

12/88

DIRECT AND INDIRECT EFFECTS OF WINTERKILL ON FRESHWATER GASTROPODA

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* = revisions indicated on 1st draft, but not responded to

ABSTRACT

In the two experiments of this study, the effects of winterkill on four species of snails were tested. These laboratory experiments employed multiple replicates, while controls were used to isolate differential effects of hypoxia and low temperature. Destructive sampling was used to monitor differential mortality between species, and the rate of DO decline. The organisms' own respiration was the means of lowering the oxygen level in these tests. In the first experiment, conditions of winterkill were mimicked by exposing the organisms to low temperature, hypoxia, and darkness. The data did not indicate threshold levels of oxygen tolerance. Further, the effect of hypoxia on mortality proved to be minimal. In the species studied, resistance to winterkill conditions proved to be greatest in *Lymnaea emarginata*, followed by *Amnicola* sp., *Helisoma campanulata*, and *Gyraulus* sp. The second experiment subjected two populations of *Amnicola* (one from a winterkill lake, and one from a non-winterkill lake) to summerkill conditions in an attempt to demonstrate the selection of stress-resistant traits in a population exposed to a severe environment. The results from this test indicated that hypoxia actually increases survival rate, and that individuals from a winterkill lake are actually more poorly adapted to conditions of stress than are those from a more environmentally stable lake.

INTRODUCTION

In many north temperate, dimictic, lakes of the United States, the arrival of winter is

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accompanied by the freezing of the water's surface. As a result, atmospheric oxygen no longer diffuses over the air-water interface. Further, as snow accumulates on the ice, less sunlight penetrates, and photosynthesis of autotrophic algae declines precipitously. Thus, the fauna of these lakes have access to a finite amount of oxygen while the lake is so covered. In shallow or eutrophic lakes, this oxygen may be quickly used by organisms (Hargrave 1969), thus yielding hypoxic, and in some cases anoxic, conditions (Nagell and Brittain 1977). Oxidation of the sediments and ice formation itself also contribute to this state of hypoxia by consuming dissolved oxygen (Brewer et al. 1977). These severe conditions of low temperature and hypoxia often result in increased rates of mortality in certain of the lakes' species. Death due to such causes is known as winterkill. Previous studies have demonstrated winterkill in fish (Casselman and Harvey 1975) and in crayfish and snails (Funk 1988).

Another ecological phenomenon, known as summerkill, occurs in the same type of lakes that exhibit winterkill. In late summer, the rate of decomposition in these lakes may exceed that of oxygen production by autotrophs. This results in hypoxic conditions which may lead to increased mortality among the lakes' species.

Experiments: This study sought to determine some of the specific effects of winterkill and summerkill conditions on organisms which typically inhabit the lakes in which these phenomena occur. Various pulmonate and prosobranch snail species constitute a large proportion of the animal biomass of these lakes, and previous studies of winterkill have involved these organisms (Funk 1988). Because of the ecological importance of the gastropods, and in an attempt to test the results of such previous experiments, these were the organisms chosen for study.

The various families in the order Pulmonata exhibit varying degrees of aquatic adaption, while the pulmonates as a whole display differing adaptive plasticity and physiological adaptations to aquatic living than do the prosobranchs. Thus, for the first experiment, several different taxa were selected for study in the hopes of

reference?

+Tommy May '82
Rahel '84

demonstrating the differential resistance to winterkill conditions resulting from such adaptations. The ecological ramifications of winterkill in these species are many and far reaching. As various snail species comprise such a significant percentage of the animal biomass in these lakes, they represent an integral component of the trophic interactions of such communities. More specifically, because snails are grazers of periphyton, and the principal food source of many crayfish (two more crucial components of the lake ecosystem), it is obvious that marked ^{as?} ~~fluctuation~~ ^vascillation of their population density as a result of winterkill can have strong implications on the community structure of the lake. Given data on the DO, temperature levels, and species composition of a given lake during the winter, quantitative knowledge of the differential effects of temperature and of hypoxia on the various denizens of such a lake would facilitate the prediction of the specific ecological effects of winterkill on that lake in the future. The acquisition of such knowledge, obtained by mimicking the conditions of a winterkill lake, was the goal of the first test of this study.

You're a little too confident here. We know none of these things.

Specifically, the ultimate goals of the first experiment were as follows: 1) to determine the existence of possible threshold DO levels at which mortality significantly increases; 2) to determine the differential effects of hypoxia versus temperature on snail mortality through the use of controls; and 3) to determine the differential resistance of various snail species to winterkill conditions.

why focus on thresholds?

The second experiment used two populations of one species of snail, *Amnicola* sp., to study the evolutionary implications of winterkill conditions. By determining the ability of populations of *Amnicola* from both winterkill and non-winterkill lakes to endure environmental stress, it was hoped that the natural selection of environmental tolerance might be demonstrated. By subjecting the snails to conditions of summerkill, the ability of these organisms to withstand environmental stress was tested.

Summerkill can similarly be expected to have significant impact on the community structure of lakes so affected, and thus is also a phenomenon worthy of attention.

Let's go further, '85

METHODS AND MATERIALS

Experiment A: This test of winterkill conditions involved three pulmonate and one prosobranch species of gastropod: *Gyraulus* (sp.), *Helisoma campanulata*, *Lymnaea* *immarginata*, and *Amnicola* (sp.) respectively. Prior to setting up the first experiment, these four species were collected over a period of several days from various lakes in northern Wisconsin (Table 1). All the individuals of a given species were collected from a single lake, and each species was maintained in a separate, aerated and filtered, ten gallon aquarium until the first experiment was initiated. Macrophytes and periphyton covered rocks were provided as a food source for the organisms.

Five individuals of each of the four species were placed in each of sixty, one-quarter jars. These were divided into six time intervals, each consisting of five experimental and five control replicates. Thirty of each species were measured using vernier calipers (Appendix 1). The jars were then completely filled with 23°C water containing 6.0 ppm DO, and the snails were added to these jars. The experimental replicates were then sealed with the jar lids, while the control replicates were covered with pieces of fiberglass mesh held on with rubber bands to allow oxygen to dissolve over the air/water interface. Using this method, the organisms themselves lowered the DO levels in the experimental replicates, while individuals in the controls had access to a constant oxygen supply. The jars were then placed in a 14°C refrigerator. The bulb was removed from the refrigerator so the organisms would not be exposed to light when the refrigerator was opened to remove the intervals. This was done because the organisms would have no contact with light under the natural conditions which we sought to mimic. Over the next five days, the temperature was gradually lowered to 4°C (Table 2), the temperature of water in the benthic zone of a lake in the winter. To accomplish this, the thermostat in the refrigerator was changed nightly using only

parvus + deflectus?

Imosa + ?

Would have been better to mix sp so that all had same conditions in captivity.

water source?

True?

indirect light from a flashlight so as not to elicit any zeitgeber responses in the organisms. This method was also used during removal of the six intervals of replicates. During the acclimation of the organisms, only the control replicates had access to atmospheric oxygen. The experimental replicates were also to be exposed to the air to eliminate the possible confounding factors that might arise if the organisms were subjected to increasingly hypoxic conditions during temperature acclimation. For in a lake, hypoxia would not occur until after the water had reached its minimum temperature. However, the potential confounding factors associated with removing replicates into the warmth of the laboratory in order to seal the experimental jars were deemed to be more significant. Intervals were removed irregularly, at times believed to yield the most revealing data concerning the mortality rates of the organisms. As destructive sampling was used, the organisms were not returned to the experiment following the removal of an interval. Certain intervals included replicates which were partially frozen due to a malfunction of the refrigerator. Upon removal, the lids and mesh were removed from the replicates, and temperature and DO readings for each replicate were immediately taken using an oxygen probe. The organisms from each replicate were then removed from their jars and placed in individual dishes containing oxygenated, room temperature water in an attempt to revive them. After a revival period, mortality was recorded for each species in each replicate.

based on previous interval's results,

right? Did you include these results in analysis?

Just letting them gradual warm to room temp would prob be better.

Experiment B: For this experiment, *Amnicola* were collected from both highly eutrophic Kickapoo Lake, which was believed to winterkill, and the larger Tenderfoot Lake which was not believed to do so. Snails from the two lakes were maintained for one day in separate aquaria, equipped as were those in experiment A. Thirty individuals from each of the two populations were measured with vernier callipers (Appendix 2.), and 300 from each tank were counted out to be used in the test. This experiment was structured as the previous one had been, using six time intervals of

five experimental and five control replicates removed at irregular intervals. Each replicate again consisted of five individuals from each of the snail populations held in a one quart jar. To separate Tenderfoot snails from Kickapoo individuals, the individuals from each of the two populations were marked with a different color permanent marker prior to the test. After marking, the snails were added to the quart jars which were once again either covered with wire mesh, or sealed with the lids of the jars. However, in order to ensure that no air was inadvertently sealed into the jars, the lids were screwed on while the jars were held under water, a procedure not employed in experiment A. Jars were maintained at room temperature (19 C) for the duration of the experiment, as the data from the previous test indicated that the quick acclimation (Table 2) of snails to winter temperatures in the middle of the summer may have been a significant confounding factor. The organisms were similarly exposed to the natural photoperiod which they had been exposed to in their native habitat before collection. As previously, when jars from a given time interval were removed, the DO was determined for each replicate using the oxygen probe, and mortality was recorded after allowing the individuals to revive in aerated water.

describe in detail - e.g., trade name

RESULTS

Experiment A: Mean levels of dissolved oxygen dropped sharply in the experimental replicates during the five day acclimation period (Fig. 1). After this period, the DO levels decreased very slowly, but never reached zero. such a trend can be explained by the fact that snails consume less oxygen at low temperatures (Buckingham and Freed 1976). The DO levels in the controls rose briefly, then also slowly decreased. An average of three ppm DO was still present in the control replicates at the conclusion of the test (Fig.1).

* = DISCUSSION

In both the experimental and the control replicates, mortality of all species was

In a paper, you should not provide more information than you use, & you should proceed in a logical way through the information you use. Do not, for example, present the same information in a table & a figure. Do not go back & forth between 2 figs or tables.

approximately proportional to time (Figs. 2 & 3). In both the control and the experimental replicates, *Gyraulus* sp. exhibited the highest mortality rate, while the patterns of mortality in the three remaining species were very similar to one another in both the control and the experimental replicates. By examining the total number of individuals surviving in all intervals combined, however, it appeared that *Lymnaea* fared best under these conditions followed by *Amnicola*, *Helisoma*, and *Gyraulus* respectively (Tab. 3c).

DO NOT ABBREVIATE 'Table' X

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When the rates of mortality in the experimental replicates were directly compared to those in the controls, there appeared to be very little difference between the two. The overall mortality rate was, however, slightly higher in the experimental replicates (Fig. 4).

Of the four snails in this test, *Helisoma* appears to be the one most adversely affected specifically by hypoxia, as the ratio of total experimental survivors compared to total control survivorship was the lowest for this species (Table 3f). Using this ratio as an indication of relative resistance to hypoxia, the second most susceptible species appeared to be *Lymnaea*, followed by *Amnicola* and *Gyraulus* respectively.

Experiment B: In the second experiment of the study, oxygen levels in the controls remained high and relatively constant for the duration of the experiment (Fig. 5). The DO levels in the experimental replicates, however, displayed a parabolic approach to zero.

In both the hypoxic, and the fully oxygenated replicates, the survival rate of the *Amnicola* collected from Tenderfoot Lake was higher than was that of the Kickapoo *Amnicola* (Fig. 9).

for 4 out of 6 intervals.

The mortality rate of both snail populations proved to be markedly lower in the experimental replicates than in the controls (Fig. 8).

In both the experimental and the control replicates, the patterns of mortality were extremely inconsistent between intervals, with a much higher mortality rate exhibited

what is this?

Confusing & disorganized

Figs shall be numbered in the order to which you refer them.

by the second interval than by the fourth.

DISCUSSION

* Both experiments tried to avoid pseudoreplication through the use of multiples of identical replicates. They further attempted to anticipate and counter confounding factors when possible. These attempts were more successful than in previous tests (Funk 1988) in several important respects: 1) the use of controls allowed the examination of the differential effects of hypoxia and low temperature on mortality; 2) lower DO levels, more closely approximating anoxic conditions, were attained; 3) the use of destructive sampling allowed for more accurate determination of death in individuals; 4) far more replicates were used, thus yielding data far more statistically significant. Thus, the data from these tests were probably more precise, more accurate, more strongly supported, and more illuminating than were those of the previous study.

are
replicates
identical by
definition?

How do you
know? You
haven't applied
any stats
yet.

The introduction of new methods, however, also resulted in further unforeseen difficulties (see discussion of individual experiments below). Such methodological problems may have artificially influenced results to a significant extent. The greatest confounding factor, however, was probably that of starvation. This factor was also believed to have confounded the data of previous tests (Funk 1988). In both experiments, the organisms were fed before the test, but provided with no food for its duration. However, over-wintering snails presumably *do* have ample detritus to feed upon under natural conditions. Although the metabolism of benthic organisms slows considerably in the winter, various studies have connected starvation with oxygen consumption in both prosobranch snails (Studier and Pace 1978) (using *Viviparus contectoides*), and in pulmonates (Callow 1974) (using *Planorbis contrortus*). Thus, lack of food may have artificially increased mortality rates in the tests by increasing the need for oxygen among the organisms, as well as by simple deprivation of nutrition.

What was
the connection

How can
data
strongly
supported?

The reason that food was not provided was to avoid the accumulation of toxic metabolites from decaying food and the organisms' waste products as it was believed that these might act as a confounding factor by artificially increasing mortality. Indeed, in an experiment involving erpobdellid leeches (Davies et al 1987), high metabolite concentrations *were* found to contribute to death. As dead organisms were not able to ~~be~~ removed during our tests, however, their decomposition may have raised the metabolite concentration of the water to unnatural levels in spite of all efforts to the contrary.

Experiment A: Three problems were specific to the first test: 1) Because the jars were sealed by filling ~~the jars~~ to overflowing and then attaching the lids, it is feasible that air bubbles may have been trapped in the jars, resulting in a heterogenous oxygen distribution which may have been differentially accessed by the various species in the test. (E.g., the pulmonates, which are capable of ~~breathing and~~ storing atmospheric oxygen, might have taken advantage of these pockets while the prosobranchs could not.). 2) As the experimental replicates were acclimated under conditions of limited oxygen, they were subjected to the additional stress of hypoxia during their period of adjustment to lowered temperature, while the control replicates were not (Fig. 1). This could have resulted in artificially heightened mortality throughout the experiment from the effects of this additional stress. 3) The malfunction of the refrigerator resulted in the partial freezing of some replicates in four of the intervals. Such freezing resulted in greatly lowered DO in certain control replicates, and may have resulted in higher mortality rates in both experimental and control replicates. As the extent of this freezing varied extensively among replicates and between intervals, there is no way to adequately interpret the effect that this had upon the data attained. The above-mentioned problems detract from an otherwise well-conceived experiment, and limit the ~~level of~~ significance ~~which can be assigned to~~ the data obtained.

Below, I

~~To determine what this experiment reveals, the data must be examined as it~~
~~pertains to the three issues which this test sought to explore:~~
 experiment

1) Threshold DO levels: In the experimental replicates of the first test, the DO levels dropped sharply during the period of acclimation and decreased much more slowly thereafter (Fig.1). However, mortality rates in all species (with the possible exception of *Gyraulus*) declined at an approximately linear rate both during and after the acclimation period in these replicates (Fig.2). These trends certainly do not support the hypothesis that the species under consideration can only survive in water of oxygen concentrations above a distinct level. The fact that mortality in the control replicates exhibits a similar trend (Fig.3) supports the idea that no threshold DO levels exist for these species. Previous tests also support this conclusion (Funk 1988).

2) Differential effects of hypoxia and low temperature on mortality: By ~~utilizing~~
~~control replicates (in which the organisms were subjected to low temperatures, but~~
~~were exposed to oxygen), and experimental replicates (which were exposed to both~~
~~low temperature and hypoxia), it was possible to isolate the effects of these two factors.~~
 temp & hypoxia
 2 3
 Figures ~~two and three~~ suggest that there was very little difference between the rates of mortality exhibited by the control and experimental replicates. ~~Indeed,~~ although more deaths did occur in the experimental jars, the difference between the two was small (Table 3d). If hypoxia was ~~indeed~~ a significant factor in the mortality of the organisms, a high ratio of death in the experimental replicates to that in the controls would be expected. This was not the case, however (Fig.4). The results of this experiment strongly suggest that hypoxia is of minimal influence on mortality in the species studied. As significant mortality did occur, however, it would appear that this must be attributed primarily to the effects of sustained exposure to low temperature. ~~Yet,~~ ^{or starvation} as truly anoxic conditions were never reached, the results obtained are consistent with the possibility that low DO could be a major factor in winterkill lakes in which DO levels consistently lower than those attained in this test are the norm.

The pattern of resistance to hypoxia (see results) is curious as Planorbid snails such as *Helisoma* possess hemoglobin in their blood, and thus are able to utilize oxygen very efficiently (McMahon, 1983). The prosobranch snails are generally more strictly aquatic than the pulmonates, and possess gills which allow them to extract dissolved oxygen from the water (Aldridge 1983). It is thus not surprising that *Amnicola* was relatively unaffected by the lowered oxygen levels. *Gyraulus* appeared to be the least affected by hypoxia in this test, but as very few survived in any intervals in either the experimental or the control replicates (Table 3c), and as the vast majority of them died before oxygen levels became greatly lowered (Fig. 1, 2, &3) the significance of this is questionable.

3) Differential resistance to winterkill conditions among the species studied:

Assuming the differential effects of confounding factors to be equal between species, *Gyraulus* clearly appears to be the least resistant species to winterkill conditions. The other three species demonstrated patterns of mortality very similar to one another. The total number of surviving individuals, however, indicates that *Lymnaea emmarginata* was the most resistant to winterkill, followed by *Amnicola* sp., *Helisoma campanulata*, and *Gyraulus* sp., respectively. In light of earlier studies of winterkill, these results were rather surprising. In a previous test, *Helisoma campanulata* was found to be significantly more resistant to winterkill conditions than was the species of lymnaeid snail used in that test, which in turn was more resistant than *Amnicola* (Funk 1988). As pulmonate snails are generally better adapted to fluctuations in temperature and DO levels than are the strictly aquatic prosobranchs, and since the planorbid pulmonates are better adapted to aquatic living than are lymnaeids (McMahon 1983), the results of those tests were as expected, while those of this are questionable.

The various problems and confounding factors, especially starvation, which influenced the results of this test ensure that any conclusions derived from them are of limited certainty. The consistently low ratio of experimental to control mortality was,



however, intriguing. Despite the difficulties encountered in this test, the apparently small influence of hypoxia on mortality is of great interest, and if accurate, of great significance.

Experiment B: The primary purpose of the second experiment was to determine whether exposure to winterkill conditions has indirect as well as direct effects upon snail populations. The *direct* effects of winterkill on the mortality of *individuals* was tested in experiment A. Experiment B, then, sought to demonstrate that winterkill *indirectly* affects *populations* through the natural selection of individuals which are more resistant to these conditions. The hypothesis was made that a population of resistant individuals would result from such selection, and that these individuals would also be more resistant to environmental stress *in general*. This hypothesis was then tested by subjecting these individuals to summerkill conditions. This test was conducted with the assumption that Kickapoo (a small, shallow, eutrophic lake) was a winterkill lake, while Tenderfoot (a comparatively large, deep, oligotrophic lake) did not exhibit this phenomenon. I hypothesized that snails from Kickapoo (in this case *Amnicola* sp.) would thus be more resistant to severe stress than would snails of the same species from a lake with relatively constant environmental conditions.

DO consumption in both the control and the experimental replicates exhibited expected patterns (Fig.5). The DO levels in the controls remained constant while those in the experimental replicates declined parabolically in accord with the fact that increased snail mortality would result in fewer individuals to consume the oxygen. [This pattern does not take into account the affect of bacterial decomposition on DO levels, but as DO levels of 0.0 ppm were not attained in either of the two tests in this study, or in a previous study (Funk 1988), it appears that the ability of decomposition to consume significant amounts of oxygen is minimal.] The rate of oxygen consumption and of mortality was considerably higher in this experiment than in the first test because temperature correlates positively with metabolic rate. The confounding effects

of starvation may also have increased in this test, however, as no food was provided in the experiment to accommodate the increased metabolic needs of the snails.

The remainder of the results, however, were completely unexpected. The most striking trend was the seeming discrepancy of the mortality rates exhibited between intervals. As stated above, the DO levels in the experimental replicates consistently dropped over the duration of the experiment. However, the number of individuals surviving actually increased significantly in the second, third, and fourth intervals, after dropping to below 10% survivorship in the first interval (Fig.6). Further, this pattern was followed by the individuals in the control replicates as well (Fig. 7). Several possible explanations for these trends exist, but none are very satisfactory. Jar effects do not seem to be a satisfactory answer, as the jars were randomized before the organisms were added at the start of the experiment. Simple chance could have played a role, but this is unlikely given the relatively large number of individuals (600) used in this test. Another possibility is that the quality of water used to fill the replicates and revive the snails may not have been consistent. The running water at U.N.D.E.R.C. contains high levels of dissolved minerals, and the concentration of these minerals varies significantly as evidenced by the ever-changing tint of the tap water. It is thus possible that the water used to fill the replicates of certain intervals, or to revive the organisms of these same intervals contained mineral levels lethal to the snails. Factors such as this could have resulted in the high mortality exhibited by the first two intervals of this experiment.

Perhaps even more striking was the fact that mortality rates in the control replicates were actually higher than those in the experimental jars (Fig. 8). Again, no rational explanation for this trend could be deduced. Jar effects are an improbable cause for the reasons given above. As the same water was used to fill both experimental and control replicates, and to revive experimental and control organisms, varying water quality is similarly an unlikely explanation. One possibility is that *Amnicola* are

capable of depending upon anaerobic respiration when DO declines to a given level, and that this ability allowed the anaerobic individuals to survive better than the aerobically respiring controls. This explanation, too, seems highly implausible.

Finally, the data indicate that the Tenderfoot *Amnicola* were actually more resistant to the experimental conditions than were the Kickapoo snails (Fig. 9). These results directly contradict the proposed hypothesis. As Kickapoo Lake *does* have an inlet providing an external water source, it is possible that the oxygen in this water is sufficient to prevent conditions of hypoxia from arising in the winter. If this is indeed the case, the assumption made at the beginning of the experiment that Kickapoo exhibits winterkill was false. The data attained could still be consistent with the notion that Kickapoo is a winterkill lake, however. Any lake provides heterogenous environments, and these various habitats may be differentially exposed to winter kill conditions (Baird et al 1987). For example, the *Amnicola* from Kickapoo Lake were collected from the underside of the lily pad *Nuphar*. These leaves float in the upper stratum of the water, far from the sediments where decomposition rapidly consumes oxygen. The *Amnicola* from Tenderfoot, however, were collected from the sediment, among very small, short macrophytes. Upon the arrival of winter, the Kickapoo snails, living in the most oxygenated portion of the lake, might not be subjected to hypoxia for some time, while the sediment-dwelling Tenderfoot snails would be be exposed to low oxygen much earlier. Thus, although the main water mass of Tenderfoot might never reach a significant state of hypoxia, the particular habitat sampled for this test might contain a population of snails relatively resistant to environmental stresses such as low oxygen.

Overall, the results from this experiment appear quite questionable, and certainly unsatisfactory. It is possible that the mineral content of the water resulted in the unusual patterns of mortality, and that the heterogeneity of lake habitats accounts for the hardiness of Tenderfoot *Amnicola*. However, even if this is the case, the high mortality of the controls cannot be accounted for, and may indicate that the data

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attained in this experiment as a whole is unreliable. Further, the effects of starvation, heightened by the high temperature at which the test was conducted, cannot be ignored.

General Discussion: The first experiment was marred by a malfunctioning refrigerator, unequal acclimation, and pockets of air which may have resulted in the unexpected patterns of winterkill resistance between species that were attained (Table 3c). Due to unknown causes, the second test yielded unexpected mortality trends and experimental/ control mortality ratios the reverse of what were expected. More importantly, the effects of starvation were unaccounted for, and possibly very significant in both tests. However, while both experiments were fraught with potential confounding factors and mysterious trends, one pattern was consistently exhibited: namely the apparently small influence of hypoxic conditions on the mortality of the organisms studied. This pattern was demonstrated both in the apparent lack of threshold DO levels, and in the low ratio of experimental to control mortality. It is possible that what appears to be a definite trend is actually no more than the combination of confounding factors and chance. However, the consistency of this trend between intervals, and in the two separate tests is disquieting, and entails that the extent of hypoxia's influence should at least be exposed to closer scrutiny.

ACKNOWLEDGEMENTS

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See lab report comments

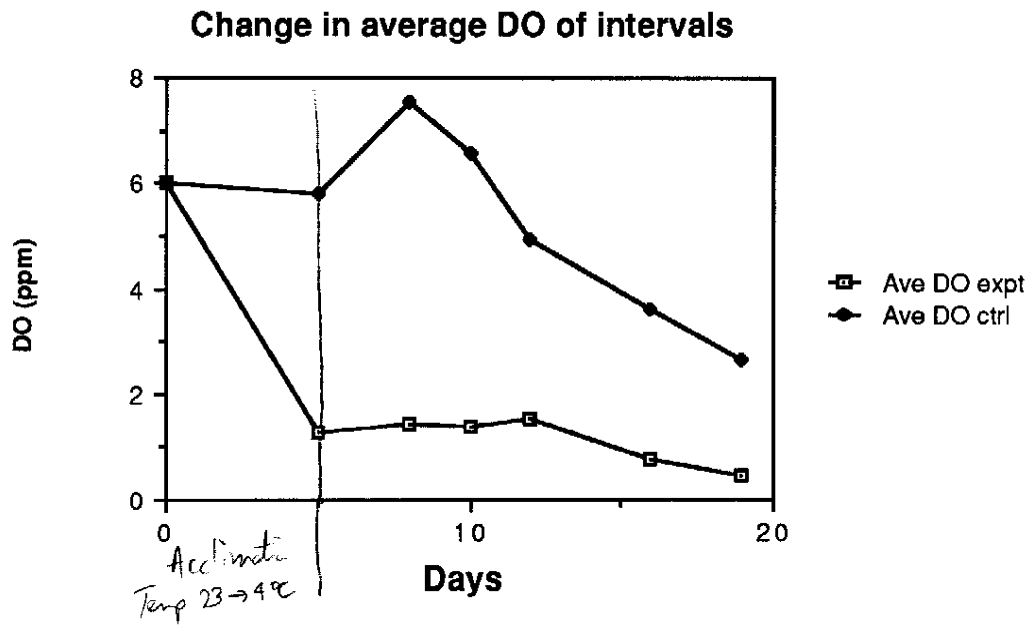


Fig. 1. Change in dissolved oxygen levels in first experiment. Average DO for each interval is plotted. First five days represent acclimation period of organisms.

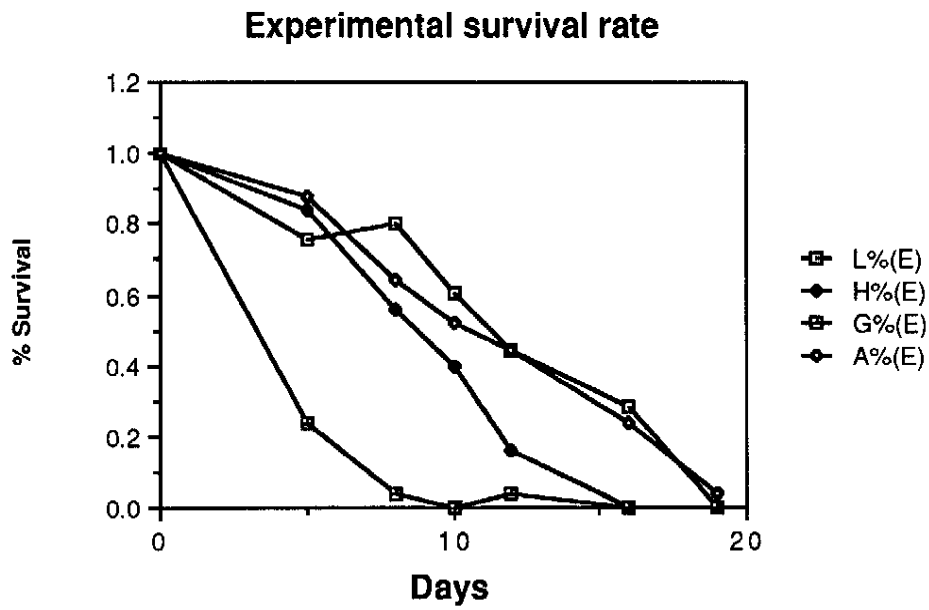


Fig. 2. Survival rate of each species in the experimental replicates of first experiment. Ratio of survivors to total individuals is plotted for each interval.

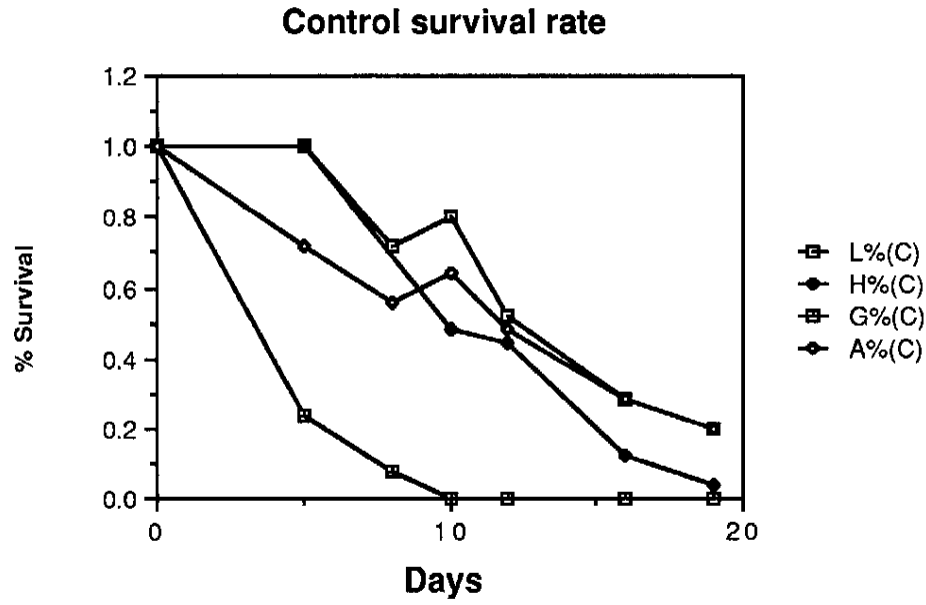


Fig. 3. Differential survival of the species used in the first experiment.
 The ratio of survivors to total individuals is plotted for each interval.

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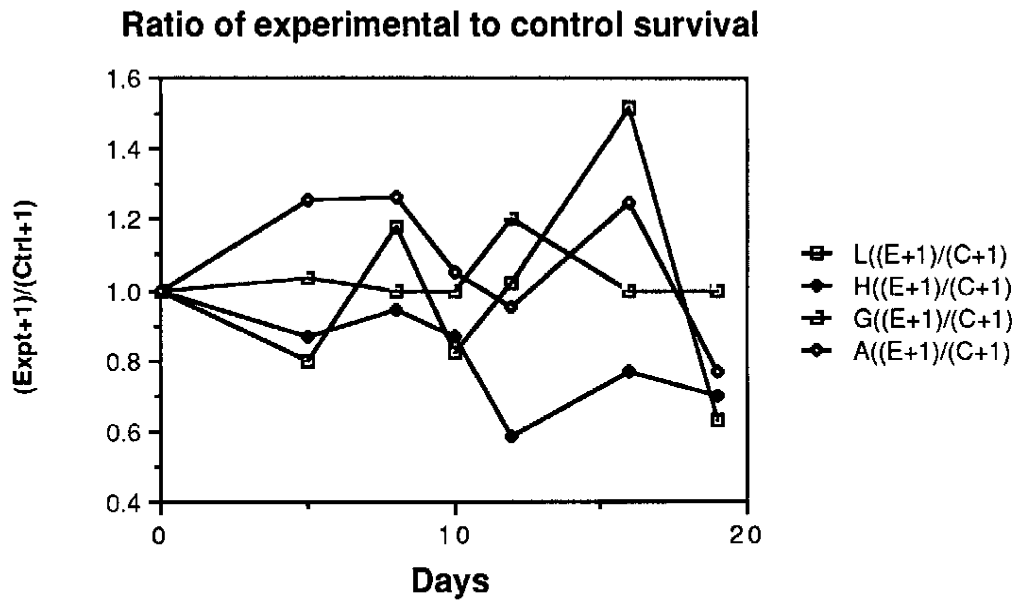


Fig. 4. The ratio of experimental to control survival in the first experiment. The average experimental to control survival ratio is plotted for each interval. Values < 1 indicate mortality due to hypoxia. L=Lymnaea emarginata; H=Helisoma campanulata; G=Gyraulus sp.; A=Amnicola sp.

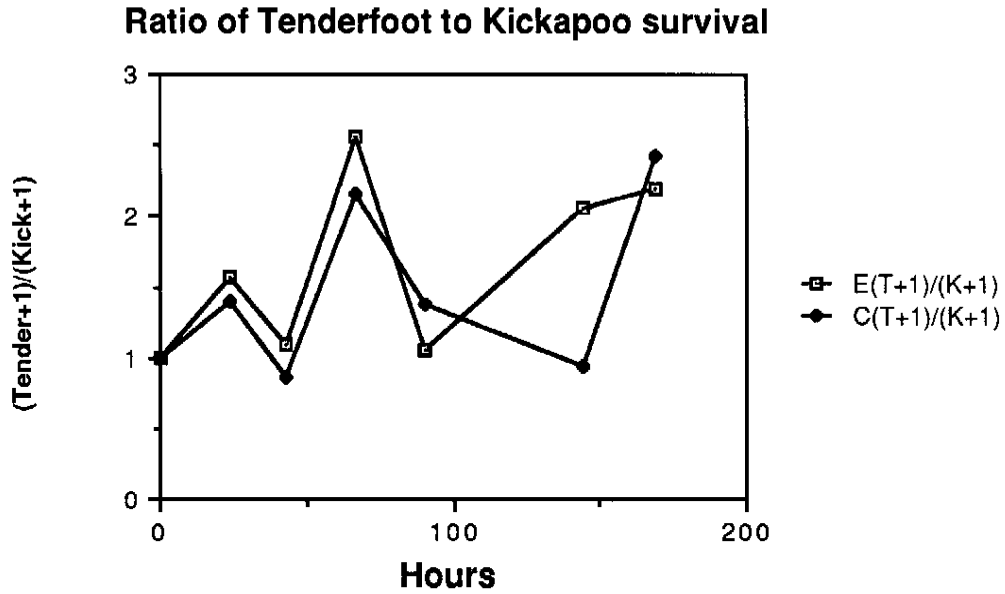


Fig. 9. Ratio of Tenderfoot Amnicola survival to that of Kickapoo Amnicola. Average ratio of T. Amnicola survival divided by K. Amnicola survival for the replicates of each interval are plotted for both experimental and control replicates.

be | figs 4 & 5?

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* See
1st Draft Comment

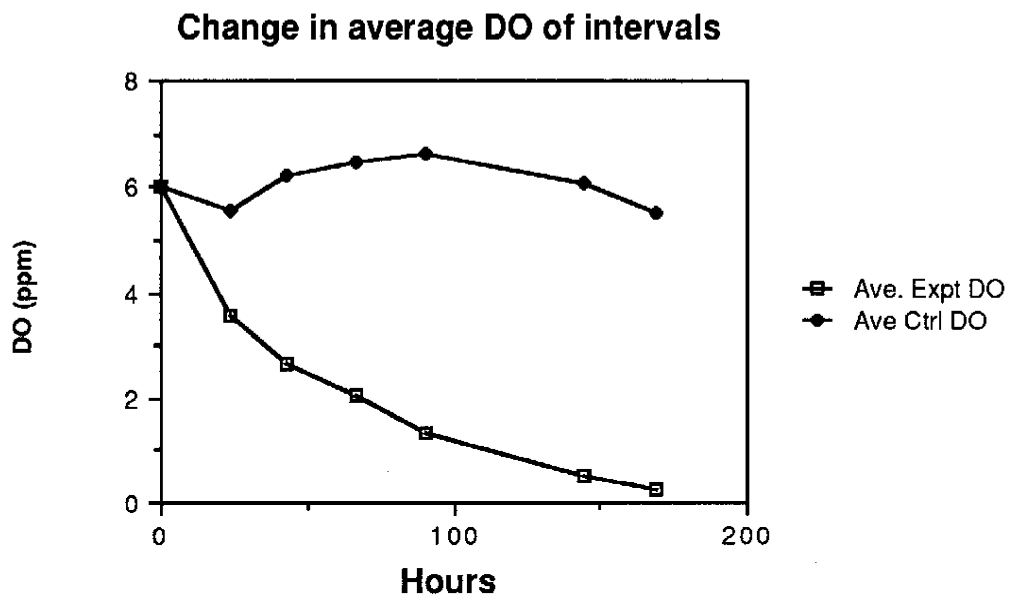


Fig 55. Change in DO in experimental and control replicates in the second experiment. Average DO is plotted for each interval.

See 1st draft

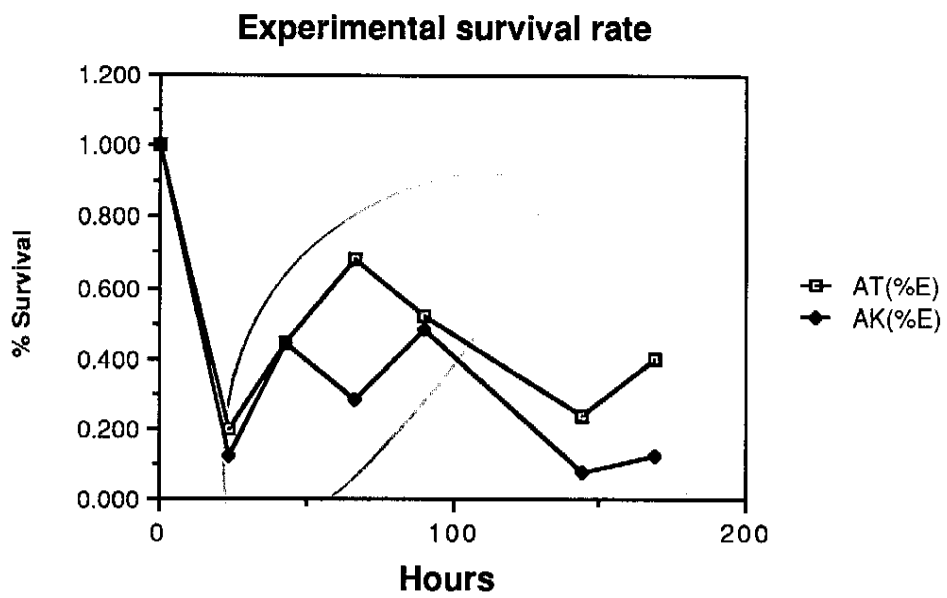


Fig.6. Survival rate of Tenderfoot and Kickapoo populations of Amnicola in in both experimental and control replicates. Ratio of surviving Amnicola to total individuals is plotted for each interval.

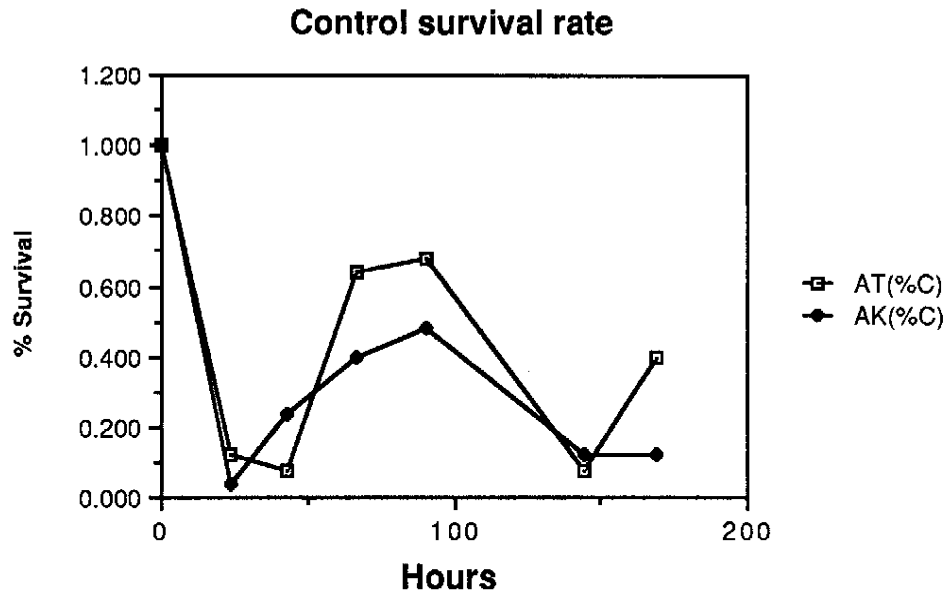


Fig. 7 Survival rate of both Tender foot and Kickapoo populations of *Amnicola* in the second experiment. Ratio of surviving snails to total individuals is plotted for each interval of control replicates.

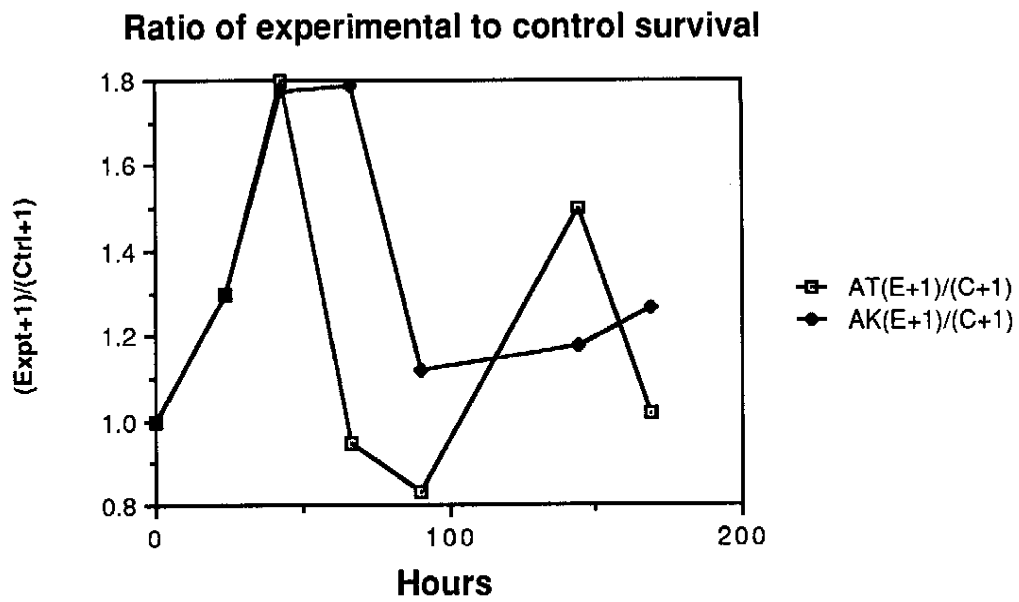


Fig. 8. Ratio of experimental to control survival in second experiment. Average ratio of experimental survival divided by control survival between replicates is plotted for each interval. Values < 1 indicate mortality due to hypoxia.

Appendices should come last,

Appendix 1. Shell lengths of snails used in first experiment. All snails were measured using vernier callipers. Lengths are given in mm.

#	<i>Gyraulus</i> sp.	<i>Helisoma campanulata</i>	<i>Lymnaea emarginata</i>	<i>Amnicola</i> sp.
1	4.625	10.950	16.875	2.700
2	4.675	11.450	16.950	2.850
3	4.725	11.925	17.300	2.850
4	4.750	12.000	17.400	3.450
5	4.775	12.150	17.800	3.550
6	4.950	12.250	19.125	3.650
7	5.050	12.300	19.250	3.675
8	5.150	12.525	19.900	3.700
9	5.175	12.525	20.050	3.700
10	5.725	12.600	20.200	3.725
11	5.775	12.650	20.225	3.750
12	5.800	12.750	20.250	3.800
13	5.825	12.850	20.325	3.850
14	5.900	12.900	20.400	3.850
15	5.900	12.950	20.925	4.900
16	5.925	13.000	21.100	4.000
17	5.925	13.150	21.350	4.050
18	5.950	13.250	21.650	4.100
19	6.000	13.725	21.650	4.100
20	6.025	13.775	21.700	4.100
21	6.050	13.850	22.850	4.200
22	6.250	13.850	22.000	4.250
23	6.275	13.950	22.050	4.250
24	6.475	14.000	22.050	4.450
25	6.650	14.050	22.125	4.500
26	6.650	14.075	22.250	4.525
27	6.675	14.150	22.725	4.550
28	6.800	14.150	22.900	4.600
29	7.250	14.250	22.950	4.600
30	7.300	14.750	24.300	4.750
<u>Mean:</u>	5.825	13.100	20.025	3.800

X

Appendix 2. Shell lengths of Tenderfoot and Kickapoo *Amnicola* in experiment B.
 Shells measured using vernier callipers. All lengths given in mm.

#	<i>Amnicola</i> (Kickapoo)	<i>Amnicola</i> (Tenderfoot)
1	2.700	2.375
2	2.950	2.500
3	3.150	2.800
4	3.275	3.000
6	3.450	3.000
7	3.450	3.025
8	3.450	3.325
9	3.450	3.450
10	3.450	3.450
11	3.525	3.550
12	3.525	3.650
13	3.550	3.725
14	3.575	3.850
15	3.625	3.850
16	3.625	4.050
17	3.650	4.100
18	3.650	4.150
19	3.725	4.175
20	3.800	4.200
21	3.825	4.275
22	3.850	4.300
23	3.875	4.550
24	4.125	4.525
25	4.150	4.550
26	4.200	4.600
27	4.225	4.650
28	4.250	4.700
29	4.275	4.700
30	4.300	4.825
<u>Mean:</u>	3.675	3.725

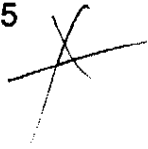


Table 1. Date, lake, and habitat in which species for experiment A were collected.

<u>Species</u>	<u>Date</u>	<u>Lake</u>	<u>Habitat</u>
<i>Gyraulus</i> sp.	June 12	Tenderfoot	Undersurface of large, flat, dark rocks in shallow water
<i>Helisoma campanulata</i>	June 9	Kickapoo	Periphyton on macrophytes and sediment
<i>Limnaea emarginata</i>	June 8	Plum Lake (at Trout Lake)	Periphyton on sediment near and among macrophytes
<i>Amnicola</i> sp.	June 7	Tenderfoot	Among gravel and small macrophytes in shallow water

Table 3. Total number of survivors of control (a) and experimental (b) replicates, and of both combined (c). Also presented are the total difference in survival between control and experimental replicates (d), the ratio between this difference and the total number of survivors (e), and the ratio of experimental to control survival. The latter two parameters indicate extent to which mortality of a given species is influenced by hypoxia. Higher values for e and lower values for f indicate greater influence. of what?

You must be much more explicit here. E.g., indicate for each index what a high # means & what a low # means.

	<i>Gyraulus</i> sp.	<i>Helisoma</i> sp.	<i>Lymnaea</i> sp.	<i>Amnicola</i> sp.
a. Total # of ctrl surviving	8	67	88	72
b. Total # of expt surviving	8	49	72	69
c. Total individuals surviving	16	116	160	141
d. a - b	0	18	16	3
e. d/c	0	0.155	0.100	0.021
f. b/a	1.0	0.731	0.818	0.941

almost equivalent to Fig 4. Why duplicate?

should be before Table 3,
what was date when placed in fridge?



Table 2. Rate at which organisms were acclimated during experiment A. When organisms first placed in refrigerator, water temperature was 23 C, and refrigerator temperature was 14 C.

<u>Date</u>	<u>Temperature</u>
June 13	14 C
June 14	10 C
June 16	8 C
June 17	6 C
June 18	1 C (first interval of experiment)

A graph would be more effective here.

