



Image courtesy of NOAA Ocean

Exploration.

# **Investigation: Methane Ice Worms**

# **Overview**

**TOPIC:** Trophic Relationships in Deep-Sea Environments

FOCUS: Methane ice worms are found in dense colonies at cold seeps where

they live on mounds of exposed methane hydrate ("methane ice").

**GRADE LEVEL:** 9-12 Life Science

TIME NEEDED: Two 45- or 50-minute class periods

How do methane ice worms obtain organic compounds and energy

**DRIVING QUESTION:** while living on methane hydrate?

OBJECTIVE/

PHENOMENON/

**LEARNING OUTCOME:** Students will:

> • Evaluate evidence and reasoning in order to construct an argument that supports a claim about how methane ice worms obtain matter and energy.

**MATERIALS: Methane Ice Worm Slides** 

**Student Handouts** 

Student Worksheet: Methane Ice Worms (one per student, print or share digital copies)

 Methane Ice Worms Evidence and Reasoning Cards (one set per group of 3-4 students, printed and cut)

Video and Image

· Cold Seeps and Methane Hydrates (4:50) Marum

Ice worms inhabiting methane hydrate image NOAA Ocean Exploration

**Poster Materials** 

Large poster paper (one per group)

**Disciplinary Core Ideas (DCIs)** 

LS2.B: Cycles of Matter and Energy

Markers

**Teacher Key:** Methane Ice Worms Evidence and Reasoning Card Sort

#### **NEXT GENERATION SCIENCE STANDARDS (NGSS)**

**HS-LS2: Ecosystems:** Interactions, Energy, and **Dynamics** 

HS-LS2-4

Performance Expectation (PEs) **Crosscutting Concepts (CCs) Energy and Matter** 

Transfer in Ecosystems

Science & Engineering Practices (SEPs) **Engaging in Argument from** Evidence

COMMON CORE CONNECTIONS ELA/Literacy - RST.11-12.1, WHST.9-12.1

OCEAN LITERACY ESSENTIAL PRINCIPLES AND FUNDAMENTAL **CONCEPTS** 

Principle 5: FC c, d, g

# Overview cont.

#### **EQUIPMENT:**

- · Computer and projector for class viewing of videos and slides or online sharing capability
- White board and dry erase marker or online platform to record class findings
- Optional: Student laptops or tablets for extensions and/or additional research

#### SET-UP INSTRUCTIONS: For online learning

 Share links or digital copies of all materials listed above with students using a preferred online platform.

### For in-person instruction

- · Cue up all videos and slides for student viewing.
- If projecting these for the class is not an option, print or share digital copies with students.
- · Print and cut card sort sets.

# **Educator Guide**

#### **Background**

Cold seeps are places throughout the global ocean where chemicals like hydrogen sulfide, methane, and other hydrocarbon-rich fluids and/or gasses escape from cracks in the ocean floor. They are referred to as cold seeps to distinguish them from the super-heated substances released from hydrothermal vents. A methane seep is just one type of cold seep and is characterized by the methane and hydrogen sulfide bubbles that come out of the seafloor. These percolating chemicals provide the energy for chemosynthetic life and new species are often discovered in seep ecosystems. A flat, pinkish "ice worm" (Hesiocaeca methanicola) was discovered in dense colonies on cold seeps in the Gulf of Mexico. These worms create and live in burrows on mounds of methane hydrate ("methane ice"). Although scientists hypothesized that bacteria might colonize gas hydrates, this was the first time animals were found living on the mounds. It is unclear how the worms feed; however, scientists suspect they may graze upon and/or depend on bacteria found on the mounds to get their food and energy.

### **LEARN MORE**

Gas hydrates are ice-like crystalline structures that form when a low-density gas, like methane, ethane, or carbon dioxide, combines but does not chemically bond with water and freezes into a solid under low temperature and moderate pressure conditions.

#### **Educator Note**

- Students should have prior knowledge of photosynthesis and energetics.
- A variety of student interaction techniques and examples of student questions are provided throughout this activity to engage students in the process of sense-making to move their learning forward.
- <u>Learn more</u> about the instructional strategies and tools included in the NOAA Ocean Exploration student investigations.

#### FOR MORE INFORMATION:

► <u>Chemosynthesis</u> Fact Sheet



► Cold Seeps Fact Sheet



Cold Seep
Communities
Fact Sheet





# **Educator Guide**

# **Experience the Phenomenon**

- · Give students a brief background on cold seeps using Slide 1: Background.
- Tell students they are going to watch a short video clip about a type of ecosystem
  that is home to some unusual animals. Play the first 3:19 min of <u>Cold Seeps and</u>
  <u>Methane Hydrates</u> video provided on <u>Slide 2</u>.
- Mention the following key takeaways.
  - Animals near the beginning of the video were in the Indian Ocean.
  - Map showed that methane hydrates can also be found in the Gulf of Mexico.
  - Focus for this lesson will be on an interesting organism that was first discovered living on the methane hydrate in the Gulf of Mexico.
- Assign students to groups of 3-4 individuals for the remainder of the lesson.

### **Making Observations**

- Distribute the Student Worksheet: Methane Ice Worms to each student.
- Before showing students the ice worm image, **project** <u>Slide 3: Think about it!</u> to guide them toward the driving question.
- Display <u>Slide 4: Methane Ice Worm Image</u> for the entire class or print colored copies for each group.
- Have students write down 2-3 observations and 2-3 questions on their worksheet.
- Have student groups discuss their work and share an observation and question on a class whiteboard or other shared space you provide.
- Talk through some of their observations/questions, and then review
   Slide 5: Methane Ice Worms of the Gulf. Emphasize that scientists had similar
   questions and observations. But the big question was "How do methane ice
   worms obtain organic compounds and energy while living on methane hydrate?"



Slide 1. Courtesy of NOAA Ocean Exploration.



Video courtesy of NOAA Ocean Exploration.



Methane Ice Worms Student Worksheet. Courtesy of NOAA Ocean Exploration.



Slide 3. Courtesy of NOAA Ocean Exploration.



Slide 4. Courtesy of NOAA Ocean Exploration.



Slide 5. Courtesy of NOAA Ocean Exploration.

# **Educator Guide** cont.

# **Investigate**

Students will analyze evidence published by scientists between the discovery of the methane ice worms in 1997 and 2022.

### **Hypotheses About the Methane Ice Worm**

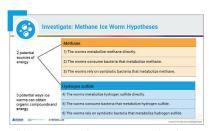
- · Prompt students to start thinking with Slide 6: Investigate: Think about it.
- · Review Slide 7: Methane Ice Worm Hypotheses.
  - Scientists identified two potential energy sources (methane or hydrogen sulfide) and three possible ways the methane ice worms could access each food source (direct, by consuming bacteria, or by relying on symbiotic bacteria).

## Methane Ice Worms Evidence and Reasoning Card Sort

- Provide each group with a set of the printed and cut <u>Methane Ice Worms</u>
   <u>Evidence and Reasoning Cards</u> (can be printed in color or black and white).
  - A <u>Teacher Key</u> is provided to show the correct pairing for each piece of evidence and reasoning.
  - Use <u>Slide 8: Card Sort</u> to go over the basics of the activity and to show them a sample matching.
- Encourage student groups to discuss and come to consensus on their matches. Check for this as you circulate.
  - If you notice errors in matching evidence and reasoning, use some of the
    questions listed below to help students diagnose/correct errors and to push
    them to think more critically.
    - Why do you think that piece of evidence goes with that reasoning?
    - Did you both/all agree on that initially? If not, then how did you come to an agreement?
    - Are there any matches that you are still uncertain about? If so, why?
    - What does that evidence and reasoning tell you about how the methane ice worms might obtain energy and matter?
    - Why is that evidence and reasoning important?
    - Does that evidence and reasoning help you evaluate the hypotheses we have? Why not? Or How so?



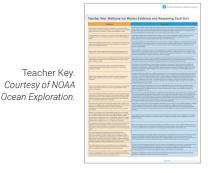
Slide 6. Courtesy of NOAA Ocean Exploration.



Slide 7. Courtesy of NOAA Ocean Exploration.



Methane Ice Worms Evidence and Reasoning Cards. Courtesy of NOAA Ocean Exploration.





Slide 8. Courtesy of NOAA Ocean Exploration.

# **Educator Guide** cont.

# Put the Pieces Together

After the group has completed the card sort, they will work together to come to a consensus on the best hypothesis and supporting evidence and reasoning to answer the question, "How do methane ice worms obtain organic compounds and energy while living on methane hydrate?" Student will use questions 1-4 to help guide them. Discuss as a class if needed (Slide 9: Reflection).

- 1. Are there any hypotheses that we can eliminate based on the class consensus?
  - There is conclusive evidence that hydrogen sulfide, rather than methane, is the likely source of energy and organic compounds for the methane ice worms.
  - The three hypotheses regarding methane can be eliminated.
  - We can also eliminate the hypothesis that the worms metabolize hydrogen sulfide directly.
     This would be a major new scientific finding and would require overwhelming evidence to accept.
- 2. Did your group come to consensus regarding any remaining hypotheses?
  - There will likely, and should, be disagreement within a group about whether the worms are consuming bacteria as prey or relying on them as symbionts.
  - The question is not completely resolved among scientists, and the true answer likely involves both concepts.
  - The focus within this lesson is that students gain practice in using scientific evidence and science ideas to evaluate hypotheses (plausible claims) and construct arguments.
- 3. Which pieces of evidence seem to be most important? Is there consensus within your group about this?
  - Answers will vary.
  - Each piece of evidence was pulled from a published scientific report, so each piece was deemed important by professional scientists.
  - The importance that students place on particular pieces of evidence is likely to depend on their levels of understanding.
- 4. What changes would you make to your initial argument after seeing the arguments from other groups?
  - Answers will vary.

#### **TEACHER NOTE**

After students have completed their written arguments, you may want to provide them with a brief overview of where scientists stand on the driving question. As stated above, the question is not settled and the truth likely does not fit cleanly into either the consumption or symbiosis boxes. Additional testing is needed to confirm this, but the methane ice worms and sulfide-metabolizing bacteria likely represent a form of mutualism referred to as **symbiotic farming**. This would mean that the worms are helping the bacteria grow at rates that the bacteria could not sustain on their own. The worms do this by creating small currents that dissolve the methane hydrate and mix hydrogen sulfide with oxygenated water, providing nutrients to the bacteria. These currents create the oval depressions in which scientists find the worms. The worms then consume the bacteria and thereby obtain energy and organic compounds. Even though the worms are consuming the bacteria, the net growth of the bacteria is greater than it would be without the worms. Thus, each species is benefitting. Again, direct testing is needed to confirm this explanation.

### Extension •

Have students generate additional questions about methane ice worms or methane ice in general.
 Then have students propose investigations and/or design studies that would collect the data/evidence needed to answer their questions.

# **Educator Guide** cont.

### Scientific Terms

**Cold seeps:** Places throughout the ocean where hydrogen sulfide, methane, and other hydrocarbon-rich fluids and/or gasses escape from cracks in the ocean floor.

Hydrogen sulfide: A colorless, toxic gas known for its pungent "rotten egg" odor at low concentrations.

**Isotope**: Atoms with the same number of protons but different numbers of neutrons.

**Metabolize:** The chemical changes in a cell or an organism that make the energy and materials needed for the cell or organism to grow and reproduce.

**Metagenome:** The recovery and complete sequencing of genetic material extracted directly from all environmental samples.

Methane hydrate: White, ice-like solids that consist of methane and water. The methane molecules are enclosed in microscopic cages composed of water molecules. Methane gas is primarily formed by microorganisms that live in the deep sediment layers and slowly convert organic substances to methane.

Plankton: Microscopic plants and animals that are the foundation of freshwater and seawater food pyramids.

Polychaete: Segmented, generally marine, worms with pairs of appendages on each body segment.

**Symbiont:** An organism living in symbiosis with another. Symbiosis is a close and prolonged interaction between organisms of different species.

#### Assessment =

- Have each student construct a **final written argument** (Slide 10) on a large, white poster that answers the driving question for the lesson. The argument should include the following components.
  - · The hypothesis selected.
  - The specific evidence and reasoning selected.
  - A rationale explaining how the selected evidence and reasoning supports the selected hypothesis.
  - A brief reflection of how the students' thinking was changed or reinforced by the gallery walk and discussion.
- After students have completed their written arguments, provide them with a brief overview of
  where scientists stand on the driving question since the question is not settled and the truth likely does not fit
  cleanly into either the consumption or symbiosis boxes. Refer to the Teacher Note above.

Opportunities for formative assessment are embedded throughout the lesson. The student models and explanations that are developed at the end of the lesson could be used as an opportunity for summative assessment of learning.

You can use the following look-fors to assess and provide feedback on students' written arguments.

- Recognition that there is evidence for both claims 5 and 6, but either claim on its own is an acceptable response.
- A rationale explaining how the selected evidence and reasoning supports the selected hypothesis is provided.
- Thoughtful reflection of how their thinking was changed or reinforced by their class discussions.



# **Teacher Key: Methane Ice Worms Evidence and Reasoning Card Sort**

Evidence	Reasoning
Observation and dissection of the methane ice worms revealed that they have a functional digestive tract. Freshly collected worms produced feces for 24 hours. (Fisher and colleagues, 2000)	Unlike most animals, tube worms collected from deep sea thermal vent communities have no digestive tract, mouth, or anus. This is definitive evidence that they must rely on symbiotic bacteria living inside the tissues of the tube worms that provide them with energy and organic compounds. An animal that has a functional digestive tract may ingest food, rely on internal symbionts, or both.
Only prokaryotes are known to use methane and sulfide gasses as energy sources or to metabolize crude oil. (Fisher and colleagues, 2000)	Animals are known to metabolize the four major groups of macromolecules: carbohydrates, proteins, lipids, and nucleic acid. An animal that could directly metabolize methane, sulfide gas, or the hydrocarbons found in crude oil would be a major scientific discovery.
Fisher and colleagues (2000) used a scanning electron microscope to examine the external surface of two methane ice worms and found "no external symbionts."	External symbionts are organisms that live on the surface of other organisms in a dependent relationship. These symbionts may act as parasites or provide the host organism with protection. Alternately, external symbionts may provide the host with nutritional resources by metabolizing compounds that the host cannot utilize directly.
When Fisher and colleagues (2000) observed and dissected the methane ice worms, they did not find any hypertrophied tissues.	Hypertrophied, or enlarged, tissues can be observed in an organism that is hosting internal symbionts. This occurs when large populations of the symbiont are present in a specific tissue. For example, some beetles have enlarged sacs in their digestive system that host bacterial symbionts. Root nodules holding nitrogen-fixing bacteria in legume plants are another example.
Examination of internal tissues via light and transmission electron microscopes revealed only scattered bacteria in the gut. (Fisher and colleagues, 2000)	Bacteria that are serving as internal symbionts would be expected to be observed in large quantities somewhere within the host organism, often in the digestive tract. For example, a large and characteristic microbial community can be found in the digestive tracts of ruminant animals like cows.
Samples collected from the surface of the hydrate revealed "an abundant and varied population of bacteria." (Fisher and colleagues, 2000)	The behavior of the methane ice worms may contribute to the growth of bacteria near the surface of the hydrates. Small water currents caused by movement of the worms' appendages may increase the breakdown of the hydrate, releasing methane and sulfide gasses into the surrounding water. This could increase the supply of oxygen needed by the bacteria to grow. This may also form the depressions in which the worms live.
Fisher and colleagues (2000) found that the sulfur isotope ratio values in two methane ice worms were 1.9 and 3.6 parts per thousand.	Average isotope ratio values for sulfate compounds found in ocean water are near 20 parts per thousand. In marine food webs based on photosynthetic plankton, sulfur isotope ratios typically range from 15 to 20 parts per thousand. Bacteria that use sulfur compounds as an energy source can have sulfur isotope ratio values as low as -30 parts per thousand. Tube worms that live at seeps and are known to have a symbiotic relationship with sulfur-metabolizing bacteria are known to have sulfur isotope ratio values that range from -20 to 5 parts per thousand. Animals tend to reflect the sulfur isotope ratios of their food sources (Kennicut II et al., 1992).
Fisher and colleagues (2000) found that the nitrogen isotope ratio values measured in methane ice worms ranged from 5.3 to 6.3 parts per thousand.	Analysis of animals living both at and away from hydrothermal vents indicates that nitrogen isotope ratios for vent animals is generally <10 parts per thousand, while all non-vent animals are generally <10 parts per thousand. The animals that live at vents are presumed to either host symbiotic bacteria or consume organisms that do.
Fisher and colleagues (2000) found that the carbon isotope values measured in the methane ice worms were approximately -24 parts per thousand. This is intermediate between the ratio found in the methane hydrate (-48 parts per thousand) and the ratio typically found in seawater (0 parts per thousand).	If the methane ice worms obtained their nutrition from methanotrophic bacteria that were metabolizing the methane hydrate directly, then their carbon isotope ratio values would be expected to be similar to the ratio found in the methane hydrate. If the worms were feeding on bacteria or other organisms that had drifted from the surrounding seawater, then their carbon isotope ratio values would be expected to be close to 0. The values found in the methane ice worms are within the range found in giant tubeworms that live at hydrothermal vents and harbor sulfur-metabolizing symbionts.
Pile and Young (2006) found that in laboratory trials, methane ice worm larvae significantly reduced the growth rates of microscopic plankton, including heterotrophic bacteria, autotrophic cyanobacteria, and phototrophic eukaryotes that were less than <3 m in size.	Reduction in growth rate of microorganisms when grown in the presence of a possible predator indicates that the larger organism is in fact ingesting the microorganisms. Evidence that a larval stage of an organism feeds on a particular food source indicates that it is possible that the adult stage also feeds on that same food source. However, adults may also utilize different food sources than larva of the same species.
Becker and colleagues (2013) analyzed isotope ratios in animals from multiple seep communities in the Gulf of Mexico. They found that ratios for methane ice worms were highly variable. Carbon isotope values ranged from -63 to -29 parts per thousand; nitrogen isotope values ranged from -6 to 5 parts per thousand; and sulfur isotope values ranged from 0.7 to -21 parts per thousand.	Large variation in the isotope ratio values for an animal species indicates large variability in the isotope compositions of the microbes on which they feed and suggests that they do not specialize on a single type of bacteria.
Gut contents removed from methane ice worms consisted of clear liquid with black particulates, and environmental exposure to hydrocarbons was indicated by a petroleum smell emanating from the worm gut when pierced during dissection. (Lim and colleagues, 2022)	Black particulates found in a deep sea animal's gut are likely to contain sulfur. Similar material has been found in the digestive tract of juvenile hydrothermal vent shrimp and was believed to be a nutrition source for these juveniles before they switch to a mainly epibiont-based diet in their adult forms.
Lim and colleagues (2022) determined the RNA genome sequences for all bacterial species found in the gut contents from two methane ice worms. Eighty-six percent (86%) of these sequences were identified as Sulfurospirillum species, which are bacterial species that are known to metabolize sulfur-containing compounds. Species that metabolize methane were rare, and there was little evidence of bacterial symbiosis outside the gut.	Feeding experiments are needed to definitively determine an organism's food sources. However, genomic studies can establish that bacteria have an important nutritional role, either as food sources or as digestive symbionts in the gut. High abundances of Sulfurospirillum species were also reported in the gut of the hydrothermal vent crab Austinograea species, which is also known to feed on bacteria.
The Sulfurospirillum species identified by Lim and colleagues (2022) through genomic analyses were genetically different from the free-living species that have been found previously.	The fact that Sulfurospirillum species found in the gut of the methane ice worms do not match previously identified species indicates that these new species may not live in the general environment. This supports the idea that Sulfurospirillum species act as intestinal symbionts in methane ice worms. This is an important finding that needs to be verified because it represents new information.
Lim and colleagues (2022) found that some bacteria identified in the gut of the methane ice worm have genes that would allow them to synthesize certain B vitamins and amino acids.	Supplying B vitamins and amino acids that cannot be synthesized by the host is a common way that symbionts contribute to the nutrition of the host organism.

# **Cold Seeps: Methane Ice Worms**

#### Page 1: Methane Ice Worm Slides: https://oceanexplorer.noaa.gov/edu/materials/methane-ice-worm-slides.pdf

- ▶ Student Worksheet: Methane Ice Worms (PDF): <a href="https://oceanexplorer.noaa.gov/edu/materials/student-worksheet-methane-ice-worms.pdf">https://oceanexplorer.noaa.gov/edu/materials/student-worksheet-methane-ice-worms.pdf</a>
- Methane Ice Worms Evidence and Reasoning Card Sort: https://oceanexplorer.noaa.gov/edu/materials/methane-worm-evidence-reasoning-cardsort.pdf
- ▶ Cold Seeps and Methane Hydrates (video): https://www.youtube.com/watch?v=ahmjHLyF9GM
- ▶ Ice worms inhabiting methane hydrate (image):

https://oceanexplorer.noaa.gov/okeanos/explorations/ex1803/background/chemo-comm/media/ice-worms-hires.jpg

### Page 2: → Ocean Exploration Facts: <a href="https://oceanexplorer.noaa.gov/facts/hydrates.html">https://oceanexplorer.noaa.gov/facts/hydrates.html</a>

- Making Sense of Deep-Sea Phenomena (PDF): https://oceanexplorer.noaa.gov/edu/materials/NOAA-NSTA-sensemaking-phenomenon.pdf
- $\blacktriangleright \ \ Chemosynthesis\ Fact\ Sheet\ (PDF): \\ \underline{https://oceanexplorer.noaa.gov/edu/materials/chemosynthesis-fact-sheet.pdf}$
- ▶ Cold Seeps Fact Sheet (PDF): https://oceanexplorer.noaa.gov/edu/materials/what-are-cold-seeps-fact-sheet.pdf
- ▶ Cold Seep Communities Fact Sheet (PDF): <a href="https://oceanexplorer.noaa.gov/edu/materials/cold-seep-communities-fact-sheet.pdf">https://oceanexplorer.noaa.gov/edu/materials/cold-seep-communities-fact-sheet.pdf</a>

#### Page 3: ▶ Cold Seeps and Methane Hydrates (video): <a href="https://www.youtube.com/watch?v=ahmiHLyF9GM">https://www.youtube.com/watch?v=ahmiHLyF9GM</a>

- ▶ Methane Ice Worm Slides: https://oceanexplorer.noaa.gov/edu/materials/methane-ice-worm-slides.pdf
- > Student Worksheet: Methane Ice Worms (PDF): https://oceanexplorer.noaa.gov/edu/materials/student-worksheet-methane-ice-worms.pdf

#### Page 4: Methane Ice Worm Slides: https://oceanexplorer.noaa.gov/edu/materials/methane-ice-worm-slides.pdf

- ▶ Methane Ice Worms Evidence and Reasoning Card Sort: <a href="https://oceanexplorer.noaa.gov/edu/materials/methane-worm-evidence-reasoning-cardsort.pdf">https://oceanexplorer.noaa.gov/edu/materials/methane-worm-evidence-reasoning-cardsort.pdf</a>
- Page 5: Methane Ice Worm Slides: https://oceanexplorer.noaa.gov/edu/materials/methane-ice-worm-slides.pdf
- Page 6: ► Methane Ice Worm Slides: https://oceanexplorer.noaa.gov/edu/materials/methane-ice-worm-slides.pdf

#### Page 7:

#### References:

Becker, E. L., Cordes, E. E., Macko, S. A., Lee, R. W., & Fisher, C. R. (2013). Using stable isotope compositions of animal tissues to infer trophic interactions in Gulf of Mexico lower slope seep communities. PloS one, 8(12), e74459.

Fisher, C. R., MacDonald, I. R., Sassen, R., Young, C. M., Macko, S. A., Hourdez, S., ... & McMullin, E. (2000). Methane ice worms: Hesiocaeca methanicola colonizing fossil fuel reserves. Naturwissenschaften, 87(4), 184-187.

Holden, C. (1997). Ice Worms from the Gulf. Science, 277(5327), 769.

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Pile, A. J., & Young, C. M. (2006). Consumption of bacteria by larvae of a deep sea polychaete. Marine Ecology, 27(1), 15-19.

# **Partners**







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# Information and Feedback

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