

## Coastal Unit 3

# Anchialine Detectives

## Overview

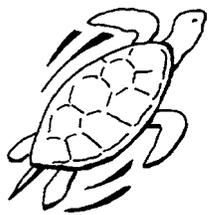
Anchialine ponds are pools containing brackish or salt water that have no surface connection with the ocean. On Maui, they dot the southwest coastline of Maui on Cape Kīnau and Cape Hanamanioa. In this unit, students explore the anchialine pond habitat, using their reasoning abilities to develop hypotheses that explain the ponds' characteristics as well as an interesting distribution pattern associated with the small red shrimp that inhabit many of these ponds. They also conduct an experiment to test the salinity tolerance of brine shrimp, which, like the red shrimp of the Maui ponds, are adapted to a range of habitat conditions.

## Length of Entire Unit

Four class periods

## Unit Focus Questions

- 1) Why are anchialine ponds a special habitat on Haleakalā?
- 2) What are the unique characteristics of anchialine ponds and what geological and biological factors may help explain these characteristics?
- 3) How do brine shrimp respond to differences in salinity, a key environmental variable in anchialine ponds?



## Unit at a Glance

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### Activity #1

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#### **Anchialine Pond Detective Story**

Students solve six “mysteries” related to anchialine ponds and red shrimp that are found in these ponds.

#### **Length**

One class period

#### **Prerequisite Activity**

None

#### **Objectives**

- Describe and explain key environmental characteristics of the anchialine pond habitat.
- Generate hypotheses to explain anchialine pond characteristics and adjust these hypotheses based on new information.
- Devise a way to test a scientist’s hypothesis about the distribution of shrimp species associated with anchialine ponds.

#### **DOE Grades 9-12 Science Standards and Benchmarks**

**DOING SCIENTIFIC INQUIRY:** Students demonstrate the skills necessary to engage in scientific inquiry.

- Formulate scientific explanations and conclusions and models using logic and evidence.

**LIVING THE VALUES, ATTITUDES, AND COMMITMENTS OF THE INQUIRING MIND:** Students apply the values, attitudes, and commitments characteristic of an inquiring mind.

- **OPEN-MINDEDNESS:** When appropriate, modify ideas, explanations, and hypotheses, based on empirical data or evidence.

### Activity #2

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#### **Salinity Tolerance Lab**

Students conduct a lab to test brine shrimp tolerance for different salinity levels.

#### **Length**

Three class periods

#### **Prerequisite Activity**

None

#### **Objectives**

- Investigate salinity tolerance among brine shrimp, accurately performing lab procedures, making observations, and recording results.

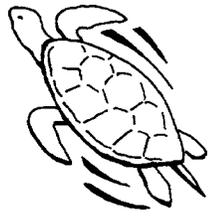
#### **DOE Grades 9-12 Science Standards and Benchmarks**

**DOING SCIENTIFIC INQUIRY:** Students demonstrate the skills necessary to engage in scientific inquiry.

- Develop and clarify questions and hypotheses that guide scientific investigations.
- Design and conduct scientific experiments to test hypotheses.

**LIVING THE VALUES, ATTITUDES, AND COMMITMENTS OF THE INQUIRING MIND:** Students apply the values, attitudes, and commitments characteristic of an inquiring mind.

- **HONESTY:** Report findings accurately without alterations and draw conclusions from unaltered findings.



## Enrichment Ideas

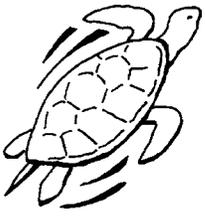
- Devise and conduct an experiment to test the effect of different light conditions and water temperatures on brine shrimp hatching rates, using the salinity concentration that was the most conducive to hatching in the first experiment in Activity #2 “Salinity Tolerance Lab.”
- Build on Activity #2 “Salinity Tolerance Lab” by devising and conducting an experiment to investigate habitat *preference* among adult brine shrimp using environmental variables such as salinity, temperature, and light. One such experiment establishes condition gradients in water contained in flexible plastic tubing, allowing shrimp to congregate in the preferred conditions. By clamping the tubing into sections, students are able to sample along this gradient to determine habitat preferences.

## Resources for Further Reading and Research

Maciolek, John A., “Distribution and Biology of Indo-Pacific Insular Hypogeal Shrimps,” *Bulletin of Marine Science*, 1983, Vol. 33, No. 3, pp. 606-618.

Science Junction, The Brine Shrimp Project at [www.ncsu.edu/sciencejunction/terminal/lessons/brine.html](http://www.ncsu.edu/sciencejunction/terminal/lessons/brine.html).

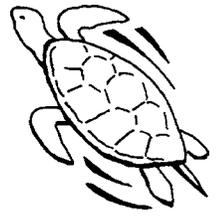
Includes a lesson plan for an investigation into the factors that affect the hatching and development of brine shrimp eggs.



Coastal Unit 3

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Activity #1

# Anchialine Pond Detective Story

## ● ● ● Class Period One *Anchialine Pond Detective Story*

### Materials & Setup

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- “The Anchialine Pond Detective Story” acetates (master, pp. 11-19)
- Overhead projector and screen

*For each student or group of three to four students*

- Student Page “The Anchialine Pond Detective Worksheet” (pp. 20-25)

*For each student*

- Student Page “Hypothetically Speaking” (pp. 26-28)

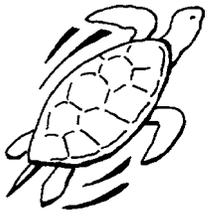
### Instructions

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- 1) Divide the class into groups of three to four students, or have students work individually. Hand out the Student Page “The Anchialine Pond Detective Worksheet.”
- 2) There are eight acetates.
  - Acetate #1 introduces anchialine ponds and the activity.
  - Acetates #2-6 each pose a mystery for students to resolve to by developing hypotheses. Most include the question, a photo, and a series of observations.
  - Acetate #7 is an “Information Interlude” that provides students with background for the next “mystery” acetate.
  - Acetate #8 is another “mystery” acetate that includes a question, a photo, and a series of observations.

Use the acetates as a “script” for this activity. Go through the acetates, one by one, giving student groups time to come up with a hypothesis for each question and write it on their worksheets. You may incorporate class discussion into this activity by asking students to discuss their ideas openly with each other before recording their hypotheses. Before moving on to the next acetate, review the correct answer using the responses and additional information provided in the teacher background for “The Anchialine Pond Detective Story” (pp. 7-10).

- 3) Hand out the Student Page “Hypothetically Speaking” as homework.



### Journal Ideas

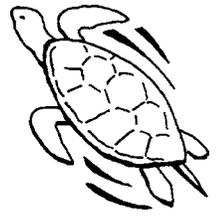
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- Do you think it's important to protect the anchialine pools on Maui? Why or why not?
- Write a short story about the adventures of a traveling shrimp.

### Assessment Tools

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- Participation in group work
- Student Page "The Anchialine Pond Detective Worksheet"
- Student Page "Hypothetically Speaking"
- Journal entries



## Teacher Background

# The Anchialine Pond Detective Story

## Background on Anchialine Ponds

The southwest coastline of Maui, dominated by rough ‘a‘ā flows, is dotted here and there by anchialine ponds. These ponds are found between Cape Kīnau and Cape Hanamanioa. Anchialine ponds exist elsewhere on the Hawaiian Islands, as well.

Anchialine ponds are pools containing brackish or salt water but that have no surface connection with the ocean. Water levels in these ponds fluctuate with the tides, offering evidence that the pools maintain subsurface connections with the ocean through tiny openings in the porous lava substrate. In some anchialine ponds, the water is almost fresh, which indicates the mixing of ocean water with fresh ground water.

Ocean water enters the pools through underground fissures in the lava rock. The fresh water comes from direct rainwater and from groundwater that filters down from mountain slopes underground. This fresh water can be reached by digging wells along the shoreline, and it also seeps into lava tubes at sea level, the ponds, and into the ocean.

Fresh ground water forms a cold freshwater lens floating on top of the warmer ocean water because fresh water is less dense than salt water. Normally one might expect the warm water to be closer to the surface, but the difference in density makes the temperature reversal happen. In deeper ponds, this layering tends to stay in place. In shallow ponds under windy conditions, the layers get disturbed.

Common plants in anchialine ponds include marine algae (seaweeds), cyanobacteria (blue-green algae), and native widgeon grass (*Ruppia maritima*). Along pond edges, non-native pickleweed (*Batis maritima*), *makaloa* (*Cyperus laevigatus*), and ‘ākulikuli (*Sesuvium portulacastrum*) are sometimes found. Close to the ocean, anchialine pond fauna includes damselflies, eels, crustaceans, mollusks, worms, the Hawaiian flagtail or *āholehole*, mullets, and gobies. In ponds that are further inland, fish and marine algae are not typically found. In these ponds, the fauna is dominated by shrimp, most commonly *Metabetaeus lohena* and the endemic ‘ōpae ‘ula (*Halocaridina rubra*).

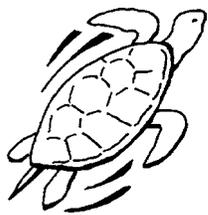
The hypogeal (which means associated with or living below the surface of the earth) shrimp species found in Maui anchialine ponds have been identified in similar habitats on a relatively small number of islands spread around the world, from Hawai‘i to the western Indian Ocean. Trying to account for this “disjunct distribution pattern” has occupied scientists since the early 1960s. In 1983, Hawaiian marine scientist John A. Maciolek proposed a new hypothesis that students will consider during this activity.

## Solving the Mysteries

Use the following notes to help students “solve” the anchialine pond mysteries presented on the “The Anchialine Pond Detective Story” acetates.

### **Mystery #1: The water in the ponds is both fresh water and salt water. Where does this water come from?**

There are fissures in the rock that allow ocean water to enter the pools from underground. The freshwater component is both rainwater and groundwater. Groundwater comes from rain that falls on the mountain slopes, then seeps underground and slowly flows down Haleakalā. This fresh



water can be reached by digging wells along the shoreline, and it also seeps into lava tubes at sea level, the ponds, and into the ocean.

In the anchialine ponds, it forms a cold freshwater lens floating on top of the warmer ocean water because fresh water is less dense than salt water. Normally one might expect the warm water to be closer to the surface, but the difference in density makes the temperature reversal happen.

**Mystery #2: What makes the water turn red each spring at Wai‘ānapanapa?**

‘Ōpae ‘ula—the red shrimp—gather in the spring, turning the rocks and pool red. According to Hawaiian tradition, this is a sign of forgiveness or the casting out of an evil spirit.

**Mystery #3: You often see small, red shrimp in the ponds. If all the water goes out of the ponds at low tide, the shrimp disappear. Where do they go?**

The shrimp live in the underground water, in cracks and fissures in the rock.

**Mystery #4: The same species of shrimp that live in the Maui ponds also live in similar ponds on the Kona coast of Hawai‘i and holes in the ancient (and dry) coral reef that is now the ‘Ewa plain of O‘ahu. How did the shrimp travel between the islands?**

Ocean currents transporting planktonic larval stages of shrimp probably account for the dispersal.

**Mystery #5: The larvae reach the shorelines of the other islands. How do they get into the ponds?**

Shrimp may find the ponds by following the trail of the fresh water that seeps into the ocean through cracks. (However, this has not been established, so other well-reasoned answers could be correct.)

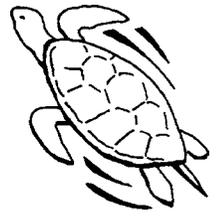
**Information Interlude**

Have students turn to pages 3-5 of the student page (pp. 21-23) that contains the map of world-wide dispersal of shrimp species, and the distribution table. Discuss these maps, highlighting the fact that certain of these shrimp species are found in widely separated geographic areas.

**Mystery #6: Theories about isolation and speciation hold that shrimp species that live thousands of miles apart should have evolved into separate species. What can explain the fact that populations of the same shrimp species are found thousands of miles apart?**

There is no answer to this question that has been proven correct. Encourage students to explain their hypotheses and their reasoning. As part of their homework (or for the remainder of the class) have students consider John Maciolek’s hypothesis to explain this phenomenon (on the first page of the Student Page “Hypothetically Speaking”).

The following section of this teacher background piece provides more information on John Maciolek’s hypothesis which may help you explain it better to students. The reading is technical and may be difficult to understand for some classes. You may use it to lead in to the homework assignment (Student Page “Hypothetically Speaking”) or you choose to hold a brief in-class discussion following the homework assignment and use some of this information as background.



## Explaining Disjunct Shrimp Populations

Excerpted from John A. Maciolek, “Distribution and Biology of Indo-Pacific Insular Hypogeal Shrimps,” *Bulletin of Marine Science*, 1983, Vol. 33, No. 3, pp. 606-618.

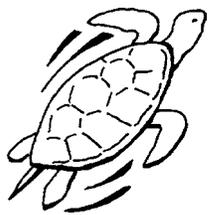
Four hypotheses explaining disjunct distributions of hypogeal [shrimp] were discussed by Smith and Williams (1981). With reference to the “extremely disjunctive” distribution of *Antecaridina lauensis*, they rejected three of them: parallel evolution (i.e., from a common ubiquitous ancestor), transport by man, and persistence as relicts. They maintained that “Passive oceanic dispersal remains the only plausible explanation for the present distribution of *A. lauensis* . . .” Dispersal as larvae or post larvae seems most likely, but adult migration is a possibility because of the known salinity tolerances and unusual longevity of the species.

Six other members of the insular hypogeal shrimp group treated here [see the Student Page “Anchialine Pond Detective Worksheet” for details] have such disrupt distributions. The most extreme hiatus is that between populations of *Caliasmata pholidota* in the central Pacific and the northern Red Sea. It is also plausible to explain many of these scattered populations as the result of passive oceanic dispersal. This concept is supported by the occurrences of populations of several species on nearby islands that are separated by deep water. For example, five species occur on the adjacent islands of Maui and Hawai‘i. Although their populations are not disjunct in a relative sense, the separation indicates the shrimps’ abilities to migrate across an interval (50 km) of deep (>2000 m) ocean that has never had a shallow or emergent bridge.

Passive oceanic dispersal does not itself explain extreme separation of populations, nor whether any observed disjunction represents more than a single migration resulting in successful colonization. The phenotypic uniformity of species in separated populations implies either a one-time migration, recently or with complete cessation of evolution, or at least occasional interchange from repetitive migrations. Genetic mixing, which seems more likely, would require yet undetected populations at closer distances. The continuing discovery of hypogeal shrimps in new locations reduces the degree of disjunction for some species. But there remain vast areas of ocean, having currents adverse to passive dispersal and without island “steps,” which would ultimately defy complete elimination of apparent disjunction. The obvious question is, do these shrimps require island—emergent land—as a habitat feature? If not, a given species could be more [widespread] in the Indo-Pacific, and oceanic dispersal would apply more universally as an explanation of all insular hypogeal shrimp distributions.

Thus far, these hypogeal shrimps have been reported only from on or within emergent land, and much emphasis has been given to their occurrence in exposed pools. Data presented earlier indicated that the essential habitat requirement for the shrimps as a group is seawater in rock interstices, and not its surface exposure in pools. Emergent land offers one unique contribution in some situations—the dilution of seawater with fresh groundwater. That these shrimps do not require such mixohalinity [low salinity levels] is shown by five of the species (*Antecaridina lauensis*, *Caliasmata pholidota*, *Halocaridina rubra*, *Ligur uveae*, and *Periclimenes pholeter*) that are known to have sustaining populations in continually euhaline (30% salinity and higher) water. No factors are evident that would prohibit these shrimps from inhabiting submerged rock that had the necessary interstitial cavities. Such habitat would be very difficult . . . to sample.

Accordingly, I submit that most if not all of these hypogeal shrimps could have widespread and probably longstanding populations in submerged as well as emergent rock of the tropical Indo-Pacific.



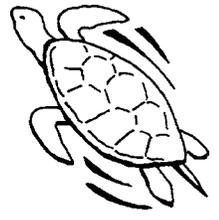
The following evidence is offered in further support of this hypothesis: (1) The common appearance of these shrimps in modern artificial exposures of groundwater (wells, quarries, and a bomb crater), as well as in very recent lava fields, more likely has resulted from localized movement of existing subterranean populations than by new migration from a distant and unspecified locality. (2) Most reported habitat localities are low islands that were probably inundated by high sea levels during the Pleistocene. It is more reasonable to assume that many or most populations existed there during submergence than that they all represent recent (post-emergence) migrations—a situation that would also raise the question of the whereabouts of Pleistocene refugia. (3) More direct substantiation is in unpublished information recently communicated to me by Dr. A. H. Banner of the University of Hawaii, and by Dr. D. Devaney and Ms. A. Fielding of the Bishop Museum. Dr. Banner's information concerns the discovery of *Metabetaeus* in limestone rubble from the reef flat of Ambon Bay and from the outer reef face near Tulear, Madagascar [locations indicated by question marks on the map in the student page]. Ambon material consisted of a single specimen of *M. lohena*, which, although not signifying an established population, does suggest the occurrence of one in the reef structure. The Tulear material consisted of five specimens obtained by an airlift pump from a depth of about 7 m. Unfortunately, [no specimen could be identified] to species. If this is either of the two established species of the genus, the finding would create a distribution nearly as disjunct as that of *Calliasmata pholidota*.

Information from the Bishop Museum concerns collections and sightings of *Ligur uveae* by scuba divers at several littoral marine locations in the central Pacific. Specimens cataloged in the Museum were collected in 1979 from a cave about 100 m offshore and 8 m deep at Oahu Island, and in 1981 from similar submerged caves at Maui and Hawai'i. *L. uveae* was seen by knowledgeable divers in the same type of habitat at Lanai, Kahoolawe, and Hawai'i, and also Kwajalein and Enewetak Atolls, Marshall Islands. The shrimp was found in the farthest recesses of the caves where it occurred on rock surfaces in groups of 10 or more. Individuals were not disturbed by close approach nor artificial light, and they had the night-time coloration described by Wear and Holthuis (1997). These occurrences suggest that *L. uveae* may well have a widespread distribution in the tropical marine littoral zone.

This broadened habitat hypothesis allows that the shrimps could occur in the groundwaters of many isolated and archipelagic islands where they have not yet been found, in shallow reefs and seamounts, and possibly in suitable rock of continental shelves. Distributions would be limited by a thermal minimum, as discussed earlier . . . [and] distributional limits become restricted with increasing depth [in the Pacific, that would occur at approximately 100 m]. Below this depth, 20°C water disappears rapidly; at 150 m, it separates into relatively small hemispheric masses separated by cool (15°C) water between 5° and 10°N latitude.

### Literature Cited

- Smith, M. J. and W. D. Williams. 1981. The occurrence of *Antecaridina lauensis* (Edmonson) (Crustacea, Decapoda, Atyidae) in the Solomon Islands—intriguing biogeographical problem. *Hydrobiologia* 85: 49-58.
- Wear, R. G. and L. B. Holthuis. 1977. A new record for the anchialine shrimp *Ligur uveae* (Borradaile 1899) (Decapoda, Hippolytidae) in the Philippines with notes on its morphology, behavior, and ecology. *Zool. Meded.* 51: 125-140.



# The Anchialine Pond Detective Story

## Acetate #1

### What are Anchialine Ponds?

Here and there in the rough 'a'ā flows that dominate the southwest coastline of Maui between Cape Kīna'u and Cape Hanamanioa, lies a scattering of anchialine ponds. Anchialine ponds are brackish or saltwater pools, a unique habitat found on Maui, O'ahu, and Hawai'i, as well as on other islands and coastal areas in tropical regions around the world.

Anchialine ponds are simple natural systems in which the balance is easily disturbed. For example, people sometimes disrupt the system by dumping aquarium fish in the ponds. The fish eat all the red shrimp, which normally feed on algae. With the shrimp essentially removed from the system, the algae can take over the pond.

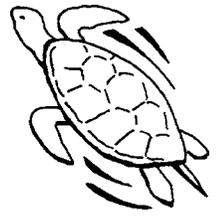


*Anchialine ponds at 'Āhiki-Kīna'u  
(Photo: Forest Starr and Kim Martz)*

### Mysteries to Solve

Anchialine ponds in Hawai'i are home to several species of tiny shrimp, some of which are found nowhere else in the world and others which have been found in anchialine ponds thousands of miles away.

You will be asked to solve six mysteries linked to these tiny shrimp. You will develop hypotheses to explain these mysteries based on observations made by scientists who have studied these unique ponds and their tiny inhabitants for many years.



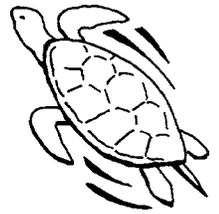
### Mystery #1

The water in the ponds is both fresh water and salt water. Where does this water come from?



*Anchialine pool at Kanaio  
(Photo: Philip Thomas)*

- Observation #1      If you taste the water, it is slightly salty but not as salty as the ocean.
- Observation #2      While the ponds in the photos are near the coast, they are far enough from the ocean that waves do not break into them.
- Observation #3      The water in the ponds rises and falls with the tides along the shoreline.
- Observation #4      If you swim along the lava rock shoreline, you will swim through water that is very blurry and colder than the surrounding ocean water.
- Observation #5      Hawaiians living along the lava shorelines from Cape Kīna‘u, Kanaio and south to Kahikinui were able to dig wells along the coast or explore coastal lava tubes and find water that was slightly salty but good enough to drink. This was the only source of water for many people living along this coastline.



### Acetate #3

## Mystery #2

What makes the water turn red each spring at Wai‘ānapanapa?

Popoalaea, a Hawaiian chiefess from the Hāna area above Wai‘anapanapa, was married to a powerful and arrogant warrior chief named Kakae. Kakae became jealous of her affection for her brother, Pi‘ilani, and threatened to kill the chiefess. Popoalaea fled for her life, along with her faithful companion, Manona.

At the last minute, Manona picked up a small *kāhili*. This feathered standard was a symbol of royalty. They fled toward the sea, travelling in lava tubes and under cover until they reached the ocean at Papaloa, near Hāna.

Popoalaea and Manona found a deep cavern in which they hid during the day, emerging only at night to look for food and a way to escape to another island. A pool of water filled the entrance to the cave, and to enter, the women had to dive into the pool and under a jutting ledge. The cavern opened into a low room where Popoalaea and Manona passed their days silently dangling their feet in the water. Manona would sometimes wave the *kāhili* slowly back and forth to distract her mistress’s thoughts.

Meanwhile Kakae searched madly for his wife. At the village of Honokalani, he heard strange tales of spirits wandering the shore at night. Nearby, he stopped to rest on the rocks just above a pool of water at the entrance to a cave. He noticed the perfect reflection of the cave roof in the still surface of the water. Suddenly he saw something move in the reflection, and recognized it as the *kāhili*.

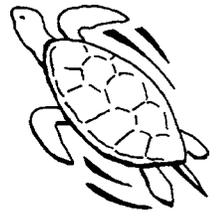
Kakae and his men dove into the cavern and there killed both women by dashing them against the rocks, then throwing their bodies into the pool. To this day, the roof and sides of the cave are dark with the women’s blood. On the nights of Kū, when the moon is in a certain stage, the waters of the pool are said to become red, and there is an eerie light in the cave.

In the spring—the time of year at which the tragedy took place—the stones are said to be a redder hue.

*Adapted from Elspeth P. Sterling, Sites of Maui, Bishop Museum Press, Honolulu, 1998, pp. 125-6.*



*Wai‘ānapanapa cave  
(Photo: Carol Gentz)*



Acetate #4

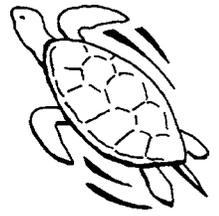
### Mystery #3

You often see small, red shrimp in the ponds. If all the water goes out of the ponds at low tide, the shrimp disappear. Where do they go?



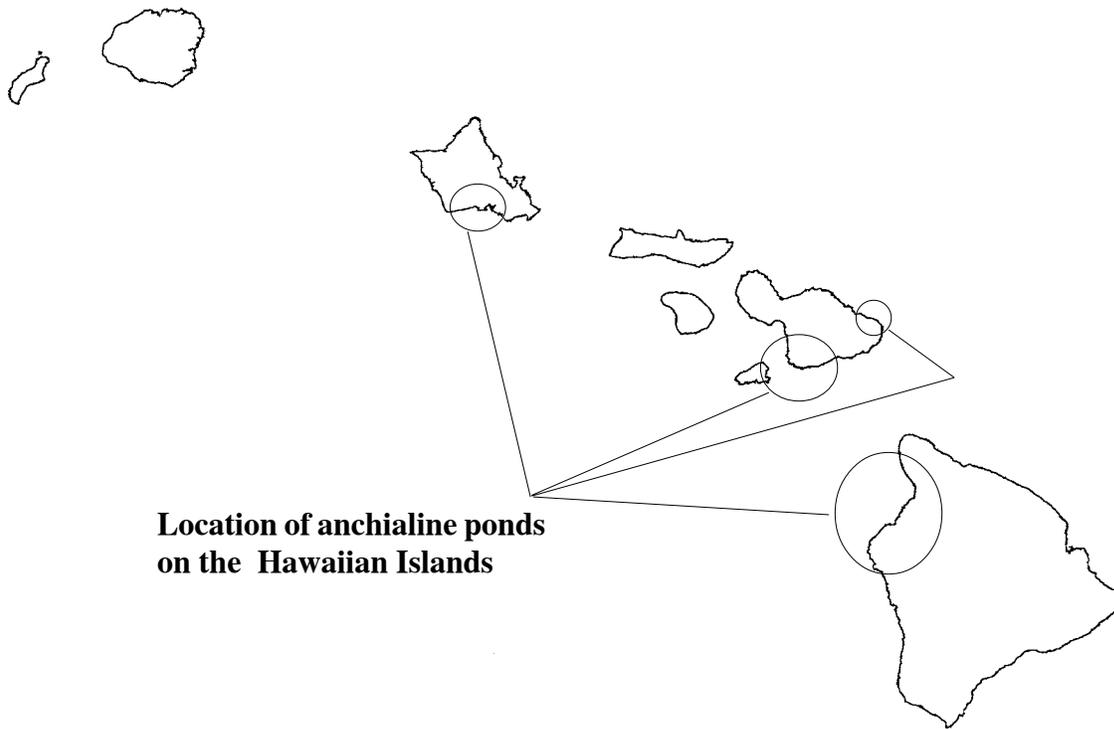
*Photo: John Hoover, Hawai'i's Sea Creatures, Mutual Publishing*

- Observation #1: When the tide starts rising and the water level rises in the ponds, too, the shrimp enter the ponds.
- Observation #2: If you dig a well along the shoreline, you are likely to have some of these shrimp in your well.
- Observation #3: So far, the shrimp found in the ponds in Hawai'i have not been seen in the ocean. But they are small, so it could be the case that they simply have not been discovered in the ocean yet. Most do not exceed 3.0 cm (1.2 in) in body length.

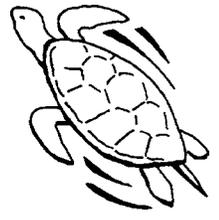


### Mystery #4

The same species of shrimp that live in the Maui ponds also live in similar ponds on the Kona coast of Hawai'i and holes in the ancient (and dry) coral reef that is now the 'Ewa plain of O'ahu. How did the shrimp travel between the islands?



- Observation #1      These shrimp can live in a wide range of salinity levels but need some saltiness in the water.
- Observation #2      Maui and the Big Island have a deep-water channel between them, and even when the sea level was lower during the last ice age about 12,000 years ago, there was still a deep channel.
- Observation #3      In their larval stages, reef fish and corals disperse to other places as part of the "plankton" (tiny organisms that float freely through the ocean).

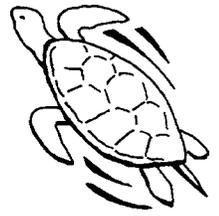


**Mystery #5**

The larvae reach the shorelines of the other islands. How do they get into the ponds?



*Anchialine ponds at Cape Hanamanoia  
(Photo: Forest Starr and Kim Martz)*



## Information Interlude

Anchialine ponds are found in coastal areas throughout the tropics. As you can see from the maps in “The Anchialine Pond Detective Story Worksheet,” certain species are found in ponds separated sometimes by thousands of miles of ocean.

Find these examples on the shrimp distribution maps:

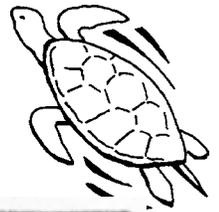
Cp = *Callinectes pholidota*

Found in ponds on Hawaiian Islands, Ellice Islands, and the north end of the Red Sea

Al = *Antecaridina lauensis*

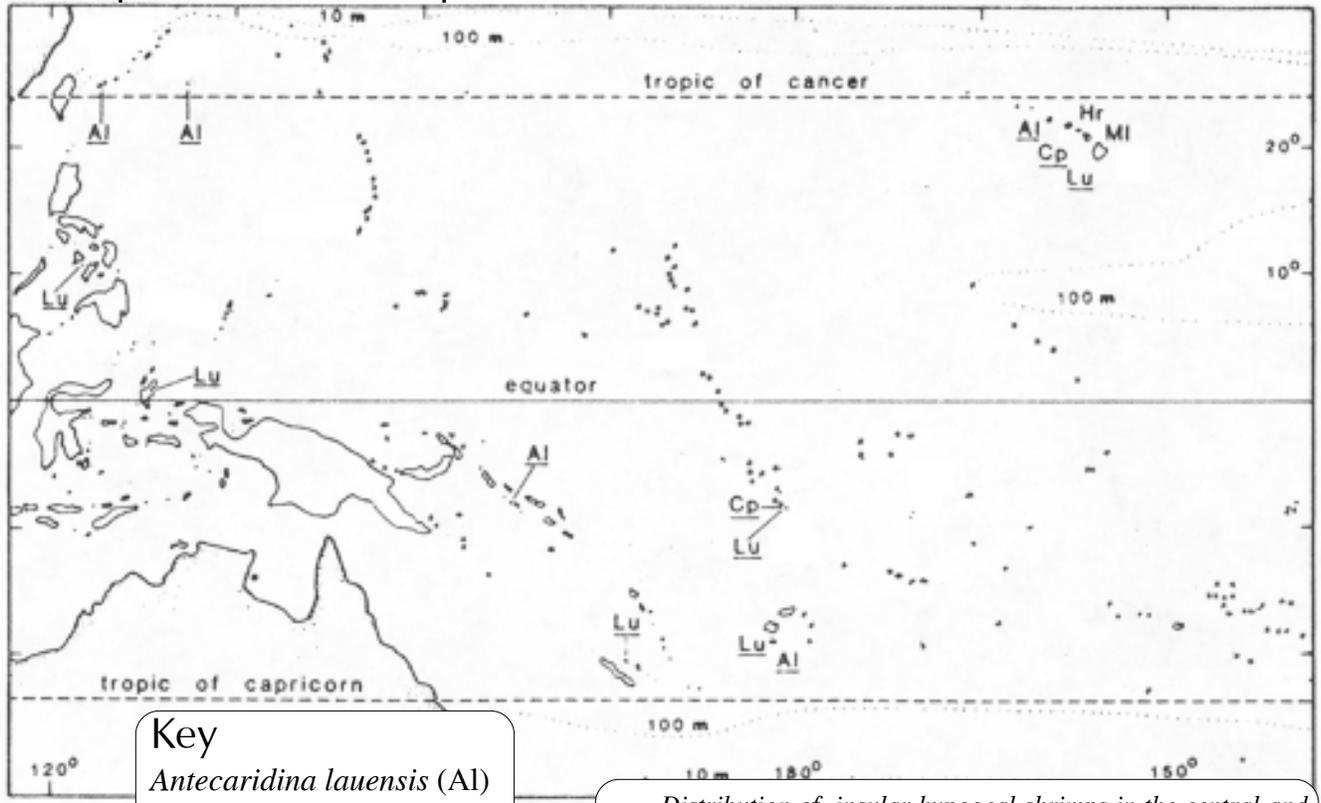
Found in ponds on Hawaiian Islands, Fiji Islands, Mozambique Channel Islands, Solomon Islands, at the south end of the Red Sea, and in Japan

According to widely accepted ideas about isolation and speciation, these widely separated populations should have diverged into separate species because of the limited pool of genetic material and specific local conditions. If these shrimp populations are as separate as they seem, scientists would expect to see more endemic shrimp species and fewer species that were scattered in anchialine ponds around the world.



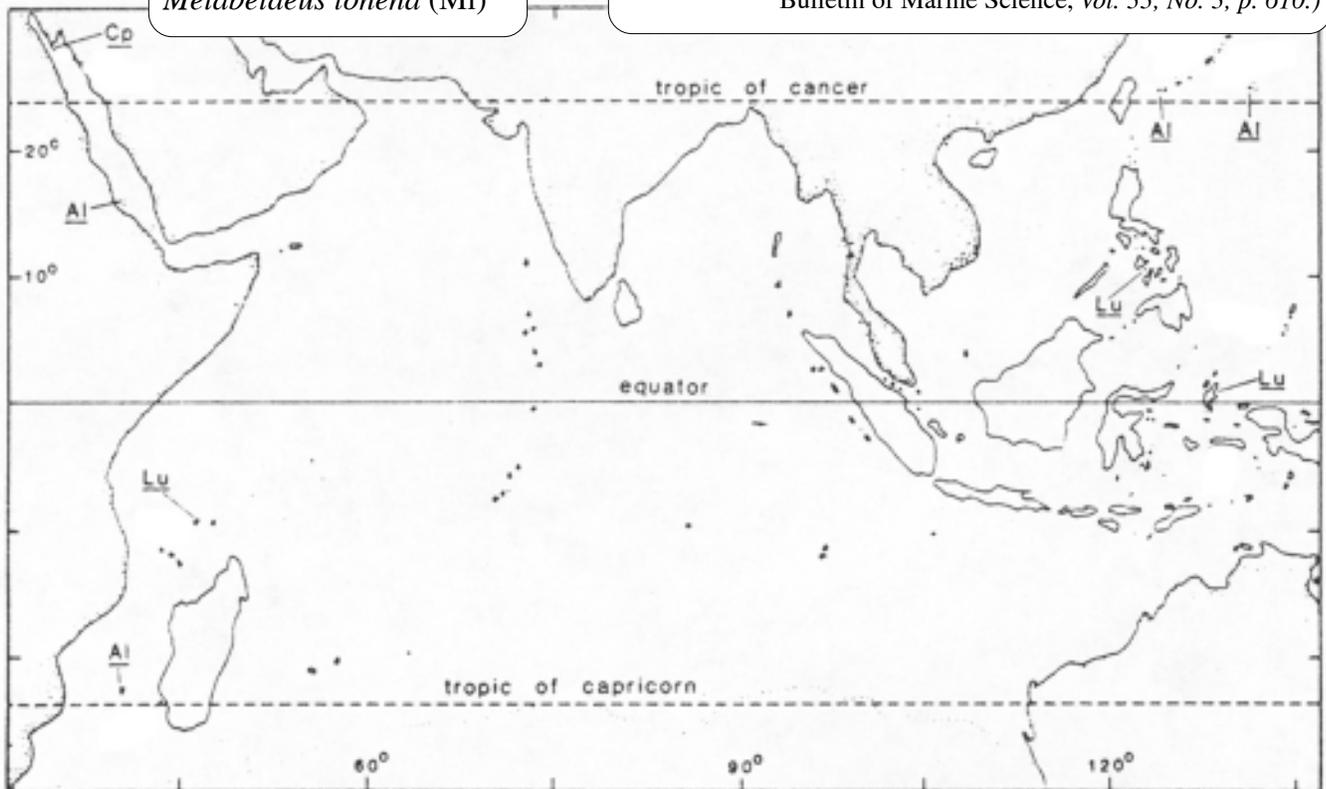
Acetate #8

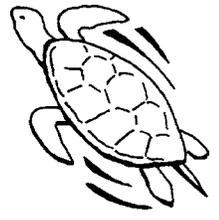
Shrimp Distribution Maps



**Key**  
*Antecaridina lauensis* (Al)  
*Calliasmata pholidota* (Cp)  
*Halocaridina rubra* (Hr)  
*Ligur uveae* (Lu)  
*Metabetaeus lohena* (MI)

*Distribution of insular hypogean shrimps in the central and western Pacific Ocean (above) and western Pacific and Indian Oceans (below) (Adapted from John A. Maciolek, "Distribution and Biology of Indo-Pacific Insular Hypogean Shrimps," Bulletin of Marine Science, Vol. 33, No. 3, p. 610.)*





## Acetate #9

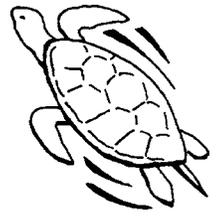
### Mystery #6

Theories about isolation and speciation hold that shrimp species that live thousands of miles apart should have evolved into separate species. What can explain the fact that populations of the same shrimp species are found thousands of miles apart?



Photo: John Hoover, Hawai'i's Sea Creatures, Mutual Publishing

- Observation #1      The shrimp *Antecaridina lauensis* (Al), *Halocaridina rubra* (Hr), and *Metabetaeus lohena* (MI) can live up to 5 years in aquariums. They live longer than other types of small shrimp.
- Observation #2.      All of the small, red shrimp found in the anchialine ponds can tolerate a wide variation in “salinity” (saltiness of the water), but they must have a little bit of seawater in the mix. They can live in pure ocean water.
- Observation #3      All these shrimp need dark, underground crevices. While human beings have mostly seen them in the ponds, the shrimp probably do not need to come into ponds. While *Ligur uveae* (Lu) is found in ponds in other parts of the world, it has never been seen in a pond in Hawai‘i. Here, it has been seen by divers only in underwater caves. While we tend to associate the shrimp with anchialine ponds, it is possible that they do not *need* to live in or on “emergent land” (land that comes out of the water, an island).
- Observation #4      On islands where populations of the shrimp have been found in anchialine ponds, the shrimp sometimes show up in new holes in the ground such as a bomb crater, wells, and quarries, as well as in ponds in recent lava flows. This suggests that they have migrated through underground crevices to enter these new holes, so there is probably an extensive underground population.
- Observation #5      Only two of the many species of small, red shrimp are endemic to an area. Since isolation generally results in the evolution of new species, this seems to indicate that larvae are passively floating in currents between these various areas. But in many places the currents don't go the right way!

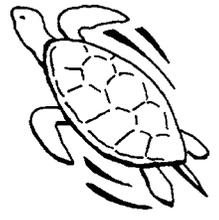


# The Anchialine Pond Detective Worksheet

Solve the following mysteries using the information your teacher will present to you. Like a detective, your job is to piece together observations or clues to arrive at a conclusion.

**Mystery #1:** The water in the ponds is both fresh water and salt water. Where does this water come from?

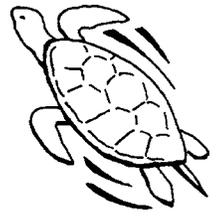
**Mystery #2** What makes the water turn red each spring at Wai‘ānapanapa?



**Mystery #3** You often see small, red shrimp in the anchialine ponds. If all the water goes out of the ponds at low tide, the shrimp disappear. Where do they go?

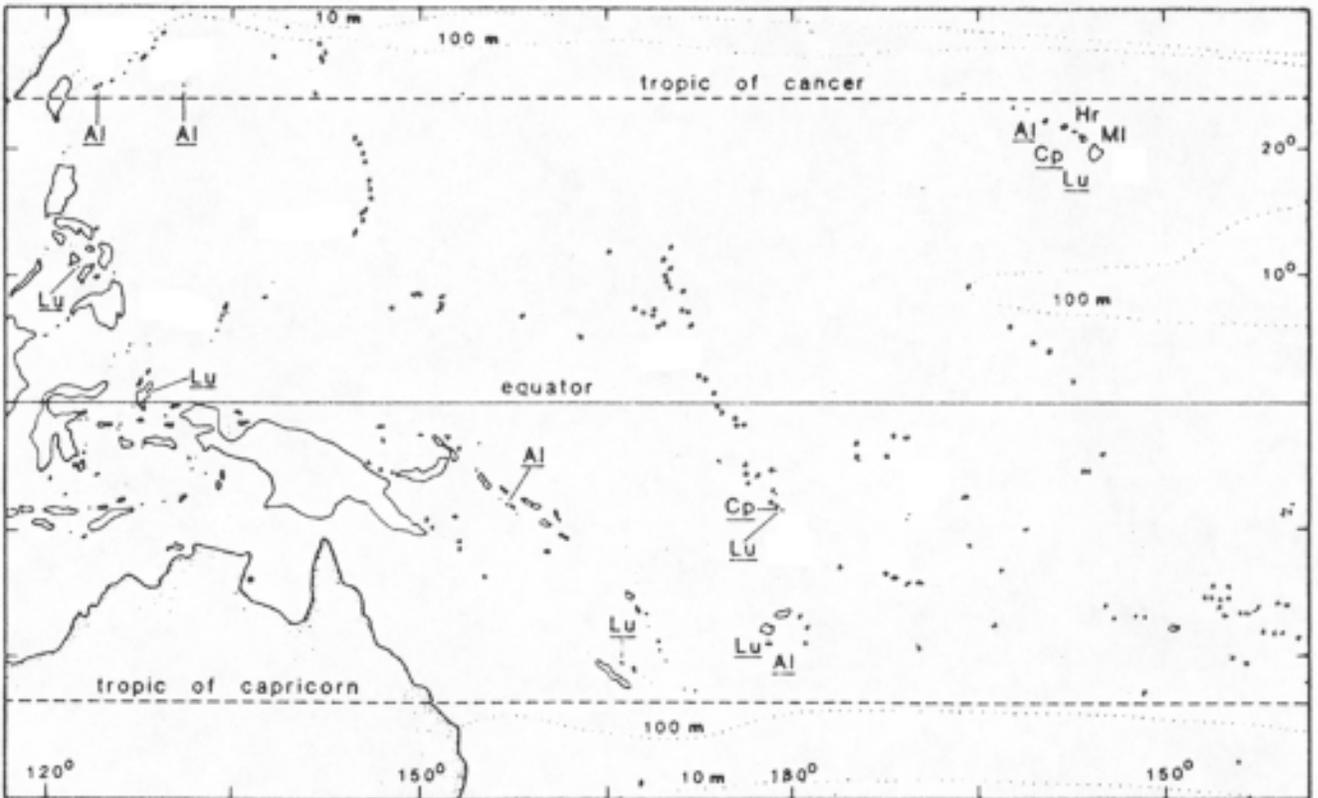
**Mystery #4** The same species of shrimp that live in the Maui ponds also live in similar ponds on the Kona coast of Hawai'i and holes in the ancient (and dry) coral reef that is now the 'Ewa plain of O'ahu. How did the shrimp travel between the islands?

**Mystery #5** The larvae reach the shorelines of the other islands. How do they get into the ponds?



## Information Interlude

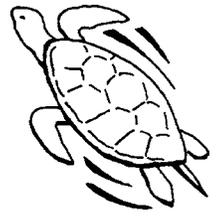
### Shrimp Distribution Map: Central and Western Pacific Ocean



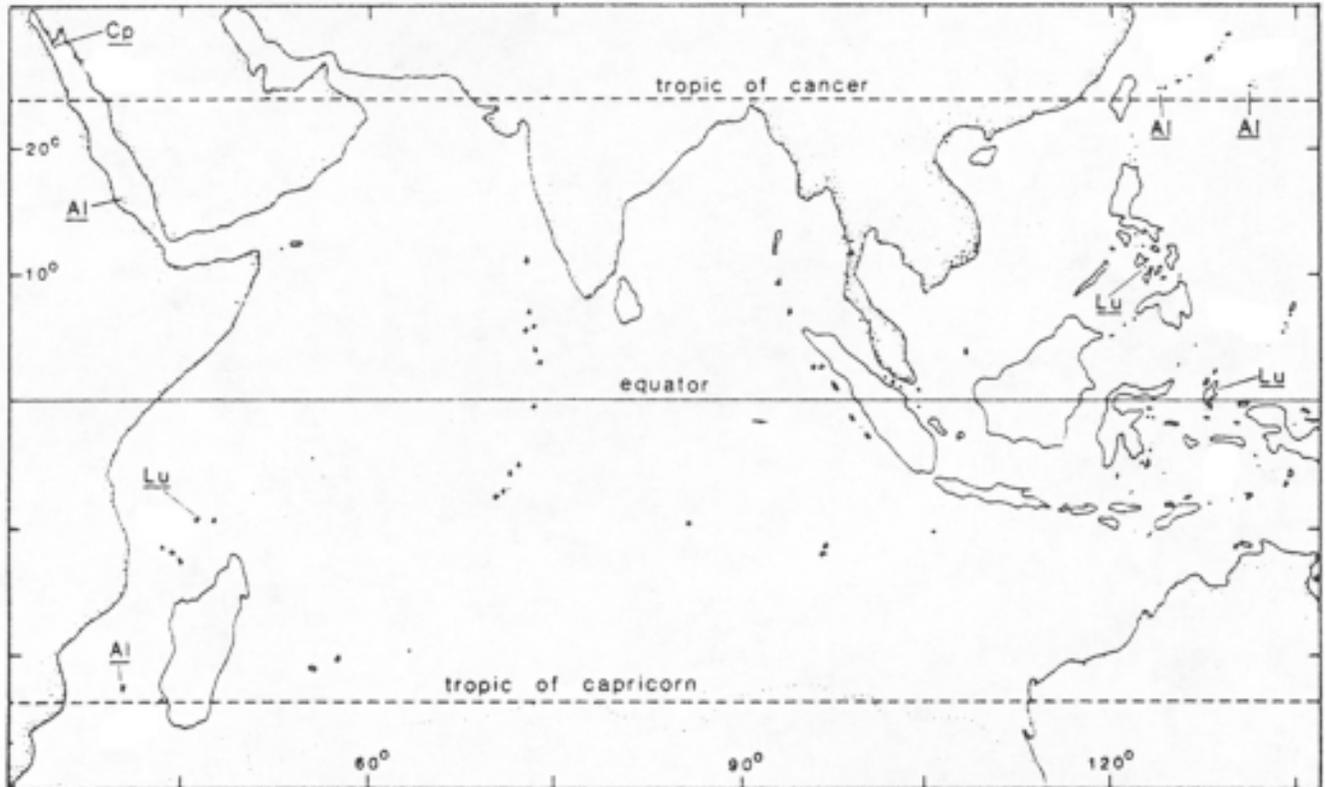
*Distribution of insular hypogeal shrimps in the central and western Pacific Ocean (Adapted from John A. Maciolek, "Distribution and Biology of Indo-Pacific Insular Hypogeal Shrimps," Bulletin of Marine Science, Vol. 33, No. 3, p. 610.)*

#### Key

- Antecaridina lauensis* (AI)
- Calliasmata pholidota* (Cp)
- Halocaridina rubra* (Hr)
- Ligur uveae* (Lu)
- Metabetaeus lohena* (MI)



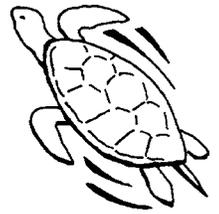
## Shrimp Distribution Map: Western Pacific and Indian Oceans



*Distribution of insular hypogeal shrimps in the western Pacific and Indian Oceans (Adapted from John A. Maciolek, "Distribution and Biology of Indo-Pacific Insular Hypogeal Shrimps," Bulletin of Marine Science, Vol. 33, No. 3, p. 611.)*

### Key

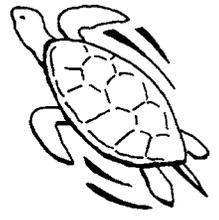
- Antecaridina lauensis* (Al)
- Calliasmata pholidota* (Cp)
- Halocaridina rubra* (Hr)
- Ligur uveae* (Lu)
- Metabetaeus lohena* (Ml)



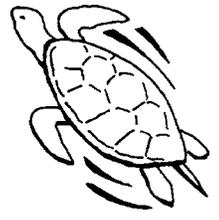
Distribution of Indo-Pacific Caridean Hypogeal Shrimp  
Species (the small, red shrimp in anchialine ponds) Found in Hawai‘i

Family, Genus, Species	Locality	Island(s)
Alpheidae		
<i>Metabetaeus lohena</i> (Ml)	Hawaiian Islands	Hawai‘i, Maui
Atyidae		
<i>Antecaridina lauensis</i> (Al)	Fiji Islands Mozambique Channel Is. Red Sea-Dahlak Hawaiian Islands Daito Islands Ryukyu Islands Solomon Islands	Namuka, Wangava Europa Entedibir Maui, Hawai‘i Minami Kuro Uipi
<i>Halocaridina rubra</i> (Hr)	Hawaiian Islands	Hawai‘i, O‘ahu, Maui, Moloka‘i
Hippolytidae		
<i>Calliasmata pholidota</i> (Cp)	Red Sea-Sinai Ellice Islands Hawaiian Islands	Ras Muhammad Funafuti Maui, Hawai‘i
<i>Ligur uveae</i> (Lu)	Molucca Islands Loyalty Islands West Indian Ocean Fiji Islands Ellice Islands Philippine Islands Hawaiian Islands	Halmahera Uvea/Sayawa Aldabra Vanua Levu, Vanua Vatu, Vatulele Funafuti Tiniguiban O‘ahu, Hawai‘i, Maui
Procarididae		
<i>Procaris hawaiiana</i>	Hawaiian Islands	Maui, Hawai‘i

Based on John A. Maciolek, "Distribution and Biology of Indo-Pacific Insular Hypogeal Shrimps,"  
Bulletin of Marine Science, 1983, Vol. 33, No. 3, pp. 606-618.



**Mystery #6** Theories about isolation and speciation hold that shrimp species that live thousands of miles apart should have evolved into separate species. What can explain the fact that populations of the same shrimp species are found thousands of miles apart?



# Hypothetically Speaking

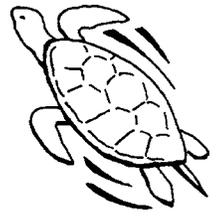
- 1) In 1983 a scientist named Dr. John Maciolek offered a hypothesis to explain the widely scattered populations of the small, red shrimp species found in anchialine ponds. Based on the evidence, he thinks that the shrimps' habitat is much broader than is commonly thought. Instead of occurring only in association with a scattering of anchialine ponds and their porous substrates, Maciolek hypothesizes that "the shrimps could occur in the groundwaters of many . . . islands where they have not yet been found, in shallow reefs and seamounts, and possibly in suitable rock of continental shelves." (John A. Maciolek, "Distribution and Biology of Indo-Pacific Insular Hypogean Shrimps," *Bulletin of Marine Science*, 1983, Vol. 33, No. 3, pp. 606-618.)

In other words, these shrimp could be hiding out in all kinds of submerged nooks and crannies—reducing the distances between what now seem to be separate populations.

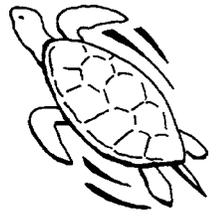
Dr. Maciolek goes on to say he thinks the distribution would be restricted by water temperature. There is evidence to suggest that the shrimp do not survive in water colder than 20° C. In the tropics, that generally means that the shrimp could survive in waters no deeper than 100 m (328 ft).

Do you think Dr. Johan Maciolek offers a reasonable hypothesis? Why or why not?





- 4) Assuming that you were unable to look directly for shrimp in underwater rock crevices, how would you study the shrimp themselves to determine whether the shrimp would be physiologically able to live in these underwater habitats? Keep in mind that these underwater habitats would have the consistent salinity of sea water and may be darker or cooler than exposed or near-surface waters depending on their depth.



Activity #2

# Salinity Tolerance Lab

## ● ● ● In Advance *Obtaining Lab Supplies*

- Order brine shrimp cysts (eggs) from a science supply house such as Carolina Biological Supply, Sargeant-Welch, Flinn, or Frey. Other sources include local pet stores and online aquaculture suppliers. (If you are unfamiliar with any of these sources, an Internet search will quickly help you identify a supplier.)

## ● ● ● Class Period One *Brine Shrimp Lab*

### Materials & Setup

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*For each student*

- Student Page “Lab Background and Procedures: Brine Shrimp Salinity Tolerance” (pp. 31-33)
- Student Page “Lab Worksheet: Brine Shrimp Tolerance for Fluctuating Environmental Conditions” (pp. 34-38)

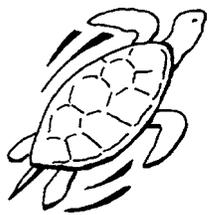
*For each lab group of three to four students*

- Brine shrimp cysts
- 1/8 tsp measure
- Four test tubes (150 ml) with stoppers
- Labels or labeling pens for the test tubes
- Test tube rack
- Sea salt or noniodized salt
- Graduated cylinder (more than 100 ml)
- Triple beam or electronic balance
- Distilled water or tap water that has been aged 24 hours in an open container to dechlorinate it

### Instructions

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- 1) Divide students into lab groups of three to four students. Hand out the student pages and make sure each group has a complete set of equipment and supplies.
- 2) Conduct the “Brine Shrimp Salinity Tolerance” lab using the procedures outlined in the student page.
- 3) If more than 24 hours will elapse between the two lab sessions, feed the shrimp on the second day by placing a tiny amount of brewer’s yeast or dried spirulina algae (available in pet stores) in each test tube.



## ● ● ● Class Periods Two and Three *Brine Shrimp Lab, Continued*

### Materials & Setup

---

*For each lab group of three to four students*

- Hand lens or dissecting microscope
- Sampling pipette (transparent 1 ml or larger)
- Four petri dishes

### Instructions

---

- 1) Continue the brine shrimp lab, covering questions as the lab proceeds.
- 2) At the end of the lab, discuss groups' findings. Bring up the following questions and points in this discussion:
  - What is the advantage of brine shrimp, like hypogeal shrimp, being able to tolerate a range of salt concentrations?
  - What is the difference between a *tolerance* of environmental conditions and habitat *preference*? (In other words, brine shrimp may be able to survive and even reproduce in certain environmental conditions but may not congregate in those conditions if there is a more favorable option.)
  - At low salinity levels and optimal food levels, female brine shrimp can produce 75 free-swimming larvae per day. At salinities above 150 percent and low oxygen levels, the female produces non-developing cysts. In such unfavorable conditions, the female can release 75 cysts. These cysts float and eventually may drift ashore. Development will not continue until the cysts are washed back into the water and reach an area of favorable salinity and oxygen levels.
  - Shrimp reproduction did not factor into this experiment because brine shrimp do not reach adulthood until they are about eight days old.
  - Brine shrimp can live in extremely harsh and variable conditions with temperatures ranging from 43° to 95° F and salinities usually in the range of 28–70 parts per thousand. They can survive in salinities up to 340 parts per thousand.

### Journal Ideas

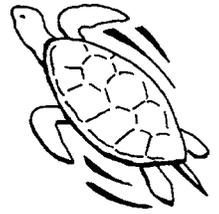
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- Explain what a variable is and what is meant by “controlling” a variable. Why is controlling variables important to scientific investigations? Use the brine shrimp experiment to illustrate your definitions and ideas.
- If you were going to study the habitat preferences of the *‘ōpae ‘ula* (endemic Hawaiian shrimp found in anchialine ponds), what variables would you test? Why?

### Assessment Tools

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- Conduct during lab
- Student Page “Lab Worksheet: Brine Shrimp Tolerance for Fluctuating Environmental Conditions”
- Journal entries



# Lab Background and Procedures: Brine Shrimp Tolerance for Fluctuating Environmental Conditions

## Introduction

Brine shrimp are crustaceans, like lobsters, crabs, and crayfish. A hard exoskeleton supports their bodies and protects them from injury. Brine shrimp are found throughout the world and are adapted to live in harsh, changing environments. These are not exactly the same environmental conditions as the red shrimp that we've been studying inhabit. However, conditions for both shrimp can vary widely in "salinity" (salt concentration) and temperature.

In this lab, you will test the tolerance of brine shrimp to variations in environmental conditions. The measure of tolerance that you will use is the hatching rate of brine shrimp eggs. The environmental condition that you will vary is salinity.

Brine shrimp eggs are initially housed within a structure called a "cyst," which is similar to an eggshell. Brine shrimp cysts can dry out and the egg inside them will remain viable (able to hatch) for many years. As the egg develops, the cyst bursts. For the first few hours after the cyst bursts, the embryo hangs beneath the cyst in the "umbrella" stage. The embryo continues to develop and will emerge as a free-swimming larva.

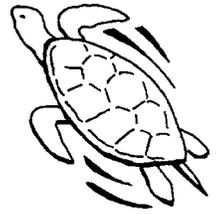
Brine shrimp tend to be brownish-orange in color in this first larval stage. After about 12 hours, they molt (shed their exoskeleton) and begin feeding on tiny algae, bacteria, or nonliving debris. They continue to feed and grow, molting 15 times before reaching adulthood in about eight days.

Adult brine shrimp are generally around 8 mm long. Adult males can be identified by large claspers near their heads, and females by the brood pouch, where the cysts develop, just below the last of their legs. Their lifespan is typically several months.

## Materials

### Lab Period One

- Brine shrimp cysts
- 1/8 tsp measure
- Four test tubes (150 ml) with stoppers
- Labels or labeling pens for the test tubes
- Test tube rack
- Sea salt or noniodized salt
- Graduated cylinder (more than 100 ml)
- Triple beam or electronic balance
- Distilled water or tap water that has been aged 24 hours in an open container to dechlorinate it



## Lab Periods Two and Three

- Hand lens or dissecting microscope
- Sampling pipette (transparent 1 ml or larger)
- Four petri dishes

### Lab Period One Procedure

- 1) On the lab worksheet, write the question or problem investigated by this experiment and your hypothesis.
- 2) Using salt and the distilled or aged tap water, mix solutions of the following concentrations:
  - 1 percent salt solution
  - 5 percent salt solution
  - 10 percent salt solution
  - 20 percent salt solution

The formula for creating solutions of a specific concentration is:

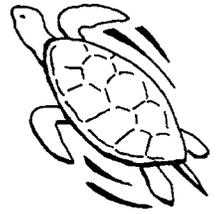
X grams of NaCl (salt) per 100 ml H<sub>2</sub>O (X=target percent concentration)

Measure 100 ml of water into the graduated cylinder and pour it into a test tube. Weigh the salt and add it to the test tube. Place the stopper on the test tube and mix until the salt is dissolved. Place the test tube in the rack and label it. Mix all of the solutions before moving on to the next step.

- 3) Unstop all of the test tubes and add 1/8 teaspoon of brine shrimp cysts to each test tube. Leave the tubes open, unless you are moving your test tubes and rack to another part of the room.
- 4) Leave your test tube rack out of direct sunlight, preferably in a dim part of the classroom. All lab groups should leave their test tube racks in the same location in the classroom.
- 5) Answer question #3 on the lab worksheet.

### Lab Period Two Procedure

- 1) Observe your test tubes. Write your observations on the lab worksheet (question #4, first row in the table)
- 2) Working with one test tube at a time, make a count of the brine shrimp in a sample volume of water. If the shrimp are not evenly dispersed throughout the water, gently swirl the test tube. Use a pipette to measure 1 ml of water and shrimp (if any have hatched) from the test tube and into a clean petri dish.
- 3) Using the hand lens or dissecting microscope, count the shrimp in that sample of water and record your findings on the lab worksheet (question #4, first row in the table).

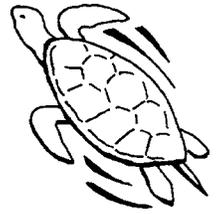


- 4) Place the shrimp and water back into the test tube they came from.
- 5) Continue until you have sampled and counted shrimp from each of your four test tubes and recorded your observations on the lab worksheet (question #5, first row of the table). Replace your test tubes and rack to the location where you kept them previously.
- 6) Answer question #6 on the lab worksheet.

### Lab Period Three Procedure

- 1) Observe your test tubes. Write your observations on the lab worksheet (question #4, second row of the table).
- 2) Working with one test tube at a time, make a count of the brine shrimp in a sample volume of water. If the shrimp are not evenly dispersed throughout the water, gently swirl the test tube. Use a pipette to measure 1 ml of water and shrimp (if any have hatched) from the test tube and into a clean petri dish.
- 3) Using the hand lens or dissecting microscope, count the shrimp in that sample of water and record your findings on the lab worksheet (question #5, second row of the table).
- 4) Replace the shrimp and water back into the test tube they came from.
- 5) Continue until you have sampled and counted shrimp from each of your four test tubes.
- 6) Answer questions #6-10 on the lab worksheet.





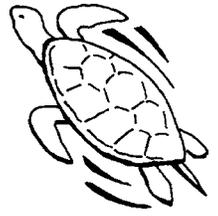
**Lab Period Two** (Date: \_\_\_\_\_ ) **and Three** (Date: \_\_\_\_\_ )

4) Test tube observations

	1% solution	5% solution	10% solution	20% solution
<b>Lab Period Two</b>				
<b>Lab Period Three</b>				

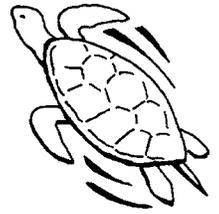
5) Shrimp count per 1 ml water

	1% solution	5% solution	10% solution	20% solution
<b>Lab Period Two</b>				
<b>Lab Period Three</b>				

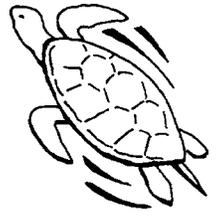


6) Did your group encounter any difficulties counting the brine shrimp in the samples? If so, how were you able to resolve them?

7) Summarize the results of your experiment.



- 8) Based on the results of your experiment, which solution was the best in which to hatch brine shrimp eggs? Explain your answer.
- 9) Design another experiment to measure the tolerance of brine shrimp to a different environmental variable such as light or temperature. Describe it here.



10) What types of field observations (made at the ponds, not in a laboratory experiment) could you conduct to test the habitat preferences of the shrimp that live in the anchialine ponds on Maui?