



## Bacteria-caused Mortality of Freshwater Shrimp ('Opae-Kala-'ole, *Atyoida bisulcata*) from the Island of Hawaii

by John G. Chan

During a routine collection of Hawaiian freshwater shrimp ('Opae, *Atyoida bisulcata*), at a site on the Wailuku River, Hawaii Island, an unusual incident led to the observation of a mass mortality of these shrimp. On 8 June 1978, my three undergraduate assistants and I were collecting 'Opae from a series of pools about 200 meters downstream from the Hilo Hospital. Our collections were made during a period of low water when these pools become isolated from the main stream. Normally, the 'Opae are caught with hand-held scoop nets made of a fine mesh. The shrimp were captured for our study of the occurrence of exoskeleton lesions. On this occasion, student Mark Greer mentioned that he was able to capture several shrimp from submerged rocks bare-handed with relative ease. This is unusual, because the 'Opae tend to be alert and dart away rapidly when alarmed. We noted that many of our net-captured specimens appeared rather lethargic and showed disoriented swimming behavior. While the 'Opae appeared moribund, no dead individuals were observed in the pond.

The catch, which totaled 315 individuals, was transported back to our laboratory in two 20-liter styrofoam cooler chests containing about 7.5 liters of pond water. No external aeration was used. This method of transport had been used many times in the past without any mortality problems. On this trip, however, 14 'Opae had died by the time we returned to the laboratory at the University — a trip of only 10 minutes at the most. Upon arrival at the laboratory, the shrimp were divided into two 76-liter aquaria containing pond water and equipped with corner filters and aeration.

It was obvious that this was an unusual catch. The shrimp exhibited agitated behavior and disoriented, erratic swimming. A shrimp would often swim vertically to the top of the tank, flip over on its back and then sink to the bottom. The shrimp would then lie on its side or back, often with its pereiopods moving, but unable to right itself or to walk. Often the shrimp would be in this immobilized state for several minutes. As the condition progressed, the shrimp would spend longer periods of immobility — often on its back with its pereiopods extended outward. If prodded, the individual might dart a short distance, but swimming was not sustained. Individuals near death were unable to swim at all; the only movement exhibited by these individuals was a twitching of the pereiopods and movement of the respiratory appendages. Death followed shortly. In addition to disoriented swimming and gradual immobilization, some internal organs, particularly the hepatopancreas, underwent color changes. By the time an individual approached death, the hepatopancreas had changed from dark brown to a bright orange-red color.

By the next day, 9 June, 68 'Opae had died in one tank and 34 in the other. Together, this amounted to about 32% of the total catch. Mortality continued until less than 30 animals of the original 315 remained in both tanks at the end of 6 weeks. During the early part of the die-off, afflicted specimens were examined and cultured for the presence of possible pathogens. The remainder were preserved in formalin.

Examination of the hemolymph by phase-contrast microscopy revealed numerous bacteria. The hemolymph is usually free of bacteria, and their presence clearly indicated a bacteremia. Similar pathological conditions are known to have occurred in American lobsters (Rabin 1965), marine penaeid shrimps (Lightner and Lewis 1975), and freshwater crayfish (Amborski and Amborski 1977). As far as we know, however, this is the first observation of a septicemic condition in a freshwater shrimp. The presence of the bacteremia was confirmed by culturing the hemolymph on trypticase soy agar. The hemolymph from afflicted specimens consistently yielded bacteria; whereas 'Opae hemolymph is normally sterile. In most cases, the bacteria were predominantly of one type. One suspected pathogen was a Gram negative, short rod of about 0.6  $\mu\text{m}$  by 1.3  $\mu\text{m}$ . Analysis of the isolate using the Roche Enterotube II system provided an identification of *Citrobacter freundii*. *Citrobacter* are commonly found in water, moist soil, and feces of animals but have not been previously reported as pathogenic for crustaceans.



Hawaiian freshwater shrimp.

Photo by John I. Ford



We were able to establish that this bacterial isolate was the probable cause of mortality. Pure culture suspensions of the bacteria with approximately  $5 \times 10^5$  cells were injected under the carapace of healthy shrimp with a syringe tipped with 28 gauge needle in an attempt to induce the disease. Shrimps averaged 35 mm in total length and 0.6 g in weight. A second group of shrimp was injected with heat-killed bacteria, and a third group with sterile saline to act as a control. Ten shrimp were used for each condition, and the experiment was repeated twice. The control specimens injected with the saline alone showed no mortality. However, the specimens injected with the suspension of live bacterial isolates began within minutes to show symptoms of erratic swimming and shock similar to those of stream caught specimens. Death occurred in these specimens as early as 3 hours after injection. The heat-killed bacteria injections also proved to be lethal. These tests showed that the heat-killed cell suspension and the live cell suspension probably contained a toxic factor — possibly an endotoxin. Most Gram negative bacteria possess a cell wall polysaccharide component which can be toxic when ingested or injected into animals (Boyd and Hoerl 1981).

The pond was revisited on 17 July 1978, and a thorough investigation using a snorkel and mask revealed fewer than a hundred shrimp as compared to the thousands of individuals seen a month earlier. The dramatic decline in the 'Opae population in the pond was probably caused by the virulent bacteremia. Despite this putative die-off, dead specimens were never observed in the pond. The presence of numerous scavenging crayfish, *Procambarus clarkii*, might explain the absence of dead 'Opae. It is also possible, however, that weakened 'Opae were simply washed out of the pond by high river flow. Indeed, U.S. Geological Survey data indicate an unusually high discharge of 88 cubic meters per second on 6 July. This was far higher than the mean flow of 7.7 cubic meters per second.

While the crayfish in the pond did not appear to be affected by the bacteremia afflicting the 'Opae, they were, nevertheless, susceptible to injections of the bacterial pathogen. When injected with the bacterial pathogen in the laboratory, the crayfish exhibited a similar syndrome of lethargy and a gradual loss of equilibrium followed by death. However, control crayfish (injected with sterile saline) survived.

Exactly why the 'Opae at this particular site should suffer from a bacteremia is not known. However, the 'Opae specimens captured from this pond during this period had numerous necrotic lesions of the exoskeleton. Indeed, 170 of 315 individuals, or 54% of the specimens captured on 8 June, had one or more lesions. Previous collections of 'Opae from the Wailuku River had incidences of shell lesions ranging from less than 5% to 92% of the specimens (Chan 1978), but we had never observed lethargic behavior in the field, nor had we ever brought specimens into the laboratory and observed mortality. It is possible that the presence of shell lesions contributed to the development of the bacteremia. The intact exoskeleton normally provides an impermeable barrier to bacteria and other pathogens. Once disrupted, however, invasion of deeper tissues and subsequent pathology can occur. It is possible that the pond, isolated by low water levels, provided conditions which promoted the development of bacteremia from the lesions.

The pools were revisited on 11 July 1981 and 6 September 1981, during the period of low river flow. No 'Opae were observed,

but crayfish were present. By contrast, pools in the adjoining main Wailuku River and pools upstream at Piionua contained numerous 'Opae.

It appears that the 'Opae population declined as the pool became isolated by low water levels. Stagnant water, disease, and predation may have been contributing factors to the 'Opae decline in the isolated pool described here. It is possible that such mortality occurs in other streams where similar conditions exist.

#### ACKNOWLEDGEMENTS

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Editor's note: This manuscript was originally accepted for publication in 1981, but the manuscript was misplaced. Our apologies to the author for the delay in publication and our thanks for his willingness to resubmit the paper.



NO NA LEO 'OLE  
GEOHERMAL ENERGY DEVELOPMENT  
ON BIG ISLAND  
CONSERVATION LANDS

Conservationists have won the lengthy battle to keep geothermal energy development out of 22,000 acres of native rain forest at Kahaualea in Puna on the Big Island. This land parcel includes a relatively intact ohia forest and the upper portion of the volcanically active Kilauea East Rift Zone. Native plants and birds dominate this wet forest ecosystem. Here the calls and songs of 'Apapane, 'Amakihi, 'Elepaio, and 'Oma'o are commonly heard. 'O'u, the extremely rare and endangered honeycreeper, and 'Io, the endangered Hawaiian Hawk, have been observed at Kahaualea.

#### Background

The Hawaii Audubon Society has been involved in geothermal development issues in the Kilauea East Rift since mid-1981. At that time Campbell Estate announced plans to produce 250 megawatts of electrical power from geothermal energy on its land at Kahaualea. The development site was right next to the east boundary of Hawaii Volcanoes National Park. Years earlier, the park had publicly expressed its intention to acquire 5,650 acres of the same land that Campbell Estate now mapped for drilling sites, power plants, transmission lines, and access roads.

#### Industrial land use

The announcement was startling because of the magnitude of such large-scale industrial development in a high-quality natural environment designated for conservation land use. To whom would the developer sell 250 megawatts of electrical power when all of the Big Island used less than 100 megawatts at peak load? It turned out that the State of Hawaii was setting goals for energy self-sufficiency because of the imported oil crisis. So Campbell Estate aimed to transport most of the 250 megawatts by land and deep seabed cables to Oahu to meet that island's high power demands.

#### Coalition

In 1982 the Society joined together with the Sierra Club, Volcano Community Association, and other public-spirited individuals in a common cause. Their goals were to reduce the scale of development to what was needed to meet

the needs of the Big Island and to move the site away from the sensitive environment of Kahaualea. The Puna Geothermal Committee emerged from this coalition to take leadership roles in political action, legal counsel and fund-raising. The committee was composed of local residents representing a spectrum of community and conservation interests dedicated to working together until the issue was favorably resolved.

#### Political action

Over the next four years the geographical area in dispute moved downrift from Kahaualea to the Puna Forest Reserve and the Wao Kele O Puna Natural Area Reserve, as the State proposed a land exchange with the Campbell Estate. The controversy moved on in a marathon of public hearings, environmental impact statements, controversial decisions by the Board of Land and Natural Resources, lobbying at the State Legislature, contested case hearings, and appeals to the Circuit Court.

#### Gains

By May 1986 the Puna Geothermal Committee and their clients had achieved a substantial portion of their goals. The difficult question was whether to pursue further a reduction in the size of the Middle East Rift Geothermal Resource Subzone and reduction in the scale of development permitted. To continue would mean a costly appeal to the Hawaii Supreme Court with no clear hope of winning anything more.

#### No ohia woodchipping

A signal event in early July was the good news in the local press that Campbell Estate had withdrawn its application to downzone its newly-acquired Puna forest lands from the Protective subzone to the Limited subzone of the Conservation District. Opposition to the application had been exceedingly strong at the January public hearing in Hilo. Logging and woodchipping of ohia forests could have been permitted in the Limited subzone, but would not be allowed in the Protective subzone. So it was a victory for the native forests that Campbell's new Puna lands could not be logged in the foreseeable future.

#### Settlement

Finally, in early October, the conservationists concluded they had won as much as they were going to win and reached a settlement with Campbell Estate and the geothermal developers, True/Mid-Pacific Venture. In exchange for the conservationists withdrawing court appeals, Campbell Estate has agreed:

a) to fund a pre-drilling baseline health survey to be conducted by the State Department



of Health of about 250 households in the upper Kalapana area, where residents are most likely to experience air pollution from geothermal energy production.

b) to send the Sierra Club and other parties (including the Society) copies of all reports regarding biological conditions, and air, noise and water quality monitoring reports.

Two plaintiffs separate from other parties, Ralph Palikapu Dedman and Dr. Emmett Aluli, are unaffected by the settlement and are moving ahead with their own appeals to the Hawaii Supreme Court.

#### Summary of outcome

Here is a brief summary of the final outcome of the 4½-year struggle between the State of Hawaii, Campbell Estate, and the community-conservation advocates:

1) The land exchange between the State and Campbell Estate has been completed. Campbell acquires about 27,000 acres that include all of the Wao Kele O Puna Natural Area Reserve, the remainder of the Puna Forest Reserve, and some agricultural land. The State acquires all of the Kahaualea land parcel of 22,000 acres plus about 3,500 acres of agriculture land--including the former ohia forest that Campbell woodchipped in 1985.

2) The State and National Park Service are negotiating a land exchange in which Hawaii Volcanoes National Park will acquire Tract 22 at Kahaualea. This amounts to 5,650 acres of native rain forest on the park's eastern boundary between Thurston Lava Tube and Pauahi Crater. Tract 22 has been top priority for acquisition because of its biological value as a native rainforest. In October 1986, both houses of Congress passed a bill into law before the month was out. But before the park adds the parcel to its boundaries, federal property in the form of land or structures must be conveyed to the State in exchange.

3) The Kilauea Middle East Rift Geothermal Resource Subzone of about 9,000 acres is established on Campbell lands in what was formerly part of the Wao Kele O Puna Natural Area Reserve.

4) The new Kahaualea Natural Area Reserve of about 16,000 acres is expected to be set aside by Executive Order of Governor Ariyoshi before the end of 1986.

5) Campbell Estate and True/Mid-Pacific Venture have been granted a Conservation District Use Permit to develop geothermal wells generating an initial 25 megawatts of power. Under the permit terms, up to 100 megawatts can be produced if needed, and if health and safety requirements are met.

6) Biological and archeological surveys must be conducted before any area is cleared for operation.

7) An "air quality monitoring program" for checking levels of hydrogen sulfide, sulfur dioxide, mercury, radon, and other gases must be approved by the Board of Land and Natural Resources.

8) Noise and gas monitoring stations must be built in communities surrounding the geothermal resource production zone.

Because of the strenuous efforts of community and conservation advocates over the long haul, geothermal development in the Kilauea East Rift in the near future will be cleaner, on a smaller scale, and less destructive of native wet forests.

*Mae Mull*

*Big Is. Representative*

### PALILA WINS AGAIN!

On 19 November, Federal Judge Samuel P. King ordered the State of Hawaii to remove Mouflon Sheep from the slopes of Mauna Kea to protect the endangered Palila. The sheep, maintained by the state for sport hunting, eat the leaves, seedlings and shoots of the mamame tree, which the Palila depends on for breeding, food and shelter. There are approximately 2,200 Palila on the upper slopes of Mauna Kea where a 200-square-kilometer ring around the mountain has been designated as the birds' "critical habitat."

The decision came in a suit filed against the state by the Sierra Club, National Audubon Society and the Hawaii Audubon Society in behalf of the Palila and is the second decision by King ordering the removal of animals to protect the Palila. In 1979, he ordered the removal of feral domestic sheep and goats, which were also destroying the mamane forests. Populations of Mouflon also occur on the upper slopes of Mauna Loa in the Kau district and on Lanai.

### SPECIES-SPECIFIC FUNGUS

### APPROVED FOR USE AGAINST

### COSTER'S CURSE

The Hawaii State Board of Agriculture has recently approved the use of a Central American fungus which attacks *Clidemia* or Coster's Curse (*Clidemia hirta*), a plant pest found on all our major islands, particularly



Oahu. The fungus was found in Panama by UH plant pathologist Eduardo Trujillo. It attacks the leaves and prevents new growth on clidemia bushes, eventually killing them off. According to Stan Higa of the Department of Agriculture, there has been quite a bit of testing on other plants, and it does not attack other members of the Melastomaceae family of which clidemia is a member. There are no native Hawaiian Melastomaceae.

The state plans to spray the fungus in mountain areas where clidemia is prevalent. A native of Central and South America, clidemia first appeared in Wahiawa on Oahu in 1941. It has since spread to nearly 100,000 acres on Oahu, largely in the Koolau Range, and is now found on all the major islands. Several hundred acres on Hawaii are infested, and smaller infestations occur on Maui, Kauai, and Molokai.

The fungus to be used to combat clidemia is called *Colletotrichum gloeostorioides* f.s. *clidemiae*.



Coster's Curse (*Clidemia hirta*)

## BISHOP MUSEUM PROMOTES PRESERVATION AND STUDY OF ITS MOLLUSK COLLECTION

Thanks to two recent grants, the Bishop Museum Department of Zoology is more than \$31,000 closer to providing improved care for its malacological (mollusk) collections and planning for active research on them.

The grants will be used to temporarily hire a conservator to study a shell deterioration condition known as "Byne's disease" and to install air conditioning in the collection

area to help reduce its effects. The conservator also is expected to recommend a new permanent storage system that will eliminate this problem.

Dr. Allen Allison, chairman of the Department of Zoology, said considerable progress has been made during the past year toward his department's goal of again becoming a major, active malacological research center.

"A significant number of donations have been received, and we are presently using these funds to employ a collections technician and to purchase needed equipment," Allison said. He credited this progress to the combined efforts of a number of individuals and organizations, including the Hawaii Malacological Society.

To realize the goal of increased research activity, Allison said, "We currently are raising funds to endow a chair in malacology; that person will initiate an active research program bringing additional use and support to the collection."

Starting with material collected in 1837 and now including more than six million specimens, Bishop Museum's mollusk collection is the eighth largest in the United States. The terrestrial mollusk (land snail) collection, which is two-thirds of the total, is the third largest in the country and also is the world's largest and most complete collection of Pacific Island land snails.

Assembled largely through the efforts of the late Charles Montague Cooke Jr., who was a Bishop Museum malacologist from 1902 to 1948, Bishop Museum's collection represents marine and land mollusks from Polynesia, Micronesia, and parts of Melanesia. A prominent American malacologist calls the collection "without equal."

Dr. Cooke envisioned the museum as a "Mecca" to which specialists might turn for study material and find practically all of the species assembled and the preliminary segregation work completed.

While Cooke used the best technology available in his time, he could not have anticipated the long-term effect upon the shells of acids in the cardboard storage boxes and wooden storage cabinets, Allison added. This has brought about Bynne's disease.

By lowering air temperature and humidity, air conditioning, when installed, will reduce the effects of Bynne's disease on the collection. However, the problem will not be eliminated completely until all shells are transferred to a better storage system. Installation of the new system, once selected and obtained, and transfer and labeling of the shells will take several years.



## DONATIONS SOUGHT FOR 'ELEPAIO COMPUTER

For the past ten years, the newsletter portion of the 'Elepaio has been typed out on the Society's IBM typewriter. This faithful machine has begun to falter in recent months. Rather than replace it with a newer model, the editorial committee has recommended, and the HAS Board has approved, the purchase of a computer, printer, and software appropriate for producing our journal. By entering the world of "desk top publishing," we hope to streamline the typing and layout while improving the style of the journal. The new machinery has been priced at \$8,000. The Society is now soliciting donations to cover part or all of the purchase. If you would like to contribute towards this improvement of our journal, please send your donation to:

'Elepaio Fund  
Hawaii Audubon Society  
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## NEW ADDRESS FOR THE 'ELEPAIO

The 'Elepaio will be moving to a new address, beginning 1 January 1987. T. Pratt's new apartment, a block-and-a-half mauka of his present residence on Spencer Street, offers to paste-up participants the same convenient, in-town location and AMPLE PARKING. Correspondence for the 'Elepaio should be sent to this new address:

The 'Elepaio  
1022 Prospect St., Apt. 1103  
Honolulu, HI 96822.

Correspondants wishing to "play it safe" until after the move may prefer to send mail to the regular HAS address:

Hawaii Audubon Society  
P. O. Box 22832  
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## POSTCARDS AVAILABLE

Post cards of the Hawaiian Monk Seal hauled out alongside a Hawaiian Green (Sea) Turtle are still for sale by the Hawaii Audubon Society. The cards come in packets of 50, and

sell for \$6.00 a packet. Please send requests and checks to:

Hawaii Audubon Postcards  
c/o Marjorie F. Ziegler  
45-636 Liula Place  
Kaneohe, Hawaii 96744

## ANNUAL MEETING ELECTION OF OFFICERS DECEMBER PROGRAM

The Hawaii Audubon Society will hold its annual meeting and election of officers on 15 December at the Moiliili-McCully Library. If you haven't mailed your ballot in, you may bring it to the meeting, where votes will be counted.

The December meeting will also feature a program entitled, "Geographic variation in Laysan Finch populations." The speaker, Dr. Sheila Conant, has been studying Laysan Finches at Laysan Is., where the natural population occurs, and at Pearl and Hermes Reef, where a population of finches was introduced by the U.S. Fish & Wildlife Service in 1967. Conant has found that the populations on the two atolls differ slightly, but significantly, in bill shape, and she is currently working with Marie Morin and Dr. Robert Fleischer to determine what some of the causes of variation might be. Slides will feature finches, a variety of seabirds, monk seals, and sea turtles, as well as biologists at work.

## 1986 CHRISTMAS COUNTS

There will be six Audubon Christmas Counts this year. These counts are always exciting, with records to be broken and new birds to be seen. We especially need people to attend the counts on the outer islands. The counts have been scheduled to facilitate a weekend visit to Kauai or Hawaii. For information on Kauai counts, contact Winona Sears at 822-3045. For Hawaii Is. counts, call Larry Katahira at 967-7416 (home). Bob Pyle (262-4046) will be coordinating Oahu counts. The counts are scheduled as follows:

### Oahu:

Waipio - Saturday, 20 December  
Honolulu - Sunday, 21 December

### Kauai:

Lihue - Saturday, 20 December  
Kapaa - Saturday, 27 December  
Waimea - Sunday, 28 December

### Hawaii:

Volcano - Saturday, 3 January



### HAWAII AUDUBON SOCIETY

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Mauī	(vacant)	572-1499
Hawai'i	Mae Mull	967-7352

### FREE ICE CREAM

Will again be served to those volunteers who help with the typing, proof-reading, or paste-up of next month's 'Elepaio at Thane Pratt's house, 954 Spencer St. on Saturday, 13 December, at 1:00 PM. Phone 524-8464 for more information. Authors of articles, notices, etc. must submit these by 7 December to be included in the January issue.

Many thanks to Sheila Conant, David McCauley and Susan Schenck.

### 'ELEPAIO

Editors	Thane Pratt	524-8464
	Sheila Conant	948-8241
Production	Robert Fleischer, Marie Morin, Robert Pyle	
Mailing	George Campbell, Susan Schenk, Alan Ziegler	

(MANUSCRIPTS of articles and newsletter items may be sent to the Managing Editor at 954 Spencer St., Honolulu, HI 96822. Articles not subject to peer review MUST be received by the 15th of each month to be considered for publication in the next month's issue.

#### IF NOT A MEMBER, PLEASE JOIN US

##### JOINT MEMBERSHIP

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*All Local Memberships and Subscriptions are for a calendar year January through December.*



CALENDAR OF EVENTS

- Dec. 8 (Mon.) Board Meeting at Bishop Museum at 7:00 PM. Call Phil Bruner, 293-3820 (work).
  - Dec. 15 (Mon.) General Meeting at McCully-Moiliili Library at 7:30 PM. Program notice on page 196.
  - Dec. 20 (Sat.) Christmas Count - Waipio, O.  
Christmas Count - Lihue, K.
  - Dec. 21 (Sun.) Christmas Count - Honolulu, O.
  - Dec. 27 (Sat.) Christmas Count - Kapaa, K.
  - Dec. 28 (Sun.) Christmas Count - Waimea, K.
  - Jan. 3 (Sat.) Christmas Count - Volcano, H.
- For all Christmas Counts, refer to notice on page 196.

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