

## Appendix A. Evaluation of Replicate, Re-sort, and Repeat Samples of Macroinvertebrates Collected from Streams on Maui, Hawai'i, 2009-2010

Multivariate statistical analyses were used to evaluate the results of the replicate, re-sort, and repeat samples collected on Maui (figs. A1–A2). Nonmetric multidimensional scaling (nMDS) was used to examine the relations among the samples (Clarke, 1993). These relations were assessed using the abundance (organisms/m<sup>2</sup>) dataset and the relative abundance (proportional) dataset. Abundance data were  $\log(x + 1)$  transformed before the analysis, and the proportional data were arcsine–square root transformed before the analysis. Hierarchical agglomerative clustering was performed on both datasets using Bray–Curtis similarity scores to compare the samples. Figure A3 displays the nMDS ordination of the results for (A) the proportional dataset and (B) the abundance dataset. Samples that plot near to each other are most similar. The results of the resemblance clustering were overlaid on the ordination plots to show the percent similarity between the samples.

### Re-sorted Samples

The unsorted material remaining from four previously sorted quantitative samples was sorted by the contract laboratory, EcoAnalysts Inc., to assess some of the variability inherent in the subsampling process. These samples were from Kōlea, Kopili'ula-B, Hanawā-A, and the replicate sample from Hanawā-A (fig. A1; tables A1–A3). The Kōlea re-sort and original sort grouped very closely on the nMDS ordination and clustered with a 90 percent similarity using the proportional data (fig. A3A). They were a little farther apart using the abundance data, with an 80 percent similarity (fig. A3B). This was because the original sort was calculated to have 23,137 organisms/m<sup>2</sup>, with only 1.82 percent of the sample sorted to achieve the 500-organism count, whereas the re-sort contained 9,869/m<sup>2</sup>, with 4.17 percent of the remaining material required to be sorted (table A1). The samples from Kopili'ula-B showed a similar trend, grouping closely together in the ordination with a 90 percent similarity using the proportional data (fig. A3A) and an 85 percent similarity with the abundance data (fig. A3B). The original sort required 10.94 percent of the sample whereas the re-sort required 18.76 percent. The Hanawā-A samples all grouped closely together, the two replicates and one re-sort had an 85 percent similarity, and all four had an 80 percent similarity with the proportional data (fig. A3A), and they also grouped closely together, with both re-sorts having an 85 percent similarity, the two replicates having an 80 percent similarity, and all four having a 75 percent similarity with the abundance data (fig. A3B). These results show that there is some variability in the subsampling process, although there is an overall high degree of similarity between the samples.

### Replicate Samples

Replicate quantitative samples were collected at eight sites, including Hanawā-A (as discussed in the previous section), Hanawā-B, Honolulu, Olowalu-B, Waihe'e-B, 'Āao-C, North Waiehu-A, and South Waiehu-A (figs. A1–A2; tables A4–A10). These paired samples all tended to group closely together using either dataset (fig. A3A and B). The percent similarity ranged from 75 to 85 percent with a mean of 81.25 percent, using the proportional data (fig. A3A) and from 70 to 90 percent, with a mean of 80 percent similarity with the abundance data. These results show that there is some variability between the pairs of samples due to the heterogeneous nature of physical habitats in tropical streams, and that some macroinvertebrate assemblages from other similar streams grouped closely to these pairings, although there is an overall high degree of similarity between the paired replicate samples.

### Repeat Samples

Three repeat quantitative samples were collected at the Waihe'e-A site over the duration of the field work on Maui (fig. A2). The first sample was collected on September 22, 2009, the second on October 23, 2009, and the third on January 29, 2010 (table A11). The ordination of the proportional data revealed that the first and third samples had a higher degree of similarity, grouping closer together with an 85 percent similarity, than either sample had with the second sample, with a 70 percent similarity (fig. A3A). The ordination of the abundance data revealed a comparable trend, with the first and third samples grouping closer together and sharing an 80 percent similarity and the second sample having a 75 percent similarity with the others (fig. A3B). The second sample had a lower total abundance of macroinvertebrates than the other two samples, 57 percent less than the first and 61 percent less than the third sample. This was mainly due to much lower abundances of the chironomids *Cricotopus* (73 and 77 percent fewer, respectively) and *Eukiefferiella* (71 and 70 percent fewer, respectively) in the second sample (table A11). This reduction caused the relative abundances of other macroinvertebrates, especially the trichopterans *Cheumatopsyche* and Hydroptilidae to be greater in the second sample (table A11). The exact cause of the decline in chironomids in the second sample is uncertain. Some of this variability is accounted for in the spatial heterogeneity demonstrated in the replicate sample comparisons, but this does not account for the sharp decline and subsequent upturn in chironomid abundances. It is possible that the sampling locations of the second sample were disturbed by a series of four relatively small spates that occurred in the 32 days between sampling, or perhaps the sampling locations were inadvertently disturbed during the collection of the first sample and the chironomids were not able to recover in the short time between sampling.

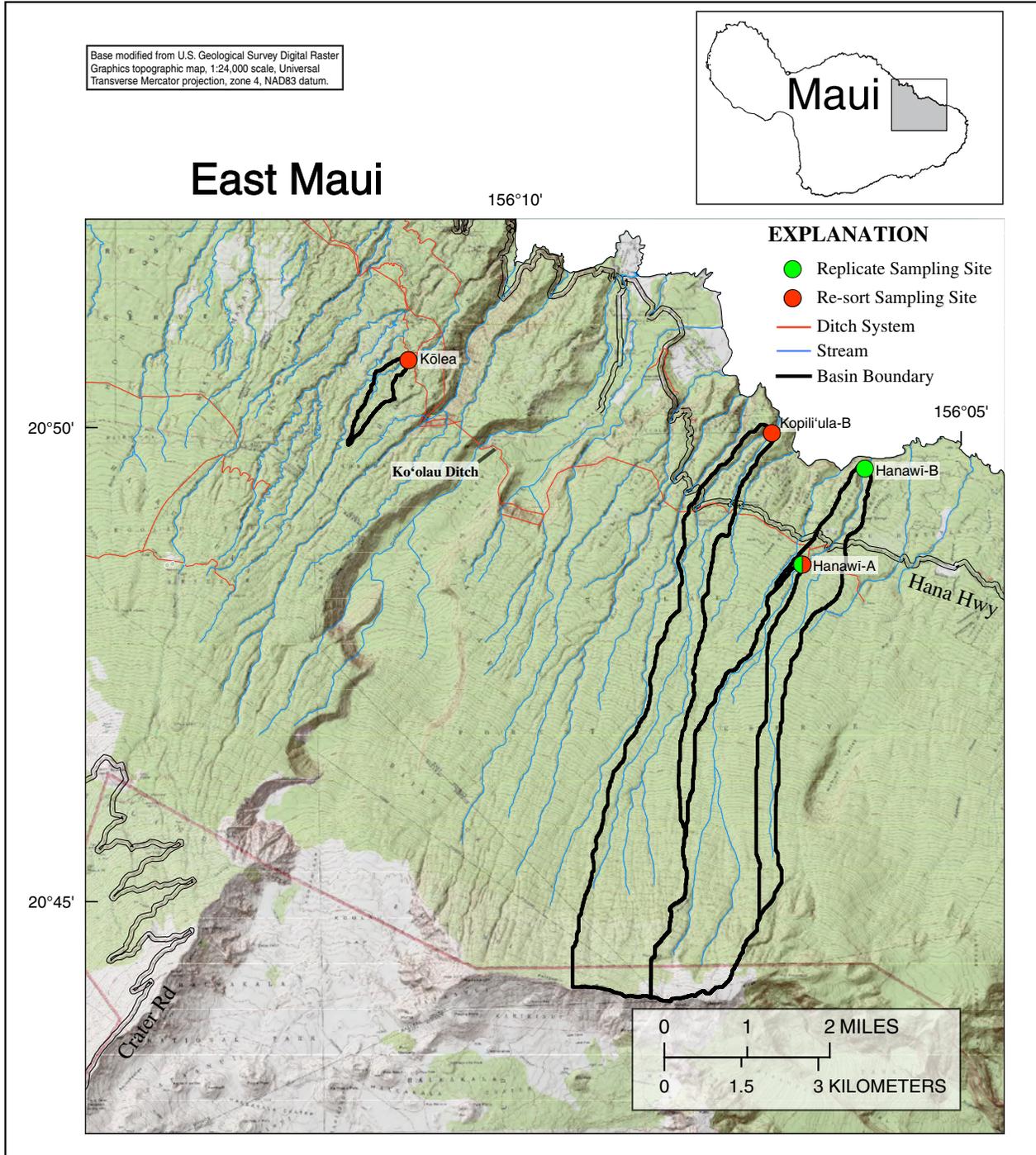
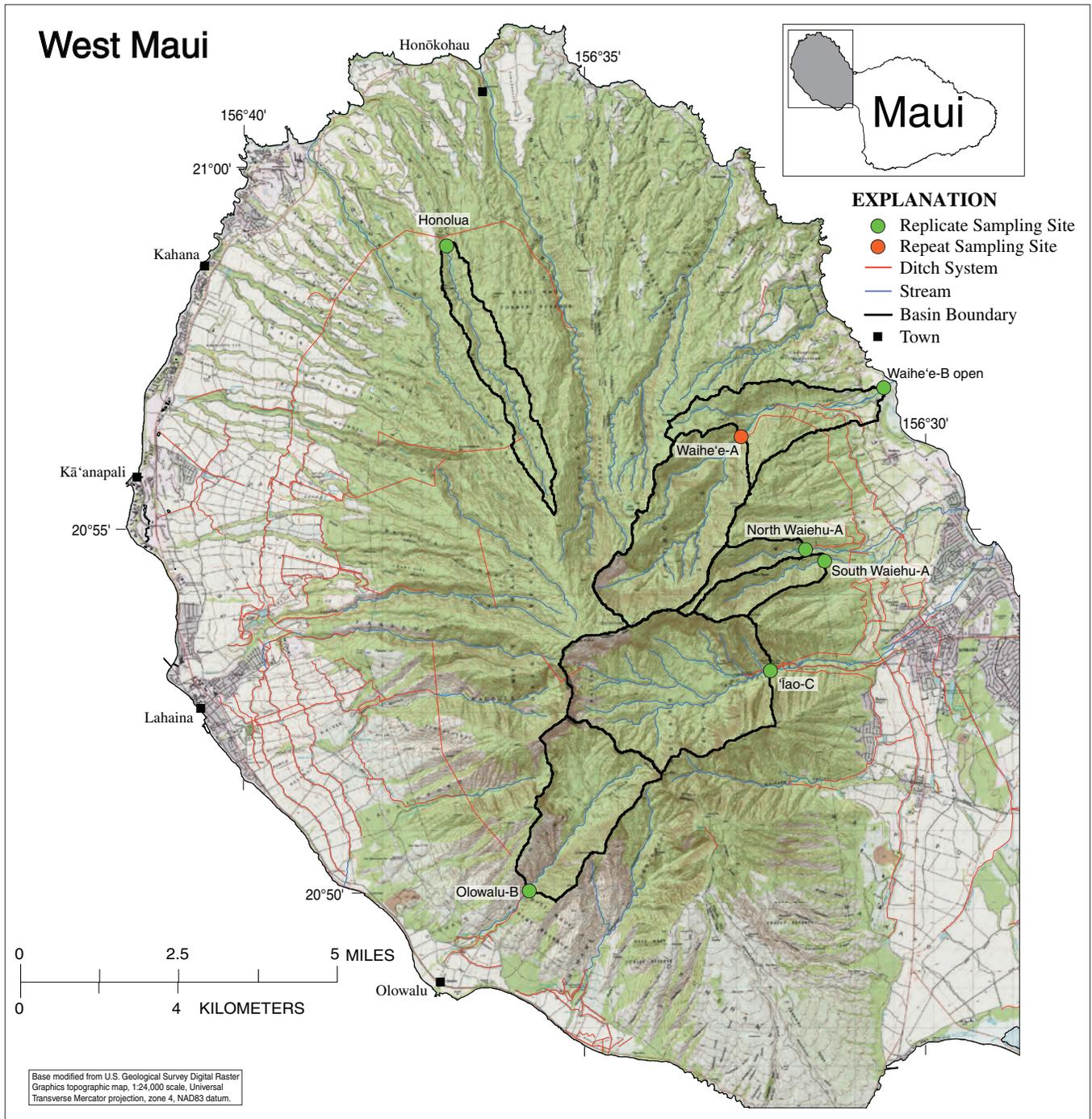
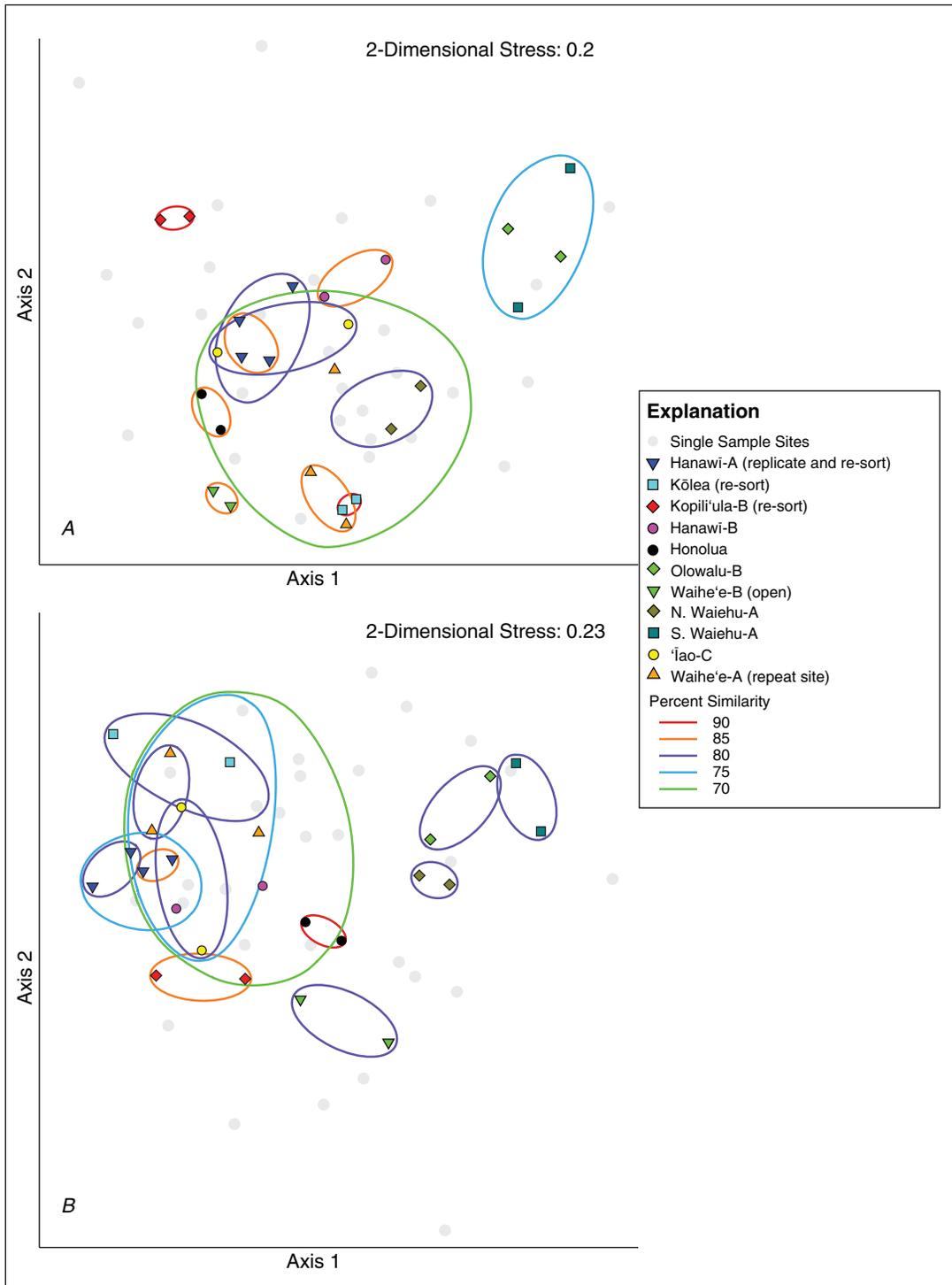


Figure A1. Locations of replicate and re-sort sampling sites on East Maui.



**Figure A2.** Locations of replicate and repeat sampling sites on West Maui.



**Figure A3.** Nonmetric multidimensional scaling (nMDS) ordinations of the Maui quantitative macroinvertebrate samples displaying the relationships between the re-sorted, replicate, and repeat samples using (A) arcsine-square root transformed proportional data and (B)  $\log(x+1)$  transformed abundance data (logarithm of the sum of the abundance plus one, with abundance in number of organisms per square meter). Colored lines are statistically derived overlay of cluster contours from a dendrogram plot determined using hierarchical agglomerative cluster analysis.

**Table A1.** Re-sorted quantitative sample from Kōlea (HI\_MAU1\_09-011).

[—, not observed in sample]

Taxon	Sample			
	A (first sort: 1.82 percent)		A (second sort: 4.17 percent)	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	131.7	0.6	38.4	0.4
Oligochaeta	877.8	3.8	211.0	2.1
<i>Ferrissia</i> sp.	131.7	0.6	38.4	0.4
Lymnaeidae	131.7	0.6	19.2	0.2
Acari	43.9	0.2	38.4	0.4
<i>Atyoida bisulcata</i>	8.0	0.0	46.4	0.5
<i>Cricotopus bicinctus</i> gr.	8,470.4	36.6	4,086.3	41.4
<i>Eukiefferiella</i> sp.	9,699.3	41.9	3,760.2	38.1
<i>Orthocladius</i> Complex	43.9	0.2	—	—
<i>Telmatogeton</i> sp.	307.2	1.3	57.6	0.6
<i>Megalagrion</i> sp.	—	—	19.2	0.2
<i>Cheumatopsyche</i> sp.	2,721.1	11.8	1,381.3	14.0
Hydroptilidae	570.5	2.5	172.7	1.8
<b>Total</b>	23,137.2	100	9,869.1	100

**Table A2.** Re-sorted quantitative sample from Kopili'ula-B (HI\_MAUI\_09-014).

[—, not observed in sample]

Taxon	Sample			
	A (first sort: 10.94 percent)		A (second sort: 18.76 percent)	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	29.3	0.8	25.6	1.1
Oligochaeta	1,162.7	30.9	665.3	29.3
<i>Ferrissia</i> sp.	—	—	17.1	0.8
Lymnaeidae	7.3	0.2	12.8	0.6
<i>Neritina granosa</i>	34.4	0.9	34.4	1.5
Tanaidacea	14.6	0.4	—	—
<i>Atyoida bisulcata</i>	1.6	0.0	1.6	0.1
<i>Apedilum</i> sp.	95.1	2.5	38.4	1.7
<i>Paratanytarsus</i> sp.	—	—	4.3	0.2
<i>Cricotopus bicinctus</i> gr.	336.4	9.0	157.8	7.0
<i>Eukiefferiella</i> sp.	73.1	2.0	59.7	2.6
<i>Orthocladius</i> Complex	7.3	0.2	4.3	0.2
<i>Cheumatopsyche</i> sp.	1,053.0	28.0	733.5	32.3
Hydroptilidae	943.3	25.1	516.0	22.7
<b>Total</b>	<b>3,758.1</b>	<b>100</b>	<b>2,270.8</b>	<b>100</b>

**Table A3.** Replicate and re-sorted quantitative samples from Hanawā-A (HI\_MAUJ\_09-004).

[—, not observed in sample]

Taxon	Sample							
	A (first sort: 5.21 percent)		A (second sort: 4.17 percent)		B (first sort: 6.25 percent)		B (second sort: 5.73 percent)	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	—	—	19.2	0.2	—	—	14	0.2
Erpobdellidae	—	—	19.2	0.2	12.8	0.2	—	—
Oligochaeta	721.7	9.1	76.7	0.7	832.0	12.5	251.3	3.3
Acari	30.7	0.4	—	—	12.8	0.2	—	—
<i>Atyoida bisulcata</i>	80.0	1.0	80.0	0.8	56.0	0.8	56.0	0.7
<i>Apedilum</i> sp.	245.7	3.1	95.9	0.9	102.4	1.5	97.7	1.3
<i>Paratanytarsus</i> sp.	—	—	—	—	12.8	0.2	—	—
<i>Pseudochironomus</i> sp.	15.4	0.2	—	—	—	—	—	—
<i>Cricotopus bicinctus</i> gr.	2,011.5	25.4	2,647.5	25.3	2,291.2	34.4	2,890.1	38.4
<i>Eukiefferiella</i> sp.	460.7	5.8	326.1	3.1	486.4	7.3	432.8	5.8
<i>Telmatogeton</i> sp.	—	—	—	—	25.6	0.4	27.9	0.4
<i>Limonia</i> sp.	—	—	38.4	0.4	—	—	27.9	0.4
Coenagrionidae	15.4	0.2	—	—	—	—	—	—
<i>Megalagrion</i> sp.	—	—	38.4	0.4	1.6	0.02	—	—
<i>Cheumatopsyche</i> sp.	2,410.8	30.5	4,278.2	40.8	1,523.2	22.9	2,192.0	29.1
Hydroptilidae	1,919.4	24.3	2,858.5	27.3	1,305.6	19.6	1,535.8	20.4
<b>Total</b>	7,911.3	100	10,478.1	100	6,662.4	100	7,525.5	100

**Table A4.** Replicate quantitative samples from Hanawā-B (HI\_MAUI\_09-015).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	38.4	0.6	23.0	0.5
Oligochaeta	153.6	2.3	23.0	0.5
<i>Ferrissia</i> sp.	—	—	7.7	0.2
Lymnaeidae	38.4	0.6	—	—
<i>Neritina granosa</i>	0.8	0.0	2.4	0.1
<i>Atyoida bisulcata</i>	40.0	0.6	8.8	0.2
<i>Apedilum</i> sp.	76.8	1.1	30.7	0.7
<i>Cricotopus bicinctus</i> gr.	1,638.4	24.1	583.5	13.8
<i>Eukiefferiella</i> sp.	371.2	5.5	215.0	5.1
<i>Telmatogeton</i> sp.	—	—	7.7	0.2
Ephydriidae	51.2	0.8	15.4	0.4
<i>Cheumatopsyche</i> sp.	3,686.4	54.1	2,963.5	70.0
Hydroptilidae	716.8	10.5	353.2	8.3
<b>Total</b>	6,812	100	4,233.9	100

**Table A5.** Replicate quantitative samples from Honolua (HI\_MAUI\_09-020).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	8.8	0.8	18.3	1.9
Oligochaeta	61.4	5.4	36.6	3.7
<i>Ferrissia</i> sp.	41.6	3.7	42.1	4.3
Lymnaeidae	188.5	16.7	263.8	26.8
<i>Apedilum</i> sp.	83.3	7.4	18.3	1.9
<i>Cricotopus bicinctus</i> gr.	309.0	27.4	225.3	22.9
<i>Eukiefferiella</i> sp.	184.1	16.3	111.8	11.4
<i>Telmatogeton</i> sp.	2.2	0.2	—	—
<i>Cheumatopsyche</i> sp.	190.7	16.9	208.8	21.2
Hydroptilidae	59.2	5.2	58.6	6.0
<b>Total</b>	1,128.8	100	983.6	100

**Table A6.** Replicate quantitative samples from Olowalu-B (HI\_MAU1\_09-022).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	—	—	2.9	0.2
Erpobdellidae	3.2	0.2	—	—
Oligochaeta	9.6	0.6	5.7	0.4
<i>Ferrissia</i> sp.	48.0	2.8	22.8	1.5
Physidae	364.8	21.6	42.7	2.8
Acari	6.4	0.4	2.9	0.2
<i>Atyoida bisulcata</i>	—	—	7.7	0.5
<i>Apedilum</i> sp.	3.2	0.2	2.9	0.2
<i>Cricotopus bicinctus</i> gr.	137.6	8.1	94.0	6.2
<i>Eukiefferiella</i> sp.	102.4	6.1	96.8	6.4
Ephyridae	—	—	2.9	0.2
<i>Megalagrion</i> sp.	—	—	5.7	0.4
<i>Cheumatopsyche</i> sp.	1,008.0	59.7	1,216.1	79.9
Hydroptilidae	6.4	0.4	19.9	1.3
<b>Total</b>	1,689.6	100	1,523.0	100

**Table A7.** Replicate quantitative samples from Waihe'e-B open (HI\_MAUI\_09-023).

[—, not observed in sample; abundance in number per square meter]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Oligochaeta	15.9	2.2	64.0	4.9
<i>Ferrissia</i> sp.	1.5	0.2	2.6	0.2
Acari	1.5	0.2	—	—
Ostracoda	4.3	0.6	—	—
<i>Atyoida bisulcata</i>	—	—	2.6	0.2
<i>Apedilum</i> sp.	140.5	19.0	189.4	14.4
<i>Paratanytarsus</i> sp.	2.9	0.4	5.1	0.4
<i>Tanytarsus</i> sp.	1.5	0.2	—	—
<i>Cricotopus bicinctus</i> gr.	324.3	43.8	657.9	50.1
<i>Eukiefferiella</i> sp.	23.2	3.1	41.0	3.1
<i>Gymnometriocnemus</i> sp.	—	—	2.6	0.2
Ephydriidae	1.5	0.2	12.8	1.0
<i>Megalagrion</i> sp.	1.5	0.2	—	—
<i>Cheumatopsyche</i> sp.	204.2	27.6	322.6	24.6
Hydroptilidae	17.4	2.4	12.8	1.0
<b>Total</b>	740.2	100	1,313.4	100

**Table A8.** Replicate quantitative samples from 'Iao-C (HI\_MAU1\_09-031).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Turbellaria	30.7	1.5	69.1	1.7
Oligochaeta	61.5	3.0	145.9	3.5
<i>Apedilum</i> sp.	11.5	0.6	7.7	0.2
<i>Cricotopus bicinctus</i> gr.	334.1	16.5	675.6	16.2
<i>Eukiefferiella</i> sp.	372.5	18.4	829.2	19.9
<i>Orthocladius</i> Complex	7.7	0.4	—	—
<i>Telmatogeton</i> sp.	—	—	7.7	0.2
Ephydriidae	7.7	0.4	23.0	0.6
<i>Limonia</i> sp.	—	—	7.7	0.2
<i>Cheumatopsyche</i> sp.	395.6	19.5	1,804.2	43.4
Hydroptilidae	806.5	39.8	591.2	14.2
<b>Total</b>	2,027.8	100	4,161.3	100

**Table A9.** Replicate quantitative samples from North Waiehu-A (HI\_MAUI\_09-028).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	2.3	0.2	—	—
Oligochaeta	15.8	1.3	188.0	13.1
<i>Ferrissia</i> sp.	2.3	0.2	17.1	1.2
Acari	2.3	0.2	—	—
<i>Atyoida bisulcata</i>	4.0	0.3	1.6	0.1
<i>Paratanytarsus</i> sp.	—	—	2.9	0.2
<i>Cricotopus bicinctus</i> gr.	419.6	35.1	373.1	26.1
<i>Eukiefferiella</i> sp.	198.5	16.6	381.6	26.7
Ephydriidae	—	—	2.9	0.2
<i>Limonia</i> sp.	2.3	0.2	—	—
<i>Megalagrion</i> sp.	54.2	4.5	42.7	3.0
<i>Cheumatopsyche</i> sp.	489.6	41.0	418.7	29.3
Hydroptilidae	4.5	0.4	2.9	0.2
<b>Total</b>	1,195.4	100	1,443.5	100

**Table A10.** Replicate quantitative samples from South Waiehu-A (HI\_MAUI\_09-030).

[—, not observed in sample]

Taxon	Sample			
	A		B	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	3.1	0.2	—	—
Erpobdellidae	3.1	0.2	3.2	0.2
Oligochaeta	31.4	1.9	6.3	0.4
<i>Ferrissia</i> sp.	9.4	0.6	9.5	0.6
Physidae	18.8	1.2	6.3	0.4
<i>Cricotopus bicinctus</i> gr.	62.7	3.8	217.5	13.6
<i>Eukiefferiella</i> sp.	59.6	3.6	252.2	15.8
<i>Megalagrion</i> sp.	—	—	6.3	0.4
<i>Cheumatopsyche</i> sp.	1,452.0	88.5	1,096.9	68.6
<b>Total</b>	1,640.1	100	1,598.2	100

**Table A11.** Repeat quantitative sampling results from Waihe'e-A (HI\_MAU1\_09-026).

[—, not observed in sample; richness, total number of taxa]

Taxon	Sample Date					
	9/22/2009		10/23/2009		1/29/2010	
	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample	Absolute abundance, in number of organisms per square meter	Relative abundance, in percentage of organisms in sample
Nemertea	—	—	7.7	0.2	—	—
Oligochaeta	68.2	0.7	15.4	0.4	95.9	0.9
<i>Ferrissia</i> sp.	—	—	7.7	0.2	—	—
<i>Atyoida bisulcata</i>	3.2	0.03	—	—	0.8	0.0
<i>Apedilum</i> sp.	—	—	7.7	0.2	115.1	1.1
<i>Cricotopus bicinctus</i> gr.	4,230.3	44.8	1,136.3	28.0	4,892.1	46.7
<i>Eukiefferiella</i> sp.	2,933.9	31.0	836.9	20.6	2,820.1	26.9
<i>Orthocladus</i> Complex	—	—	—	—	95.9	0.9
<i>Telmatogeton</i> sp.	187.6	2.0	69.1	1.7	—	—
Ephydriidae	170.6	1.8	46.1	1.1	19.2	0.2
<i>Cheumatopsyche</i> sp.	1,518.1	16.1	1,335.9	32.9	2,033.6	19.4
Hydroptilidae	341.2	3.6	598.9	14.7	402.9	3.9
<b>Total</b>	9,453.1	100	4,061.7	100	10,475.6	100
<b>Richness</b>	8		10		9	