

Teacher Resource for:
Nanomole-scale high-throughput chemistry for the synthesis of complex molecules



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GENERAL USE OF Science in the Classroom

Student Learning Goals:

“One fundamental goal for K-12 science education is a scientifically literate person who can understand the nature of scientific knowledge.”¹

The U.S. National Academy of Sciences defines science as: “Any new finding requires independent testing before it is accepted as scientific knowledge; a scientist is therefore required to honestly and openly report results so that they can readily be repeated, challenged, and built upon by other scientists. Proceeding in this way over centuries, the community effort that we call science has developed an increasingly accurate understanding of how the world works. To do so, it has had to reject all dogmatic claims based on authority, insisting instead that there be reproducible evidence for any scientific claim.”

An important student learning goal, central to any understanding of “the nature of scientific knowledge,” is to give each student an appreciation of how science is done.

This includes knowing why:

- Scientists must be independent thinkers, who are free to dissent from what the majority believes.
- Science can deal only with issues for which testable evidence can be obtained.
- All scientific understandings are built on previous work
- It is to be expected that one scientist’s conclusions will sometimes contradict the conclusions of other scientists.
- Science is a never-ending venture, as the results from one study always lead to more questions to investigate.

¹ *A Framework for K-12 Science Education*, National Research Council, 2012

Using This Resource

Learning Lens:

The Learning Lens tool can be found on the right sidebar of each resource and is the source of annotations. Click on the headings to highlight portions of the text of the corresponding research article. A subsequent click on the highlighted text will produce a text box containing more information about that particular piece of text. Below is an example of the Glossary function of the Learning Lens.

ABSTRACT

White-Nose Syndrome (WNS) is an emerging disease affecting hibernating bat mortality and precipitous population declines in winter hibernacula. First discovered spreading rapidly across eastern North America and currently affects seven species, WNS is a regional population collapse and is predicted to lead to regional extinction of the little brown myotis (Myotis lucifugus), previously one of the most common bat species in North America. Novel diseases can have serious impacts on naïve wildlife populations, which in turn can have substantial impacts on ecosystem integrity.

REPORT

Emerging infectious diseases are increasingly recognized as direct and indirect agents of extinction of free-ranging wildlife (1–4). Introductions of disease into naïve wildlife populations have led to serious declines or local extinctions of different species in the past few decades, including amphibians from chytridiomycosis (5, 6), rabbits from myxomatosis in the United Kingdom (7), Tasmanian devils from infectious cancer (3), and birds in North America from West Nile virus (8). Here we demonstrate that White-Nose Syndrome (WNS), an emerging infectious disease, is causing unprecedented mortality among hibernating bats in eastern North America and has caused a population collapse that is threatening regional extinction of the little brown myotis (*Myotis lucifugus*), a once widespread and common bat species.

WNS is associated with a newly described psychrophilic fungus (*Geomyces destructans*) that grows on exposed tissues of hibernating bats, apparently causing premature arousals, aberrant behavior, and premature loss of critical fat reserves (9, 10) (Fig. 1). The origin of WNS and its putative pathogen, *G. destructans*, is uncertain (9). A plausible hypothesis for the origin of this disease in North America is introduction via human trade or travel from Europe, based on recent evidence that *G. destructans* has been observed on at least one hibernating bat species in Europe (11). Anthropogenic spread of invasive pathogens in wildlife and domestic animal populations, so-called pathogen pollution, poses substantial threats to biodiversity and ecosystem integrity and is of major concern in conservation efforts (1, 2).

A



B



LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

- GLOSSARY
- PREVIOUS WORK
- AUTHOR'S EXPERIMENTS
- CONCLUSIONS
- NEWS AND POLICY LINKS
- CONNECT TO LEARNING STANDARDS
- REFERENCES AND NOTES

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An example of the resource with the Glossary, Previous Work, Author's Experiments, News and Policy Links, and References and Notes tools turned on. The Glossary tool is in use.

Learning Notes:

Learning Notes accompany each figure and are designed to help students deconstruct the methods and data analysis contained within each figure.

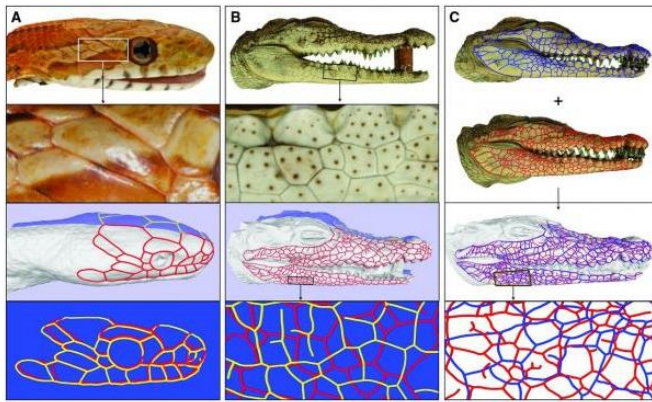


Fig. 1. Spatial distribution of head scales. (A) Head scales in most snakes (here, a corn snake) are polygons (two upper panels) with stereotyped spatial distribution (two lower panels): left (yellow) and right (red) scale edges overlap when reflected across the sagittal plane (blue). **(B)** Polygonal head scales in crocodiles have a largely random spatial distribution without symmetrical correspondence between left and right. **(C)** Head scales from different individuals have different distributions of scales' sizes and localizations (blue and red edges from top and bottom crocodiles, respectively).

Method: 3D geometry and color-texture reconstruction

Panel A

Panel B

Panel C

The authors took 120 color pictures of each animal to create detailed, three-dimensional models of reptile heads. Watch this video in which the authors further explain their modeling methods:

<http://www.sciencemag.org/content/suppl/2012/11/29/science.1226265.DC1/1...>

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- REFERENCES AND NOTES

References:

The Reference section of each resource is annotated with a short statement about how or why each reference relates to the current research study.

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LEARNING LENS

Click on a category below to display annotations. You can find more information by clicking the highlighted text to the left.

GLOSSARY

PREVIOUS WORK

AUTHOR'S EXPERIMENTS

CONCLUSIONS

NEWS AND POLICY LINKS

CONNECT TO LEARNING STANDARDS

REFERENCES AND NOTES

Learning Lens

This paper showed that while both physical activity, like running, and living in an enriched environment can result in the generation of new hippocampal neurons in mice, a combination of the two activities leads to even greater rates of neurogenesis.

flexible use of spatially precise

brain plasticity. *Front Neurosci* 4,

Thought Questions

Thought Questions are located above the Learning Lens in the right sidebar of each resource. These questions were written to be universal and applicable to any primary research paper. Thought questions do not have a single answer, or a correct answer for that matter, and can be used to stimulate discussion among students.

Science in the Classroom

A collection of annotated research papers and accompanying teaching materials

Audience High School **University** **Edit** TOPIC Biological

Lemmings: They're What's for Dinner



EDITOR'S INTRODUCTION
Cyclic Dynamics in a Simple Vertebrate Predator-Prey Community.
Gilg et al.

Scientific studies often involve more than one discipline. In this case of lemming population dynamics, scientists use both ecology-related methodology to collect data in Greenland, and mathematical equations to construct a predictive model. Similar to the cyclic dynamic described in this study, this interdisciplinary research would not have been complete if one of these two scientific disciplines had been missing.

annotated by Fanny Bernardon
original paper published 10/31/2003
annotations posted on 1/31/14

ABSTRACT
The collared lemming in the high-Arctic tundra in Greenland is preyed upon by four species of predators that show marked differences in the numbers of lemmings each consumes and in the dependence of their dynamics on lemming density. A predator-prey model based on the field-estimated predator responses robustly predicts 4-year periodicity in lemming dynamics, in agreement with long-term empirical data. There is no indication in the field that food or space limits lemming population growth, nor is there need in the model to consider those factors. The cyclic dynamics are driven by a 1-year delay in the numerical response of the stoat and stabilized by strongly density-dependent predation by the arctic fox, the snowy owl, and the long-tailed skua.

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THOUGHT QUESTIONS

1. Why is this study important?
2. What is the objective?
3. What are the conclusions?
4. What is the supporting evidence?
5. Are there any doubts that this conclusion is right?
6. What would you do next?

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Suggestions for Classroom Use:

In addition to the thought questions discussed above, other resources are provided for use in the classroom. These can be found toward the end of the teacher guides associated with each specific article and include:

1. Discussion questions specific to the article, related to the standards, and/or associated with the figures.
2. Activities tied to the articles.

Some ways to use the *Science in the Classroom* articles:

1. Assign to student groups to read and discuss during class.
2. Assign small sections of the article to student groups to read and discuss during class, with the expectation that they will present or use jigsaw to teach the entire class what is in their part of the article.
3. Assign to individual students to complete during class or as homework.
4. Assign reading as an extra credit project.

Some ideas for interactive student engagement after reading the article:

1. Students write answers to discussion questions (for example, those linked to the standards or those linked to the diagrams).
2. Go over the abstract, as well as information about the purpose and structure of an abstract, and have students write their own abstracts for the articles in language that could be understood by their peers.
3. Have students edit the article, or parts of the article, to a simpler reading level.
4. Have students, alone or in small groups, use the annotated list of references to explain how the scientists who wrote this article built on the published work of at least one independent group of scientists in making their discoveries. In the process, did they produce data that supports the findings of the earlier publication that they have cited in the text? In what way does this article support the statement that scientific knowledge is built up as a “community effort”?

5. Use the article and discussion questions linked to the standards and the diagrams for a teacher-led classroom discussion. The discussion can focus on the nature of science and scientific research, as well as on the science in the article itself.
6. Have students give a classroom presentation about the article, parts of the article, or their answers to discussion questions.

ARTICLE-SPECIFIC MATERIALS

Connections to the nature of science from the article

- Why do scientists want to develop methods that consume low quantities of material per reaction and enable faster screening of suitable reaction conditions?
- How can these targets be achieved?

The importance of this scientific research

- This research highlights a powerful miniaturized approach to overcome material limitations at the front end of drug discovery or natural product synthesis processes.

The actual science involved

- Nanoscale chemistry
- Drug discovery
- High-throughput evaluation of successful reactions and promising molecules

Connect to Learning Standards:

[The Next Generation Science Standards](#)

- Science and Engineering Practice 2: Developing and using models
- Science and Engineering Practice 4: Analyzing and interpreting data
- Science and Engineering Practice 6: Constructing explanations (for science) and designing solutions (for engineering)

[The AP Chemistry Standards](#)

- Essential knowledge 2.A.3: Solutions are homogenous mixtures in which the physical properties are dependent on the concentration of the solute and the strengths of all interactions among the particles of the solutes and solvent.
- Essential knowledge 3.A.2: Quantitative information can be derived from stoichiometric calculations that utilize the mole ratios from the balanced chemical equations. The role of stoichiometry in real-world applications is important to note, so that it does not seem to be simply an exercise done only by chemists.
- Essential knowledge 4.A.1: The rate of a reaction is influenced by the concentration or pressure of reactants, the phase of the reactants and products, and environmental factors such as temperature and solvent.

[JSTOR Research Basics](#)

This is an online self-study program that can be completed at one's own pace; it teaches research basics that are helpful for high school and beyond.

Summary of the Article for the Teacher:

It is recommended that this not be used by students in place of reading the article.

General Overview:

The problem that researchers are trying to address in this study is that of unavailability of large quantities of material at the front end of the drug discovery process. To overcome this limitation, the potential of high-throughput experimentation was explored and it was found that it can serve as the much needed tool for identification of promising reaction conditions, on microgram or nanomole scale, for synthesis of highly functionalized drug molecules. The reactions were carried out at room temperature using palladium as the catalyst and dimethyl sulfoxide (DMSO) as the solvent. This miniaturized and automated platform, coupled with mass spectrometry-based high-throughput analysis, resulted in a capability of running more than 1500 reactions in less than a day, using as little as 0.02 milligrams of material per reaction with product yields that were reproducible when the scale was increased by a factor of 1000 (0.02 to 25 mg).

Topics Covered:

- High-throughput experimentation
- Reaction chemistry in drug discovery

Methods used in the Research:

- Chromatography-based quantification techniques (MISER LC-MS)
- Nanoliter robotics
- Design of experiments for optimization of reaction conditions

Conclusions:

- Various ambient-temperature coupling conditions in DMSO solvent were identified and translated to large scale (50 mg), giving 68% to 98% product yield.
- Reaction performance (relative conversion) was found to be highly reproducible as the scale of reaction volume was increased from 1 μL to 25 μL .
- Out of the initial 32 failed reactions, this methodology, which first looked at catalyst-based combinations and then at reaction stoichiometries, resulted in 21 successful substrate combinations, out of which 16 combinations yielded a pure product after a 1000-fold scale up.

Areas of Further Study:

- Would this platform provide similar results while evaluating Pd-catalyzed reactions at room temperature in other solvent systems besides the model DMSO? Maybe NMP (N-methyl-2-pyrrolidone)?

Discussion Questions:

Discussion questions associated with the learning standards

Essential knowledge 2.A.3: Distinction between homogeneous and heterogeneous solutions and the separation principle of chromatography.

- The researchers suggest that engineering complexities can be overcome by conducting reactions at room temperature. What are some of these typical complexities that researchers face during laboratory experimentation? What kind of mixtures are scientists dealing with in this study and why is chromatography the ideal analytical technique to assay their reaction outcomes?

Essential knowledge 3.A.2: Role of mole concept and stoichiometry in real-world applications.

- Every chemical reaction utilizes specific moles of reactants and produces specific moles of products. What is the concept of limiting reactant? What concentration of substrates was used for each reaction in this study? Do you think the researchers had the stoichiometric knowledge of each of these reactions and could that be one of the reasons that so many reactions fail and do not produce the desired product?

Essential knowledge 4.A.1: Dependence of reaction rate on various variables.

- What are the different variables that affect the rate of a reaction? In this study, what was the optimum temperature and solvent system they used and why? What is the role of catalyst in reaction rate? What kind of catalysts did researchers use in this study and why?

Practice 2: Developing and Using Models

Practice 4: Analyzing and Interpreting Data

Practice 6: Constructing explanations (for science) and designing solutions (for engineering)

What is the importance of the statistical technique of design of experiments (DOE)? What were the main DOE conclusions in this study? What are some other fields of science in which this tool is highly useful? Did experimental results prove their hypothesis or, in other words, did their proposed solution (i.e., automated miniaturized platform) improve the material-limited front end of the drug discovery process? Evaluate certain merits and limitations of their proposed model, if any.

Discussion questions associated with the figures

Fig. 1:

- Panel A and B: What are electrophiles and nucleophiles?
- Panels C, D, and E: How were successful reactions screened in the laboratory? What are microvials? What are microtiter plates? What different plate configurations were used in the screening process? What is MISER LC-MS and what was it used for?
- Panel F: What are the typical variables under consideration in a reaction?
- Panel G: What does this graph represent? Reproducibility and strong correlation of results obtained at microtiter/small (1 μ L) and large/microvial scale (25 μ L).

Fig. 2:

- Panels A and B: What are heat maps and what do the colors blue, green, and yellow signify? What is the difference between UPLC and MISER, besides processing time?
- Panel C: What is a chromatogram and what information does it give regarding a particular reaction? Hint: product yield! What are the variables on the x-axis and y-axis?
- Panel D: How did relative conversion change with the charge on the nucleophile and base? What does the three factorial four-level response curve represent? In other words, what are the three factors and the four levels of each factor in this design? Hint: The electrophile (6) was not changed but the other three factors (nucleophile-10, base-29, and catalyst-42) were tested at four different levels each (such as four charge levels of nucleophile and base and four levels of catalyst loading; third factor is hidden in this curve) to optimize for highest conversion.