Comments on Manuscript of Mitts and Reynolds

1. The title "influence of eutrophication" is an extrapolation of results, which may or may not be justified. In this paper, the authors test two levels of dissolved oxygen and two levels of algae. Both of these can be components of eutrophication, but they are not 'eutrophication'. A more accurate title would be "Effects of dissolved oxygen and algae concentrations on reproductive success of the bitterling in freshwater mussels". A definition of eutrophication or reference to a standard definition would be helpful.
2. Water temperature is a key factor in mussel physiology (feeding rate, ventilation rate, etc.) yet the only reference to water temperature is at the bottom of p. 9 (16-22°C). What temperature was used in the experiments?
3. It would be helpful if the authors translated their chlorophyll a values to #cells/ml. Feeding experiments with mussels typically use a standard cells/ml.
4. Perhaps as important as the effects of "eutrophication" on quantity of algae (chlorophyll a) is the effect on quality (species composition) of algae for mussel nutrition. Typically, algal diversity declines as eutrophication increases. There is a trend toward more green and blue-green algae with eutrophication and away from the more nutritious diatoms, dinoflagellates, and chrysophytes. Polyunsaturated fatty acids (PUFAS) are important for mussels; green algae have less and blue-greens have little or no PUFAs or sterols when compared to the preferred mix of algae.
5. The cell walls of Chlorella vulgaris are thick and cellulosic, difficult to digest for many mollusks. The internal protoplasm is nutritious, but the cells are typically ball-milled or otherwise processed to fracture the wall for human use, for example. Thus, the use of this species in the experiment is not a 'best choice'. The recorded ventilation rate may have had nothing to do with quantity of algae but rather a behavioral response to the poor quality of the alga. How does one partition the effects of oxygen level, algae abundance, and algae unsuitability in these experiments? Mussels often reduce filtration rates or close their valves if poor quality algae are available. Did the authors confirm digestion of the algae, examine pseudofeces for ejected undigested cells, or use some other means to demonstrate that mussels were assimilating this thick-walled species?
6. The 'low oxygen levels', 55-75% saturation, may not have been low enough to generate a response by A. anatina. A recent paper by Chen et al. (2001) in Hydrobiologia comments on oxygen consumption of a related species (Pyganodon grandis) at two temperatures. The authors need to address this paper in their Discussion. The methods for oxygen conditions describes DO levels in terms of % air saturation, yet no water temperature data are provided. I see no water temperature mentioned in either of the main experiments. They need to present % air saturation in terms of mg/L O_2.
7. All 3 experiments show that of the two variables, the data are aggregated more by algae level than O_2 level. The 'high algae' variable is always on the left,

and the 'low algae' variable is always on the right in the Figures. Yet, the authors essentially ignore this in their narrative.

Figure 1- Why were more eggs ejected in the high O_2/low algae than the low O_2/high algae? This doesn't make sense if O_2 level is the presumed stressor causing ejection. According to page 10, "eggs were ejected significantly more quickly from mussels in the high algae treatments, than those in the low algae treatments". Figure 1 shows just the opposite; more eggs were ejected in both low algae treatments.

Figure 2- Why would ventilation rate be highest in the treatment with highest O_2? This is the opposite of what would be expected if O_2 were the important variable. High algae trials had the higher ventilation rates than the low algae trials. Why would the 'low O_2/low algae' have the lowest ventilation rate? Mussels typically increase their ventilation rate to compensate for low O_2 in the water column. Results in Figure 2 do not agree with expected outcomes, if O_2 is the most important variable.

Figure 3- Again, there seems to be a response to algae level and not O_2. Mussels are open more because of greater algae abundance (feeding response?) How would the authors refute my claim that algae level (low versus high) had a greater effect on the mussels than O_2 level?

8. I could make a case that the responses in all three Figures can be explained by algae alone and its effect on mussel behavior. If eutrophication results in speciose algal communities becoming a uni-algal soup of C. vulgaris, then the authors have made their case strictly on this factor. If not, then the conditions resulting from eutrophication have not been shown to explain the results of their experiments, and subsequent linkage to the reproductive success of bitterlings.

The authors need to re-assess their data and convince phycologists and malacologists that they have logically explained their results relative to expected paradigms (quantity versus quality of algae, mussel respiratory and feeding behavior) and a cause-effect relationship in their experimental design.