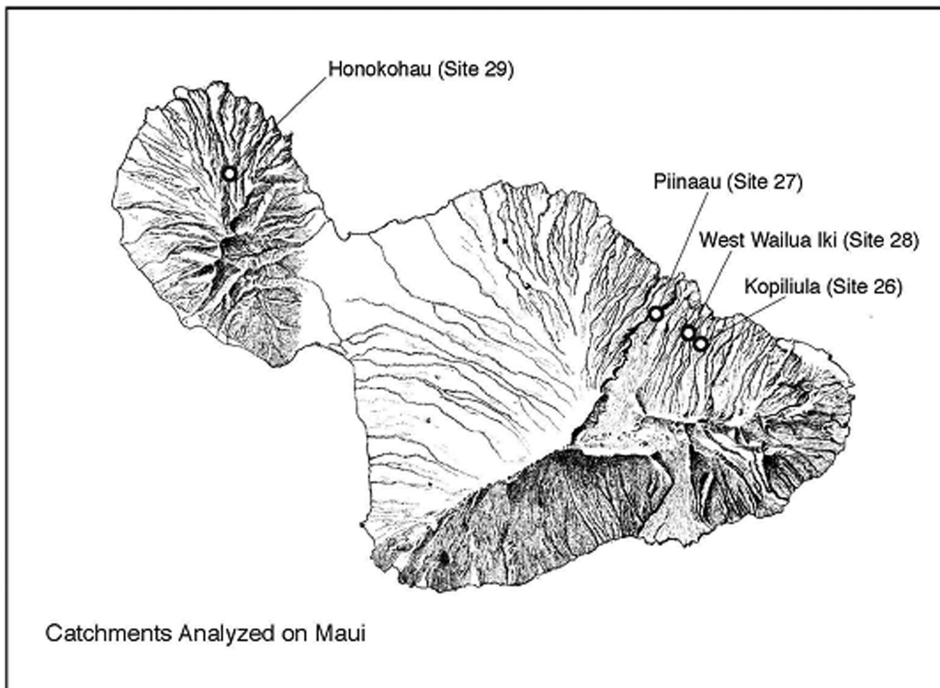


Figure 4. Streams sampled for aquatic biota during this study on the island of Maui.

(Leps & Smilauer, 2003). The analyses were conducted using CANOCO 4.5 and CanoDraw software (Ter Braak & Smilauer, 2002). CANOCO performs multivariate ordination on species data, calculating chi-square distance between samples, and plotting sample and species scores these on canonical (constrained) axes, determined by correlations between specified environmental variables and species scores. Plots of ordinations are generated by CanoDraw (Ter Braak & Smilauer, 2002).

The ordinations were initially done for all species, and then broken down by insect family. Families were analyzed separately as each has different ecological characteristics, and meaningful

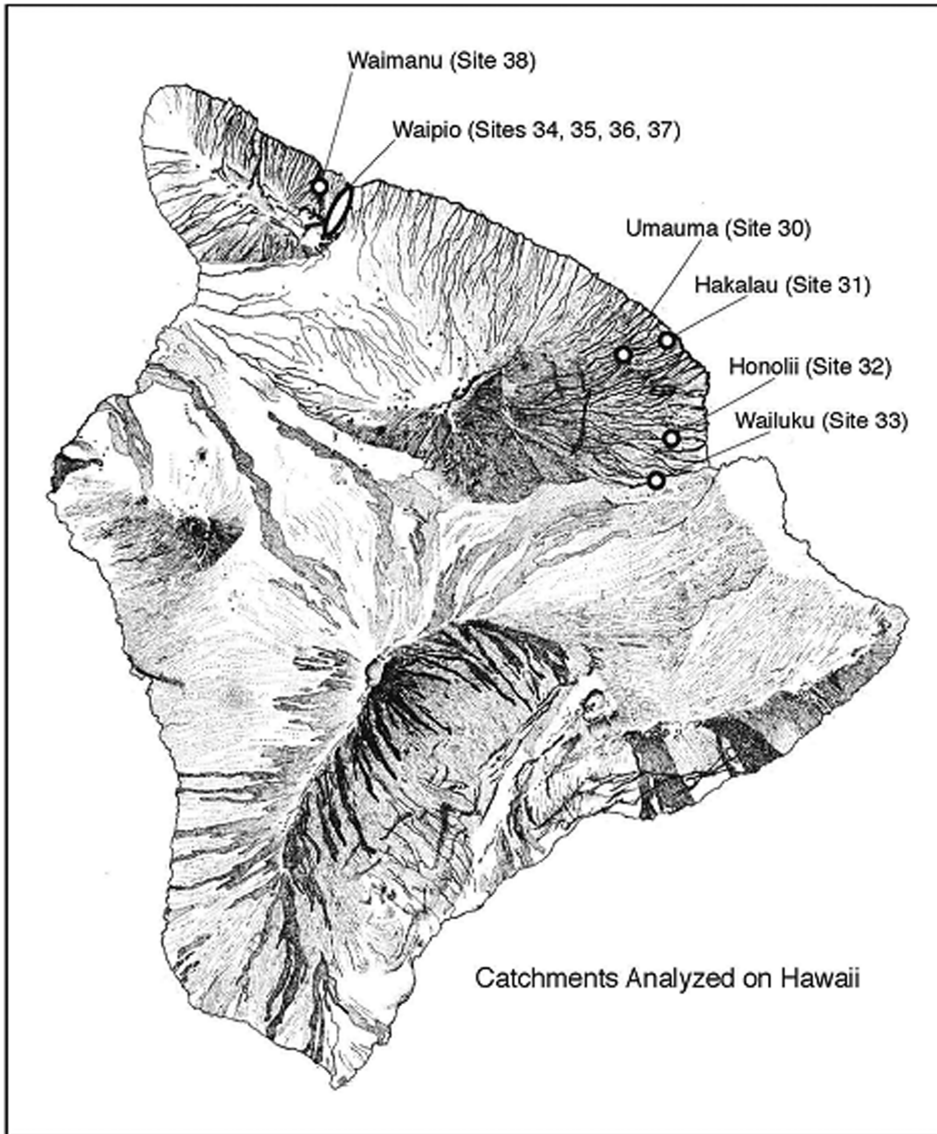


Figure 5. Streams sampled for aquatic biota during this study on the island of Hawai'i.

graphical analyses could be presented with the reduced data sets. Environmental variables that were selected were: island (coded as 1–5, Kaua'i = 1; Hawai'i = 5); elevation (m.a.s.l.); type of stream (coded as 1 = undiverted, not channelized; 2 = concrete channel; 3 = channelized no concrete; 4 = diverted, below diversion but not channelized); presence or absence of indigenous and exotic fish species; and presence or absence of exotic frogs. Exotic fish species and frogs were included as environmental variables because they may impact indigenous insects negatively, or in some cases, they may be associated with either positive or negative environmental conditions that are suitable for certain communities of aquatic insects. The former situation is the case for poeciliid fish that are often the only fish species found in concrete channelized Hawaiian streams, while the latter situation is

Table 1. Island, stream sampling sites, sampling date, and stream type assessed for native and introduced aquatic insects, fishes, crustaceans, and amphibians in the Hawaiian Islands. The Hawaii Division of Aquatic Resources stream database was also consulted for fish species composition. Stream type at sample reach: 1 = undiverted, not channelized, 2 = concrete channel, 3 = channelized no concrete, 4 = diverted, below diversion.

Site No.	Stream (elevation surveyed- m)	Stream Type	Date(s) Sampled	Reference
Kauai				
1	Hanalei (380 m)	1 (Undiverted)	Nov 1994	Polhemus (1995)
2	Kauaikimana (1035 m)	1 (Undiverted)	Aug 1997, Aug 1998, Jan 1999	Englund and Polhemus (2001)
3	Kawaikoi (1035 m)	1 (Undiverted)	Aug 1997, Aug 1998, Jan 1999	Englund and Polhemus (2001)
4	Koaoie (1175 m)	1 (Undiverted)	Aug 1997, Jan 1999, July 2000	Englund and Polhemus (2001)
5	Lumaha'i (430 m)	1 (Undiverted)	Nov 1994	Polhemus (1995)
6	Waiakoali (1035 m)	1 (Undiverted)	Aug 1997, Aug 1998, Jan 1999	Englund and Polhemus (2001)
7	Waialae (1095 m)	1 (Undiverted)	Jan 1999	Englund and Polhemus (2001)
O'ahu				
8	Manoa (5-75 m)	2 (Concrete Channel)	April 2004	Englund and Arakaki (2004)
9	Makiki (2 m)	2 (Concrete Channel)	April 2004	Englund and Arakaki (2004)
10	Makiki (45 m)	2 (Concrete Channel)	April 2004	Englund and Arakaki (2004)
11	Punaluu (31 m)	1 (Undiverted)	Nov 2002	Englund <i>et al.</i> (2003a)
12	Punaluu (275 m)	1 (Undiverted)	Nov 2002	Englund <i>et al.</i> (2003a)
13	Kawainui (Anahulu trib) (305 m)	1 (Undiverted)	Apr 2003	Englund <i>et al.</i> (2003a)
14	Kaluanui (100 m)	1 (Undiverted)	Nov 2002	Englund <i>et al.</i> (2003a)
15	Kaluanui (762 m)	1 (Undiverted)	Jan 1994	Englund <i>et al.</i> (2003a)
16	Waiekele (0-1 m)	3 (Channelized, no concrete)	Mar 1993, Dec 1997, Aug 1998	Englund and Filbert (1999), Englund <i>et al.</i> (2000)
17	Waiekele (381 m)	1 (Undiverted)	Mar 1993	Englund (1993)
18	Tripler Stream (79 m)	1 (Undiverted)	Mar 1995-Jan 2005	Evenhuis <i>et al.</i> (1995), Englund 2001
19	Waiahole (Waianu trib) (60 m)	1 (Undiverted)	Feb-Aug 1995, Feb, May, Nov 2002, Apr 2003	Filbert and Englund (1995), Englund <i>et al.</i> (2003b)
20	Waikane (210 m)	1 (Undiverted)	Feb-Aug 1995, Feb, Nov 2002, Mar 2003	Filbert and Englund (1995), Englund <i>et al.</i> (2003b)
21	Halawa (0-1 m)	2 (Concrete Channel)	Nov, Dec 1997, Mar-Aug 1998	Englund <i>et al.</i> (2000)
22	N. Halawa (300 m)	1 (Undiverted)	Jan 1991-Feb 1994	Polhemus (1994)

Table 1. (continued)

Site No.	Stream (elevation surveyed- m)	Stream Type	Date(s) Sampled	Reference
Moloka'i				
23	Pelekunu (0-1 m)	1 (Undiverted)	Jan 1991, Apr 2000, May 2001, May 2002	Englund & Arakaki (2003)
24	Pelekunu (182-237 m)	1 (Undiverted)	Apr 2000, May 2001, May 2002	Englund & Arakaki (2003)
25	Waialeia (0-60 m)	1 (Undiverted)	Nov 1998	Englund, unpublished data
Maui				
26	Kopihulu (610 m)	1 (Undiverted)	Jan 2003	Englund <i>et al.</i> (2003a)
27	Piinaau (731 m)	1 (Undiverted)	Jan 2003	Englund <i>et al.</i> (2003a)
28	W. Wailua Iki (493)	1 (Undiverted)	Jan 2003	Englund <i>et al.</i> (2003a)
29	Honokohau (450 m)	1 (Undiverted)	Jan 2003	Englund <i>et al.</i> (2003a)
Hawai'i				
30	Umauna (713 m)	1 (Undiverted)	Mar 2003	Englund <i>et al.</i> (2003a)
31	Hakalau (0-10 m)	1 (Undiverted)	Dec 1993	Polhemus (1995)
32	Honolii (536-640 m)	1 (Undiverted)	Feb 2002, Mar 2003	Englund <i>et al.</i> (2002), Englund <i>et al.</i> (2003a)
33	Wailuku (670 m)	1 (Undiverted)	Mar 2003	Englund <i>et al.</i> (2003a)
34	Wailoa (Waipio) (0-1 m)	4 (Diverted)	Mar 2001, 2003-2005 (quarterly)	Englund <i>et al.</i> (2001)
35	Hilawe (Waipio) (15 m)	1 (Undiverted)	Mar 2003-2005 (quarterly)	Englund <i>et al.</i> (2001)
36	Wailoa (Waipio) (190 m)	4 (Diverted)	Oct 1996, Nov 1998	Englund & Filbert (1997), Englund & Preston (1999)
37	Kawainui (Waipio) (425 m)	1 (Undiverted)	Oct 1996	Englund & Filbert (1997)
38	Waimanu (90 m)	1 (Undiverted)	Dec 1998	Englund & Preston (1999)
Kauai'i				
39	Kapa'a (80-120 m)	1 (Undiverted)	Nov 1994	Polhemus (1995)

also true for the indigenous fish species included. Their association with certain insect species, demonstrated by their correlation as “environmental variables” may serve as a surrogate for true environmental variables that were not directly measured. For each analysis, environmental variables that significantly affected variation in community structure were selected by Monte Carlo simulation (499 permutations), with the six best predictors selected automatically by CANOCO.

The results of these analyses provide extensive information about the communities analyzed (Leps & Smilauer, 2003). Sample sites are arranged in space (the ordination) based on similarity of insect communities; species are similarly arranged, and their proximity to sample sites and other species in the ordination are indicative of their association with sites, and other species. Environmental variables are plotted as vectors on the ordinations, each indicating the relative contribution it makes toward defining each axis plotted. The longer the vector, the greater the effect it has in explaining an environmental gradient; the smaller the angle between a vector and an axis, the more closely correlated that variable is with the gradient of points plotted. Finally, canonical correspondence axes (CCA) can be viewed as linear combinations of environmental variables along which insect community data are plotted according to similarity of species composition.

A primary objective of this study was to determine what insect species are typically associated with pristine or disturbed habitats. The availability of presence / absence data for fish and frogs in these habitats allowed us furthermore to assess the contribution that they might have on shaping insect communities, and also to determine whether any are specifically associated with pristine habitats.

Results

A list of species collected during this study can be found in the Appendix, along with a code number for each species as shown in Figs. 6–13. The ordination of all species in the data set, from all sites, showed that there were patterns along gradients, but these could not be clearly explained from the full data set (which produced a complex graph with many overlaid points, and no distinct patterns). To better understand the patterns within the data, each family of native insects was analyzed separately. The cumulative percentage of variation on species composition and species environment relationship for families and selected genera is shown in Table 2. Higher variance and species environment relationship accounted for with large, diverse taxonomic assemblages (e.g., Dolichopodidae) provide more robust and meaningful results when compared to smaller taxonomic assemblages such as Telmatogeton.

Coenagrionidae

Figure 6 shows the ordination for the native Coenagrionidae (*Megalagrion*) and the three introduced damselfly species (numbered 16, 17, and 18 on Fig. 6), where 84.5% of the species-environment relation was explained (Table 2) by the first three correspondence axes. This ordination clearly defines the sample sites along a gradient defined by “island” and “elevation”. Elevation was auto-correlated with stream type, and they are thus largely functionally equivalent in these analyses. The Kauai samples were grouped in a clearly defined cluster along CCA1, with the Hawai‘i samples at the other end of that spectrum, for a loosely defined cluster. O‘ahu, Moloka‘i and Maui are distributed along the gradient (Fig. 6). The second axis (CCA 2) was defined by introduced Mexican molly *Poecilia mexicana*, and the introduced bullfrog *Rana catesbeiana* and elevation. This may be interpreted as the Kauai sites being associated with highest elevation and absence of *P. mexicana* and *R. catesbeiana*; clearly there is a negative correlation of the presence of these two species and the absence of native *Megalagrion* damselflies. Of particular interest was the fact that the three introduced damselfly species *Ischnura posita*, *Ischnura ramburii*, and *Enallagma civile*, (numbers 16-18 on Fig. 6) were also closely clustered around axis of the introduced *P. mexicana* and the disturbed streams and sites associated with this fish species. These results were also encouraging as it indicates that our CCA results were sensitive at delineating communities of introduced taxa, even though all of these introduced damselflies are commonly caught with native *Megalagrion* damselflies.

It is interesting to note that O‘ahu streams at elevations lower than 200 m.a.s.l. were clustered

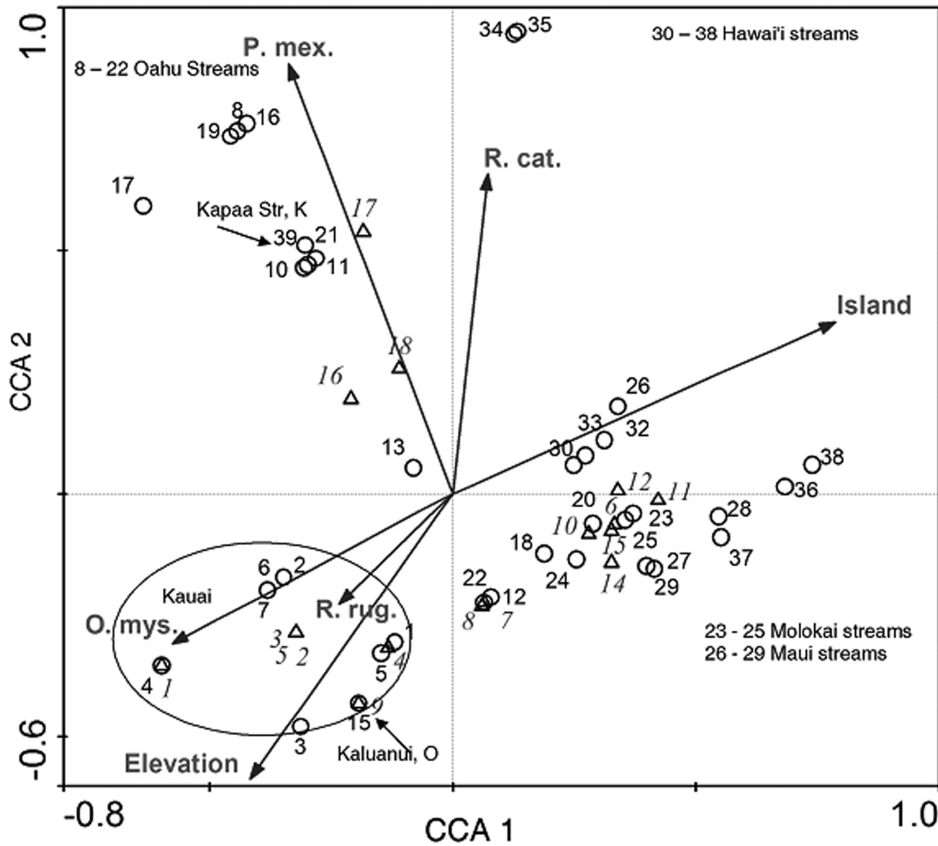


Figure 6. Canonical correspondence (CCA) ordination of sites and species-environment relationship for native Coenagrionidae (*Megalagrion*).

in a distinct group, and that Kapa‘a Stream, Kaua‘i (site 39) was grouped with them, rather than with the other Kaua‘i streams. In contrast, Kaluanui Stream (O‘ahu, 762 m.a.s.l.) was grouped with the Kaua‘i sample sites (Fig. 6). Indeed, most higher elevation sites from O‘ahu, such as Kawainui (upper Anahulu) (site 13), Waikane (site 20), and North Halawa (site 22), had greater similarity with less disturbed islands than low elevation sites on O‘ahu. A number of species such as *Megalagrion eudytum*, *M. heterogamias*, *M. oresitrophum*, *M. orobates*, and *M. vagabundum* (all Kauai endemics) were closely associated with the pristine sites on Kaua‘i.

Dolichopodidae

“Island” and elevation were the major determinates of CCA 1 for these aquatic flies (Fig. 7) with the high elevation Kaua‘i sites forming a distinct group, O‘ahu also distinct, and the other islands showing a spread along the axis. CCA 2 was largely a function of the presence of indigenous fish, depending on their presence or absence. While not as high damselflies, 65% of the cumulative variance in species composition (Table 2) was explained by the first three correspondence axes. This ordination clearly identifies Dolichopodidae as being effective indicators of stream quality, for example, there are certain species associated with the Kaua‘i sites that could perform such a function. What was especially striking, was the occurrence of indigenous fish species being correlated with certain Dolichopodidae species, particularly for the Moloka‘i, Maui, and Hawai‘i sample sites. Of great

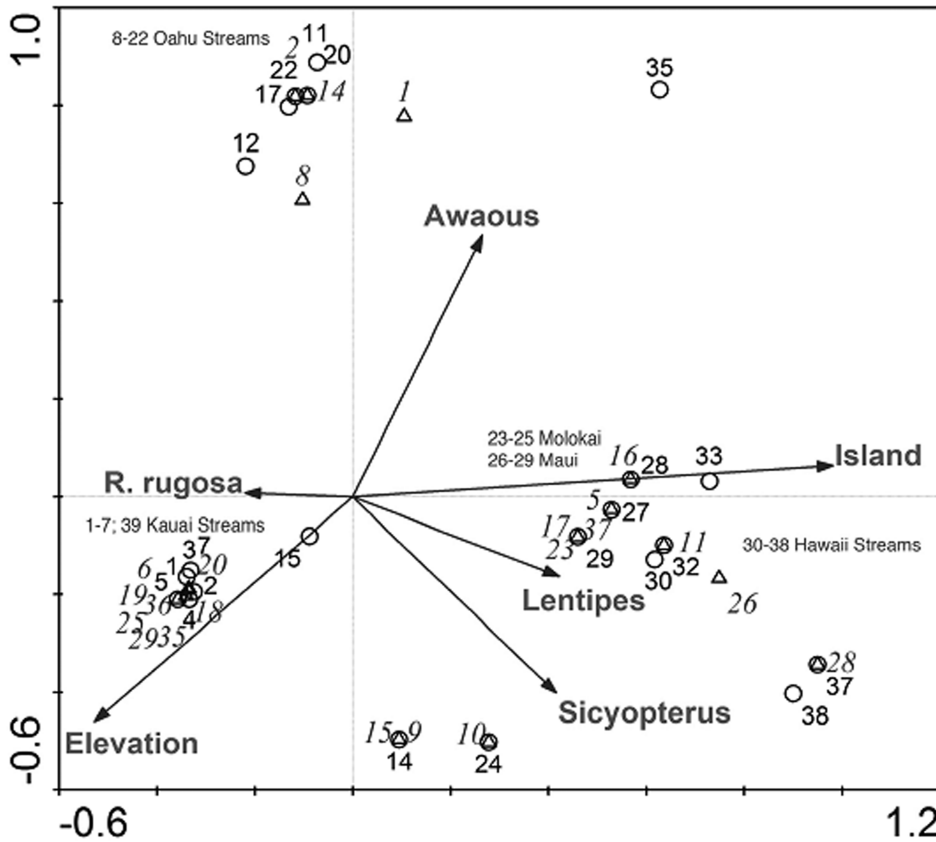


Figure 7. Native Dolichopodidae (all taxa) sites and species-environment relationship using CCA (CCA1 vs. CCA2).

interest was that the top three fish species associated with the Dolichopodidae were the 3 native stream species; *Lentipes concolor*, *Awaous guamensis*, and *Sicyopterus stimpsoni* (Figure 7).

Chironomidae

The ordination for this family of aquatic flies provided 70.4% explanation of variability by the first two axes, with all environmental variables retained (Fig. 8, Table 2). The resolution of this ordination is relatively high; however, it shows strong associations of these flies with alien taxa (Fig. 8). If the analysis was reduced to only the genus *Telmatogeton*, and excluding crustaceans as environmental variables, yet adding indigenous fish, the ordination (Fig. 9) shows clear separation of samples by “island”, and strong associations of *Telmatogeton abnormis*, *T. fluviatilis*, *T. hirtus*, and *T. williamsi* with indigenous fish species (e.g. *Lentipes concolor*).

Ephydriidae

The ordination (CCA1 vs. CCA2) (Fig. 10) was severely skewed by sample site 21 (Halawa Stream at Pearl Harbor) and the native *Atissa oahuensis* (species 99) (Fig. 11); CCA 2 and 3 accounted for 83.1% of the variation (Table 2), with the samples forming groups defined primarily by “island”. However, it would appear that the Ephydriidae may be less responsive to the environmental variables we examined. Site 39 (Kapa’a Stream, an impacted, low elevation stream), for example, is included with other Kaua’i sites, which from an overall indicator species perspective offers little in terms of

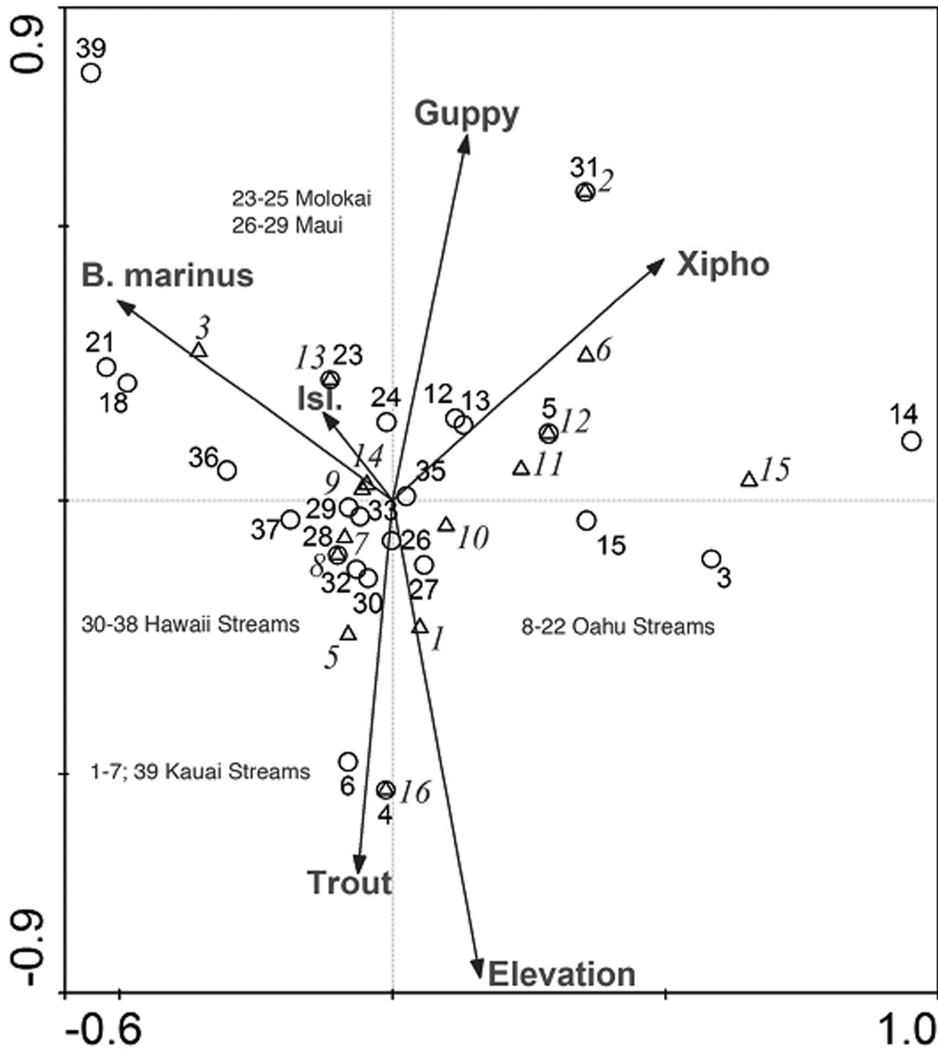


Figure 8. Native Chironomidae (all taxa) sites and species-environment relationship using CCA (CCA1 vs. CCA2).

identifying impacted habitats. It was also of interest to examine the genus *Scatella* because it is one of most dominant native aquatic insect groups in Hawaiian streams. CCA 2 and 3 accounted for 82.8% of the variation (Fig. 11, Table 2), thus *Scatella* by itself is responsive to environmental variables. They were, however, most strongly associated with alien fish and amphibian species, and low elevation native fish species. In contrast to the native species where environmental associations were not always clear, certain introduced ephydriids were clearly associated with disturbed environments, such as *Placopsidella marquesana*, *Scatella stagnalis*, and *Donaceus nigronotatus*.

Canacidae

Because of their association with torrenticolous habitats it was hypothesized that the endemic genus *Procanace* would be sensitive to disturbed habitats or introduced aquatic taxa. The ordination for this family provided 86.5% explanation (Table 2) of variability by the first two axes, with all environ-

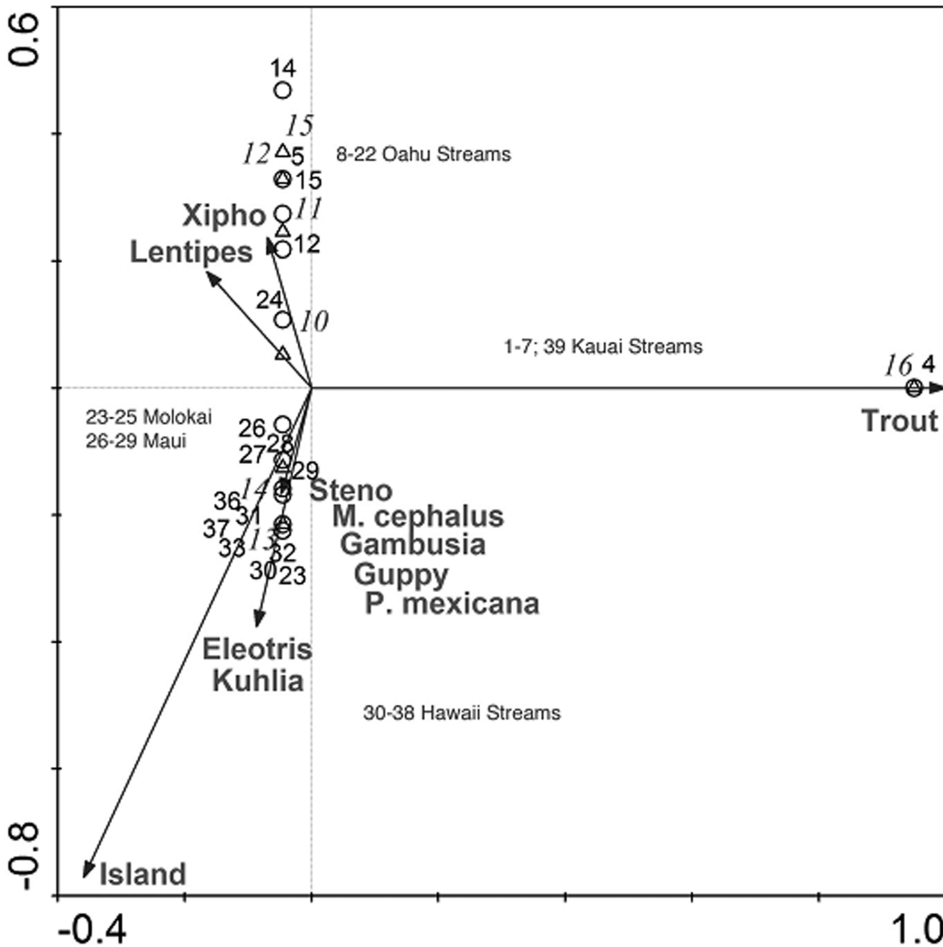


Figure 9. Native Chironomidae (*Telmatogeton* spp. only) sites and species-environment relationship using CCA (CCA1 vs. CCA2).

mental variables retained (Fig. 12). Running CCA for only the genus *Procanace* increased the level of variability to 92.3% (Fig. 13, Table 2), with good resolution by island, and accounted for relatively strong associations with indigenous fish species.

Amphibian Impacts

Hawai'i currently has three species of introduced aquatic amphibians, *Bufo marinus*, *Rana catesbeiana*, and *R. rugosa*. Of greatest concern according to CCA analysis was *R. catesbeiana*, with the other two species showing little impact in regard to native insect taxa. This is because *B. marinus* is found in mainly highly disturbed low elevation areas, while *R. rugosa* is found in high elevation areas and is often co-associated with endemic aquatic insects. As shown on Fig. 6, *R. catesbeiana* was associated with *Poecilia mexicana* in degraded habitats, while *R. rugosa* was by contrast usually found in high quality habitats on Kaua'i and O'ahu (see Figs. 6 or 12), and likely because of its small size is having little impact on dolichopodid Diptera or native damselflies, except perhaps to exclude certain species of the latter from preferred fast water breeding sites with its gelatinous egg masses.

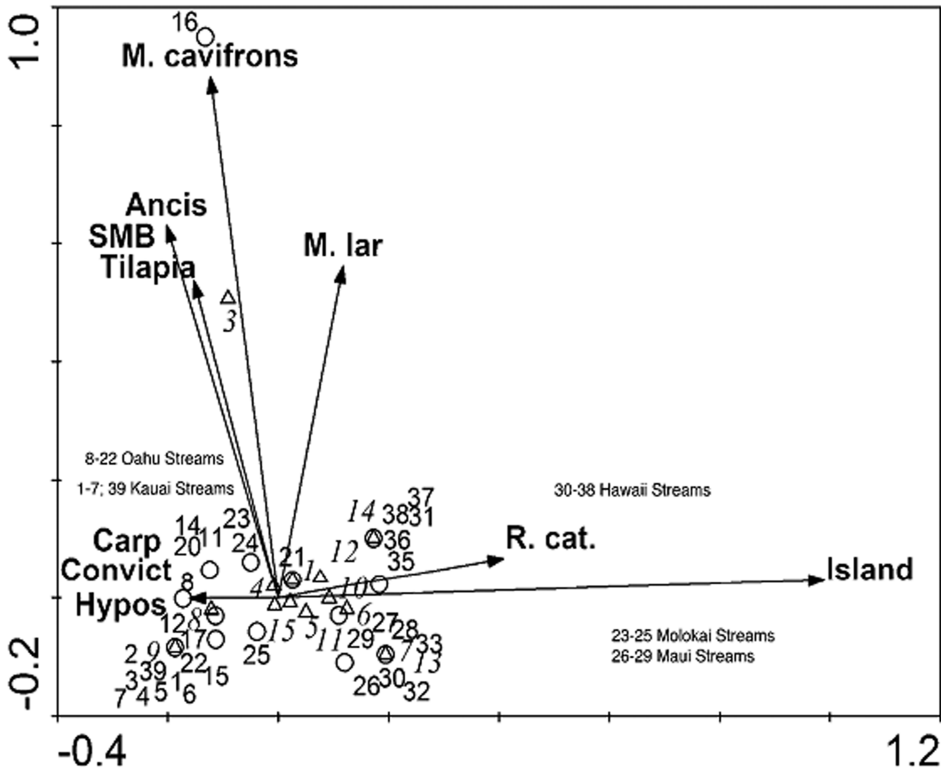


Figure 10. Native Ephydriidae site and species-environment relationship using CCA (CCA2 vs. CCA3).

Discussion

These findings represent the first attempt at elucidating statistical associations of native aquatic insect faunas with environmental variables such as alien fish species, elevation, and stream disturbance. Our results have also allowed us to explore relationships between native aquatic insects and indigenous stream fish. The primary objective of this study was determine what, if any, species of aquatic insects are associated with pristine or disturbed habitats. A significant finding was that at least two groups, the native *Megalagrion* damselflies and dolichopodid flies, exhibited statistical relationships that appear to reflect correlations with disturbed and undisturbed environments (Figs. 6 and 7). Several aquatic insect families also exhibited obvious groupings, with sites from Kaua'i and O'ahu often clustered together, while Maui and Moloka'i sites often grouped together with the Hawai'i sites, as shown in Figs. 6 and 7. That these patterns may reflect the evolutionary history of the *Megalagrion* and dolichopodid species is of great future research interest; as is the fact that these patterns also show consistency in identifying sites with similar levels of impact among the different islands. *Telmatogeton* spp. was another assemblage of taxa showing clear separation by islands and strong associations with native fish taxa such as *Lentipes concolor*.

These findings then lend credence that *Megalagrion* damselflies, dolichopodid flies, *Procanace* spp., and *Telmatogeton* spp. (giant Hawaiian midges) are all suitable as indicator species for diverse aquatic habitats worthy of preservation and conservation attention. Ephydriidae also had high resolution, but this associated with disturbance rather than with pristine conditions. At the family level, dam-

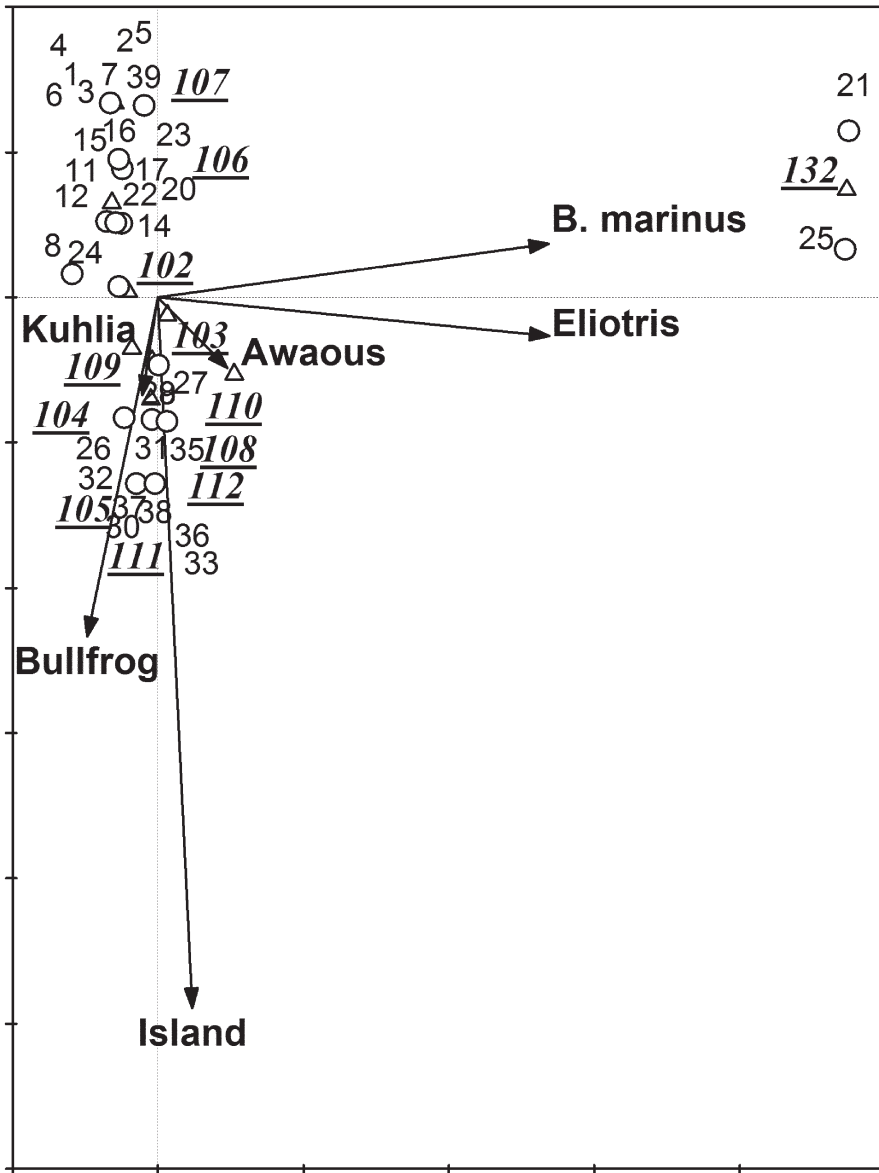


Figure 11. Native Ephydriidae (*Scatella* spp. only) species-environment relationship using CCA.

selflies and canacid flies received the highest species-environment relationship score (84.5%) for the CCA. Because Hawaiian damselflies have a larger species assemblage than canacids (18 vs. 12 species analyzed here) their results are more meaningful than canacids, suggesting that damselflies have the most easily detected sensitivity of the aquatic insect taxa we assessed, and show the clearest patterns in community composition and responses to environmental factors. Odonata are well known to the public because of their large size and stunning appearance, and because of this would certainly qualify as the most charismatic of the aquatic insects in Hawaii, and thus could also be considered a flag-

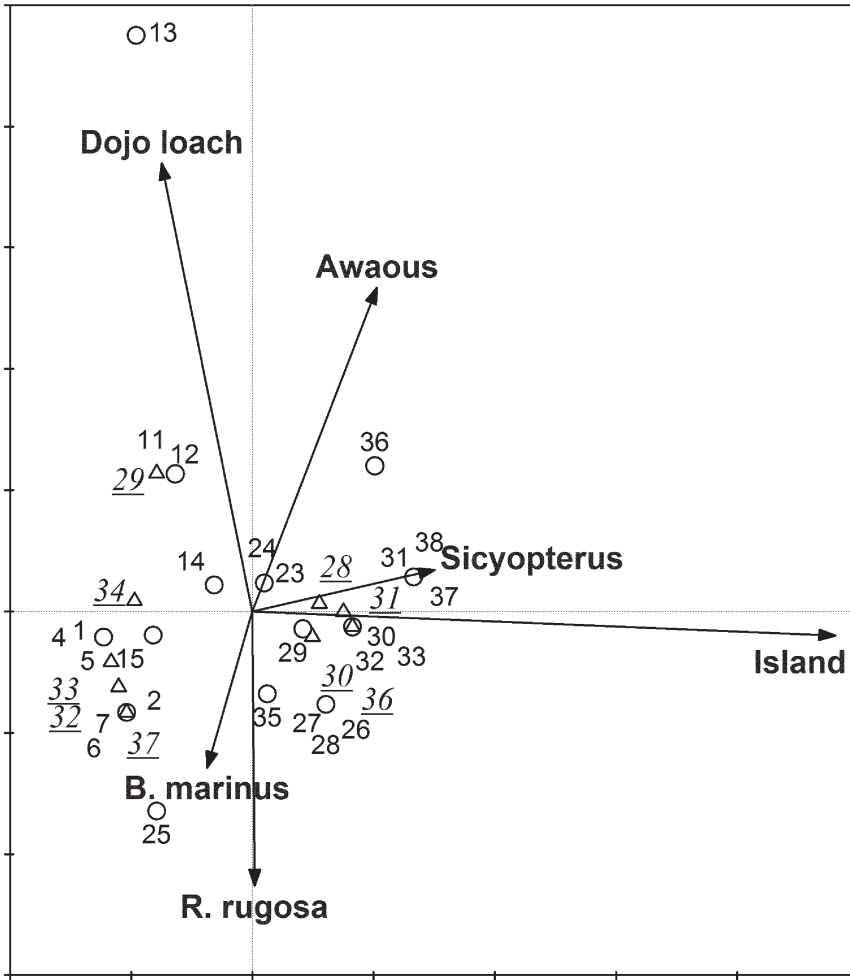


Figure 12. Native Canacidae (all taxa) site and species-environment relationship using CCA (CCA1 vs. CCA2).

ship species (Andelman & Fagan, 2000). On a more controversial note, our data suggest that Hawaiian damselflies would fall under the dual role of an umbrella species (Andelman & Fagan, 2000), or species defined as requiring such large areas of habitat that their protection might simultaneously protect other aquatic species. Because native damselflies will only be found in areas with little disturbance, this would in turn lead to healthy populations of native stream fish species being found in the same area. In contrast, ephydriids and all chironomids had well defined axes and groupings associated with disturbed habitats in our analyses (Figs. 10–13), suggesting these species are more resistant to both a disturbed environment and alien aquatic species, and are thus not good indicator candidates for pristine conditions. One of the weaknesses of the current study, which used presence / absence data rather than abundance data. The availability of abundance data would make the CCA considerably more robust. Nonetheless, the analyses provide credible characterizations of the streams surveyed.

Field observations indicate that *Telmatogeton* spp. are now found only in exceedingly pristine, high volume, and high water quality environments. Because of these requirements and the prevalence of water diversions on Hawaiian streams *Telmatogeton* are now difficult to find in the

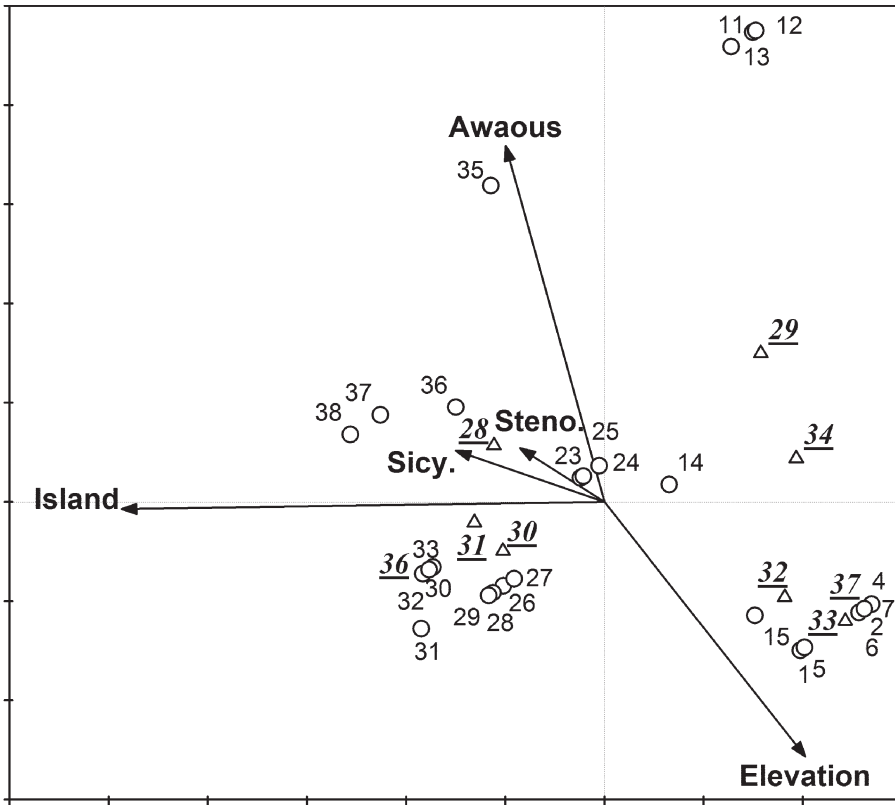


Figure 13. Native Canacidae (*Procanace* spp. only) species-environment relationship using CCA.

Hawaiian islands and are becoming increasingly rare, and for example, this genus is now found in only 4 of 57 streams on O‘ahu (Englund & Polhemus, unpubl. data). The current rarity and naturally low species richness (7 spp.) in the genus *Telmatogeton* resulted in an inflated degree of variance accounted for; rarity of species in this genus precludes them from being an effective indicator species. These giant Hawaiian chironomids may not be as charismatic as the Hawaiian damselflies, they are easy for untrained observers to identify in the field because of their large and distinctive white larval cases on stream boulders, and hence could make an ideal suite of indicator species if they were more common.

The conservation community has recently had heated debates on the various conceptual and practical values of indicator, umbrella, flagship, and keystone species (Simberloff, 1998; Andelman & Fagan, 2000; Rubinoff, 2001) when it comes to the assessment and preservation of biodiversity. In the Hawaiian Islands there has also been some degree of controversy, with inappropriate attempts to use Index of Biotic Integrity (IBI), developed for continental salmonid streams, to rank and assess Hawaiian streams (Parham, 2005). The shortcomings of the use of IBI in tropical insular streams with low natural fish diversity were well recently documented (Parham, 2005), but further problems exist with IBI as used in Hawai‘i in that native aquatic insects are excluded from the metrics. Thus, in Hawai‘i the dominant component of native aquatic biodiversity, the 400+ species of native aquatic insects, have been overlooked. Our findings that certain native insect taxa such as the *Megalagrion* damselflies, canacid, and dolichopodid flies are correlated with the presence of native

Table 2. Cumulative percentage of variation in species-environment and species composition explained by correspondence axes 1–3 by family or genus.

Insect family / taxon (# spp. included in analysis)	% Variance account for by first three axes	
	Spp.-environment	Species only
Coenagrionidae (18)	84.5	30.4
Dolichopodidae (38)	65.0	22.9
Chironomidae (16)	70.4	26.3
Canacidae (12)	84.5	32.7
Ephydriidae (15)	83.1	40.9
<i>Telmatogeton</i> (genus level) (7)	78.9	54.3
<i>Procanace</i> (genus level) (10)	92.3	29.5
<i>Scatella</i> (genus level, native only) (14)	82.8	40.1

indigenous stream fish indicates that any assessment of native streams should necessarily be conducted in a more holistic fashion than has been practiced with IBI in Hawai'i (e.g., Parham, 2005).

While the indicator species concept has received considerable criticism because it is both difficult to determine which species are the best indicators, or even what a species should indicate (Simberloff, 1998), we feel the indicator concept still has value for Hawaiian streams, especially in light of our findings from the present study indicating certain native aquatic insect taxa are sensitive to physical disturbance and alien species. For example, with funding for habitat conservation measures likely to remain at a low level, these findings can be used to identify taxa and stream areas that have high conservation value, thus prioritizing allocation of resources. In this case, we define areas of high conservation value as Hawaiian streams and adjacent wetlands with high biodiversity of native aquatic taxa. The presence of native species from the highly diverse groups such as dolichopodid and *Megalagrion* damselflies in a Hawaiian stream indicates that the stream has not been greatly disturbed by alien species or physically altered. In addition, if these two groups of taxa are present it usually means that many endemic and indigenous species will be co-associated with them, and that there will often be healthy populations of native stream fish as well.

We therefore conclude that for the highly endemic and diverse aquatic insect fauna in Hawaiian streams the indicator species concept still has value. Until now, most attention and resources have been focused on freshwater fish as indicators (Parham, 2005), but our results indicate the nearly exclusive use of native Hawaiian stream fish as indicator species in models such as is the current practice with IBI in Hawai'i should be re-examined.

Our results indicate that there are certain advantages to using certain aquatic insect taxa as indicators for highly diverse Hawaiian aquatic habitats, and streams that maintain these indicator species should have a high conservation priority. Although the use of aquatic insects as indicator species in Hawaiian streams has both advantages and drawbacks (Table 3) as compared to native fish, advantages include greater specificity and increased sensitivity to external disturbances.

While data for this research of necessity was collected in a species presence or absence format, future directions in Hawaiian aquatic insect research could focus on developing techniques to further quantify specific aquatic insect populations. This study is the first to shed light on the fact that Hawaiian aquatic insects and native stream fish populations are closely linked, yet we are only just beginning to understand the relationships between different groups of native aquatic insects, let alone the interactions between stream fish and insects. Two major obstacles remain in obtaining quantitative data on native Hawaiian aquatic insect populations, taxonomic and ecological. Most of the taxonomic descriptions and illustrations of native aquatic insect taxa have been from the adult aerial stage, and few systematic larval descriptions exist for most taxa. Even some of the well-studied groups such as the genus *Megalagrion* have numerous undescribed larval stages. Very few descriptions exist for the other aquatic insect groups, and some taxa such as the diverse Chiro-

Table 3. Summary of advantages and disadvantages of using native aquatic insects versus native freshwater fish as species for monitoring the health of an aquatic ecosystem.

Taxa	Advantages	Disadvantages
Fish	<ol style="list-style-type: none"> 1. Easily identifiable 2. Charismatic species 3. Culturally important 	<ol style="list-style-type: none"> 1. Open system: impacts outside watershed have great influence 2. Broad habitat preferences (less sensitive to disturbance) 3. Found only at lower elevations (900 m max) 4. Usually not above diversions/dams 5. Migratory: impacts outside watershed influence Population 6. Only 5 species
Insects	<ol style="list-style-type: none"> 1. Closed system: impacts outside of watershed have no influence 2. Certain groups easily identifiable 3. Charismatic species (a few) 4. Narrow habitat Preferences 5. More sensitive to disturbance 6. Found above diversions 7. Occurrence correlated with indigenous fish 8. 400+ species 	<ol style="list-style-type: none"> 1. Many groups difficult to identify (taxonomic knowledge required) 2. 400+ species

nomidae are taxonomically quite difficult in the larval stage. With the exception of the *Megalagrion* damselflies, most native aquatic insects evolved in wave-swept marine habitats and have secondarily invaded and radiated into freshwater habitats (Howarth & Polhemus, 1991). These native insects are almost exclusively then found in torrenticolous riffle and cascade habitats, which are difficult to quantify with benthic enumeration devices such as a Surber or Hess sampler. Future research should be directed at further refining quantitative sampling methods for such taxa. For instance, new technologies such as DNA extraction from larval aquatic insects, statistically sound methods of collecting adults, and new methods to sample torrenticolous habitats would increase our knowledge of this highly endemic fauna, thus helping to ensure its ultimate preservation.

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APPENDIX

Biota found during this study and their native or introduced status. [Status taken from Yamamoto (2000) and Nishida (2002).]

Taxa	Species (Ind = Indigenous; End = Endemic; Int = Introduced)	Species Number on Figures 6–13
Native (Endemic)		
Aquatic Insects		
Odonata		
Aeshnidae	<i>Anax strenuus</i> (End)	1
Libellulidae	<i>Nesogonia blackburni</i> (End)	2
Coenagrionidae	<i>Megalagrion eudytum</i> (End)	3
	<i>Megalagrion heterogamias</i> (End)	4
	<i>Megalagrion oresitrophum</i> (End)	5
	<i>Megalagrion orobates</i> (End)	6
	<i>Megalagrion vagabundum</i> (End)	7
	<i>Megalagrion hawaiiense</i> (End)	8
	<i>Megalagrion leptodemas</i> (End)	9
	<i>Megalagrion nigrohamatum nigrolineatum</i> (End)	10
	<i>Megalagrion oceanicum</i> (End)	11
	<i>Megalagrion xanthomelas</i> (End)	12
	<i>Megalagrion blackburni</i> (End)	13
	<i>Megalagrion calliphya</i> (End)	14
	<i>Megalagrion nesioties</i> (End)	15
	<i>Megalagrion nigrohamatum nigrohamatum</i> (End)	16
	<i>Megalagrion oceanicum</i> (End)	17
Heteroptera		
Nabidae	<i>Nabis gagneorum</i> (End)	18
	<i>Saldula exulans</i> (End)	19
Saldidae	<i>Saldula oahuensis</i> (End)	20
	<i>Saldula procellaris</i> (End)	21
Veliidae	<i>Microvelia vagans</i> (End)	22
Coleoptera		
Dytiscidae	<i>Rhantus pacificus</i> (End)	23
Hydrophilidae	<i>Limnoxenus semicylindricus</i> (End)	24
Lepidoptera		
Cosmopterigidae	<i>Hyposmocoma</i> sp. (End)	25
	<i>Hyposmocoma</i> sp. nr <i>montivolans</i> (End)	26
	<i>Hyposmocoma</i> sp. nr <i>saccophora</i> (End)	27
Diptera		
Canacidae	<i>Procanace acuminata</i> (End)	28
	<i>Procanace bifurcata</i> (End)	29
	<i>Procanace confusa</i> (End)	30
	<i>Procanace constricta</i> (End)	31
	<i>Procanace nigroviridis</i> (End)	32
	<i>Procanace quadrisetosa</i> (End)	33
	<i>Procanace wirthi</i> (End)	34
	<i>Procanace</i> new sp. 1 (End) (Oahu - Rare Alien Survey)	35
	<i>Procanace</i> new sp. 1 (End) (Hawaii koa timber survey)	36
	<i>Procanace</i> sp. (End)	37

Taxa	Species (Ind = Indigenous; End = Endemic; Int = Introduced)	Species Number on Figures 6–13	
Native (Endemic)			
Aquatic Insects			
Ceratopogonidae	<i>Dasyhelea digna</i> (End)	38	
	<i>Dasyhelea hawaiiensis</i> (End)	39	
	<i>Dasyhelea</i> sp. (not <i>hawaiiensis</i>) (End)	40	
	<i>Dasyhelea platychaeta</i> (End)	41	
	<i>Dasyhelea</i> sp. (End)	42	
	<i>Forcipomyia hardyi</i> (End)	43	
	<i>Forcipomyia kaneohe</i> (End)	44	
	<i>Forcipomyia</i> sp. (End)	45	
	Chironomidae	<i>Chironomus</i> sp. (End)	46
		<i>Chironomus hawaiiensis</i> (End)	47
		<i>Clunio</i> sp. nr. <i>vagrans</i> (End)	48
		<i>Micropsectra</i> sp. (End)	49
		<i>Micropsectra hawaiiensis</i> (End)	50
		<i>Orthocladius</i> sp. (End)	51
		<i>Orthocladius grimshawi</i> (End)	52
		<i>Pseudosmittia paraconjuncta</i> (End)	53
		<i>Telmatogeton abnormis</i> (End)	54
		<i>Telmatogeton fluviatilis</i> (End)	55
		<i>Telmatogeton hirtus</i> (End)	56
		<i>Telmatogeton japonicus</i> (End)	57
<i>Telmatogeton torrenticola</i> (End)		58	
<i>Telmatogeton williamsi</i> (End)		59	
<i>Telmatogeton</i> sp. (End)	60		
Dolichopodidae	<i>Campsicnemus brevipes</i> (End)	61	
	<i>Campsicnemus gloriosus</i> (End)	62	
	<i>Campsicnemus labilis</i> (End)	63	
	<i>Campsicnemus lepidochaites</i> (End)	64	
	<i>Campsicnemus longitibia</i> (End)	65	
	<i>Campsicnemus nigricollis</i> (End)	66	
	<i>Campsicnemus modicus</i> (End)	67	
	<i>Campsicnemus miritibialis</i> (End)	68	
	<i>Campsicnemus patellifer</i> (End)	69	
	<i>Campsicnemus ridiculus</i> (End)	70	
	<i>Campsicnemus tibialis</i> (End)	71	
	<i>Campsicnemus</i> nr. <i>truncatus</i> (End)	72	
	<i>Campsicnemus williamsi</i> (End)	73	
	<i>Campsicnemus</i> sp. (End)	74	
	<i>Campsicnemus</i> new sp. 1 (End) (Oahu- Rare Alien Surveys)	75	
	<i>Campsicnemus</i> new sp. 2 (End) (Maui - Rare Alien Surveys)	76	
	<i>Campsicnemus</i> new sp. 3 (End) (Maui- Rare Alien Surveys)	77	
	<i>Campsicnemus lawakua</i> (End) Kokee	78	
	<i>Eurynogaster mediocris</i> (End)	79	
	<i>Major minor</i> (End)	80	
	<i>Elmoia multispinosa</i> (End)	81	
	“ <i>Eurynogaster</i> ” sp. (End)	82	
	“ <i>Eurynogaster</i> ” new sp. (End) (Maui - Rare Alien Surveys)	83	
	“ <i>Eurynogaster</i> ” new sp. (End) (Koa Timber survey -Hawaii)	84	
	<i>Paralicanthus metallicus</i> (End)	85	
	<i>Sigmatineurum englundii</i> (End)	86	
	<i>Sigmatineurum iao</i> (End)	87	
	<i>Sigmatineurum meaohi</i> (End)	88	
<i>Sigmatineurum napali</i> (End)	89		
<i>Sigmatineurum nigrum</i> (End)	90		

Taxa	Species (Ind = Indigenous; End = Endemic; Int = Introduced)	Species Number on Figures 6–13
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Native (Endemic)**Aquatic Insects**

Dolichopodidae (cont.)

	<i>Sigmatineurum parenti</i> (End)	91
	<i>Sigmatineurum mnemogagne</i> (End) (Hanawi)	92
	<i>Sigmatineurum omega</i> (End)	93
	<i>Sigmatineurum puleloai</i> (End) (Moloka'i)	94
	<i>Sigmatineurum</i> n. sp. 1 (End) Koke'e	95
	<i>Sigmatineurum</i> n. sp. 2 (End) Koke'e	96
	<i>Sigmatineurum</i> n. sp. (End) (Maui - Rare Alien Surveys)	97
	<i>Thambemyia acrostichalis</i> (End) (formerly <i>Paraphrosylus</i> sp.)	98
Ephydriidae	<i>Atissa oahuensis</i> (End)	99
	<i>Hydrellia tritici</i> (End)	100
	<i>Notiphilia insularis</i> (End)	101
	<i>Scatella cilipes</i> (End)	102
	<i>Scatella clavipes</i> (End)	103
	<i>Scatella (Apulvillus) femoralis</i> (End)	104
	<i>Scatella fluvialis</i> (End)	105
	<i>Scatella hawaiiensis</i> (End)	106
	<i>Scatella kauaiensis</i> (End)	107
	<i>Scatella (Apulvillus) mauiensis</i> (End)	108
	<i>Scatella oahuense</i> (End)	109
	<i>Scatella warreni</i> (End)	110
	<i>Scatella williamsi</i> (End)	111
	<i>Scatella</i> new sp. (End) (Waimanu)	112
Muscidae	<i>Lispe</i> sp. (End)	113
	<i>Lispocephala</i> new sp. 1 (End) (Maui - Rare Alien Surveys)	114
	<i>Lispocephala</i> new sp. 2 (End) (Maui - Rare Alien Surveys)	115
Psychodidae	<i>Psychoda</i> sp. (End)	116
	<i>Trichomyia hawaiiensis</i> (End)	117
	<i>Trichomyia</i> sp. (End)	118
Limoniidae	<i>Dicranomyia hawaiiensis</i> (End)	119
	<i>Dicranomyia grimshawi</i> (End)	120
	<i>Dicranomyia jacobae</i> (End)	121
	<i>Dicranomyia kauaiensis</i> (End)	122
	<i>Dicranomyia nigropolita</i> (End)	123
	<i>Dicranomyia perkinsi</i> (End)	124
	<i>Dicranomyia swezeyi</i> (End)	125
	<i>Dicranomyia stygipennis</i> (End)	126
	<i>Dicranomyia</i> sp. (End)	127
	<i>Gonomyia</i> sp. (End)	128

Native (Indigenous)**Insects**

Odonata

Aeshnidae	<i>Anax junius</i> (Ind)	129
Libellulidae	<i>Pantala flavescens</i> (Ind)	130

Diptera

Chironomidae	<i>Chironomus esakii</i> (Ind)	131
Ephydriidae	<i>Scatella sexnotata</i> (Ind)	132
Tethinidae	<i>Dasyrhicnoessa insularis</i> (Ind)	133
	<i>Dasyrhicnoessa</i> (Ind)	134
	<i>Dasyrhicnoessa vockerothi</i> (Ind)	135

Taxa	Species (Ind = Indigenous; End = Endemic; Int = Introduced)	Species Number on Figures 6–13
Introduced Aquatic		
Insects		
Odonata		
Libellulidae	<i>Tramea abdominalis</i> (Int)	136
	<i>Tramea lacerata</i> (Int)	137
	<i>Crocothemis servilia</i> (Int)	138
	<i>Orthemis ferrugenia</i> (Int)	139
Coenagrionidae	<i>Ischnura posita</i> (Int)	140
	<i>Ischnura ramburii</i> (Int)	141
	<i>Enallagma civile</i> (Int)	142
Heteroptera		
Mesoveliidae	<i>Mesovelia amoena</i> (Int)	143
	<i>Mesovelia mulsanti</i> (Int)	144
Notonectidae	<i>Notonecta indica</i> (Int)	145
	<i>Buenoa pallipes</i> (Int)	146
Coleoptera		
Dytiscidae	<i>Rhantus guttulatus</i> (Int)	147
	<i>Copelatus parvulus</i> (Int)	148
Hydrophilidae	<i>Tropisternus lateralis</i> (Int)	149
Diptera		
Canacidae	<i>Procanace williamsi</i> (Int)	150
	<i>Canaceioides angulatus</i> (Int)	151
Ceratopogonidae	<i>Forcipomyia</i> sp. (Int)	152
	<i>Atrichopogon jacobsoni</i> (Int)	153
Chironomidae	<i>Cricotopus bicinctus</i> (Int)	154
	<i>Polypedilum nubiferum</i> (Int)	155
Dixidae	<i>Dixa longistyla</i> (Int)	156
Dolichopodidae	<i>Condylostylus longicornis</i> (Int)	157
	<i>Chrysosoma globiferum</i> (Int)	158
	<i>Chrysotus longipalpus</i> (changed from <i>pallidipalpus</i>) (Int)	159
	<i>Chrysotus</i> sp. 1 (Int)(Waipio)	160
	<i>Dolichopus exsul</i> (Int)	161
	<i>Pelastoneurus lugubris</i> (Int)	162
	<i>Syntormon flexibile</i> (Int)	163
	<i>Tachytrechus angustipennis</i> (Int)	164
	<i>Thinophilus hardyi</i> (Int)	165
Empididae	<i>Hemerodromia stellaris</i> (Int)	166
Ephydriidae	<i>Brachydeutera ibari</i> (Int)	167
	<i>Ceropsilopa coquilletti</i> (Int)	168
	<i>Discocerina mera</i> (Int)	169
	<i>Hecamede granifera</i> (Int)	170
	<i>Hydrellia williamsi</i> (Int)	171
	<i>Donaceus nigronotatus</i> (Int)	172
	<i>Lytogaster gravaida</i> (Int)	173
	<i>Ochthera circularis</i> (Int)	174
	<i>Paratissa pollinosa</i> (Int)	175
	<i>Placopsidella marquesana</i> (Int)	176
	<i>Typopsilopa</i> sp. (Int)	177
	<i>Scatella stagnalis</i> (Int)	178
Muscidae	<i>Lispe assimilis</i> (Int)	179

Taxa	Species (Ind = Indigenous; End = Endemic; Int = Introduced)	Species Number on Figures 6–13
Introduced Aquatic		
Insects		
Diptera (cont.)		
Psychodidae	<i>Clogmia albipunctata</i> (Int)	180
Sciomyzidae	<i>Sepedon aenescens</i> (Int)	181
Tethinidae	<i>Tethina variseta</i> (Int)	182
Limoniidae	<i>Dicranomyia advena</i> (Int)	183
	<i>Erioptera bicornifer</i> (Int)	184
Trichoptera		
Hydropsychidae	<i>Cheumatopsyche analis</i> (Int)	185
Hydroptilidae	<i>Hydroptila icona</i> (Int)	186
	<i>Hydroptila potosina</i> (Int)	187
	<i>Oxyethira maya</i> (Int)	188
Fish		
	<i>Lentipes concolor</i> (End)	189
	<i>Sicyopterus stimpsoni</i> (End)	190
	<i>Awaous guamensis</i> (Ind)	191
	<i>Stenogobius hawaiiensis</i> (End)	192
	<i>Eleotris sandwicensis</i> (End)	193
	<i>Mugil cephalus</i> (Ind)	194
	<i>Kuhlia xenura</i> (End)	195
	<i>Gambusia affinis</i> (Int)	196
	<i>Poecilia reticulata</i> (Int)	197
	<i>Poecilia mexicana</i> (Int)	198
	<i>Poecilia latipinna</i> (Int)	199
	<i>Limia vittata</i> (Int)	200
	<i>Poecilia</i> (misc) spp. (Int)	201
	<i>Xiphophorus helleri</i> (Int)	202
	<i>Micropterus dolomieu</i> (Int)	203
	<i>Oncorhynchus mykiss</i> (Int)	204
	<i>Misgurnus anguillicaudatus</i> (Int)	205
	<i>Mugilogobius cavifrons</i> (Int)	206
	<i>Tilapia/Oreochromis</i> spp. (Int)	207
	<i>Cichlasoma managuense</i> (Int)	208
	<i>Archocentrus</i> (<i>Cichlasoma</i>) <i>nigrofasciatus</i> (Int)	209
	<i>Hemichromis elongatus</i> (Int)	210
	<i>Melanochromis johanni</i> (Int)	211
	<i>Hypsophrys nicaraguensis</i> (Int)	212
	<i>Amphilophus citrinellum</i> (Int)	213
	<i>Ancistris temminicki</i> (Int)	214
	<i>Hypostomous watwata</i> (Int)	215
	<i>Cyprinus carpio</i> (Int)	216
Amphibians		
	<i>Bufo marinus</i> (Int)	217
	<i>Rana catesbeiana</i> (Int)	218
	<i>Rana rugosa</i> (Int)	219
Crustaceans		
	<i>Procambarus clarkii</i> (Int)	220
	<i>Macrobrachium lar</i> (Int)	221