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Local Prevalence and Transmission of Avian Malaria in the Alakai Plateau

Report by Anouk Glad, Hawai’i Audubon Society Educational Grant Recipient

Anouk Glad is a graduate student from University of Montpelier conducting an internship and research project with the University of Hawaii at Manoa and Kauai Forest Bird Recovery Project. This article is an overview of her thesis project.

The isolation of the Hawaiian Islands led to a unique evolution and speciation of the avifauna, which flourished in a predator and disease-free environment until human colonization of the islands ca. 1800 years ago. During this evolution, many native Hawaiian forest birds lost immunity to mosquito-borne diseases and now have a high susceptibility to the recently introduced avian malaria. Avian malaria is today one of the most important threats to native forest bird populations, along with habitat loss and non-native predators. This disease is caused by the parasite *Plasmodium relictum,* which infects red blood cells. This parasite is transmitted between birds mostly by the mosquito *Culex quinquefasciatus* (the southern house mosquito), which was first introduced on Maui in 1826. Across the archipelago, many native bird populations have declined dramatically and have almost disappeared from lower and mid-elevations as a result of malaria. However, above 1500m in elevation, ambient temperature historically has been sufficiently cool to prevent both mosquito and *P. relictum* development and survival. Thus, malaria is less prevalent and native forest birds are still present in high elevation forests.

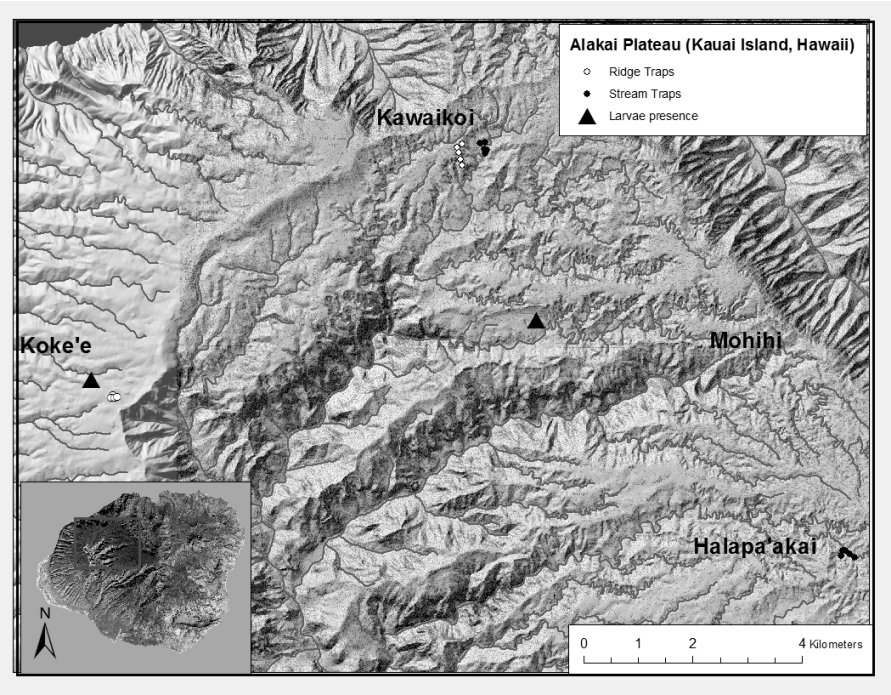

Figure 1: Study sites, on the Alakai Plateau, Kauai, Hawaii, USA

On Kauai, the Alakai Plateau historically has been known as a refuge for forest birds as temperatures during most of the year were cool enough to prevent transmission of malaria. The Alakai Plateau constitutes the entire remaining range of three critically endangered endemic species: Akikiki (*Oreomystis bairdi*), Akeke’e (*Loxops caeruleirostris*) and Puaiohi (*Myadestes palmeri*). The three other endemic species on the island (Kauai Amakihi (*Hemignathus kauaiensis*), Kauai Elepaio (*Chasiempis sclateri*), and Anianiau (*Magumma parva*)), and two widely distributed native forest bird species (Apapane (*Himatione sanguine*) and I’iwi (*Vestiaria coccinea*)) are also found only on the Alakai Plateau.

However, a recent study showed that the Alakai Plateau might not be a malaria-free refuge anymore. By comparing bird’s blood samples collected from 1994-1997 and 2007-2013, the researchers determined that prevalence of malaria increased between the two sampling periods in both native and non-native species, which suggests that malaria is now established on the Plateau. Furthermore, in spring 2011, mosquito larvae were detected in three streams (Halepa’akai, Mohihi and Kawaikoi streams), while larvae were found only in Kawaikoi Stream in summer 1999. Those alarming results suggest that both vector and parasite had now reached the Alakai Plateau. We know that at high elevations on Hawaii Island, altitude/temperature and precipitation

were the two major factors affecting *C. quinquefasciatus* abundance and transmission of *P. relictum*. Thus, temperatures increases and precipitation declines due to climate change over the last decade may be responsible for the expansion of avian malaria to the Alakai Plateau.

The aim of this study is to examine the possibility that *P. relictum* can now complete its life cycle in the Alakai Plateau, thus allowing "local" (within the Alakai) transmission of avian malaria to birds to occur. We also examined local variation in mosquito abundance and *P. relictum* prevalence across the Plateau. To assess these hypotheses, mosquitoes were trapped at three sites representing different elevations and meteorological conditions on the Plateau, and were screened for *P. relictum* infection. Furthermore, assessment of mosquito breeding habitat is important to determine if mosquitoes can complete their life cycle, we conducted larval surveys at two sites. The three study sites are located in the Alakai Wilderness Preserve and Na Pali Kona Forest Reserve on the Alakai Plateau. In the east, Halepa'akai (1280m) is situated in a wet montane forest, whereas Kawaikoi (1100m) is located in the west of the Alakai Plateau and is characterized by a lower rainfall average. (Figure 1). Koke'e State Park (1052m) is located in the far west side of the Alakai Plateau and is characterized by an intense human activity (trails, roads, human habitations). (Figure 1). Furthermore, we trapped mosquitoes in two different microhabitats (ridge and stream) in Kawaikoi to examine the effect of topography on mosquito distribution.

Adult mosquitoes were sampled with CO_2 traps (designed for trapping females seeking a blood meal) and Reiter oviposition traps (designed for trapping females ready to lay eggs) and light traps. Traps were checked daily. Trapping stations were located every 100m along streams or along the ridge trail. DNA from mosquito tissues were extracted and analyzed for *P. relictum* infection by Polymerase Chain Reaction (PCR) in December. Since January, *P. relictum* infection in mosquitoes has been assessed by dissecting mosquitoes under a microscope.

We surveyed larval presence on 500m to 1-km transects along Halepa'akai (October) and Kawaikoi (October, November, January and March) Streams, using the same protocol as earlier larvae surveys. Sampling involves dipping a larva cup at least 10 times per 10 meters into slow-moving or stagnant water along the stream margins.

Adult mosquitoes were trapped during field trips (5 to 6 days each) from October 2013 to March 2014. We only caught adult *C. quinquefasciatus*. (Tables 1 and 2).

We set out light and Oviposition traps for four days (February) in Koke'e. Despite the presence of human activities and habitation in Koke'e, we didn't catch any mosquitoes.

Month	Habitat	Number of *C.Quinquefaciatus* (Oviposition traps)	Number of *C.Quinquefasciatus* (CO2 traps)	Number of C.Quinfasciatus (LIGHT trap)	Mean number of mosquitoes per trap (oviposition trap)	Mean number of mosquitoes per trap (CO2 trap)	Mean number of mosquitoes per trap (LIGHT trap)
November	stream	3	12	NA	**0.17**	**0.55**	NA
January	stream	16	NA	1	**0.76**	NA	**0.25**
March	stream	46	NA	0	**1**	NA	**0**
November	ridge	0	4	NA	**0**	**0.16**	NA
January	ridge	0	NA	0	**0**	NA	**0**
March	ridge	0	NA	0	**0**	NA	NA

Table 1. Total and mean number of adult mosquitoes per field trip and trap type in Kawaikoi Stream in stream-side and ridge traps

We analyzed 44 mosquitoes by PCR and 37 mosquitoes by dissection. Only one of them (from Halepa'akai) was positive for *P. relictum* (PCR result).

We did not find any mosquito larvae along Halepa'akai Stream in October and Kawaikoi Stream in November, January and March. However, in January we found *C. quinquefasciatus* and *Aedes albopictus* (Tiger mosquito) larvae in Koke'e in an old fireplace and we found *C. quinquefasciatus* larvae in pools of stagnant water along the Camp 10 road in October 2013 and March 2014.

In this study we observed that *C. quinquefasciatus* seems to breed even during the colder winter months in the Alakai Plateau, as we caught mosquitoes on each field trip from October 2013 to March 2014. As expected, we also observed differences among and within sites, as in total we caught more mosquitoes in the lower elevation Kawaikoi than Halepa'akai. Only four mosquitoes (all in CO_2 traps) were caught on the ridge compared to 12 (CO2 traps) and 65 mosquitoes (Oviposition traps) along the stream at Kawaikoi. Also, we caught fewer mosquitoes in the warmer fall season than in cooler January and March at Kawaikoi, while we caught more mosquitoes in November at the higher elevation Halepa'akai. These preliminary results suggest that the remaining mosquito-free area on Kauai is quickly disappearing, but Halepa'akai may still provide birds some refuge, especially in the winter months. Fortunately, the low prevalence of *P. relictum* in mosquitoes suggests that the disease agent still may be somewhat temperature-limited.

Contrary to previous observations, we didn't find any larvae along Kawaikoi and Halepa'akai streams, but instead found *C. quinquefasciatus* larvae only in small artificial pools. This difference may partly be explained by seasonal differences: during the winter months of our study, the streams regularly flood so there is little standing water, but during the summer months of earlier studies, there is less flooding along streams while artificial pools largely have dried up. The mosquito preference for stream might indicate that streams might be more protected from wind and also may offered more standing water than ridge. This might also be the reason why we didn't catch any mosquitoes in Koke'e as the sampling site wasn't near a stream. Unfortunately, since larvae were found on road and artificial containers, human activities appear to be creating multiple breeding places for mosquitoes near and on the Plateau.

To assess the prevalence of malaria, we need a larger sample size, as we observed only one positive mosquito. An analysis of temperature, rainfall and topography at each site might help us to better understand how mosquito demography is influenced by those factors. Furthermore, temperature data might give us good indication on the possibility of parasite development and survival in the vector *C. quinquefasciatus*. Additional mosquito trapping and larval surveys are essential to improve our understanding of the factors that contribute to local increases in malaria. This information will help inform our efforts to conserve species such as the Akikiki, Akeke'e, and I'iwi in the Alakai Plateau, their last refuge on Kauai.

Month	Habitat	Number of *C.Quinquefaciatus* (Oviposition traps)	Number of *C.Quinquefasciatus* (CO2 traps)	Number of C.Quinfasciatus (LIGHT trap)	Mean number of mosquitoes per trap (oviposition trap)	Mean number of mosquitoes per trap (CO2 trap)	Mean number of mosquitoes per trap (LIGHT trap)
October	Stream	3	8	NA	**0.1**	**0.3**	NA
January	Stream	1	NA	3	**0.08**	NA	**0.38**

Table 2. Total and mean number of adult mosquitoes caught per field trip and trap type in Halepa'akai Stream

Recent Bird Sightings

By Lance Tanino

MARCH 15

Snow Goose – Two observed at Ohiapilo Wetland, Moloka'i Island by Arleone Dibben-Young, Lance Tanino and Jean Campbell

Whimbrel – One observed at Koheo Wetland, Moloka'i Island by Arleone Dibben-Young, Lance Tanino and Jean Campbell

MARCH 26

Rhinoceros Auklet – Freshly dead specimen found by Naomi Worcester and Scott Freeman at Kure Atoll State Wildlife Sanctuary, Papahanaumokuakea Marine National Monument

APRIL 05

Laysan Albatross – One observed at Chain of Craters Road, Hawai'i Volcanoes National Park, Hawai'i Island by Cindy Granholm

APRIL 06

Laysan Albatross – One observed at Cape Kumukahi, Hawai'i Island by Lance Tanino and Howard Mayo

Band-rumped Storm Petrel – One observed at Kauhola Point, Hawai'i Island by Lance Tanino

APRIL 11

Kermadec Petrel – One observed at Kilauea Point, Kaua'i Island by Kim Uyehara

APRIL 12

Laysan Albatross – One observed at Cape Kumukahi, Hawai'i Island by Lance Tanino

APRIL 13

Bulwer's Petrel – One observed at Keahole Point, Hawai'i Island by Lance Tanino

Hawai'i Audubon Society and Kawainui Marsh

By Director Talia Starkey and President Linda Paul

Volunteer cutting off cattail heads in pond #10
Photo by Liz Kumabe

The Kawainui-Hamakua Marsh Complex on Windward Oahu is the largest remaining freshwater wetland in the main Hawaiian Islands and serves as habitat for several endemic and endangered water birds. Hawaii Audubon Society is part of a cooperative effort to protect the Marsh, which in 2005 was designated a Ramsar Wetland of International Importance. In 2014, we adopted Pond #10, one of eleven new shallow-water ponds built by the U. S. Army Corps of Engineers (ACE) in Kawainui Marsh to provide water bird habitat. We would like to encourage HAS members and their families to join us as we care for it.

The Society's involvement in the Kawainui Marsh dates back to the early 1990s, when its members worked on the 2004 Kawai Nui Marsh Master Plan, and then joined hands with the National Audubon Society to successfully lobby the Hawaii State Legislature for matching state funds to meet the 3:1 federal matching grant to construct the water bird ponds. The ponds were planned and the land was reserved until they could be built by the ACE, which completed the terraced ponds project in 2013.

Our contact with Kawainui Adopt-a-Pond Program is Jim Cogswell, Wildlife Biologist with the State of Hawaii Department of Land and Natural Resources (DLNR), Division of Forestry and Wildlife. The Adopt-a-Pond Program is a new effort and is envisioned as a means of involving community groups and stakeholders in the adaptive management process. DLNR hopes that the Program will help to build a connection and sense of ownership of the ponds project among the community while raising awareness and understanding.

Every first Saturday of the month, HAS members and other interested volunteers can come join us at Pond #10 at Kawainui Marsh. Activities include:

- Planting native Hawaiian wetland plants
- Clearing and controlling non-native plants, such as cattails and water hyacinth
- Creating floats for waterbird habitat
- Seining for fish, frogs and algae
- Monitoring water, vegetation, and nesting, etc.
- Other approved modifications

HAS members and volunteers have already participated in two Adopt-a-Pond Saturdays. The State continues to identify other Adopt-a-Corner partners for its remaining ponds; Le Jardin Academy in Kailua is our neighbor in Pond #9.

Our volunteers at the March and April Adopt-a-Pond sessions saw a variety of wetland birds at Kawainui Marsh, including the black-necked stilt or ae'o *(Himantopus mexicanus knudseni*), the Hawaiian coot or 'alae ke'oke'o (*Fulica alai*), Hawaiian duck or koloa maoli (*Anas wyvilliana*), and the Pacific golden-plover or kolea (*Pluvialis fulva*). Other widespread and non-native birds spotted at Kawainui Marsh included the cattle egret (*Bulbulcus ibis*) and the mallard duck. We found and tagged several floating nests made of woven reeds, hidden among the cattails that we were clipping.

The marsh complex is ringed by the stunning green backdrop of the Koolau Mountains. The closest street landmark is the Castle Medical Center of Kailua, but the marsh area feels secluded and wild. Volunteers work directly in the ponds, wading up to knee height in the runoff water that is captured

Floating nest in pond #10, *Photo by Richard Quinn*

there. In addition to the abundant bird life, there are crayfish, dragonflies, apple snails, tadpoles, and many other natural wonders for volunteers of all ages to appreciate and enjoy as they work.

The Hawaii Audubon Society is a founding member of Ho'olaulima Ia Kawainui, a network of conservation and community organizations whose collective mission is to foster public awareness and understanding of the natural, historical, cultural, and scenic resources of the Kawainui-Hamakua Marsh Complex. Our goal is to ensure the long-term protection, restoration, and stewardship of its resources. In addition to planning an annual World Wetlands Day Celebration at Kawainui Marsh, the network has been working on a resources interpretive plan and the update of the Master Plan.

We expect the DLNR and its consultants to publish its updated Draft Master Plan for the Kawainui-Hamakua Marsh Complex on May 14, 2014. The project area for the updated master plan includes not only Kawainui Marsh, but also nearby Hāmākua Marsh and a portion of the Hāmākua Marsh watershed, the hillside of Pu'uoehu (a total of nearly 1,000 acres). The environmental review process is continuing with an Environmental Impact Statement expected later this summer.

Please join us every first Saturday of the month at 9:00 a.m. to noon for the HAS Adopt-a-Pond activities at Kawainui Marsh.

We look forward to seeing you at Pond #10.

Kawainui-Hamakua Marsh Complex Tour

Please join the Hawaii Audubon Society and Jason Misaki, the O'ahu Wildlife District Manager, for a tour of the Kawainui-Hamakua Marsh Complex!

Members will be given a unique guided walk through the marsh complex to learn about the native wetland birds and their habitats as well as the restoration work that is currently being done in the complex.

Please meet at the Kawainui DOFAW Baseyard on Ulukahiki St at 9:00 am
Saturday, June 21st

Please RSVP to hiaudsoc@pixi.com

Alakai Wilderness Preserve Birding Opportunity

Hawai'i Audubon Society is pleased to offer a unique birding opportunity in the Alakai Wilderness Preserve to our members!

Jim Denny is an experienced bird guide on the island of Kauai and has been photographing the birds of Hawaii for more than 35 years. His work has been published in renowned publications such as The National Audubon Society, The Smithsonian and National Geographic magazines. Mr. Denny has offered to take small groups of HAS members to the Alakai Swamp Trail and lead them on a unique guided tour in search of Kauai's native and elusive honeycreepers.

Mr. Denny is able to accommodate 4 people per tour and can pick up members at the airport if needed. Available dates are: May 17th and 31st and July 5th and 9th. You can contact Mr. Denny directly to work out details at jimdenny@hawaii.rr.com or to HAS at hiaudsoc@pixi.com. Must be a current HAS member to participate.

Photo by Tom Dove: Kauai endemic Anianiau

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Hawai'i Audubon Society Awards for Student Research

By Wendy Johnson
Education Committee Chair

The Hawai'i Audubon Society presented two awards for outstanding research relating to Hawaii's natural history at the 57th Hawaii State Science and Engineering Fair held at the Hawaii Convention Center. In early April representatives of the Hawaii Audubon Society's Education Committee joined other agency judges in viewing the exhibits and interviewing students on the subject of their original research.

Sarah Jenkins, who is a senior at Molokai Intermediat/ High Schoo,l received the award for outstanding Senior Division research in the field of natural history. Her project, entitled "Hot Biology-Use of Thermal Imaging to Detect Nesting Behaviors of the Endangered Hawaiian Coot (Fulica Alai)", was the result of years of field work and a passion for native Hawaiian wildlife. This fascinating research project was based on the need to improve the reproductive success of endangered Hawaiian coots living at Pipi'o Pond on Molokai. Sarah's efforts involved many hours of field observations both on Molokai annd at Lokoaka Pond in Hilo, trying to define the ideal circumstances and habitat for nesting and successful chick rearing for the coots. With the innovative use of thermal imaging, Sarah was able to identify nesting sites in the dense mangrove roots surrounding Pipi'o pond, where predation by herons, egrets and mongoose is a serious problem. Using her extensive knowledge of coot behaviour, she has carefully engineered and tested Artificial Floating Islands which are large enough for coot nesting and yet small enough to discourage predators from landing on the platforms. These foating nests show great potential as a method of increasing nesting site options and reproductive success for Molokai's Hawaiian coots.

The Hawai'i Audubon Society award for outstanding Junior Division Research was awarded for a project submitted by Jared Goodwin, who is a seventh grader at Hilo Intermediate School. His project was entitled "Bacterial levels at 4-Mile Beach". Jared is a frequent visitor to this picturesque site near Hilo, and he was curious about the effect of tides, human activity, and the adjacent fish pond on bacterial levels in the ocean. He collected water samples at various stations in the area over a 15 day period at both high and low tide. After incubating the test samplers, Jared counted Enteroccocus and Clostridum colonies in each one and created impressive graphic displays to depict his results. These two types of bacteria are commonly tested as environmental markers of conatamination by sewage or other sources. Jared found that bacteria levels were higher in low tide than at high tide, indicating that bacteria are being generated from up-stream or nearshore activities and that the high tide dilutes the bacterial counts, or that the higher salinity results in die-off of bacteria. Jared plans to learn more about the water quality at one of his favorite swimming spots.

Left: Native coots nesting on floating islands, Right: Sarah Jenkins and Jared Goodwin, courtesy of Hawai'i Academy of Science

Upcoming Field Trips, Volunteer Opportunities & Events

Please RSVP with name and contact information to
Hawai'i Audubon Society at 808-528-1432 or hiaudsoc@pixi.com

Kawainui Marsh Restoration
Saturday, May 3rd from 9:00 am – noon
Saturday, June 7th from 9:00 am – noon
Volunteer at the monthly Kawainui Volunteer Day led by DLNR/DOFAW. Support some of Hawaii's most endangered waterbirds and contribute to the success of the new restoration ponds. For more information about Pond #10 contact HAS. Please meet behind Castle Medical Center – Follow Ulukahiki St. and turn right onto gravel road and continue straight towards the parking area at the end. Please RSVP.

Kawainui-Hamakua Marsh Complex Tour
Saturday, June 21st from 9:00 am – 1:00pm
Join Hawai'i Audubon Society on a guided tour through the marsh to learn about wetland birds and their habitat! Please meet at the Kawainui DOFAW baseyard. Please RSVP.

Visit to Red-Footed Booby Colony at Mokapu Peninsula
Saturday, May 31st at 7:45 am
Join HAS on a guided walk to the Red-Footed Booby Colony at Mokapu Peninsula on the Marine Corps Base Hawai'i. Please meet at H-3 Passhouse parking lot at **7:45 am** to carpool on base. All participants must bring their signed liability waiver; have current vehicle registration, insurance and safety check cards; and provide name and social security number to check in. All participants must wear close-toed shoes. Space is limited to 15 people. Please RSVP.

Palikea Forest Restoration Service Trip
Saturday, June 7th from 8 am – 5 pm
Hike to a restoration site in the native forest at Palikea, located on the south east side of the Waianae Mountain Range. This mesic forest is home to a large number of native species, including several endangered plants and native birds. The hike is 2 hours (round trip) and work will focus on controlling invasive weeds using saws and pruners and a caution-labeled herbicide using drip applicator bottles. All weeding tools and gloves will be provided. Please wear long sleeves and hiking boots with good traction. Led by the Oahu Army Natural Resources Program. Space is very limited. Sign up and fill out mandatory RCUH Volunteer Application at http://oanrp.ivolunteer.com/has.

Paiko Lagoon Low Tide Reef Walk
Saturday, June 12th at 9:15 am
Check out birds, limu, and sea creatures that may be at Paiko Lagoon! Remember to wear protective shoes, clothing and sunblock. Meet at Kuli'ou'ou Road. Please RSVP to Alice with your name and phone number at 808-864-8122.

Alakai Wilderness Preserve Birding Opportunity
Saturday, May 17th and Saturday, May 31st
Saturday, July 5th and Wednesday, July 9th
Unique guided hike on the Alakai Swamp Trail of Kauai with renowned bird guide and photographer Jim Denny. Open to current HAS members. Space limited to 4 per day. Contact Mr. Denny at jimdenny@hawaii.rr.com to arrange details.

Hawai'i Audubon Society Membership Renewal/Donation Form

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☐ $40 Family Membership
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Calendar of Events

Saturday, May 3rd & Saturday, June 7th
Kawainui Marsh Restoration

Saturday, May 17th and Saturday, may 31st
Saturday, July 5th and Wednesday, July 9th
Alakai Wilderness Preserve guided bird hike

Saturday, May 31st
Red Footed Booby Colony Tour at Mokapu Peninsula

Saturday, June 7th
Palikea Forest Restoration Service Trip

Saturday, June 12th
Paiko Lagoon Low Tide Reef Walk

Saturday, May 21st
Kawainui-Hamakua Marsh Complex Tour

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