

## Save Your Breath



### Focus

Metabolic adaptations to low-oxygen environments and technical reading

### Grade Level

9-12 (Life Science)

### Focus Question

Does the metabolism of organisms living in anchialine caves change in response to low-oxygen conditions?

### Learning Objectives

- Students will be able to discuss the basis for hypothetical metabolic adaptations to low-oxygen environments in anchialine caves.
- Students will be able to evaluate evidence from a research report that tests this hypothesis.

### Materials

- Copies of *Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide*, one for each student

### Audio-Visual Materials

- (Optional) Computer projector or other equipment for showing images of underwater caves

### Teaching Time

One or two 45-minute class periods

### Seating Arrangement

Groups of 3-4 students

### Maximum Number of Students

32

### Key Words

Anchialine cave  
Metabolism  
Oxygen  
Respiration  
Lanzarote Island



Image captions/credits on Page 2.

# lesson plan

## Background Information

*NOTE: Explanations and procedures in this lesson are written at a level appropriate to professional educators. In presenting and discussing this material with students, educators may need to adapt the language and instructional approach to styles that are best suited to specific student groups.*

Anchialine caves are partially or totally submerged caves in coastal areas. Anchialine (pronounced “AN-key-ah-lin”) is a Greek term meaning “near the sea,” and anchialine caves often contain freshwater and/or brackish water in addition to seawater. These caves may be formed in karst landscapes as well as in rock tubes produced by volcanic activity. Karst landscapes are areas where limestone is the major rock underlying the land surface, and often contain caves and sinkholes formed when acidic rainwater dissolves portions of the limestone rock. Volcanic caves are formed when the surface of flowing volcanic lava cools and hardens, while molten lava continues to flow underneath. If the molten lava continues to flow away from the hardened surface, a hollow tube will be formed that becomes a lava tube cave.

Water in anchialine caves tends to stratify according to salinity, with the heavier seawater below the level of fresh and brackish water. This stratification produces distinctive habitats occupied by a variety of species that are endemic to these locations. (Endemic means that these species are not found anywhere else). Some of these species are “living fossils” known as relict species, which means that they have survived while other related species have become extinct.

Animals that live only in anchialine habitats are called stygofauna or stygobites. Investigations of these species have revealed some puzzling relationships, including:

- Some stygobite species appear to have been in existence longer than the caves they inhabit, which implies that these species must have arrived in the caves from somewhere else; but how could this happen if these species are only found in caves?
- Some stygobite species are found in caves that are widely separated, such as crustacean species found in caves on opposite sides of the Atlantic Ocean and species in Australian anchialine caves that are also found in Atlantic and Caribbean caves.
- Geographic distribution of some species suggests a possible connection with mid-ocean ridges. For example, shrimps belonging to the genus *Procaris* are only known from anchialine habitats in the Hawaiian Islands, Ascension Island in the South Atlantic, and Bermuda in the North Atlantic.
- Some anchialine species are most closely related to organisms that live in the very deep ocean.
- Some anchialine species are most closely related to organisms that live in deep sea hydrothermal vent habitats.

### Images from Page 1 top to bottom:

Water in inland tidal cave pools in Bermuda is brackish at the surface, but reaches fully marine salinity by a depth of several meters. Image credit: NOAA, Bermuda: Search for Deep Water Caves 2009.

[http://oceanexplorer.noaa.gov/explorations/09bermuda/background/bermudaorigin/media/bermudaorigin\\_5.html](http://oceanexplorer.noaa.gov/explorations/09bermuda/background/bermudaorigin/media/bermudaorigin_5.html)

Divers swim between massive submerged stalagmites in Crystal Cave, Bermuda. Such stalactites and stalagmites were formed during glacial periods of lowered sea level when the caves were dry and air-filled. Image credit: NOAA, Bermuda: Search for Deep Water Caves 2009.

[http://oceanexplorer.noaa.gov/explorations/09bermuda/background/bermudaorigin/media/bermudaorigin\\_3.html](http://oceanexplorer.noaa.gov/explorations/09bermuda/background/bermudaorigin/media/bermudaorigin_3.html)

Ostracods are small, bivalve crustaceans that can inhabit underwater caves. The ostracod genus *Spelaeoecia* is known only from marine caves and occurs in Bermuda, the Bahamas, Cuba, Jamaica and Yucatan (Mexico). Image credit: Tom Iliffe, NOAA, Bermuda: Search for Deep Water Caves 2009.

<http://oceanexplorer.noaa.gov/explorations/09bermuda/background/plan/media/spelaeoecia.html>

Prof. Tom Iliffe, diving with a Megalodon closed-circuit rebreather, tows a plankton net through an underwater cave to collect small animals. Image credit: Jill Heinerth, NOAA, Bermuda: Search for Deep Water Caves 2009.

<http://oceanexplorer.noaa.gov/explorations/09bermuda/background/plan/media/plankton.html>

- An unusually large proportion of anchialine cave species in Bermuda are endemic to these caves, suggesting that these habitats have been stable for a long period of time.

Most investigations of anchialine caves have been confined to relatively shallow depths; yet, the observations described above suggest that connections with deeper habitats may also be important to understanding the distribution of stygobite species. Bermuda is a group of mid-ocean islands composed of limestone lying on top of a volcanic seamount. Because they are karst landscapes, the islands of Bermuda have one of the highest concentrations of cave systems in the world. Typical Bermuda caves have inland entrances, interior cave pools, underwater passages, and tidal spring outlets to the ocean. Bermuda's underwater caves contain an exceptional variety of endemic species, most of which are crustaceans. Most of these organisms are relict species with distinctive morphological, physiological, and behavioral adaptations to the cave environment that suggest these species have been living in caves for many millions of years. Yet, all known anchialine caves in Bermuda were completely dry only 18,000 years ago when sea levels were at least 100 m lower than present because of water contained in glaciers. Such observations suggest the possibility of additional caves in deeper water that would have provided habitat for anchialine species when presently-known caves were dry.

In this activity, students will investigate the metabolic adaptations of stygobites to low-oxygen conditions in anchialine caves.

### Learning Procedure

- To prepare for this lesson:
  - Review introductory essays for the Bermuda: Search for Deep Water Caves 2009 expedition at <http://oceanexplorer.noaa.gov/explorations/09bermuda/welcome.html>. You may also want to visit <http://oceanexplorer.noaa.gov/technology/subs/rov/rov.html> for images and discussions of various types of ROVs used in ocean exploration. If you want to explain multibeam sonar, you may also want to review information and images at <http://oceanexplorer.noaa.gov/technology/tools/sonar/sonar.html>.
  - Download a few images of anchialine caves from <http://www.tamug.edu/cavebiology/index2.html>.
  - Review the *Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide*.
- Briefly introduce the Bermuda: Search for Deep Water Caves 2009 expedition, and show some images of marine caves. Tell students that Bermuda has an unusually large number of species living in marine caves that are not found anywhere else, and that some are called living fossils because they have survived while other related species have become extinct. Explain that very little is

known about deep water marine caves, and discuss why scientists might want to find and explore these caves. Briefly describe how anchialine caves may be formed.

3. Provide each student with a copy of the *Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide* and explain that their assignment is to extract specific information from a condensed research report. If students are not familiar with basic statistical methods, you should explain that regression is a statistical technique for finding the best fit for a line between a series of graphed data points. You also may want to briefly explain the last paragraph under "Materials and Methods," or suggest that they ignore this information.
4. Lead a discussion of students' results. The following points should be included:
  - Anchialine refers to partially or totally submerged caves near the sea, and that often contain freshwater and/or brackish water in addition to seawater.
  - Stygofauna are animals that are adapted to living in caves and cannot survive outside of the cave environment.
  - Oxic, hypoxic, suboxic, and anoxic refer to the concentration of dissolved oxygen in an aquatic environment. In marine environments, oxic conditions correspond to dissolved oxygen concentrations between 8.0 and 2.0 ml O<sub>2</sub>/L; hypoxic conditions correspond to dissolved oxygen concentrations between 2.0 and 0.2 ml O<sub>2</sub>/L; suboxic conditions correspond to dissolved oxygen concentrations between 0.2 and 0.0 ml O<sub>2</sub>/L; anoxic conditions exist when there is no dissolved oxygen. Students' answers may vary somewhat, depending upon references used.
  - It is important to maintain a constant temperature during respiration measurements because temperature affects respiration in many species, and also affects the amount of oxygen that can dissolve in water (as temperature increases, oxygen solubility in water decreases).
  - The investigators decided that microbial respiration had no effect on oxygen consumption in the syringes. It was necessary to measure this because microbial respiration might obscure the measurement of oxygen consumption by the animals being tested.
  - Logarithmic numbers were used because the values of mass and oxygen consumption varied over several orders of magnitude;

mass ranged from 0.01 mg to 270 mg, while oxygen consumption ranged from 0.0002 to 0.0145 ml O<sub>2</sub>/hr. If these numbers were plotted on an arithmetic scale it would be difficult to see the relationship between the data points (if students try to plot several mass values within the range described, they will get the point!).

- A species is said to be endemic to a particular location when that species is not found anywhere else.
- Results shown in Figure 1 support the theory that organisms in oxygen-poor habitats have reduced metabolism and body size. But these results do not prove this hypothesis. In fact, most hypotheses can never be absolutely proven, because it is usually impossible to test every possible instance of a given hypothesis; but a single exception is enough to disprove a hypothesis. In this case, metabolism and body size might also be affected by the availability of food, which is usually in short supply in anchialine caves.

### The BRIDGE Connection

[www.vims.edu/bridge/](http://www.vims.edu/bridge/) – Scroll over “Ocean Science Topics,” then “Biology,” then “Invertebrates” for links to resources and activities about the physiology and ecology of marine invertebrate groups.

### The “Me” Connection

Have students write a brief essay describing ways in which they are adapted to their own habitat, and what adaptations they would like to have to make them better suited to the habitat in which they would most like to live.

### Connections to Other Subjects

English/Language Arts, Mathematics, Chemistry

### Assessment

Written reports and class discussions provide opportunities for assessment.

### Extensions

1. Visit <http://oceanexplorer.noaa.gov/explorations/09bermuda/welcome.html> for more about the Bermuda: Search for Deep Water Caves 2009 expedition.

### Other Resources

*The Web links below are provided for informational purposes only. Links outside of Ocean Explorer have been checked at the time of this page’s publication, but the linking sites may become outdated or non-operational over time.*

<http://oceanexplorer.noaa.gov/explorations/09bermuda/welcome.html> – Bermuda: Search for Deep Water Caves 2009 expedition

<http://celebrating200years.noaa.gov/edufun/book/welcome.html#book> – A free printable book for home and school use introduced in 2004 to celebrate the 200th anniversary of NOAA; nearly 200 pages of lessons focusing on the exploration, understanding, and protection of Earth as a whole system

Iliffe, T. M. and R. E. Bishop. 2007. Adaptations to Life in Marine Caves. In Fisheries and Aquaculture. Patrick Safran, ed., in Encyclopedia of Life Support Systems. UNESCO. EOLSS Publishers. Oxford, UK; available online at <http://www.tamug.edu/cavebiology/reprints/reprint-176.pdf>

Bishop, R. E. and T. M. Iliffe. 2009. Metabolic rates of stygobiontic invertebrates from the Túnel de la Atlántida, Lanzarote Mar. Biodiv. 39:189-194.

<http://www.tamug.edu/cavebiology/index2.html> – Anchialine Caves and Cave Fauna of the World

<http://www.goodearthgraphics.com/virtcave/index.html> – Virtual Cave Web site

### **National Science Education Standards**

#### **Content Standard A: Science As Inquiry**

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

#### **Content Standard C: Life Science**

- Matter, energy, and organization in living systems
- Behavior of organisms

### **Ocean Literacy Essential Principles and Fundamental Concepts**

#### **Essential Principle 1.**

##### **The Earth has one big ocean with many features.**

*Fundamental Concept g.* The ocean is connected to major lakes, watersheds and waterways because all major watersheds on Earth drain to the ocean. Rivers and streams transport nutrients, salts, sediments and pollutants from watersheds to estuaries and to the ocean.

#### **Essential Principle 2.**

##### **The ocean and life in the ocean shape the features of the Earth.**

*Fundamental Concept a.* Many earth materials and geochemical cycles originate in the ocean. Many of the sedimentary rocks now exposed on land were formed in the ocean. Ocean life laid down the vast volume of

siliceous and carbonate rocks.

*Fundamental Concept b.* Sea level changes over time have expanded and contracted continental shelves, created and destroyed inland seas, and shaped the surface of land.

**Essential Principle 5.**

**The ocean supports a great diversity of life and ecosystems.**

*Fundamental Concept d.* Ocean biology provides many unique examples of life cycles, adaptations and important relationships among organisms (such as symbiosis, predator-prey dynamics and energy transfer) that do not occur on land.

*Fundamental Concept e.* The ocean is three-dimensional, offering vast living space and diverse habitats from the surface through the water column to the seafloor. Most of the living space on Earth is in the ocean.

*Fundamental Concept f.* Ocean habitats are defined by environmental factors. Due to interactions of abiotic factors such as salinity, temperature, oxygen, pH, light, nutrients, pressure, substrate and circulation, ocean life is not evenly distributed temporally or spatially, i.e., it is “patchy”. Some regions of the ocean support more diverse and abundant life than anywhere on Earth, while much of the ocean is considered a desert.

*Fundamental Concept h.* Tides, waves and predation cause vertical zonation patterns along the shore, influencing the distribution and diversity of organisms.

**Essential Principle 7.**

**The ocean is largely unexplored.**

*Fundamental Concept a.* The ocean is the last and largest unexplored place on Earth—less than 5% of it has been explored. This is the great frontier for the next generation’s explorers and researchers, where they will find great opportunities for inquiry and investigation.

*Fundamental Concept b.* Understanding the ocean is more than a matter of curiosity. Exploration, inquiry and study are required to better understand ocean systems and processes.

*Fundamental Concept f.* Ocean exploration is truly interdisciplinary. It requires close collaboration among biologists, chemists, climatologists, computer programmers, engineers, geologists, meteorologists, and physicists, and new ways of thinking.

**Send Us Your Feedback**

We value your feedback on this lesson.

Please send your comments to:

[oceaneducation@noaa.gov](mailto:oceaneducation@noaa.gov)

### **For More Information**

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## Save Your Breath

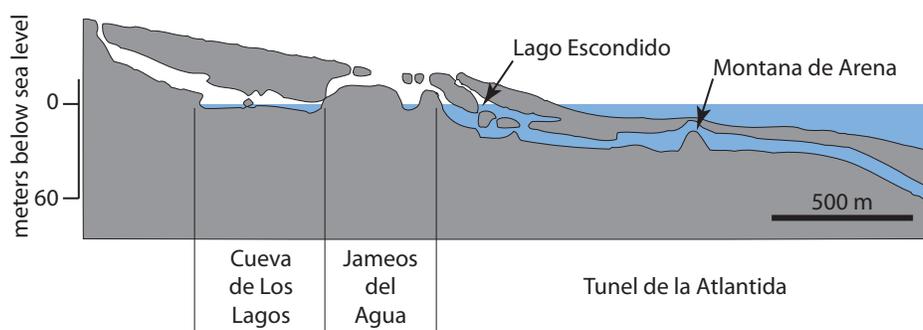
# Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide

### Introduction

Anchialine caves offer a unique set of challenges for the organisms that reside there. Because the lack of light results in little or no diurnal or seasonal fluctuation in productivity, stygofauna have to cope with constant low oxygen conditions in an energy limited environment. As a result of both food scarcity and hypoxia, there is a high selective advantage for economy of energy. Adaptations may be morphological (such as eliminating unused body parts and reducing physical size), behavioral (such as swimming slowly or intermittently while searching for food), and physiological (such as reducing metabolic rates).

This study examines the metabolism of invertebrate specimens collected from the Túnel de la Atlántida, Lanzarote, Canary Islands, the completely submerged, seaward-most segment of a 7-km-long lava tube. Unlike many cave systems, the Túnel de la Atlántida is an oxic environment. The metabolism of invertebrates from this environment was compared to the metabolism of invertebrates collected from two suboxic to anoxic anchialine limestone cave systems in the Bahamas. The body mass of amphipods *Spelaeonicippe buchii* found in the Túnel de la Atlántida was also compared to the body mass of specimens of *Spelaeonicippe provo* from anchialine caves in the Bahamas.

**Figure 1**



### Materials and Methods

Organisms were visually located by divers, prior to being collected individually in glass vials. Water chemistry was monitored at the collection site using a Yellow Springs Instruments 600XLM sonde. Although the oxygen fluctuated with the tidal flow within the cave, oxygen levels did not fall below 3.8 ml/L.

Metabolism was examined by measuring oxygen consumed by the organisms. Individual specimens were placed in modified 10-ml plastic syringe barrels with the tops removed. Oxygen electrodes were custom designed to be inserted with an airtight seal into the syringe barrels. The syringes were filled with filtered cave water and submerged in a refrigerated waterbath with the experimental temperature maintained at  $20 \pm 0.3^\circ\text{C}$ . Oxygen was monitored continuously. After an acclimation period of 1 hour, respiratory rates were obtained from the slope of the regression of the change

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### Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide – 2

in oxygen content in the respirometers versus the time elapsed between 1 and 6 hours of incubation time. Following the incubations, specimens were removed from the syringes and frozen in cryogenic vials. Mass determinations were made within days of collection.

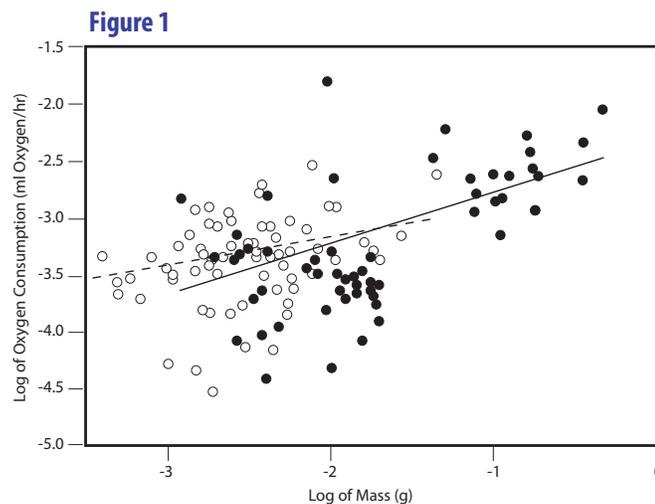
To determine the impact of microbial respiration on the overall decrease in oxygen concentration within the syringes, selected syringes were filled with filtered cave water and sealed with an electrode. The oxygen content was monitored during the entire respiration analysis and ended when the final respiration incubation was completed. The slopes of change in oxygen concentration over time were not significantly different from zero.

Regressions of log of mass and log of oxygen consumption were generated using the least-squares method with all significance at  $P < 0.05$ . A two-tailed t test was used to test for differences between the two population regressions using log of wet mass and log of oxygen consumption for Lanzarote specimens and specimens collected from caves in the Bahamas to determine if significant differences existed in oxygen consumption rates between the cave systems. A two-tailed t test was used to determine if differences existed between wet mass values of *Spelaeonicippe* from Lanzarote and the Bahamas.

#### Results

The metabolism of the Lanzarote cave invertebrates was compared with the metabolism of invertebrates from two Bahamian anchialine caves. Figure 1 shows a regression of log of mass and log of oxygen consumption of all of the Túnel de la Atlántida specimens and those from the Bahamas.

Since many caves have their own endemic fauna, it is difficult to compare organisms between caves. However, the anchialine caves in the Bahamas and the Túnel de la Atlántida both have the amphipods belonging to the genus *Spelaeonicippe*, *S. provo* and *S. buchi*, respectively. A comparison of the amphipods from both systems indicated that *S. buchi* have a significantly greater mass than *S. provo*.



**Oxygen consumption of organisms from the Túnel de la Atlántida (solid line) and Bahamas (dashed line).**

~ adapted from Iliffe and Bishop (2007) and Bishop and Iliffe (2009)

## Save Your Breath

# Metabolic Adaptations in Anchialine Cave Fauna Inquiry Guide – 3

### Research, Analyze, and Infer

1. What does anchialine mean?
2. What are stygofauna?
3. What is the difference between oxic, hypoxic, suboxic, and anoxic?
4. Why did the investigators maintain a constant temperature during the respiration measurements?
5. What did the investigators decide about the impact of microbial respiration? Why did they want to measure this?
6. Why did investigators plot the log of mass and oxygen consumption, rather than simple arithmetic numbers?
7. What does endemic mean?
8. Do the results comparing metabolism and body size support the theory that organisms in oxygen-poor habitats have reduced metabolism and body size? What other conditions in these habitats might also be related to metabolism and body size?