Survey of Immature Mosquito Predators from Taro Fields on the Island of Kauai, Hawaii

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Abstract. A survey of predators of immature mosquitoes was conducted on the island of Kauai, Hawaii, in taro fields, major larval mosquito habitat of *Culex quinquefasciatus* Say. The survey consisted of examinations of samples from taro field water in two series: monthly at five locations and weekly at two of the same five locations. Copepods (*Macrocyclops albidus* Jurine), mosquito eating fish (*Gambusia affinis* Baird and Girard and *Poecilia reticulata* Peters) and aquatic insects, including backswimmers (*Buenoa pallipes* Fabricius) and larvae of Odonata spp., were the most-commonly observed predators. While copepods were observed at all locations, backswimmers and mosquito fish were variably present. Copepod populations from all locations fluctuated during the surveys. For the two sites sampled on a weekly basis, adult mosquito counts were higher at Lihue (65.60 per gravid-trap-day) than at Hanapepe (39.91) while larvae were more frequently present at Hanapepe (79% of weeks) than Lihue (33%). There was no clear relationship at these sites between the relative abundance of the most frequently collected mosquito-feeding insects, copepods, and numbers of adult mosquitoes trapped.

Key words: mosquito, taro field, biological control, copepods, mosquito fish

Introduction

Wetland taro (*Colocasia esculenta* (L) Schott) farming is an important part of cultural and agricultural traditions in the state of Hawaii, where the yearly production is 2–3 x 10^6 kilograms (Hudson 2009). Seventy to 80% of this taro production is from the island of Kauai (Hudson 2009). Approximately 121 ha (300 acres) of taro fields on Kauai are located in lowlands near residential areas, where the flooded fields also serve as major larval mosquito habitat for the southern house mosquito, *Culex quinquefasciatus* Say. The southern house mosquito is a medically important pest because of the potential to vector pathogens to humans, livestock, and wildlife (Gubler 1966, Mullen and Durden 2002, Atkinson et al. 2005).

Mosquito control in Kauai taro fields is very limited because the mosquito larvae are not a pest of taro. A few taro farm owners periodically disperse mosquito fish in their flooded fields in order to control mosquitoes, as suggested by the Hawaii Department of Health, Vector Control (VC). VC inspects a minority (~20%) of taro fields to monitor mosquito breeding and employs a variety of control measures in those fields. VectoBac has been used by VC for mosquito larvae control on Kauai, including in taro fields with the owner's permission. Mosquito fishes (*Gambusia* spp.) and guppies (*Poecilia* sp.) are often used by VC and taro farmers for biological control of mosquitoes and the fishes are generally considered effective biological control agents for this purpose (Walton 2007).

No studies of mosquito larvae predators in Hawaii taro fields have been published. Casual observations, among major areas of taro cultivation, of an apparent difference in predatory insect abundance in conjunction with differences in immature mosquito abundance, led to the examinations presented here. With principal interest in the occurrence of aquatic invertebrates that may be unappreciated predators of immature mosquitoes in taro fields, we surveyed five locations in major areas of wet land taro cultivation on the island of Kauai.

Materials and Methods

Survey of mosquito larvae and their predators. Surveys were conducted in 2 series: monthly at 5 locations (2 in Hanalei, 1 in Lihue, 1 in Hanapepe, and 1 in Waimea) from December 2007 to November 2008; and weekly at two locations (Lihue and Hanapepe) from June to November 2009 (Figure 1). Weekly surveys were intended to investigate the patterns of occurrence of immature and adult mosquitoes and their predators, while the monthly surveys examined predators only. The 2 weekly-sampled locations, Lihue and Hanapepe, were the same as used in the monthly-sampled series.

Sampling protocol. At each taro field location, there were five sampling sites. The same five sites were used across sampling events. The places available for sampling were constrained to be within 1 m of the edge of a field, to provide minimal disruption to the field and the habitat within. There were additional site-specific constraints on access to sampling imposed by property ownership and farming activities.

Sampling sites were around field perimeters and were systematic, random samples of the accessible areas. An initial sampling point was randomly chosen within a field's perimeter, and the other sampling sites were fixed to establish approximately equal-spacing along the accessible perimeter. Sampling sites were not intentionally located with respect to irrigation water in-flow or out-flow, or with respect to visible presence of mosquito larvae or their potential predators.

Two categories of sampling were used in both monthly and weekly surveys. For counts of invertebrate predators, a 300 mL dipper (Clarke, Roselle, IL) was used to accumulate samples for laboratory examination. The dipper was submerged bottom-up to within 0–5 cm of the substrate. It was rotated to draw-in water and immediately removed above the surface. After 0.25 min passed, to allow settling of material in the dipper, 225–250 mL of water was carefully poured off, to retain visible invertebrates in the dipper. The retained water was added to a sample bottle. This process was repeated until a 1.5 L sample volume was accumulated.

The 1.5 L sample from each location was brought in a plastic bottle to the laboratory and examined for predatory invertebrates. The abundances of predators were assessed by examining aliquots of the water in a Petri dish using a Luxo KMF magnifier (22 watts, 3 diopter) (LUXO, Norway). Each aliquot contained approximately 50 mL of sample. The entire 1.5 L was examined.

The second category of sampling was for the presence or absence of mosquito larvae and mosquito fishes. At each location, up to 50 dips (10 per sampling site) were inspected visually in the field. If any mosquito larvae and mosquito fish were seen in one of these dipper samples, the animal was scored as present in the location. Presence-absence results from these dipper-based visual surveys always accorded with presence-absence observations as could be noted by walking around the taro field at each site. To investigate associations between the presence and absence of fish and mosquito larvae at the sites sampled weekly, contingency tables were constructed with time-lags introduced in the mosquito larvae presence data of 0 to 5 weeks.

Beside mosquito larvae and the predators mentioned in this study, tadpoles, water-striders, snails and water beetles were observed and collected by dipper: in the samples

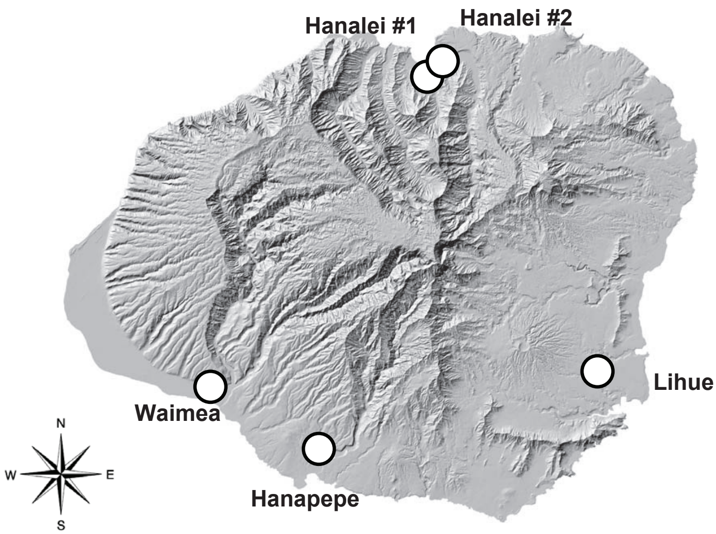

Figure 1. The island of Kauai. Taro patch locations are shown by circles.

used to quantify abundances and in the samples used to score presence–absence. These were ignored in the latter samples and removed from the former.

Adult mosquito collection. Adult *Culex quinquefasciatus* mosquitoes were monitored using gravid traps (John W. Hock, CDC type) at Lihue (June-November) and Hanapepe (July-November) in 2009. Gravid traps were placed over 2 gallons of horse manure water fermented for 4 days. Mosquitoes were collected twice per week. Gravid traps were placed within 30 m of a field-water sampling site. Gravid trap locations were chosen to conform with accessibility restrictions and with the intention to safeguard the traps from disturbance. The Hanapepe trap was placed under a shelter where the famer stores equipment. The Lihue trap was placed under a banana tree.

Statistical tests. Two-sided Fisher's exact test was used with data from the weekly sampling series at the Lihue and Hanapepe sites: (1) to evaluate the differences in the proportion of weeks for which mosquito larvae and mosquito fishes were scored as present or absent; and (2) to test for associations between the presence of mosquito-feeding fish and mosquito larvae in dipper-based visual surveys. A Welch two-sample t-test was used to test for equality of mean adult mosquito collections by gravid traps.

Results

Sampling was conducted with the permission of taro field farmers and landowners. Cultivation practices were not modified due to this study: fields were subjected to farming practices by field managers *ad libitum*. This meant that there were introductions of mosquito-predatory fish and there were dry–downs of taro fields that were not under our control. When field dry-downs occurred, the rate and duration of dry-down were unknown to us except as occurring between sampling events. Taro field dry-downs in the study area typically involve < 1 day of draining the field of irrigation water, 1–2 days of leaving the field unflooded, then < 1 day to reflood the field.

Monthly mosquito predator survey. From monthly surveys from December 2007 to November 2008, the most commonly-recorded predators from taro fields were mosquito-eating fish (*Gambusia affinis* Baird and Girard and *Poecilia reticulata* Peters), copepods (*Macrocyclops albidus* Jurine) and backswimmers (*Buenoa pallipe* Fabricius). Table 1 shows the numbers of these predators counted from 1.5 L samples.

Mosquito fish were always observed at Hanalei #1, variably observed at Hanalei #2 and Hanapepe, and never observed at Lihue and Waimea. Although mosquito-feeding fishes were seen in the irrigation water ditch at Waimea, no fishes were observed in the taro field sampling. In the Lihue taro field, no fishes were seen in the irrigation water ditch.

Copepods were always collected in Lihue and Hanapepe, collected in 9 of 10 months at Hanalei #2 and Waimea, and 7 out of 10 months at Hanalei #1 (Table 1).

Backswimmers were collected in all months at Lihue, 5 and 6 of 10 months at Waimea and Hanalei #2 (respectively), and 3 of 10 months at Hanalei #1 and Hanapepe (Table 1).

Odonate larvae were present variably at all five locations. Green hydra (*Chlorohydra viridissima* Pallas) were collected only in Lihue, but were seasonally abundant there (December 2007, April 2008).

Weekly mosquito predator survey. Table 2 shows the predators collected from 1.5 L samples from 4 June to 24 November 2009 at the two locations sampled weekly. Over the 24-week survey, copepods, backswimmers and odonate larvae were collected from the Lihue site (Table 2; Figure 2 and 3). At Hanapepe, copepods, fish, odonate larvae, green hydra and backswimmers were collected (Table 2; Figure 2 and 3).

Figure 3 shows copepod counts from Lihue and Hanapepe taro fields from June - November 2009. At Lihue, copepods were especially collected nearer to the irrigation water entry to the field. The high count (726) of copepods observed the third week of August was in large measure due to a high density of copepods near the irrigation water entry (Figure 3). At Hanapepe, copepods were not usually concentrated at any particular place of the taro field. However when strong trade winds were present, a high density of copepods was found down-wind.

Green hydra were collected only four times, all at Hanapepe. Three of the four collections were in June. No green hydra were collected from Lihue, the only place where hydra were collected during monthly surveys.

The weekly presence of mosquito larvae, by dipper observations (Table 3), was significantly higher at Hanapepe (79.2%) than Lihue (33.3%; $p < 0.01$). Fish (*P. reticulata*) were scored present significantly more often at Hanapepe (37.5%) than at Lihue (0%; $p < 0.01$) in the weekly samples (Table 3). Contingency table analyses showed that the only significant association between fish and mosquito larvae presence was when mosquito presence data were lagged by two weeks: under those circumstances, the proportion of weekly observations with fish present and larvae absent was maximized. Those proportions, for time-lags of 0 to 5 weeks, respectively, were 4, 13, 23, 5, 0, and 5%; with corresponding p values 0.36, 0.62, < 0.01, 0.60, 0.12, and 0.59.

Adult mosquito collection. The gravid traps used in this study selectively collected female mosquitoes: 10,443 (99.5%) of the 10,497 collected at Lihue during 160 days and 4,682 (96.9%) of the 4,830 collected at Hanapepe during 121 days. The Lihue site showed higher adult mosquito counts than Hanapepe (65.6 vs 39.9/trap day; with a significant [$t_{0.05(2), 85.8} = 2.457$, $p = 0.016$] difference in mean trap collections), even though Hanapepe showed significant higher, weekly, larval mosquito presence (79.2%) than did Lihue (33.3%).

Since copepods were the most abundant predator of mosquito larvae sampled from both sites, we compared their abundances with those of adult mosquitoes (Figure 3). Both copepod and mosquito populations fluctuated over time with no clear relationship between two.

Table1. Monthly surveys of copepods, fish and backswimmers from 5 Kauai taro fields, December 2007 to November 2008*.

	Hanalei #1			Hanalei #2			Waimea			Lihue			Hanapepe		
	Cop	BS	Fish	Cop	BS	Fish	Cop	BS	Fish	Cop	BS	Fish	Cop	BS	Fish
Dec	+ (34)	-	+	+ (12)	+ (3)	-	+ (69)	-	-	+ (5)	+ (3)	-	+ (113)	-	+
Jan	+ (2)	-	+	+ (1)	-	+	+ (3)	+	-	+ (80)	+	-	+ (16)	+ (1)	-
Apr	+ (14)	-	+	+ (6)	-	-	+ (3)	+	-	+ (20)	+ (12)	-	+ (1)	+ (1)	+
May	-	-	+	+ (152)	+	-	+ (37)	+ (2)	-	+ (180)	+	-	+ (92)	-	+
Jun	+ (27)	+	+	+ (43)	+ (6)	-	+ (50)	+ (1)	-	+ (22)	+	-	+ (4)	-	-
Jul	+ (104)	+ (1)	+	+ (86)	+ (3)	-	+ (74)	-	-	+ (119)	+	-	+ (1)	-	-
Aug	-	-	+	+ (2)	-	+	+ (27)	-	-	+ (205)	+ (7)	-	+ (200)	-	+
Sep	-	-	+	+ (7)	+ (1)	+	+ (1)	-	-	+ (215)	+	-	+ (30)	-	+
Oct	+ (3)	-	+	+ (4)	+ (1)	+	-	+ (1)	-	+ (80)	+ (2)	-	+ (14)	-	+
Nov	+ (18)	+	+	-	-	+	+ (6)	-	-	+ (240)	+ (6)	-	+ (56)	+	-
Total	7/10	3/10	10/10	9/10	6/10	5/10	9/10	5/10	0	10/10	10/10	0	10/10	3/10	6/10

* Presence (+) and absence (-) of the following in 1.5 L samples: Cop – copepods (*Macrocyclops albidus* Jurine); BS – backswimmer (*Buenoa pallipes* Fabricius); Fish – fish *Gambusia affinis* Baird & Girard and *Poecilia reticulata* Peters. Individual counts are shown in parentheses. An arthropod presence shown without a count is a presence determined from visual inspection alone.

Table 2. Total number of mosquito larvae predators counted from weekly 1.5 L samples at Lihue and Hanapepe taro field sites during the 24 weeks from June to November 2009.

		Copepods	Backswimmers	Larvae of Odonata	Green hydra
Lihue					
	Total	2,743	136	26	-
	Mean±SD	114.3±81.0	5.7±4.9	1.08±1.1	-
Hanapepe					
	Total	2,422	12	38	54
	Mean±SD	100.9±83.2	0.5±0.7	1.6±1.1	2.3±3.8

Discussion

Our surveys revealed in Kauai taro fields the presence of predators well known to feed on mosquitoes elsewhere. Variability in the occurrence and relative abundance of these predators was documented in taro fields that were not under any restrictions on cultivation practices due to the occurrence of this study. The predator collected in greatest number was the copepod (*Macrocyclops albidus* Jurine). We also collected backswimmers (*Buenoa pallipe* Fabricius), larvae of Odonata, hydra (*Chlorohydra viridissima* Pallas) and fish (*Gambusia affinis* Baird and Girard and *Poecilia reticulata* Peters).

Fish were observed at three surveyed locations during monthly surveys, Hanalei #1, Hanalei #2 and Hanapepe. During our observations, mosquito fish were introduced (by VC workers and farmers) at one sampling site at Hanalei #1 in order to control mosquito larvae. Fish were present at all survey occasion but mosquito larvae were never present. Mosquito-eating fish have been used as a biological control agent for mosquitoes for more than 100 years and they have been observed to virtually eliminate the presence of *Culex* mosquito larvae in some cases (Marten and Reid 2007, Walton 2007).

Comparing Hanapepe and Lihue, mosquito larvae were present on twice as many weekly survey dates at Hanapepe, while mosquito adults were 50% more abundant (per trap day) at Lihue. It is very difficult to attribute such differences to effects of aquatic predators alone, since many factors are likely involved. Such a caveat holds for any number of extrapolations that might be attempted using these survey data. In this comparison of Hanapepe and Lihue locations surveyed once per week for 24 weeks, the more frequent presence of mosquito larvae was associated with the presence (as opposed to absence) of mosquito-feeding fish. With the presence of fish in the Hanapepe taro field, there was a significantly lower abundance of mosquito adults, as assessed by gravid trap collection at the taro field. These associations are from one comparison of field locations as these were the only trap locations where adult mosquito abundances were measured.

There are qualitative differences in the habitat surrounding these taro field locations. The Hanapepe site is surrounded by other taro fields. The Lihue location is surrounded by dryland agricultural fields (growing papaya, bananas, corn). There could also be within-field differences important to the observed, contrasting data on frequency of larval mosquito presence and local abundance of adult, female mosquitoes: for example, we made no assessments of the nutrient content available in these fields to immature mosquitoes.

Copepod abundance from all locations fluctuated during the surveys. However there was no clear relationship between copepods and adult mosquito abundance from weekly surveys (Figure 3). While fish prey on all stages of mosquito larvae, copepods kill mainly

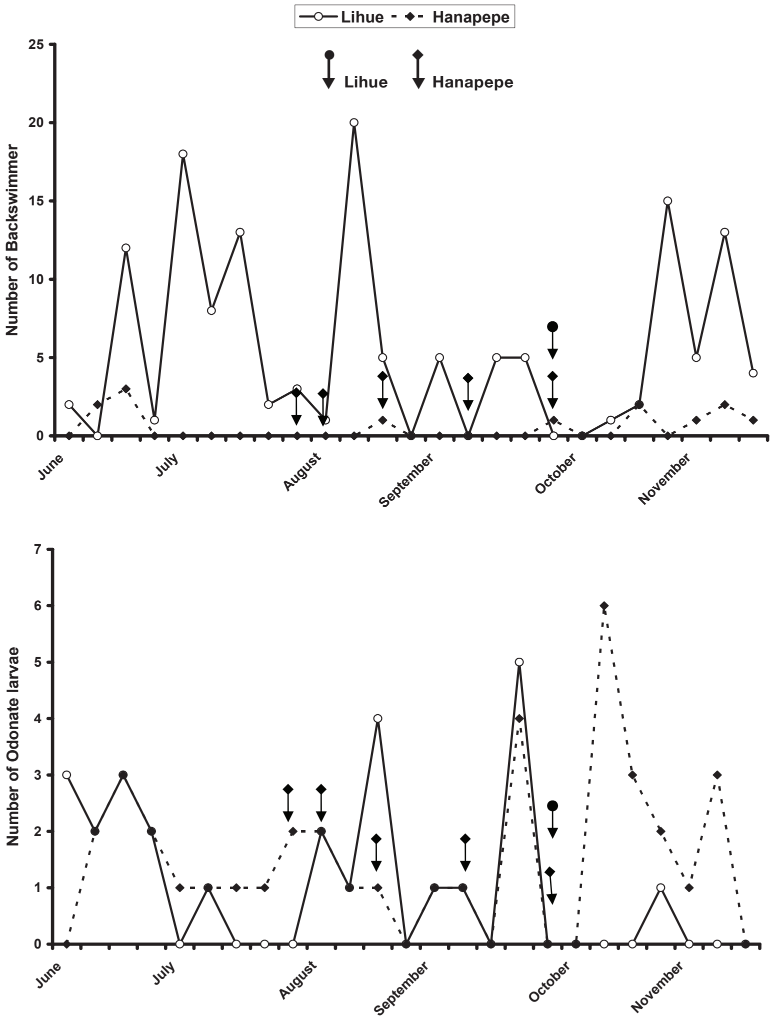

Figure 2. Comparison of counts of backswimmer and larvae of Odonata from Lihue and Hanapepe taro fields, June to November 2009. Dry-down occasions are shown by arrows.

1^{st} stage larvae (Marten and Reid, 2007). At the Hanapepe site, on several occasions when fish were not present and copepods were at high density, there was still a high density of mosquito larvae. Since it was clear copepods were not consuming or killing all the mosquito larvae at early stages, biological control would require a predator capable of consuming later-stage larvae. This observation agrees with other work, finding that the role of copepods in controlling *Culex* mosquitoes in habitats like taro fields is not in mosquito elimination but in reinforcing or augmenting other control methods (Marten and Reid, 2007). It is also reasonable to expect that there could be predator-prey interactions among

Table 3. Counts and percentages of weeks in which immature mosquitoes and their predatory fish were observed in taro fields locations on the island of Kauai, from weekly surveys in 2009. * shows occurrence of dry-down prior to field observations.

	Lihue			Hanapepe	
	Fish	**Mosquito larvae**		**Fish**	**Mosquito larvae**
2-Jun	-	-	4-Jun	+	+
9-Jun	-	-	10-Jun	+	+
16-Jun	-	+	19-Jun	+	-
23-Jun	-	-	23-Jun	+	+
30-Jun	-	-	2-Jul	-	+
7-Jul	-	-	9-Jul	-	+
14-Jul	-	-	16-Jul	-	+
21-Jul	-	-	23-Jul	-	+
28-Jul	-	-	30-Jul	-	+*
4-Aug	-	-	7-Aug	-	+*
14-Aug	-	+	13-Aug	-	+
18-Aug	-	+	20-Aug	-	-*
25-Aug	-	-	27-Aug	+	+
1-Sep	-	-	1-Sep	+	+
8-Sep	-	+	8-Sep	-	-*
15-Sep	-	-	15-Sep	+	+
22-Sep	-	-	22-Sep	-	+
2-Oct	-	+*	29-Sep	-	+*
6-Oct	-	+	6-Oct	-	+
13-Oct	-	-	13-Oct	-	+
20-Oct	-	+	20-Oct	+	+
26-Oct	-	+	26-Oct	+	+
2-Nov	-	-	2-Nov		
9-Nov	-	-	6-Nov	-	-
Total	0/24 (0%)	8/24 (33.3%)		9/24 (37.5%)	19/24 (79.2%)

the taro field predators of mosquito larvae. For example, in the monthly survey of 5 taro field locations, copepods were present at 9 or 10 of the 10 survey dates, except at the one location where mosquito-feeding fish were always seen. Even with the consistent presence of fish at that latter site, copepods were still observed on 7 of 10 survey dates. In the same survey series, backswimmers were present ≥ 50% of survey dates only when fish were present < 50% of survey dates.

On Kauai, farmers often allow their wetland taro patches to dry-down in order to maximize fertilizer efficiency. We observed dry-down at all fields. These periodic dry-downs clearly impacted abundances and interactions among taro field mosquitoes and their predators. When water was reintroduced to the taro fields, the immature mosquito populations established very quickly. The return of invertebrate predators was more delayed. For example, one week after reflooding at the Hanapepe site all stages of mosquito larvae were well established but copepod abundance was still low. The copepod population took about two weeks to recover to high density (Figure 3).

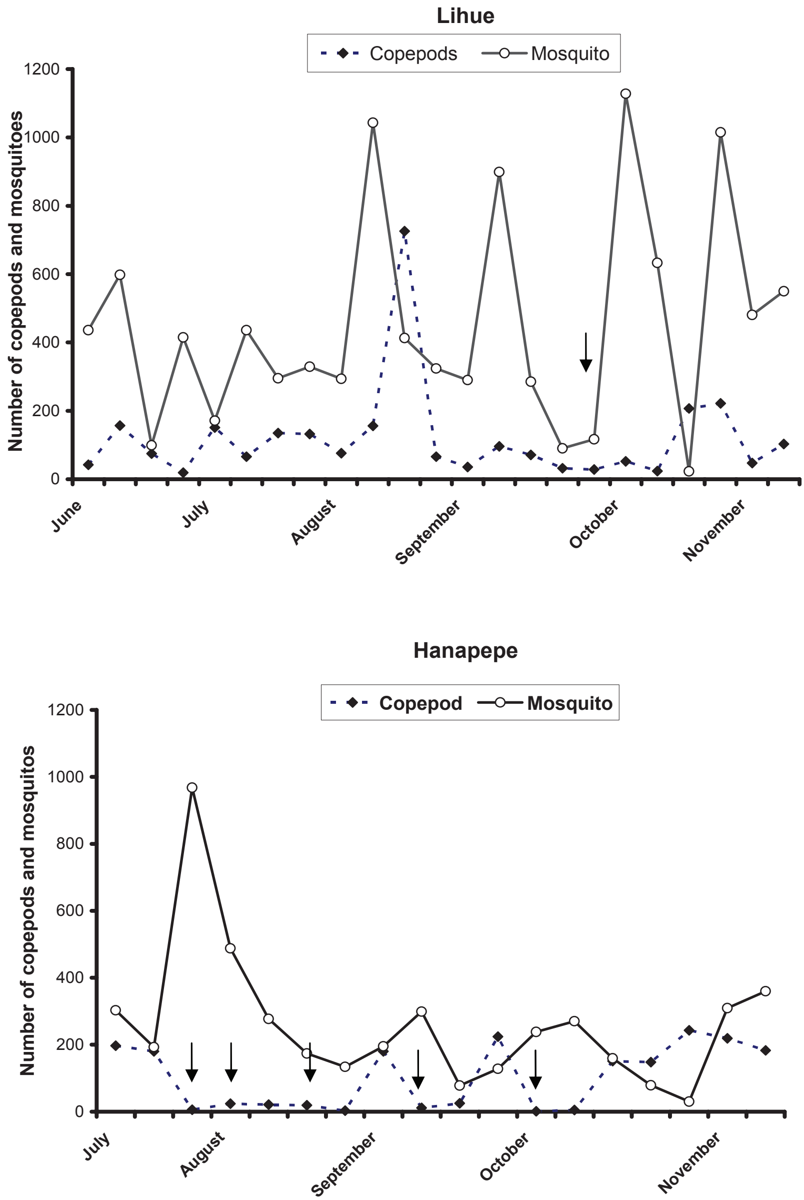

Figure 3. Comparison of copepod and adult mosquito counts from Lihue and Hanapepe taro fields from June to November 2009. Dry-down occasions are shown by arrows.

This study provides information on the differential occurrence and relative abundances of mosquito predators in taro fields under normal wetland cultivation on Kauai. These taro fields are major breeding sites of *C. quinquefasciatus* on the island. Predatory fish are viewed by many taro farmers as effective contributors to mosquito control in these habitats. Taro farmers who make use of mosquito-feeding fish report often—but not invariable—decreases in the apparent abundance of taro field mosquito larvae after intentional introductions of the fish. The farmers who abstain from use of mosquito-feeding fish report doing so because the dead fish left after dry-downs create undesirable, foul odors and attract nuisance flies. The predatory invertebrates observed in this study may also contribute to mosquito biological control in taro fields, but their contributions have not been studied. Important distinctions between predatory fishes and invertebrates include that the presence of invertebrate predators is not dependent on intentional introduction efforts by people and their presence generally goes unnoticed.

Working taro fields are unquestionably important sites for mosquito breeding on Kauai, with analogs in other geographic locations where flooded-field agriculture is practiced. A better understanding of how invertebrate predators in these habitats contribute to the biological control of mosquito populations could be helpful to mosquito control programs that seek to limit mosquito-borne diseases with limited materiel and labor investments. Manipulative studies will clearly be important for better understanding of this (for example, to facilitate controls resulting in informative, replicated, variation in predator-prey occurrences), but the findings from such research will ultimately be tested for applied value by attempts to translate the findings to unmanipulated field settings.

We know of no published work focused on mosquito predators or mosquito predation in Hawaii taro fields, despite a long history of applied, agricultural, entomological research in the islands, and ongoing interest in the occurrence and control of mosquito-vectored diseases. Observational studies can provide field-collected data on the variability in invertebrate predator distributions and abundances that will have to be addressed in future projects, in order to advance our understanding of predator-prey interactions in these habitats.

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