Part B Answer Key

(6) Clarity – Are the hypothesis and experiment(s) clearly stated?
* 2 points: Do you discuss how your hypothesis explains nitrogen fixation under slow growth?
* 2 points: Is your hypothesis clearly stated and easy to find?
* 2 points: Is your experiment clearly stated and easy to find?

(9) Logic – Do they make biological sense? Do the experiments directly address the hypothesis?
* 3 points: Does your experiment address your hypothesis.
* 2 points: Is it clear what your experiment tests?
* 1 points: Controls and techniques.
* 3 points: Does your hypothesis make biological sense and address the question?

(6) Depth of thought – Do the hypothesis & experiments demonstrate a non-trivial understanding of core principles taught in the class?
* 2 points each: discussion of niche, energy balance, and why the archaea fix nitrogen

(4) Completeness – Are multiple relevant ideas discussed?
* 4 points: did you go into depth in one hypothesis, or gloss over several possible ideas?

The biggest problem people faced in this assignment is that they didn't answer the question as we asked it! You are getting the exam 2 questions ahead of time – please make sure you understand what the question asks and that you answer this completely.

Some students had good hypotheses and experiments based on a wrong understanding of the paper. When you study in a group, please be confident that your interpretations of the papers and themes are correct and you aren't leading each other astray – taking a quick trip to a TA to double check that you understand the background of the question is a good way to prepare for Exam 2!

Possible ways someone might have approached the question (these are not complete answers, just ideas in need of your development and discussion):

- Discussion of energetics during slow growth: is it fundamentally different from fast growth? Are processes more efficient (you could imagine that less heat is lost for any given biochemical reaction, thus less ATP would be turned over per unit time)? If this were true, might there be differences in enzymes between these organisms and their faster-growing relatives? This is relevant to the question because it questions the assumption that N2-fixation is "costly". Related to this, it would be good to note the environmental conditions (high pressure, cold temperature).
- What does "costly" mean? Something can only be costly if you can't afford it (i.e. the cells wouldn't have enough ATP around to spend on N2-fixation)—but what if they have a lot of ATP around? How do they get their ATP? Perhaps

there is an unknown catabolic pathway at play unaccounted for by the investigators (beyond just AOM)? Could it be fermentation? Something else? Could you measure ATP/cell for the archaea in the AOM consortia? How does it compare to the steady-state amounts of ATP found in the sulfate reducers? Other archaea? …

- Even when something is costly, if there's no other way to get it and it's essential, you have to pay the cost! Although the question says fixed nitrogen is available in the environment, can you assume the archaea can get it? If they don't have the machinery to transport it in, or to do whatever they need to do with it inside the cell to make it biologically useful (e.g., they're lacking a key anabolic enzyme to incorporate the N into DNA or protein) it doesn't matter how much is around! Could you look bioinformatically at the archeal genome to see if they have the right genes for N-assimilation? What if they have the genes, but there's a mutation in one of them that makes it dysfunctional? Could you try to culture the consortia on other labeled N-sources and see if archaea take them up?
- What if there's something in the environment of the AOM consortia that reacts with fixed N in such a way that it is unavailable to the organism? Could you do a complete chemical characterization of N in the environment to figure out what is accessible to the organisms in situ?

Here are examples of essays that students turned in which we feel answered the questions. Note we did not grade them on beautiful prose but rather on biological content and answering the question **as it was posed** in the problem set.

Example 1

I hypothesize that despite the energetic cost for nitrogen fixation, the archaea continue to fix nitrogen because they are semi depended on the products of their sulfate-reducing bacterial symbionts which required fixed nitrogen. These products could be the hydrogen sulfides that are the result of the sulfur-reducing cycle. Several organisms are known to utilize sulfides as fuel as an electron donor. This hypothesis fits in with the experimental results where the archaea refused to fix nitrogen under inhibition of the sulfate reduction process in their accompanied bacteria. Since the bacteria were not producing the molecules the archaea needed, the archaea ceased producing fixed nitrogen. This type of refusal to perform a process under absence of a accompanied symbiont has been discussed in class with the legumes and their fixation bacteria. Only after a series of complex signal pathways is complete do the legumes grow nodules for the rhizobia.

This signal pathway could be present in the interaction between the bacteria and archaea studied in this paper. The bacteria and archaea could only form there symbiont relationship after the bacteria and archaea exchange signals in the form of increased concentrations of the products of sulfur reduction and methane oxidation processes.

In this deep sea environment, the archaea are able to fix nitrogen in order to provide it for its symbiont. It fulfills this niche because it receives by-products from the metabolic processes that utilize its fixated nitrogen. A possible product it could use is the sulfide discussed above which then gets converted back into sulfur. This is then utilized once more by the bacteria completing the energy cycle utilization of the sulfur in this deep sea environment.

The archaea sacrifice energy in order to fix nitrogen in this fashion because the products it relies on from the bacteria are more efficient and less complex to metabolize. Because of this possible reduction in complexity to metabolize, the archaea would prefer to utilize it over other fuels even though it slows their growth. This reduction in complexity could lead to cellular energy being spent on other biological processes such as storing energy for future fuel lacking periods.

An experiment that could test this hypothesis could be done as follows. By simply introducing the by-products of the sulfur-reduction process into the environment of the these archaea and utilizing the same test as the paper's authors to determine the rate of nitrogen fixation utilizing nitrogen isotopes. This would use the same N 15 and N 14 labeled in conjunction with the fluorescence in situ hybridization(FISH) and nanometer secondary ion mass spectrometry(nanoSIMS) A control could be used and compared to by simply not adding the by-products of the sulfur-reduction process. By changing the exact products introduced into the environment, one can determine if it is infact a product that the archaea utilize as a signal to begin nitrogen fixation. This would be true if the archaea begin to fix nitrogen even in the lack of bacteria simply with the products of the process added into their environment.

Example 2

It is possible that the archaea resort to fixing nitrogen because, despite the presence of reduced nitrogen, something in their environment keeps them from being able to use it. I hypothesize that these consortia live close to another (unknown) microorganism that, as a mechanism for ensuring the availability of reduced nitrogen for itself, exudes a chemical compound that actively blocks the NH_3 receptors of other nearby organisms. Thus, as long as this amensal was present in the environment, the consortia would not consume reduced nitrogen even if it was plentiful, so the archaea were pressured into evolving a metabolism that does not rely on NH_3.

This hypothesis is admittedly far-fetched, as it relies in a mechanism that has not been observed so far. Because of that, in order to test the hypothesis experimentally, it is necessary to include some additional assumptions. First, we can assume that any organism that evolved such a nitrogen denial mechanism is, itself, a big reduced nitrogen consumer. After all, if it were able to subsist on smaller quantities of nitrogen, it would not have had the need to hinder other species' nitrogen consumption. We also assume that the hypothetic amensal's evolutionary strategy was quite successful, since it exerted enough selective pressure on ANME-2/DSS consortia for it to focus on the energetically taxing process of nitrogen fixation; thus, the amensal should not be rare in its environment.

The first step of the experiment would be to use a deep sea probe to collect samples from sites known to harbor ANME-2/DSS consortia; from those samples, a list of the most abundant species would be obtained. Each of these species would be separately placed in an NH_3 saturated aqueous culture that otherwise mimics the deep sea water composition as closely as possible; the NH_3 should be made of ^{15}N in order to track its consumption. Some common NH_3 consuming archaea would be inserted into each of these cultures, and its growth would be measured in each culture by tracking its radioactive ^{15}N intake. It is expected that, in some cultures, the archaea will not grow as much as in others, due to the competitiveness of some of the abundant species.

NH_3 saturation should prevent the archaeal population from stagnating altogether in all such cultures if our hypothesis is incorrect. However, if in one culture the archaeal ^{15}N intake is practically zero, we will have found in it the organism that not only can out-compete it, but actually prevent it from consuming NH_3. If such an organism is found, then a possible follow-up experiment would be to sequence its genetic code and see what proteins it is capable of producing; it is likely that one or more of them would have a blocking effect on the NH_3 receptor proteins of archaea.

Example 3

Dekas et. al. observed that ANME-2/DSS consortia fix nitrogen through anaerobic oxidation of nitrogen coupled with sulfate reduction to produce energy. However, this metabolism is one of the least energetically favorable known, requiring 16 moles of ATP per mole of nitrogen to yield 40 kJ/mole of methane to be shared throughout the consortium. Many groups have questioned if the ubiquitous ANME-2/DSS relies on such an energetically poor metabolism. I hypothesize that this metabolism is more energetically favorable at the extreme pressures at the bottom of the ocean due to structural alterations of the fixation mechanism.
The intense pressure associated with the cold seeps inhabited by the consortia could radically change its structure. Sequencing of the *nif* genes revealed that the nitrogen fixation mechanism used by ANME-2/DSS differs significantly from other characterized N_2-fixing systems, suggesting some environmental adaptations of nitrogen fixation.

Under the hypothesized mechanism, AMNE-2/DSS consortia would either require fewer than 16 ATP molecules for each molecule of N_2 or produce more energy for the same amount of ATP. In either case, more energy per ATP molecule would be produced by the consortia. Previous experiments have demonstrated that the organisms compensate for the energetic burden associated with nitrogen fixation by slowing growth. The hypothesized modification would therefore predict increased growth by the consortia since more energy would be available. The DSS bacteria, which reduce sulfate in the metabolism, would increase reduction to compensate for the increased energy dedicated to growth.

Since diazotrophy has never been observed in deep-sea methane seeps, measurements of growth or metabolism have never been recorded for the consortia in their natural habitats. Considering the difference in *nif* genes and the discrepancy of the oceanic nitrogen fixation, it is conceivable that AMNE-2/DSS consortia have different metabolic rates than those observed under laboratory conditions. To test this idea, consortia of AMNE-2/DSS would be placed inside vessels under pressure comparable to that present at the depth of cold seeps. The environment for the consortia would be anaerobic and ^{15}N would be distributed in $^{15}N_2$, $^{15}NO_3^-$, $C^{15}N^-$, and $^{15}NH_4^+$, matching the procedure used by Dekas et. al. and varying only the pressure. The depletion of these nitrogen forms would be monitored and compared to the results of Dekas et. al. If the hypothesis is correct, at least some forms of nitrogen should be depleted more quickly. In addition to differing rates of nitrogen consumption, growth of the consortia would be measured. Assuming that ^{15}N is a major proxy for growth, the consortia should grow more quickly.

If growth is observed, the consortia growth should slow under laboratory conditions similar to those described by Dekas et. al. If the AMNE-2/DDS growth slows significantly, we could deduce that the increase in growth is a direct result of the analogue for the deep-sea conditions. This experiment would test only the effect of pressure independent of other factors that may be present at the cold seeps but not in the laboratory. Its test of pressure isolated from other factors, though, would be conclusive.

Finally…

Please note that these essays were on the longer side. There were some great essays that included everything and were much shorter. There was a lot of rambling in these essays; remember that we already know what the question is asking. You do not need to restate it☺.