NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Aliens on Earth? The #arseniclife Affair

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Part I – NASA's Big News

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NASA Sets News Conference on Astrobiology Discovery; Science Journal Has Embargoed Details Until 2 p.m. EST on Dec. 2, 2010

WASHINGTON -- NASA will hold a news conference at 2 p.m. EST on Thursday, Dec. 2, to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life. Astrobiology is the study of the origin, evolution, distribution and future of life in the universe.

The news conference will be held at the NASA Headquarters auditorium at 300 E St. SW, in Washington. It will be broadcast live on NASA Television and streamed on the agency's website

(Source: http://www.nasa.gov/home/hqnews/2010/nov/HQ_M10-167_Astrobiology.html)

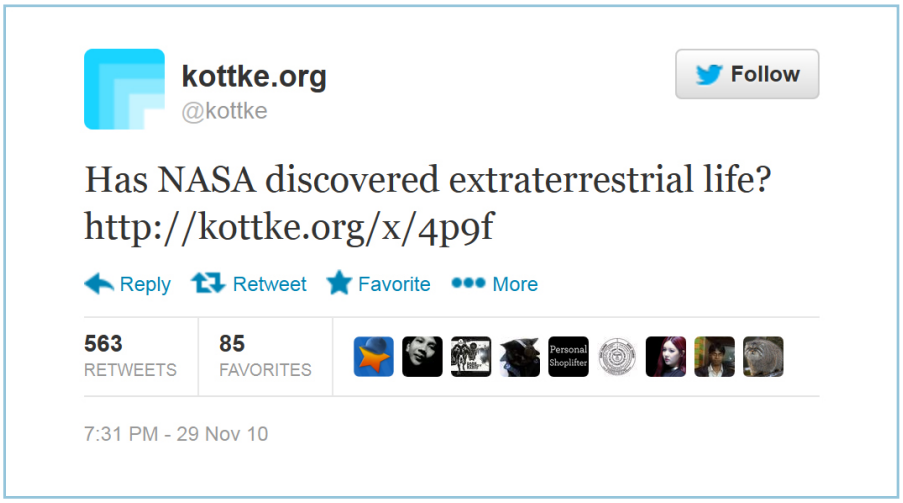
It's November 29, 2010, and being the NASA junkie that you are, you just stumbled on this NASA press release. The statement that the news event will be used as a platform "to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life" catches your eye. You eagerly anticipate the news conference, which is scheduled to be held a few days later. Meanwhile, your imagination runs wild about the possible meanings of this statement.

Questions

1. What do you think NASA discovered? Brainstorm what the statement "to discuss an astrobiology finding that will impact the search for evidence of extraterrestrial life" suggests. Create your own Twitter post about your thoughts (less than 140 characters).
2. The document states that the journal *Science* has embargoed the paper describing the finding until the time of the news conference. What does that mean? What's an embargo?

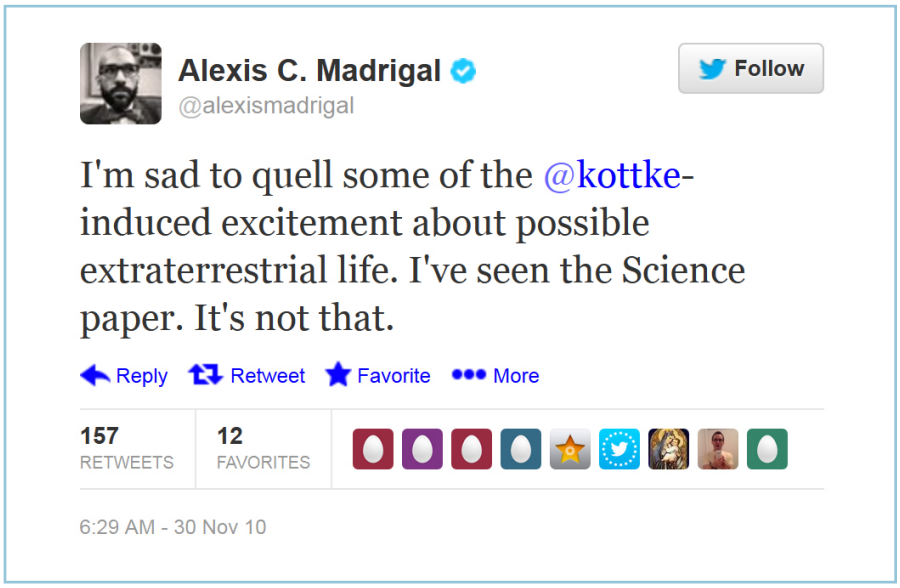
Right away, the blogosphere goes wild with speculations that NASA has found evidence for life on Mars or on one of the moons of Saturn or Jupiter. One Twitter post captures the general excitement about what's on everybody's mind (Figure 1).

Figure 1. Kotke.org tweet.



A senior editor at *The Atlantic*, who was given an advanced copy of the *Science* article ahead of the news conference, tries to calm everyone and temper their expectations:

Figure 2. Madrigal tweet.



Question

1. Did this reporter break his agreement with the journal *Science* by releasing this statement? If you were responsible for *Science's* public relations division, would you revoke his access to future *Science* articles ahead of the embargo? Why or why not?

Part II – Life as We Don't Know It

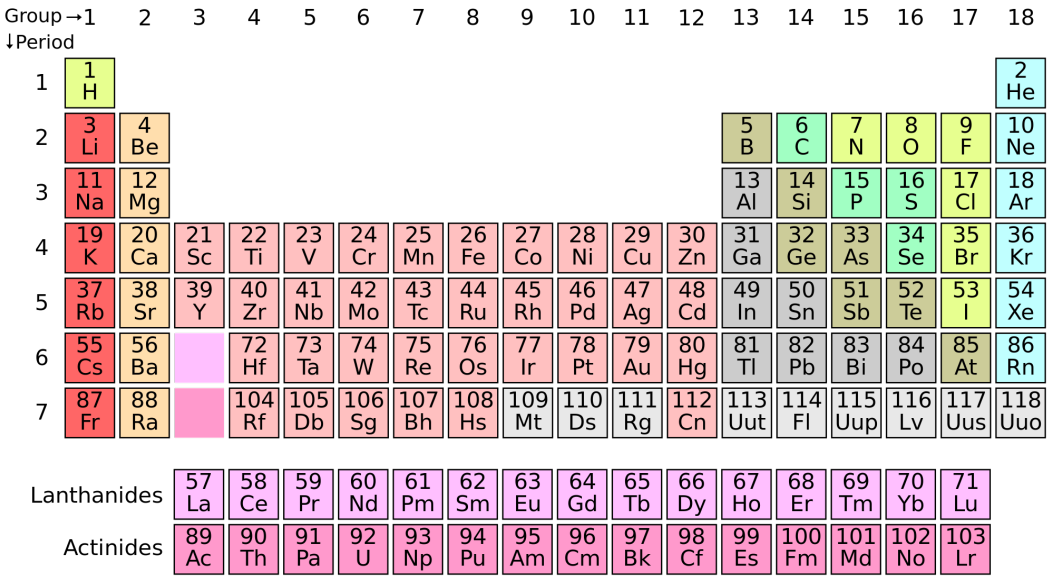
Unfazed, you are glued to your computer monitor on December 2, 2010, as you stream the NASA news conference. Sadly, NASA did not find evidence for extra-terrestrial life on Mars or on one of the moons of Saturn or Jupiter. Bummer! Nonetheless, the announced discovery is thought-provoking.

On stage is young postdoctoral researcher, Felisa Wolfe-Simon. Felisa's interests in the "big questions" about life (e.g., why life is the way that it is, and why its chemistry is the way that it is) lead her to consider why phosphorus, often in the form of phosphate in the cell (that is, a phosphorus atom linked to four oxygen atoms), was chosen during the course of evolution to form many biological molecules. Phosphorus is found in DNA, RNA, ATP, and phospholipids (a common lipid in membranes), and is used to "tag" proteins and change their activity (i.e., protein phosphorylation).

In particular, she considered the fact that phosphorus is not very abundant at the bottom of the ocean, where life is commonly believed to have originated (Tawfik & Viola, 2011). Could another atom have done the job early in the evolution of life? Might some life forms have evolved using another atom in the place of phosphorus and still exist today? If so, which atom are they using?

Knowing a bit of chemistry, it's easy to predict which atom could take on a similar role in the molecules of life. The Periodic Table of Elements classifies atoms based on their characteristics, and atoms in the same column (called group) often share similar properties.

Figure 3. Periodic table.



Questions

1. What is the symbol for phosphorus?
2. Find this symbol in the Periodic Table of Elements. Record the symbols for the elements (atoms) which are directly above and below in the column.

The reason that arsenic is so poisonous to us is precisely because it "looks like" phosphorus to our cells. Arsenic and phosphorus share many chemical properties, but they are also different, and when our cells try to use arsenic, things go badly.

But this might not be the case for an organism that has evolved to use arsenic. Felisa's goal is to find life on Earth that might be able to use arsenic instead of phosphorus.

Questions

3. Where might Felisa begin her search for these life forms? Describe the characteristics of this environment.
4. What might she do to detect an arsenic-using life form apart from a phosphorus-using life form? (Assume that the life forms are bacteria.)
5. What would Felisa need to show in order to convince other researchers that a life form uses arsenic in its cells and does not merely survive in the presence of (or tolerate high levels of) arsenic?

Part III – Felisa's Paper

Back to the NASA news conference of December 2, 2010: Felisa is explaining to a roomful of journalists that she went to eastern California's Mono Lake for her investigations. She chose Mono Lake because the water it receives from its tributary (the Sierra Nevada) can only escape by evaporation. This concentrates the salts and minerals present in that water, some of which precipitate to form stalagmite-like structures called "tufa towers" that rise from the water's surface during times of drought. The water is very salty, has a high pH (it is basic), and… is steeped in arsenic.

Figure 4. Tufa towers rising above Mono Lake's surface. http://en.wikipedia.org/wiki/File:Mono-lake-tufa-1981-003.jpg

Felisa collected bacteria from the mud at the bottom of the lake. She named one of the organisms GFAJ-1, a tongue-and-cheek acronym for "Give Felisa A Job" (remember that Felisa is a postdoctoral fellow, so she does not have a permanent position as a researcher yet and is attempting to establish herself professionally at this point in her career).

Watch a brief (3.5-min) video of Felisa explaining her work here: http://www.youtube.com/watch?v=5GKmKyfXuFw.

Back at the lab, Felisa grew GFAJ-1 in test tubes, providing them with all the nutrients that bacteria enjoy. Over a period of months, she progressively added more arsenic to the growth medium until the amount the bacteria were able to tolerate surpassed the amount found in Mono Lake. She then tested the nutrients that these bacteria needed to survive: she added different amounts of phosphorus and arsenic to three test tubes and attempted to grow cells (Simon-Wolfe et al., 2010). Here is what she found:

- When the growth media was supplemented with phosphate (1.5 mM PO_4^{3-}), the bacteria grew heartily.
- When the growth media contained arsenate (one atom of arsenic bonded to four oxygen atoms) (40 mM AsO_4^{3-}) but no added phosphate, the bacteria also grew, albeit more slowly.[1]
- When the growth medium contained neither added phosphate nor arsenate, the bacteria failed to grow.
- Felisa noted that there were trace amounts of phosphate present to 3.1 µM (that's 0.0031 mM) in all growth media, due to impurities in the salts used to make the media. This concentration is almost 500-fold less than in the "phosphate-containing" test tubes.

[1] Note: Mono Lake contains arsenic at a concentration of roughly 200 µM (200-fold less concentrated than the arsenic concentration used in the growth medium).

Question

1. What can you conclude from the results of this first experiment?

Felisa then grew the cells on medium containing either added phosphate or arsenate, washed them to remove any of the phosphorus or arsenic from the surrounding medium, and measured the fraction of atoms in the cells that were arsenic and phosphorus.

Questions

2. What is her hypothesis?
3. Given her hypothesis, would you expect any phosphorus to be present in the cells that were grown in the arsenate-supplemented medium, and vice-versa?
4. Given her hypothesis, do you expect the arsenic content in the bacteria grown in the presence of arsenate to be less, the same, or more than the phosphorus content of cells grown in phosphate-containing media? What is your rationale?

Table 1 presents the data that Felisa obtained. The numbers represent the percentage of atoms in the cell that are arsenic or phosphorus (percent dry weight).

Table 1. Percentage arsenic or phosphorous

Growth Condition (Medium)	*Arsenic*	*Phosphorus*
Added arsenate	0.19 ± 0.25	0.019 ± 0.009
Added phosphate	0.001 ± 0.0005	0.54 ± 0.21

Questions

5. Taking these numbers (and their ± values) into account, do these data support Felisa's hypothesis (that is, do they support your predictions made in response to Questions 3 and 4)?
6. Where is the arsenic and phosphorus in these cells? How do you know this?

Felisa wanted to know if there is arsenic in DNA. To find out, she grew GFAJ-1 cells on medium supplemented with either arsenate or phosphate. She then purified the nucleic acids (DNA and RNA) from these two types of cells. To separate DNA from RNA, she passed them through a sieve of agarose[2] in a procedure called "agarose gel electrophoresis." Long DNA molecules become trapped in the agarose and go through the sieve slowly, while short RNAs go through it quickly, separating the two nucleic acids. The DNA and RNA remain embedded in the agarose sieve at the end of the separation. Felisa cut out pieces of agarose containing the DNA of the cells grown on the two different media to determine the arsenic-to-carbon content of these samples. The results are shown in Table 2.

Table 2. Arsenic-to-carbon ratio in DNA of samples

	Cells grown on media containing arsenate	*Cells grown on media containing phosphate*
Arsenic-to-carbon ratio[3]	13.4 (± 2.5)	6.9 (± 1.6)

Questions

7. Why is the arsenic content provided as a ratio to carbon content?
8. Comment on these results. What do they show? Do they match your expectations? What hypothesis do they support?

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[2] Agarose is a carbohydrate (a complex sugar) derived from seaweed.

[3] Number is × 10^{-6}. In other words, a value of 1 would mean that there is one atom of arsenic for every 1,000,000 atoms of carbon.

9. Identify a potential source of error in this analysis (*Hint:* What are potential sources of carbon and arsenic in these samples?)

Felisa performed a few other experiments that are described in her paper. However, this gives you the gist of her findings and how she arrived at her conclusions.

Questions

10. Create a Twitter post (140 characters or less) that summarizes what Felisa and her collaborators have shown in this paper.
11. How convincing is the data? Are you persuaded that GFAJ-1 uses arsenic in its DNA instead of phosphorus? Is there any other experiment you might like done? What would this additional experiment need to show to convince you?

In answer to this, NASA tweets:

Figure 5. NASA tweet.

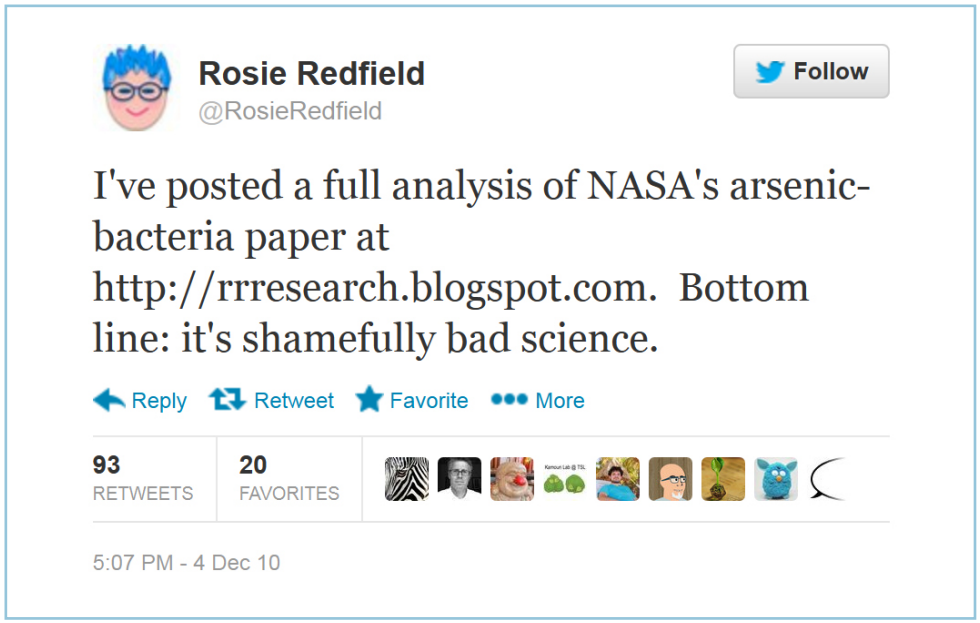
Question

12. What do you think of NASA's statement about this finding? Will this research fundamentally change our biology textbooks?

Part IV – Replication Factor

Two days after the paper was released on the *Science* website, Rosie Redfield, a microbiologist at the University of British Columbia, posted her critique of the paper on her blog (Redfield, 2010). Her tweet announcing this new post did not mince words:

Figure 6. Rosie tweet.



Here are some of the highlights of Rosie's critique of the paper's methodologies and conclusions:

A. Felisa's team claims that the cells grown in a medium with no added phosphate or arsenate failed to grow. A closer inspection of the data shows this to be inaccurate; the cells did grow, albeit more slowly. Also, Rosie calculated that the trace amount of phosphorus contaminating the medium ingredients was sufficient to sustain growth of the bacteria in the "no phosphate" condition.

B. A bacterial genome composed of 2×10^6 base pairs (a midrange genome size for bacteria) contains 4×10^6 phosphorus atoms. Using "back of the envelope calculations," the data available in Table 2, and the known ratio of phosphorus and carbon in a DNA molecule, Rosie calculates that the genome of a GFAJ-1 bacterium grown in the presence of arsenate contains 400 atoms of arsenic while bacteria grown in the presence of phosphate have 200 atoms of arsenic in their genome.

C. Using agarose pieces to calculate the arsenic-to-carbon ratio is a mistake, because agarose (a carbohydrate) contains many atoms of carbon, and therefore the arsenic-to-carbon ratio will depend on factors such as the size of the agarose chunk used in the experiment.

D. Rosie brings up the chemical instability of arsenate compared to phosphate. The bonds between the phosphorus atoms and the oxygen atoms in phosphate are very stable and break down in timescales measured in thousands of years (300,000 years is the half-life of that bond). Meanwhile, arsenate is known to be much less stable and to break down in less than a second (0.06 seconds is the half-life) (Fekry et al., 2011).

Rosie presented a few other criticisms, but these should suffice for the purpose of this discussion.

Questions

1. Concerning point A above, how damaging is this critique to Felisa's conclusions? Is it a minor point or a major one?
2. Concerning point B, does the estimated arsenic content of cells suggest a complete replacement of phosphorus by arsenic in the cells grown in the presence of arsenate?
3. Concerning point C, propose a proper control for this experiment.
4. Concerning point D, when Felisa used agarose to separate the DNA of cells grown in the presence of arsenate from its RNA, she saw one solid band for the DNA, not a smear that would indicate that the DNA population was broken-up into pieces of various sizes. Suggest at least two possible interpretations for this observation.
5. Given that Rosie's musing are "theoretical" (inasmuch as she has not conducted any experiment in the lab to refute this paper), how convincing are her arguments?

The amount of attention that this blog posting received surprised Rosie. As a consequence, she was pulled into a very public debate about the validity of the arsenic life claim.

To "meet Rosie," watch this brief (4 min) video describing the arsenic life story and Rosie's involvement. http://www.youtube.com/watch?v=RFdYL9-myqo (Dunning et al., 2012).

Months later, Rosie tried to reproduce Felisa's results in her own lab, using slightly different methods to overcome what she perceived as some of the methodological flaws of the original experiments. Her results show that:

- GFAJ-1 cells grown with 3 μM phosphate were able to grow, confirming that the "trace phosphate contaminants" in Felisa's medium ingredients provided a sufficient amount of phosphate to support the growth of these cells.
- Arsenate could not substitute for phosphate in the medium to allow the cells to grow (note that Rosie's medium contained no trace amounts of phosphate); therefore Felisa's results could not be reproduced.
- The DNA of cells grown in the presence of arsenate and limited phosphate did not break down rapidly.
- Mass spectrometry (a method of determining atomic composition) showed that GFAJ-1 grown in the presence of abundant arsenate only contained arsenic atoms "glued" to the DNA as contaminants, and none as part of the DNA backbone (this was shown by "washing" the purified DNA in water before subjecting it to mass spectrometry analysis to remove the contaminating arsenic).

This paper was published in *Science* (Reaves et al., 2012), and a paper by another research group published in the same issue of *Science* confirmed these results (Erb et al., 2012). Thus, two independent research teams have now refuted Felisa's work.

Questions

6. Given that Rosie used slightly different techniques to replicate Felisa's work, does this refute the original arsenic life results?
7. A *"retraction is a mechanism for correcting the [research] literature and alerting readers to publications that contain such seriously flawed or erroneous data that their findings and conclusions cannot be relied upon. Unreliable data may result from honest error or from research misconduct"* (COPE, 2009). Should Felisa's paper be retracted?
8. How do you expect other researchers to react to Felisa's work? Aren't "fits and starts" the way that science progresses? Is she likely to suffer a professional penalty? Should she be penalized? Why or why not? Should her colleagues, supervisors, sponsoring agency (i.e., NASA), and the reviewers of the paper bear as much responsibility as she, if any?

Part V – Post-Publication Peer Review of Science?

In the days following the NASA news conference, several scientists, like Rosie, posted their critique of Felisa's paper on their blog and on Twitter. Science journalists interviewed researchers from other universities to ask for their assessment of the paper. They also contacted Felisa's group to give them a chance to respond. Felisa and her team declined to comment, saying that it is inappropriate to debate the science of the paper using news media and blogs. They argued that the proper forum for discussion is the peer review process. Here is a tweet from Felisa to this effect:

Figure 7. Felisa tweet.

Questions

1. What is peer review in science?
2. What are some of the strengths of this system?
3. Why might it be an imperfect system?
4. What experts accept as "proof" varies with each discipline, which is problematic in an interdisciplinary field such as astrobiology. Physicists who reviewed Felisa's paper were enthusiastic about its results while biochemists considered the evidence inadequate (Benner et al., 2013). Given this information, was this paper appropriately peer-reviewed? Do you think that Felisa's paper would have been published if it had been submitted to a less prestigious journal that focused strictly on biological research?

Chris Rowan, a geologist at Kent State University, wrote on his blog *Highly Allochthonous* a sentiment that is succinctly captured in his Twitter post about it:

Figure 8. Allochthonous tweet.

In his blog, he expands on this point by adding that *"the pre-publication stuff is just a quality filter, a check that the paper is not obviously wrong—and an imperfect filter at that. The real test is what happens in the months and years* after *publication"* (Rowan, 2010).

David Dobbs, a science writer at *Wired* magazine, commented on his blog that "Rosie Redfield is a peer, and her blog is peer review" (Dobbs, 2010).

But some researchers cautioned against giving the blogosphere too much weight. Dr. Hazel Barton at the University of Northern Kentucky remarked: *"It's important that the reviews of individuals who are not experts in the field do not have as much weight as that of the original reviewers, no matter how public the review"* (Zimmer, 2010b).

Question

5. Once published, should the science be debated in the public realm or should science be debated in a "closed discussion forum" among scientists until a consensus can be delivered to the public? Why?

"If question remains about the [veracity] of these authors' findings, then the only thing that is going to answer that doubt is data. Data cannot be generated by blog discussion. [..] more experiments need to be done. Talking about digging a ditch never got it dug" (Dr. Isis, 2010).

Question

6. Given this quote, what is the value of an online discussion to the progression of science?

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