Laboratory 1: Diffusion and Osmosis

*describe the mechanisms of diffusion and osmosis
*describe how solute size and molar concentration affect the process of diffusion through a selectively permeable membrane
*design an experiment to demonstrate and measure water potential
*relate osmotic potential to solute concentration and water potential
*describe the effects of water gain or loss in animal and plant cells
*calculate the water potential of living plant cells from experimental data

kinetic energy = atoms and molecules constantly in motion
diffusion = random movement of molecules from area of higher concentration to area of lower concentration
osmosis = diffusion of water through a selectively permeable membrane

Procedure 1A.
You indirectly measure diffusion of small molecules through a semipermeable membrane (dialysis tubing) [remember, small solutes and water can move freely!] The size of pores in the tubing determines what size can pass through (dialyze) and what size barred.

Measured amounts of glucose ($C_6H_{12}O_6$) and starch $(C_6H_{12}O_6)^{2000}$ are added to dialysis tubing bag which is then tied off to seal. After 30 minutes in distilled water
a. travel of glucose checked with Tes Tape
b. travel of starch with Lugol's iodine solution

****

TesTape will turn from yellow to green, showing glucose has diffused from bag into the water. Iodine will <u>not</u> give a positive change to blue-black in the water (starch molecules too large!) A check on amount of H_2O will show more in bag (it was in a <u>hypotonic</u> solution!)
*What would happen if you had started with glucose and iodine inside the bag, starch and water outside?

Procedure 1B.
Six dialysis bags have 25 ml solution added to them as follows:
a. distilled water
b. 0.2-M sucrose
c. 0.4-M sucrose
d. 0.6-M sucrose
e. 0.8-M sucrose
f. 1.0-M sucrose

Be sure you understand
isotonic
hypotonic
hypertonic

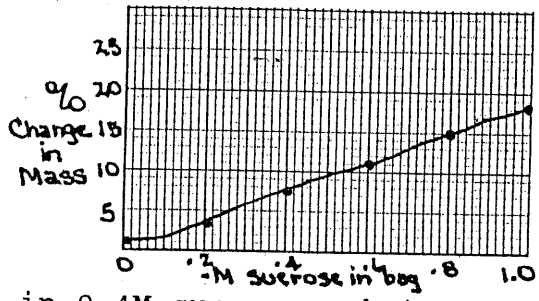
Bags closed, blotted and weighed, put in distilled water for 30 minutes; removed, blotted and reweighed.

****

Sugar bags gained weight (they had been in hypotonic solutions): here is the graph and data....

Contents in Dialysis Tube	Initial Mass	Final Mass	Percent Change in Mass
a) distilled water	24.0	24.3	+1.2%
b) 0.2-M sucrose	26.2	27.0	+3.1%
c) 0.4-M sucrose	26.1	28.1	+7.7%
d) 0.6-M sucrose	26.4	29.3	+11.0%
e) 0.8-M sucrose	24.3	30.2	+14.8%
f) 1.0-M sucrose	26.4	31.2	+18.2%

Mass in grams



Change in Mass of Dialysis Bag vs. Sucrose Molarity

*If all the bags had been put in 0.4M sucrose, what would have happened?

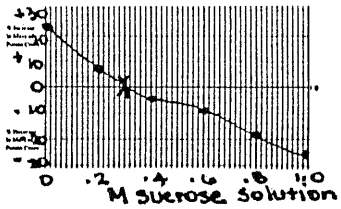
Procedure 1C

You can determine the water potential of potato cells by putting pre-weighed cubes of potato into 6 different sucrose solutions (see above) overnight. remember: water potential (ψ) = pressure potential (cell wall's force, change in barometric pressure; often not considered!) + osmotic potential (tendency of water to move toward area of lesser concentration). ψ of distilled H_2O = 0, everything else is negative!

****

Contents in Beaker	Temperature	Initial Mass	Final Mass	Percent Change in Mass
a) distilled water	23°C	4.6	5.7	+23.9
b) 0.2 M sucrose		4.7	5.1	+ 8.5
c) 0.4 M sucrose		4.7	4.6	- 2.1
d) 0.6 M sucrose		4.5	4.1	- 8.9
e) 0.8 M sucrose		4.7	3.8	-19.5
f) 1.0 M sucrose		4.6	3.5	-23.9

Change in Mass of Potato Cells vs. Sucrose Molarity



To determine the osmolarity of the potato, see where the lines cross!

To compute osmotic potential = -iCRT = - (1) (concentration) (0.0831 liter bar/mole °K) (295°K) =

Laboratory 2: Enzyme Catalysis

*graph data from an enzyme experiment
*determine the rates for enzymatically catalyzed reactions
*discuss a method for determining enzyme activity
*discuss a relationship between dependent and independent variables
*discuss the effect on initial reaction rates produced by changes in temperature, pH, enzyme concentrations, and substrate concentrations
*design an experiment to measure the effect on enzyme activity produced by changes in temperature, pH, enzyme concentration, and substrate conc.

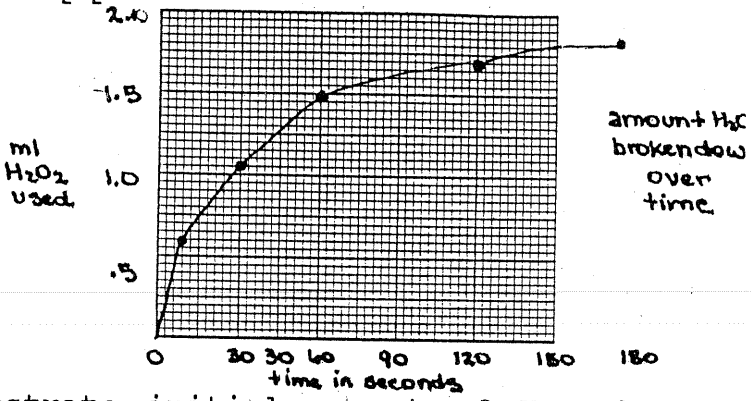
enzymes = large, tertiary or quartenary proteins with active sites where substrate(s) fit to allow accelerated catalysis of chemical reaction
Catalase, present in most cells, breaks down poisonous hydrogen peroxide H_2O_2 to H_2O and O_2 (gas bubbles). To determine the "rate of reaction", you measure the disappearance of reactants or the production of products over a period of time.

Procedure
Throughout the lab, measured amounts of hydrogen peroxide solution are added to a beaker (cup); measured enzyme is added and the reaction allows to proceed X time. To "stop" the reaction, sulfuric acid (H_2SO_4) is added. The solution is then titrated by adding drops or Burette-measured amounts of potassium permanganate....as long as there is peroxide left, it will decolorize the dark purple permanganate to clear! However, when the titration is complete, any excess permanganate will remain dark, and calculations can be made on amount of perman used and/or amount H_2O_2 still present. Since time is known, rate can also be calculated.

****

This table and graph shows the results for titration in ml permanganate of the catalase-catalyzed breakdown of H_2O_2 over time intervals 0-180 seconds.

$KMnO_4$ (mL)	Time (seconds) 10	30	60	120	180
A. Baseline = 0 seconds	3.1				
B. Final reading	8.6	10.6	12.2	13.6	14.9
C. Initial reading	6.1	8.6	10.6	12.2	13.6
D. Amount of $KMnO_4$ used (B minus C)	2.5	2.0	1.6	1.4	1.3
E. Amount of H_2O_2 used (A minus D)	0.6	1.1	1.5	1.7	1.8



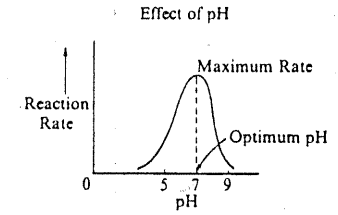
To figure the rates of reaction, substrate initial amount of H_2O_2 from time X amount of H_2O_2, and divide by time X.

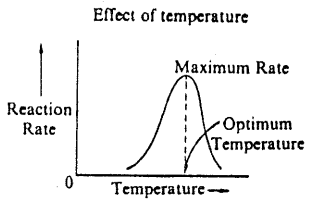
initial rate = (0 ml H_2O_2 used at time 0 − 0.6 ml H_2O_2 used at time 10 sec) / 10 sec = 0.06 ml H_2O_2 per minute

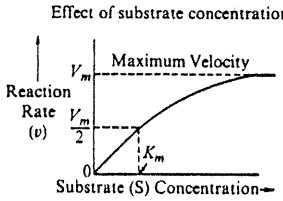
Time intervals (seconds)				
Initial (0-10)	10-30	30-60	60-120	120-180
0 − 0.6 / 10	0.6-1.1 / 20	1.1-1.5 / 30	1.5-1.7 / 60	1.7-1.8 / 60

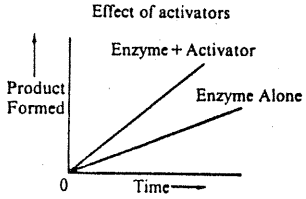
*Reaction rate (mL H_2O_2/sec)

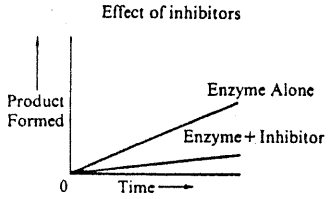
When you get the rates for each interval, you will find that the rate is highest at the start where there are many molecules to be worked on, but drops as substrate decreases (becomes "harder" to find molecules to work on). The reason you use sulfuric acid to "stop" the reaction is that extreme pH changes often denature enzymes. Lowering of temperature will slow enzymes as it does all molecular events, and raising of temperature will speed up the reaction to a point of denaturing and abrupt decline. Many other factors may affect enzymes, as seen below.


Effect of pH
Reaction Rate
Maximum Rate
Optimum pH
0
5
7
9
pH


Effect of temperature
Reaction Rate
Maximum Rate
Optimum Temperature
0
Temperature


Effect of substrate concentration
Reaction Rate (v)
Maximum Velocity
V_m
$\frac{V_m}{2}$
K_m
0
Substrate (S) Concentration


Effect of activators
Product Formed
Enzyme + Activator
Enzyme Alone
0
Time


Effect of inhibitors
Product Formed
Enzyme Alone
Enzyme + Inhibitor
0
Time

Laboratory 3: Mitosis and Meiosis

*compare the events of mitosis in plant cells with those of animal cells
*demonstrate a procedure to stain tissue for the identification of cells in the various stages of mitosis
*calculate the relative duration of the phases of mitosis
*manipulate chromosome models to demonstrate the events of meiosis I and II
*calculate the map distance between a gene for ascospore color and the centromere of the same chromosome
*explain how meiosis and crossing over result in the different arrangements of ascospores within asci
*describe the role of meiosis and mitosis in the formation of the ascospores within the asci of *Sordaria fimicola*
*use chromosome models to demonstrate segregation and independent assortment in the process of meiosis
*discuss how crossing over can introduce additional genetic variability into the products of meiosis

mitosis = identical replication of the chromosomes from a nucleus through the stages prophase, metaphase, anaphase, telophase/ two identical daughter nuclei (diploid or haploid) for growth, repair, asexual reproduction
meiosis = reduction of a replicated diploid nucleus into four non-identical haploid nuclei through two sets of divisions/ for gamete or spore production

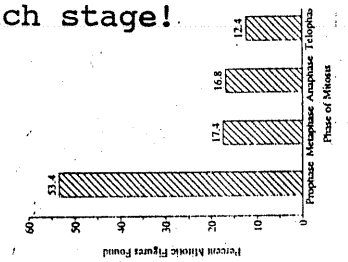
To study mitosis, observe microscopically the fastest mitosing tissue possible (in an onion, the root tip!). Chromosome squash will show cells in all cell cycle phases (remember interphase is G1, S, G2):

****

Time estimates are made by counting 100 cells or several "fields of view" at and assuming the % cells observed in each stage is an accurate representation of the actual amount of time spent in each stage!

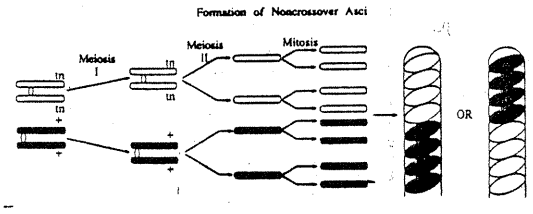
	Number of Cells				Percent of Total Cells Counted
	Field 1	Field 2	Field 3	Total	
Prophase	42	37	34	113	53%
Metaphase	7	17	13	37	17%
Anaphase	15	11	10	36	17%
Telophase	7	10	9	26	13%

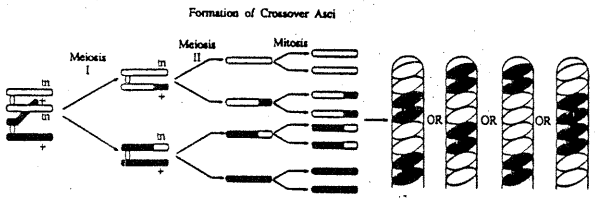
212 total cells counted...



Animal cell mitosis differs only in presence of centrioles at ends of spindles, and in cytokinesis after telophase -- they form a cleavage furrow rather than a cell plate.

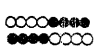
Meiosis produces genetic variation in the population because of independent assortment of chromosomes during meta- and anaphase I, and because of crossing over of homologous chromosomes during prophase I in synapsis. In the fungus *Sordaria*, meiosis followed by quick mitosis forms eight haploid ascospores contained within a sac called an ascus (rather like a sack of seeds). See the two possibilities below of crossover and non-crossover asci:





If asked to determine the "map distance" between the gene for spore coat and the centromere,

a. calculate the % crossover by dividing number of crossover asci by total number of asci
b. then divide the percent of crossover asci by 2 (remember, only half the spores in a sac are really crossovers!)

Number of 4:4 Asci	Number of Asci Showing Crossover	Total Asci	% Asci Showing Crossover Divided by 2	Gene to Centromere Distance (Map Units)
			$17.3/2 =$	
62	13	75	8.7%	8.7

Laboratory 4: Plant Pigments and Photosynthesis

*understand the principles of chromatography
*calculate R_f values
*design an experiment in which chromatography is used as a separation technique
*describe a technique for determining photosynthetic rate
*understand the relationship between dependent and independent variables
*describe how light intensity, light wavelength, and temperature can affect photosynthesis
*design an experiment to measure how light intensity, light wavelength, and temperature can affect photosynthesis

Paper chromatography allows you to separate molecules (plant pigments in this case) based on solubility in the particular solvent, differing attractions to the cellulose of the paper (due to H bonds), molecular size and weight. Chlorophyll a is the primary photosynthetic pigment at the reaction center of all photosystems: other pigments (chlorophyll b, carotene, xanthophyll) are parts of the antennae system to funnel extra energy to "a" plus carotenoids function like "sunscreen" to protect pigments from damage by bright light. In chromatography, pigments can be identified by color, but also by R_fs - ratio of fronts - the distance the molecule traveled divided by the distance the solvent traveled. R_fs remain constant for the same molecule given the same conditions (the largest R_f possible is 1.00 -- why???)

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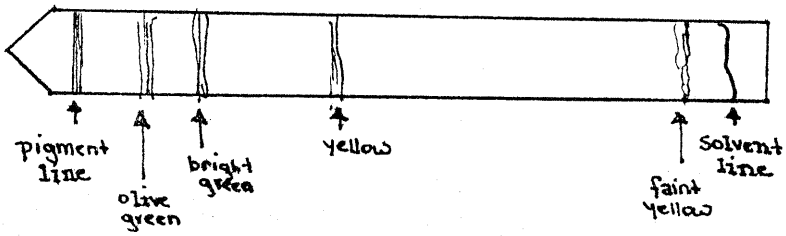
Here are the visual results of the chromatograph:



Beta carotene is carried quickly, close to the solvent front because it is very soluble in this solvent and no atoms sticking out to form H bonds.....xanthophyll is slowed by the H bonds it forms. Chlorophylls contain more exposed O and N, and are therefore bound more tightly to the paper and travel more slowly. Measurements yielded the following R_fs:

$\frac{8.2}{8.7} = .94$ carotene $\frac{3.5}{8.7} = .40$ xanthophyll $\frac{1.7}{8.7} = .20$ chlorophyll a $\frac{1.0}{8.7} = .12$ chlorophyll b

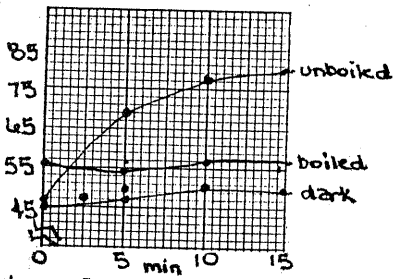
	1 Blank	2 Dark	3 Unboiled	4 Boiled
Phosphate Buffer	1 mL	1 mL	1 mL	1 mL
Distilled H_2O	4 mL	3 mL	3 mL	3 mL
DPIP	—	1 mL	1 mL	1 mL
Unboiled Chloroplasts	3 drops	3 drops	3 drops	—
Boiled Chloroplasts	—	—	—	3 drops

Wavelengths of light in the visible spectrum are used for photosynthesis. There are two photosystems in the light reactions: during the non-cyclic phase, electrons are passed from photosystem II to photosystem I and finally to NADP (which also accepts an H+ from the splitting of water). The rate of these reactions might be measured several ways, but one involves dye reduction. The dye DPIP (2,6-dichlorophenol indophenol) more readily accepts e^- and H+ than NADP, and in the process will change from a dark blue to a clear solution. This change can be quantified visually by relative color intensity changes, or more precisely with a spectrophotometer (a machine that measures % light transmittance through a sample (dark blue will transmit little light, clear almost all the light).

This experiment will involve a control (no chlorophyll present so no photosystems, no change of reducing the dye), a chloroplast setup kept in the dark (how essential is light to the process?), a chloroplast setup boiled before starting (does high heat affect the process?), and a normal chloroplast setup.

****

Cuvette	Time (minutes) 0	5	10	15
2 Dark	45.3	48.5	50.3	49.8
3 Unboiled	47.9	70.3	78.5	79.8
4 Boiled	56.2	54.0	57.1	56.4



Conditions vs. Rate of Photosysthesis

This is a Spec20 machine; solutions to be tested are placed in special tubes called "cuvettes" and entered into the chamber; wavelength of light to be shown through it is chosen (this lab involved 680 nm) and a meter reads what % actually gets through (% transmittance)!

Obviously, the control shows constant transmittance; the dark may receive some light as the tube is put into the Spec20 for testing, so may show some change but not much; the boiled should show no significant change; and the normal setup should show significant dye reduction and lightening over the time period!

Laboratory 5: Cell Respiration

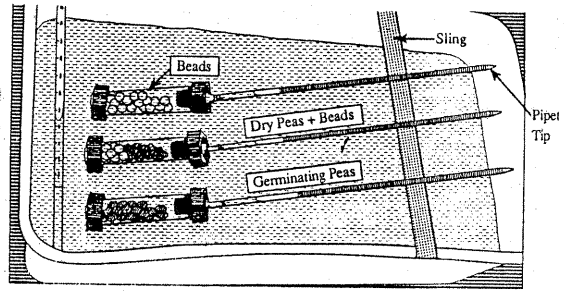
*discuss the gas laws as they apply to the function of a respirometer
*interpret data related to the effects of temperature on cell respiration
*interpret data related to the effects of germination or nongermination on cell respiration
*explain or determine the significance of a control
*explain the relationship between dependent and independent variables
*calculate a rate of cell respiration by utilizing graphed data
*design an experiment to use a respirometer to measure cellular respiration

This experiment will examine the difference between rates of respiration of germinating peas and those still in "suspended animation" (dry peas). It is possible to measure many factors of respiration: consumption of glucose, production of CO_2, of the use of O_2 -- the later will be used here. Since there are two gases in the equation, use of soda lime to absorb CO_2 produced will make it possible to check on level of oxygen only. [Note: to be able to figure <u>actual</u> amounts of O_2, gas used must be adjusted if any changes in barometric pressure occurred -- the purpose of the third tube with glass beads or gravel! The gas law that counts is PV = nRT or the pressure X volume = number of molecules X gas constant X temperature: if you change any of the parts of the equation, you must change something on the other side!]

This lab will also explore how temperature affects the rate of respiration; therefore, two identical setups will be run -- one at room temperature (25°C), one at approximately 10°C.

Twenty-five germinating peas are placed in a large test tube with soda lime to absorb the CO_2 and a weight to keep it underwater. A stopper is inserted with a graduated pipette to make a water-tight seal. The same is done with a tube of 25 dry peas (plus gravel to give an equal volume to that of the germinating ones -- remember: only one variable in the experiment, and a difference in volume could be a significant variation!) and with a third tube containing an equal volume of gravel only (will serve as the control and thermobarometer).

All tubes are submerged after equilibrating and as the O_2 is used in the respiration process, a loss in gas volume should cause more water to enter the tube, which can be measured. Measurements are taken at 0 time and then every 5 minutes for 20 minutes for all three tubes in each temperature situation.



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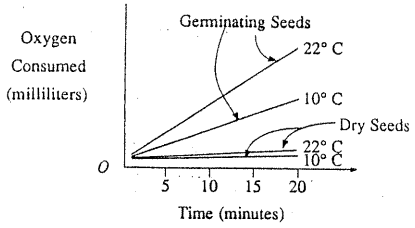
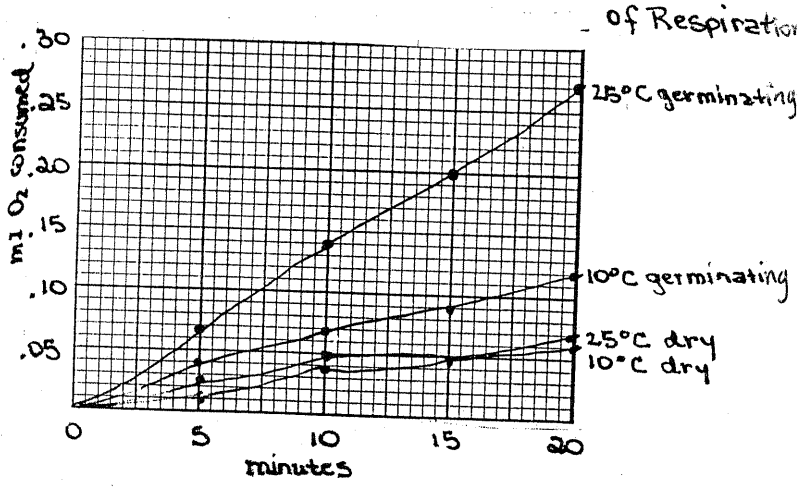
Temp. (°C)	Time (min)	Beads Alone		Germinating Peas			Dry Peas and Beads		
		Reading at time X	Diff.*	Reading at time X	Diff.*	Corrected diff.[a]	Reading at time X	Diff.*	Corrected diff.[a]
25	Initial - 0	0.93		0.91			0.92		
	0-5	0.91	0.02	0.84	0.07	0.05	0.89	0.03	0.01
	0-10	0.90	0.03	0.77	0.14	0.11	0.87	0.05	0.02
	0-15	0.90	0.03	0.71	0.20	0.17	0.87	0.05	0.02
	0-20	0.90	0.03	0.64	0.27	0.24	0.85	0.07	0.04
10	Initial - 0	0.95		0.92			0.91		
	0-5	0.94	0.01	0.88	0.04	0.03	0.90	0.01	0.00
	0-10	0.92	0.03	0.85	0.07	0.04	0.87	0.04	0.01
	0-15	0.93	0.02	0.83	0.09	0.07	0.86	0.05	0.03
	0-20	0.93	0.02	0.80	0.12	0.10	0.85	0.06	0.04

* Difference = (initial reading at time 0) − (reading at time *X*)

[a] Corrected difference = (initial pea seed reading at time 0 − pea seed reading at time *X*) − (initial bead reading at time 0 − bead reading at time *X*)



Temperature vs. Rate of Respiration



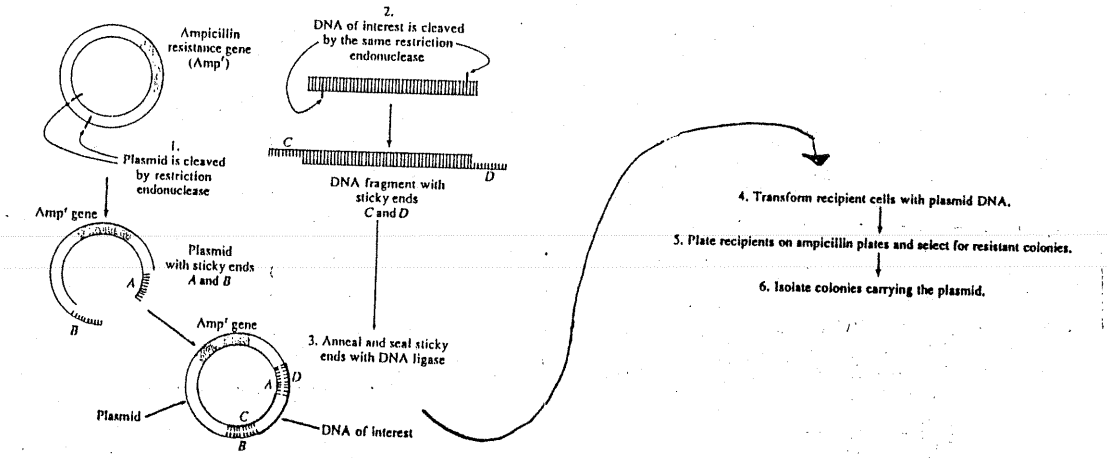
Obviously, the rate of reaction and the graph indicate that germinating peas respire at a much higher rate, no matter what the temperature: this makes sense because they are actively growing, while dry peas are in "arrested development" (NOT the rap group). Cold inhibits chemical reactions in any system, and it does also here!

Laboratory 6: Molecular Biology

*discuss the rpinciples of bacterial transformation
*describe how to prepare competent *E. coli* cells
*discuss the mechanisms of gene transfer using plasmid vectors
*discuss the transfer of antibiotic resistance genes and tell how to select positively for transformed cells that are antibiotic resistant
*discuss the mechanisms of action for restriction endonucleases
*discuss how a plasmid can be engineered to include a piece of foreign DNA that alters the phenotype of the teransformed cells
*understand and be able to explain the principles of electrophoresis as they pertain to separating and identifying DNA fragments

E. coli is an ideal organism for use in molecular biology; grown easily, its circular chromosome contains 5 million base pairs (1/600 that of single human sperm). These bacteria also contain plasmids, small circular DNA molecules of 1000 to 200,000 base pairs; extrachromosomal, they replicate with the cell, and can be transferred during bacterial transformation. This phenomenon of transfer of DNA from one bacterium to another is normall rare, but it occurs best when cells are in a competence stage: humans have figured out a way to make cells competent by adding Ca^{+2} or Mg^{+2} . It also seems as if they take up DNA best after a short pulse of heat, so humans manipulate the temperature at which parts of this process are done.

A gene that would be easy to detect if transferred is antibiotic resistance. For example, *E. coli* is normally killed by ampicillin, but if a gene for ampicillin resistance was placed into it and worked, the bacteria should be able to thrive, even on plates with the antibiotic present. You can purchase plasmids which already have this gene inserted into them (see below the process).



To get the plasmid into the bacterial cells, you must treat them carefully with $CaCl_2$ to make competent, add plasmid while solutions are on ice; heat shock with "pulse of heat" in 42°C water bath for 90 seconds, then return to ice. Now streak bacteria on agar plates with nutrients plus ampicillin antibiotic and incubate for two days.

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If cells were "transformed", they should now contain the gene for resistance to ampicillin and should grow well on antibiotic-containing plates while non-transformed cells are killed. Visual inspection will show some colonies that are thriving; transformation efficiency is expressed as the number of resistant colonies per microgram of plasmid. That can be calculated if you observe 70 colonies after placing only 100 µL of broth there (originally there was 500 µL diluting 0.05 µgram of plasmid).

.05 µgram / 500 µliters = 0.0001 µgram/µliter

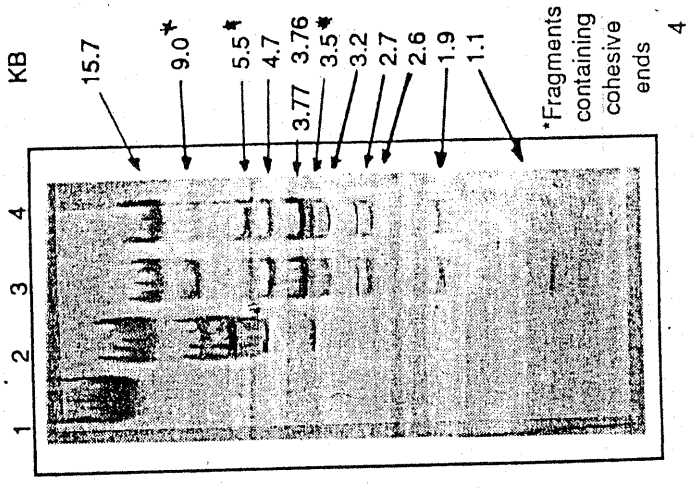
70 colonies from 100 µliters = .01 µgrams

∴ you could expect 7000 colonies per µgram

The restriction enzymes used to introduce fragments of "foreign" DNA into plasmids are more specifically restriction endonucleases. Several are very famous (EcoRI and HaeII...the first letter stands for the genus of the organisms from which it was isolated, the next two letters are the first of the species name, the fourth letter the strain -- may or may not have this -- and the Roman numerals indicate whether it was the fist enzyme isolated, the second, or so on....) These restriction endonucleases recognize specific 4-6 base pair DNA sequences, and many cleave the helix off-center to produce "sticky ends" or overhangs which allow recombination with other pieces with the same "sticky ends". If plasmids are cut with a particular enzyme and a wanted gene is spliced out with the same one, they may re-anneal and form a single circular strand of "recombinant DNA"!

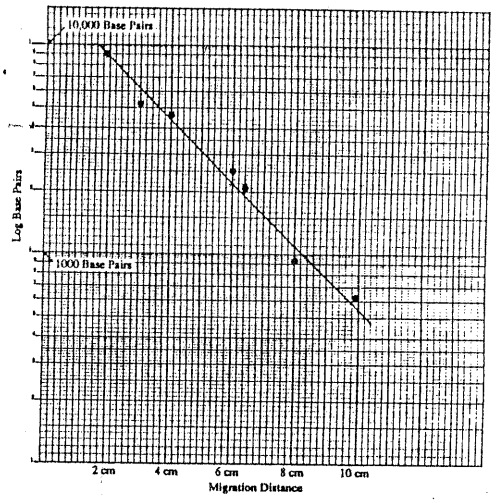
Restriction enzymes can be used to digest a sample of DNA and then the pieces electrophoresed; after migration of the DNA through an electrical field, the gel can be stained and a banding pattern corresponding to the number of sites recognized by the enzyme will be seen and an estimation of the base pair size can be made when compared to a known standard.

When any molecule enters an electrical field, the mobility or speed at which it will move is influenced by the charge of the molecule, the strength of the field, the size and shape of the molecule, and the density of the medium (gel in this case) through which it is migrating. All this makes it possible to separate heterogeneous populations of molecules. If all molecules start at the same place (parallel wells in the gel) and are run under identical conditions (in buffer, toward a positive electrode which attracts the negative phosphate groups of DNA), DNA fragments should separate into bands that migrate in inverse proportion to their size: the smallest run the farthest and fastest! Dyes help the molecule bands (groups of similar molecule fragments) show up more clearly.



By knowing how far DNA pieces of known base-pair size run, you can estimate the molecular size of all the bands on a gel! For example, if a 9,000 base-pair piece ran 2 cm from the start well, and a 620 base-pair piece ran 10 cm, you could estimate how large most other pieces were by plotting those points on semi-log paper/ a straight line there will predict the size by distance run with fair accuracy!

DNA Fragment Size vs. Migration Distance



Laboratory 7: Genetics of Drosophila

*conduct a genetics experiment for a number of generations
*compare predicted results with actual results
*explain the importance of chi-square analysis
*design genetic crosses in an experiment to illustrate independent assortment and sex-linkage
*discuss the life cycle of the fruit fly, recognize the sex of fruit flies, and recognize several classic types of mutations

Drosophila melanogaster (literally, "dew-lover, black-bellied") is a "perfect" lab animal on which to perform experimental genetic crosses. It is small, requires minimal upkeep and food, is quiet and odorless, reproduces very quickly (new generation in two weeks), and has many easily observable phenotypes resulting from different alleles.

****

A fly house is made (blue media mixed in a plastic vial, yeast added to provide food for maggots, netting inserted as structure for pupation) and pure-breeding (homozygous) parents are introduced.....

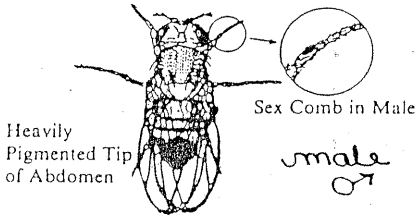
P_1 = 3 vestigial-winged males X 3 normal-winged females

These flies mate, are removed after 48 hours and maggots are observed 5 days later.....pupae are observed after 10 days. When the F_1 generation begins to hatch out, several are observed, and all exhibit normal wings.

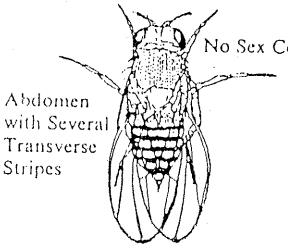
Therefore, you can deduce that normal is dominant over vestigial!

A new fly house is prepared and 3 male and 3 female F_1s are added. After several days, they are removed....at the end of two weeks, the F_2 generation begins to hatch out.

To make calculations easy, 100 F_2 flies are counted. For ease of observation, foam plugs are quickly removed and vials placed upside down on containers of ice: the flies fall to the surface and become "chilled" which renders them immobile and easy to sex (see below) and count.



male ♂



female ♀

At the end of the count, 43 normal females
33 normal males
8 vestigial females
16 vestigial males are observed.

Remember, there are two possibilities: this trait may be autosomal or sex-linked. If autosomal, the Punnett squares for the two generations should look like this:

N = normal
n = vestigial

♂ \ ♀	N	N
n	Nn	Nn
n	Nn	Nn

F_1 generation

♂ \ ♀	N	n
N	NN	Nn
n	Nn	nn

F_2 generation

If sex-linked, the Punnett squares should look like this:

♂ \ ♀	X^N	X^N
X^n	X^NX^n	X^NX^n
Y	X^NY	X^NY

F_1 generation

♂ \ ♀	X^N	X^n
X^N	X^NX^N	X^NX^n
Y	X^NY	X^nY

F_2 generation

Observing the results and comparing, it seems as if the trait must be autosomal. Therefore, the expected results should have been 3/8 normal males, 3/8 normal females, 1/8 vestigial males, 1/8 vestigial females: using this information, a Chi-square analysis can be run to determine how close the actual results are to the expected!

Degrees of Freedom	P = .99	.95	.80	.50	.20	.05	.01
1	.000157	.00393	.0642	.455	1.642	3.841	6.635
2	.020	.103	.446	1.386	3.219	5.991	9.210
3	.115	.352	1.005	2.366	4.642	7.815	11.345
4	.297	.711	1.649	3.357	5.989	9.488	13.277
5	.554	1.145	2.343	4.351	7.289	11.070	15.086
6	.872	1.635	3.070	5.348	8.558	12.592	16.812
7	1.239	2.167	3.822	6.346	9.803	14.067	18.475
8	1.646	2.733	4.594	7.344	11.030	15.507	20.090
9	2.088	3.325	5.380	8.343	12.242	16.919	21.666
10	2.558	3.940	6.179	9.342	13.442	18.307	23.209
15	5.229	7.261	10.307	14.339	19.311	24.996	30.578
20	8.260	10.851	14.578	19.337	25.038	31.410	37.566
25	11.524	14.611	18.940	24.337	30.675	37.652	44.314
30	14.953	18.493	23.364	29.336	36.250	43.773	50.892

$$\chi = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$\chi = \frac{(43-37.5)^2}{37.5} + \frac{(33-37.5)^2}{37.5} + \frac{(8-12.5)^2}{12.5} + \frac{(16-12.5)^2}{12.5} =$$

$1.13 + 0.54 + 1.62 + 0.9$

★ 4.27 at 3 degrees of freedom

the level of significance is close to .2 / acceptable!

Laboratory 8: Population Genetics and Evolution

*calculate allele and genotype frequencies using the Hardy-Weinberg theory
*discuss the effect of natural selection on allelic frequencies
*explain and predict the effect on allelic frequencies of selection against the homozygous recessive
*discuss the relationship between evolution and changes in allele frequencies, as measured by changes from the Hardy-Weinberg law of genetic equilibrium

In 1908 G. H Hardy and W. Weinberg independently suggested a scheme whereby evolution could be viewed as changes in the frequency of alleles in a population of organisms. Two formulas were used:

$p(\%\text{ dominant allele}) + q(\%\text{ recessive allele}) = 1$ referring to allele frequencies

$p^2(\%\text{ homozygous dominant}) + 2pq(\text{hetero}) + q^2(\%\text{ homo recessive}) = 1$ referring to genotype frequencies

These will remain constant from generation to generation only if five conditions are met:

1. the population is large
2. mating is random
3. no mutations occur
4. there is no immigration nor emigration
5. there is no natural selection

If any of these conditions are NOT met, then one should observe a change in the allele and genotype frequencies for the population....

****

The class is tested for ability to taste the chemical PTC (phenylthiocarbamide); a bitter-taste indicates presence of the dominant allele -- individuals will be homozygous dominant or heterozygous. Non-tasters are homozygous recessive! Once data is obtained, the frequency of each allele should be calculable.....

	Phenotypes		Allele Frequency Based on the H-W Equation	
	% Tasters ($p^2 + 2pq$)	% Nontasters (q^2)	p	q
Class Population	.91	.09	.7	$\sqrt{.09} = .3$
North American Population	0.55	0.45	.33	$\sqrt{.45} = .67$

****

A simulation of Hardy-Weinberg can be made by having class members assume genotypes based on original allele frequencies and then "mating" with other class members to produce new combinations of genes....If an AA student mates with an aa student, all the offspring will be Aa; but if both parents are Aa, there are several possibilities for offspring. If the two offspring produced are then allowed to randomly produce two other offspring and so on, at the end of several generations, we should be able to see if frequencies remain stable.

32

Generation	Offspring's Genotype	Class Totals for Each Genotype		
	(*AA*, *Aa*, or *aa*)	*AA*	*Aa*	*aa*
1		8	17	7
2		9	18	5
3		9	19	4
4		9	18	5
5		7	18	7

A
16+17
33

a
17 + 14
31

****

It is possible to simulate selection by "killing off" one of the genotypes (for example, aa cannot survive) and noting the change in frequency of alleles after several generations.

Generation	Offspring's Genotype	Class Totals for Each Genotype	
	(*AA* or *Aa*)	*AA*	*Aa*
1		12	20
2		14	18
3		17	15
4		18	14
5		21	11

A
24+20

a
20

A
42+11

a
11

Laboratory 9: Transpiration

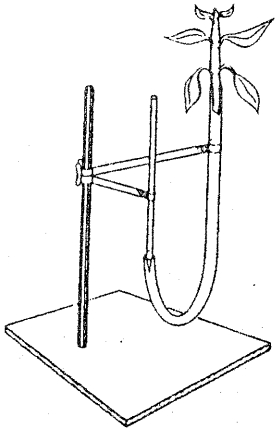
*describe how differences in water potential affect the transport of water from roots to stems to leaves
*relate transpiration to the overall process of water transport in plants
*discuss the importance of the properties of water -- including hydrogen bonding, adhesion, and cohesion -- to the transport of water in plants
*quantitatively demonstrate the effects of different environmental conditions on the rate of transpiration in plants
*identify the vascular tissues of the plant stem and describe their functions

Transpiration is the loss of water from plant surfaces, and it drives the transport of water up xylem as part of the tension-cohesion theory (TACT). As water leaves the leaves (ho-ho) through the stomata, hydrogen bonds help pull other water molecules up behind with adhesion to narrow xylem walls and cohesion to itself important contributing factors. Water potential also drives transpiration: water **always** moves from higher to lower water potential (remember, pure water is 0 potential, dry air may be -300).

****

To measure rate of transpiration, a potometer is set up (see right). A carefully cut and sealed plant stem is inserted into a water-filled tube and pipette/ any water lost should result in reduction of water in tube system. Over 30 minutes, readings are made under these condition:

a *normal room conditions
b *increased light (floodlight)
c *breeze (low speed fan)
d *mist (water spray inside plastic bag)



Total water loss could be graphed/ you can also estimate the water loss per square meter by figuring the leaf area by weight (grams leaf/160 grams per meter$_2$ = leaf area m$_2$) or by tracing leaf on 1 cm$_2$ graph paper and adding area.

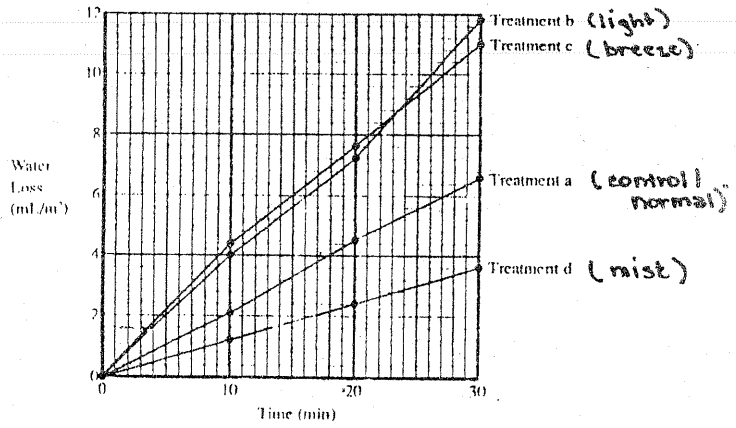
If weight of leaves = 2.25g
and leaf surface area = weight (g) / 160 g/m^2
than leaf surface area = 2.25 / 160 = .014m^2

Leaf surface area 0.014 m^2

Class Averages
Cumulative water loss in mL/m^2 at times (min)

Treatment	0	10	20	30
a	0	2.19	4.56	6.57
b	0	4.16	7.57	11.73
c	0	4.50	7.58	11.00
d	0	1.30	2.44	3.65

Amount of Transpiration vs. Physical Factors



****

The structure of a plant stem and its xylem and phloem can be observed by preparing a plant cross-section. Obtaining a nut-and bolt microtome, a piece of plant stem can be placed in the nut and melted paraffin added. As the wax hardens, it should hold the stem securely and allow it to be slices into thin pieces with a razor blade. The sections can be moved to dishes of ethanol for dehydration; then to stain for clarification. The following cell types should be visible:

parenchyma: most abundant cell type/ unspecialized, include mesophyll, fruit flesh, pith and cortex of roots and stems

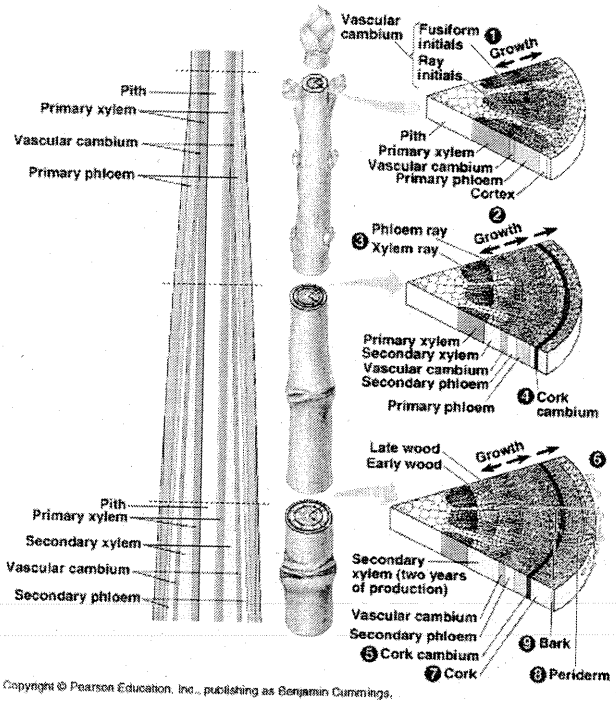
sclerenchyma: fibers in leaves, stems, fruits, usually found in bundles and often just outside the vascular tissue

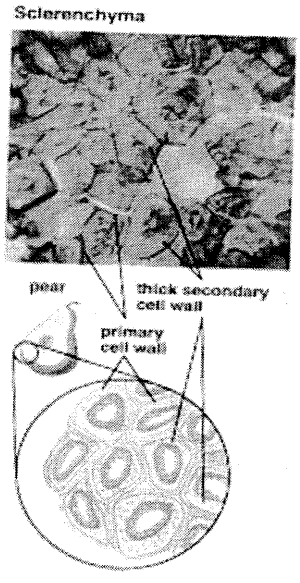
collenchyma: support cells with thickenings at corners

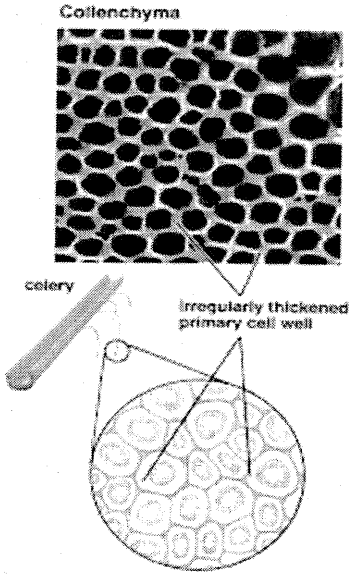
xylem: water conducting tissues of tracheid and vessel cells, dead when they function

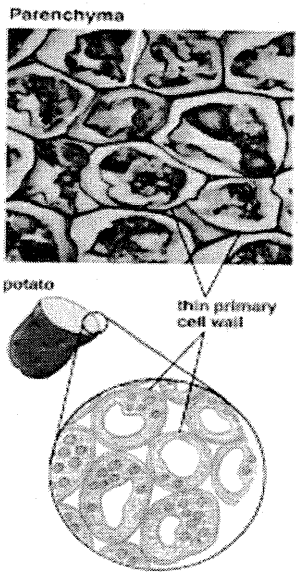
phloem: nutrient conducting tissues of sieve tube and companion cells, alive when functional

epidermis: outermost protective cell layers/ often have cutin; guard cells are specialized members







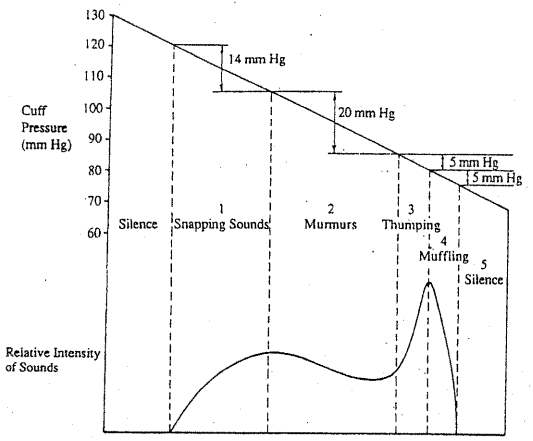


Laboratory 10: Physiology of the Circulatory System

*measure pulse rate
*measure blood pressure
*describe the relationship between changes in heart rate and blood pressure relative to changes in body position
*describe the relationship between changes in heart rate and exercise
*determine the "fitness index" for an adult human
*perform statistical analysis on class data
*define Q_{10}
*determine the Q_{10} of heart rate in a living organism such as *Daphnia*

Blood pressure tell one much about physiology; when the ventricles contract, blood pressure (directly dependent on amount of blood pumped per minute and the resistance to flow) is increased throughout the arteries. A sphygmomanometer allows you to temporarily cut off blood flow in the brachial artery, then listen through a stethoscope for Korotkoff's sounds. The first sound marks the point at which greatest force is being exerted = systolic pressure/ the last sound heart marks the point of lowest force = diastolic pressure.

The Five Phases of the Sounds of Korotkoff



During physical exertion, the cardiac rate (beats per minute) increases. You can measure this by pulse rate. Please note that physically fit individuals may have the same pulse rate as sedentary ones, but they will have higher stroke volume (milliliters delivered per beat) so their cells receive more oxygen. Under exertion, their rate will go up more slowly, and their rate return to "normal" more quickly.

An interesting test of fitness can be done by

(a) measuring two-minute reclining rate, then measure immediately upon standing; record the change in systolic pressure
(b) record pulse rate after two minutes of easy standing
(c) record pulse rate after reclining for five minutes
(d) measure the pulse rate immediately after standing from part c/ record the increase in pulse rate between the two
(e) do a step test on an 18-inch stool for 5 repetitions/ record pulse rate from 1-15 seconds, 16-30. 31-60. 61-90, and 91-120 seconds/ also record the time it takes for pulse to return to normal

Fitness Data

		Measurement	Points
Test 1.	Change in systolic pressure from reclining to standing	_____ mm Hg	_____
Test 2.	Standing pulse rate	_____ beats/min	_____
Test 3.	Reclining pulse rate	_____ beats/min	_____
Test 4.	Baroreceptor reflex Pulse rate increase on standing	_____ beats/min	_____
Test 5.	Step Test Return of pulse to standing rate after exercise	_____ seconds	_____
	Pulse rate increase immediately after exercise	_____ beats/min	_____

TOTAL SCORE _____

Total Score	Relative Cardiac Fitness
18-17	Excellent
16-14	Good
13-8	Fair
7 or less	Poor

Change in Systolic Pressure from Reclining to Standing

mm Hg	points
rise of 8 or more	3
rise of 2-7	2
no rise	1
fall of 2-5	0
fall of 6 or more	−1

Standing Pulse Rate

Beats/min	Points
60-70	3
71-80	3
81-90	2
91-100	1
101-110	1
111-120	0
121-130	0
131-140	−1

Reclining Pulse Rate

Beats/min	Points
50-60	3
61-70	3
71-80	2
81-90	1
91-100	0
101-110	−1

Reclining Pulse (beats/min)	Pulse Rate Increase on Standing (# beats)				
	0-10	11-18	19-26	27-34	35-43
	Points				
50-60	3	3	2	1	0
61-70	3	2	1	0	−1
71-80	3	2	0	−1	−2
81-90	2	1	−1	−2	−3
91-100	1	0	−2	−3	−3
101-110	0	−1	−3	−3	−3

Time Required for Return of Pulse Rate to Standing Level after Exercise

Seconds		Points
0-30		4
31-60		3
61-90		2
91-120		1
121+		1
1-10	beats above standing pulse rate	0
11-30	beats above standing pulse rate	−1

Pulse Rate Increase after Exercise

Standing Pulse (beats/min)	Pulse Rate Increase Immediately after Exercise (# beats)				
	0-10	11-20	21-30	31-40	41+
	Points				
60-70	3	3	2	1	0
71-80	3	2	1	0	−1
81-90	3	2	1	−1	−2
91-100	2	1	0	−2	−3
101-110	1	0	−1	−3	−3
111-120	1	−1	−2	−3	−3
121-130	0	−2	−3	−3	−3
131-140	0	−3	−3	−3	−3

The relationship between heart rate and temperature can be represented graphically and/or by the calculation of a value known as Q_{10} . This value is an exponential expression of the effect of temperature change. Q_{10} is a value representing the effect of raising the temperature 10° C. For example, a Q_{10} of 2 indicates the raising the temperature 10° will cause the rate being studied to double.

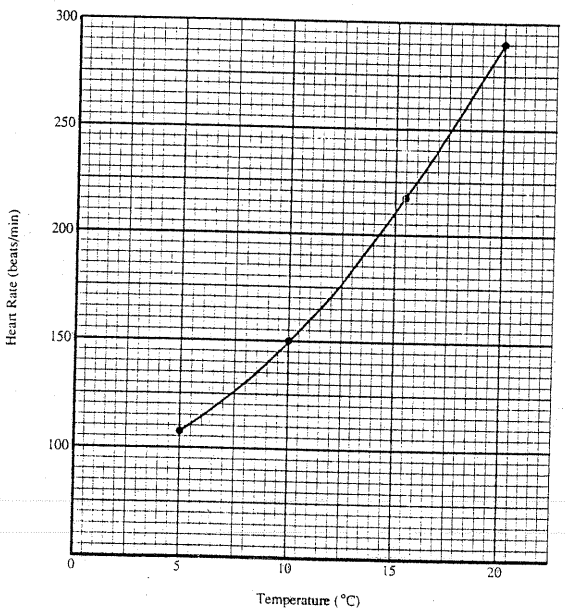
The general formula is:

t_2 = higher temperature
t_1 = lower temperature
k_2 = rate at temperature t_2
k_1 = rate at temperature t_1

Immobilize a *Daphnia* by placing it in a small section of capillary tube and sealing it at both ends. Place in a bowl of room temperature water and record the number of heartbeats for 10 seconds (multiply by 6 for beats / minute). Move the capillary tube to a bowl at 0-5° stable temperature, and let stabilize for 1 minute. Record the heart rate. Add warm water until the temperature is 5° higher, record the rate and so on until you get back to room temperature. Determine a Q_{10}.

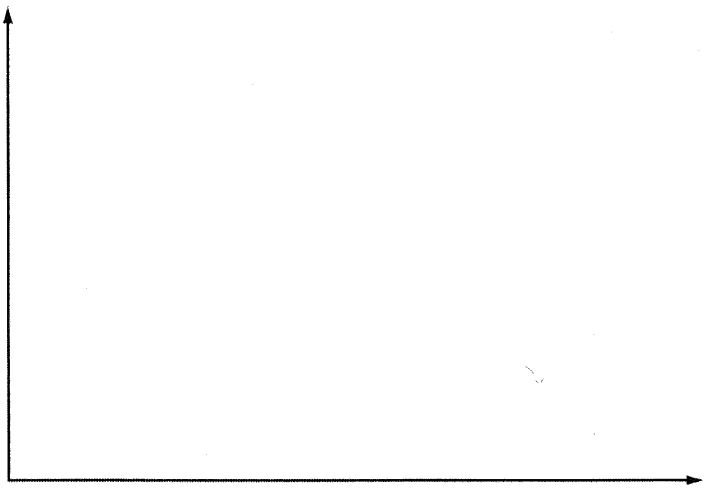
Temperature (°C)	*Daphnia Heart Rate (beats/min)*
5	108
10	152
15	211
20	290

Heart Rate and The Effect of Temperature



Lab 10
Circulation/Respir.

2. In mammals, heart rate during periods of exercise is linked to the intensity of exercise.
   (a) **Discuss** the interactions of the respiratory, circulatory, and nervous systems during exercise.
   (b) **Design** a controlled experiment to determine the relationship between intensity of exercise and heart rate.
   (c) On the axes provided below, **indicate** results you expect for both the control and the experimental groups for the controlled experiment you described in part B. Remember to label the axes.



3. The physical form of cells and organisms is often influenced by special structural polymers. Choose **one** polymer from **each** of the following three pairs of polymers:

   Pair 1: tubulin . . myosin

   Pair 2: cellulose . . chitin

   Pair 3: messenger RNA . . transfer RNA

   For each of the three polymers you have chosen, **describe** its
   (a) structure, and
   (b) role in a cell or organism.

4. A triploblastic animal is one in which three germ layers form during embryonic development. Triploblastic animals include acoelomate, pseudocoelomate, and coelomate (eucoelomate) organisms.
   (a) **Identify** the three germ layers of a triploblastic embryo and **discuss** the fates of these germ layers in embryonic development.
   (b) **Describe** acoelomate, pseudocoelomate, and coelomate body plans. **Identify** an animal that is representative of **each** of these types of body plan.
   (c) **Compare and contrast** the digestive systems of an acoelomate and a coelomate organism.

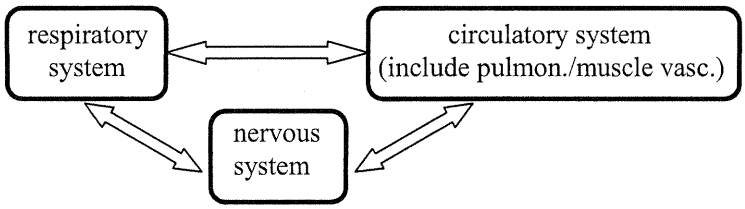
END OF EXAMINATION

AP® BIOLOGY
2002 SCORING GUIDELINES (Form B)

Question 2

2. (a) **Discuss** the interactions of the respiratory, circulatory, and nervous systems during exercise. (**4 points maximum**)

Note: Must have a "detail" on one side or the other of the interaction
non-inclusive list of possible examples . . .



nervous system medulla oblongata ANS/sympathetic, adrenalin chemosensory neurons (pH, O_2, CO_2, etc.)	← *interacting with* →	respiratory system diaphragm/ intercostals bronchodilation
respiratory system alveoli (small, thin air sacs)	← *interacting with* →	circulatory system capillaries, erythrocytes, hemoglobin
circulatory system blood containing O_2 /glucose	← *interacting with* →	exercising muscle produces ATP using O_2/glucose anaerobic – lactic acid
nervous system accelerator nerve ANS, sympathetic neurons chemosensory neurons (pH, O_2, CO_2, etc.)	← *interacting with* →	circulatory system SA/AV node (heart rate) vasodilation/vasoconstriction stroke volume

2. (b) **Design** a controlled experiment to determine the relationship between intensity of exercise and heart rate. (**4 points maximum**)

- hypothesis statement/prediction of results
- correctly describe the concept of a "control" group (baseline, resting)
- specify matched subjects (age, sex, fitness, twins, etc.)
- describe parameters of the exercise protocol
- describe how the heart rate will be measured (e.g., pulse, EKG, etc.)
- specify all other conditions stay the same (only one independent variable)
- statistical analysis
- large sample size/repetition (reliability)

Question 2 (cont'd.)

2. (c) On the axes provided below, **indicate** results you expect for both the control and the experimental groups for the controlled experiment you described in part B. Remember to label the axes. (**3 points maximum**)

- axes labeled with continuous scalar values and correct unit
- independent variable on X axis, dependent (results) on Y axis
- plots indicate correct relationship between control and experimental group

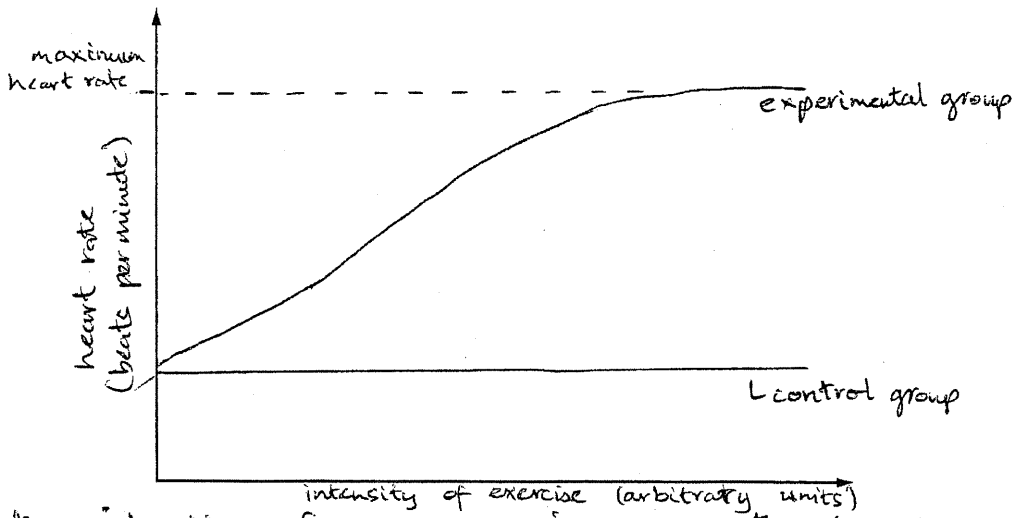
Q2-A
p.1 of 2

2. In mammals, heart rate during periods of exercise is linked to the intensity of exercise.

   (a) **Discuss** the interactions of the respiratory, circulatory, and nervous systems during exercise.

   (b) **Design** a controlled experiment to determine the relationship between intensity of exercise and heart rate.

   (c) On the axes provided below, **indicate** results you expect for both the control and the experimental groups for the controlled experiment you described in part B. Remember to label the axes.



a) As the intensity of exercise increases, the heart rate increases in order to provide oxygen for respiring muscle cells and to remove carbon dioxide and heat. ~~The~~ Chemoreceptors and barroreceptors in the carotid bodies and aorta measure CO_2 concentrations and blood pressure. The breathing rate and depth of breathing increases. Ventilation in the lungs increases. The sympathetic nerves of the autonomic nervous system stimulate the sinoatrial node of the heart. There is faster depolarization and so the rate of heartbeat increases. There is an increased blood flow to the muscle cells. This provides them with more oxygen for aerobic respiration. ~~Expr~~ Respiration releases energy, in the form of ATP, for muscle contraction. The hypothalamus is also involved. If CO_2 concentrations rise above normal, the hypothalamus stimulates the heart to beat faster.

GO ON TO THE NEXT PAGE.

ADDITIONAL PAGE FOR ANSWERING QUESTION 2

b)

b) The independent variable is the intensity of exercise while the dependent variable is the heart rate. ~~The intensity of exercise can be measured~~ Ask someone to step onto a treadmill and connect wires to his body that allow his heart rate to be measured. The heart rate is measured in beats per minute. Change the speed and inclination of the treadmill to alter the intensity of exercise. The control, in this experiment, is another person of the same age, gender and state of health, sitting down and exerting no physical effort.

Take readings of the heart rate at different levels of exercise intensity. Plot a graph of the results. Repeat the experiment with a different person. Again this person should be of the same age, gender and state of health. The results should concur. Take the average of the results and hypothesize. The experiment shows that as intensity of exercise increases, the heart rate increases. There is a direct relationship between the two variables. The results also show that the experimental group reach a certain maximum heart rate beyond which there is no increase, no matter what the level of exercise intensity is. In addition, the control group should show virtually no change in heart rate. The graph for the control group remains fairly constant. This is known as the resting heart rate.

Laboratory 11: Animal Behavior

*describe the relationship between dependent and independent variables
*discuss the value of comparing experimental results with control results
*graph and interpret histogram data
*measure volumes, distances, and temperature using metric scales
*design and conduct an experiment to measure the effect of environmental variables on habitat selection

An organism's habitat is made up by both biotic factors (life forms) and abiotic factors (wind, moisture, temperature, pH, etc.). Brine shrimp are small crustaceans that occur in salt lakes or brine ponds worldwide; they are very hardy, and can be hatched from eggs in about 2 days. This lab allows students to find the most favored physical conditions of the brine shrimp *Artemia* by allowing them to migrate along a range of conditions until they congregate at the point best suited to their physiology.

Each setup will involve filling a 1-meter long tygon plastic hose with about shrimp in solution. Three clamps are placed 25 cm apart long the hose so that individual sections can be later closed off. All experiments will be run in the dark (under a black felt cloth) unless light is the variable.

One tube is set up as the "control"/ no range of conditions is created. A second tube has an ice bag placed at one end, a hot water bottle at the other so that a temperature gradient is set up. A third tube has 1 ml 1M HCl placed in one end, 1 ml 1M NaOH in the other. A fourth tube is placed under a florescent light with sections covered by 8 layers of screening, 4 layers, 2 layers, and no screen at all.

After 30 minutes, clamps are fastened in all the tubes, trapping the shrimp in one of four quadrants. The sections are individually emptied into test tubes and shrimp counted (usually just 1 ml is sufficient; count several and take the average). Dead shrimp are not counted since they could not migrate.

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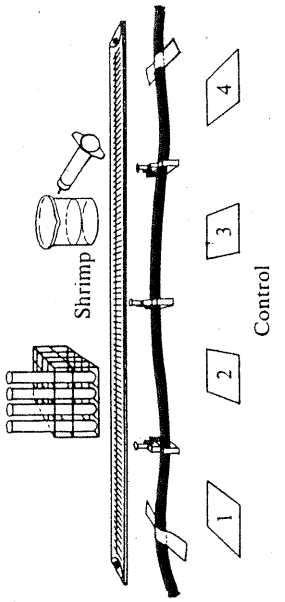
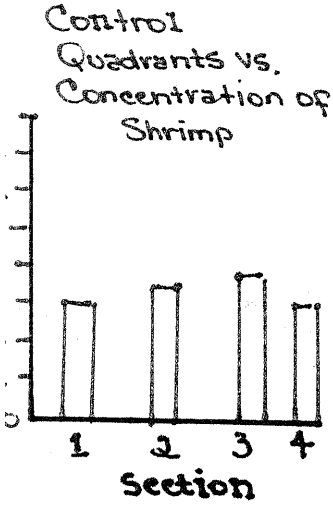
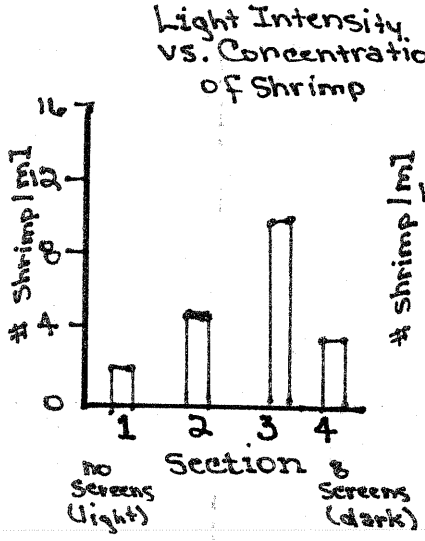


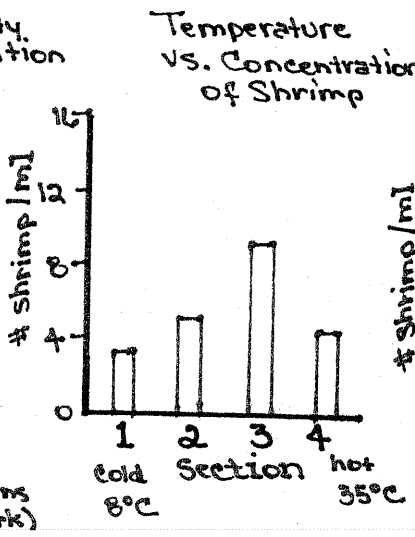
Sample Table for Reporting Data

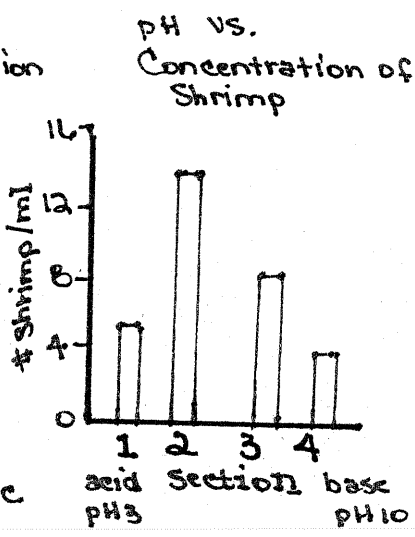
	Section																							
	light cold acid		1					2					3					4 dark hot base					
	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.
Control	5	7					8	5					7	9					6	5				
	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.
Light Gradient	2	2					4	7					12	10					5	3				
	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.
Temperature Gradient	2	4					4	7					7	14					3	7				
	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.	1	2	3	4	5	Av.
pH Gradient	6	5					16	13					7	9					1	6				

Shrimp / ml









Free-Response Question 3

*A scientist working with **Bursatella leachi,** a sea slug that lives in an intertidal habitat in the coastal waters of Puerto Rico, gathered the following information about the distribution of the sea slugs within a ten-meter square plot over a 10-day period.*

DISTRIBUTION OF SLUGS WITHIN A TEN-METER SQUARE PLOT

Time of Day	*Average Distance Between Individuals (cm)*
Midnight	*8.0*
4 A.M.	*8.9*
8 A.M.	*44.8*
NOON	*174.0*
4 P.M.	*350.5*
8 P.M.	*60.5*
Midnight	*8.0*

a. *For the data above, provide information on each of the following.*
   - *Summarize the pattern.*
   - *Identify THREE physiological or environmental variables that could cause the slugs to vary their distance from each other.*
   - *Explain how each variable could bring about the observed pattern of distribution.*

b. *Choose ONE of the variables that you identified and design a controlled experiment to test your hypothetical explanation. Describe results that would support or refute your hypothesis.*

Question 3 Standards

Overall Commentary for Question 3

Question 3 was composed of two discrete parts in which part a asked the student to analyze and interpret data; and part b directed the student to choose one of the variables identified and design an experiment. Three bullets in part a directed the student to: (1) summarize the pattern; (2) identify three physiological or environmental variables that would account for the observed data; and (3) explain how each variable would support the observed pattern of distribution. Part b asked the student to design a controlled experiment and describe how the experiment would support or refute the hypothesis.

Part a: From the data

Maximum for part a = 6 points

1 point — Summarize pattern (dispersal — day / clumped — night)
1 point — 3 physiological or environmental variables (1st three **only** and **TESTABLE**)

carbon dioxide	light	rhythms
competition	mating	salinity
desiccation	metabolism	taxis
endogenous	oxygen	temperature
feeding	pH	tidal exchange
foraging	predation	water depth
hormonal	protection	(**Others possible**)

1 point each — For a clear and plausible explanation of variable as it influences
(3 maximum) the observed distribution pattern (vary)

1 point — Elaboration

Part b: Controlled experiment for one variable

Maximum for part b = 6 points

1 point — Control — constants (explicit)
1 point — Manipulation of variable
1 point — Measurement (quantitative)
1 point — Verification (sample size / repetition)
1 point — Hypothesis (if:then) **TESTABLE**
1 point — Statistical analysis of data
1 point — Results as related to hypothesis
1 point — Elaboration

Only ONE extra elaboration point may be earned in either part a or part b — for extensive, unique, or exceptional effort.

Samples of Student Wording

Hypothesis — I hypothesize that as the temperature of the water increases the distance between the slugs will increase.

Manipulation of variable — One tank of slugs was kept at 20 degrees C and the other tank at 25 degrees C.

Measurement — A ruler was used to measure the distance between the slugs — initial-final. (This point was given when a student explicitly describe how to measure the effect of the variable. Implication was insufficient.)

Verification — The experiment was repeated five times to verify the results.

Control — Both tanks of slugs were exposed to the same amount of light. The water in both tanks was held at a constant pH, dissolved oxygen and salinity. (We considered that it was necessary to explain how the control was to function for this point. Additionally, we were looking for a discrete if:then hypothesis prediction or a close equivalent for describing the hypothesis rather than the implied hypothesis resulting from part a explanations.)

Statistical Analysis — The results from the five trials were averaged (or students could cite the use of Chi-square).

Results as related to hypothesis — If the slugs in the tank at 25 degrees C had a larger mean distance between them the hypothesis would be supported.

Elaboration — of any of the above such as a reference to the literature, an exceptional design, or use of tables.

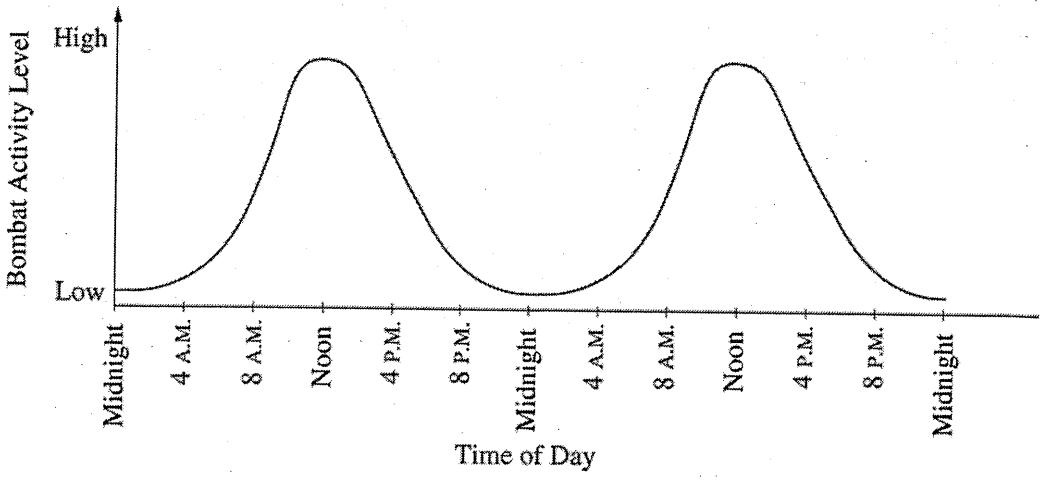
EXCELLENT ESSAY (10 points)

(a) In the experiment with the sea slug, there is a definite pattern involved around midnight the slugs are the closest together and they then proceed, as the day goes on to move farther and farther from each other until 4:00 pm when they are at their farthest. They then proceed to quickly move closer together until midnight when they are again very close together. Three physical or environmental variables that could cause the slugs to vary their distance could be the temperature, the amount of light available, and the tide as it comes in and goes out. Temp. differences could cause changes in the slugs metabolic rate of activity and colder night air could cause them to move closer and less as their systems are slowed down. Light could cause them to not move as much and congregate because they are sensitive to the light. When there is more light, they are more active. Finally, the tide because it cycles every 24 hours, could have been out at night causing the slugs to congregate in tide pools whereas during the day they could go wherever they wanted as long as they could stay in the water.

(b) In a controlled experiment to test my hypothetical explanation of the Tides part in the slugs distance, I would have 3 three different boxes all with sand and algae and the components of the ocean floor that the slugs need to live on. I would keep the temperature of the air and water at a constant 32°C, and the light would be constantly equal to that of mid day. In one of the three boxes there would be plenty of water to cover the entire box. In the second, there would be only tidal pools. In the third the water level would be changed as that of an incoming and outgoing tide. For a couple weeks I would watch the slugs and take information regarding their position at 4 hour intervals. If the slugs did not congregate in the pools of tank two and tank 3 when the "tide" is out, then my hypothesis would be incorrect, or if the congregated in the tank 1 or in Tank 3 when the "tide" was in, I would be wrong as well. If I was correct, In tank 1, the slugs would be dispersed in tank two they would be clustered, and in Tank 3 they would alternate depending if the tide was in or out.

Lab #11
Animal Behavior

2. The activities of organisms change at regular time intervals. These changes are called biological rhythms. The graph depicts the activity cycle over a 48-hour period for a fictional group of mammals called pointy-eared bombats, found on an isolated island in the temperate zone.



(a) **Describe** the cycle of activity for the bombats. **Discuss** how **three** of the following factors might affect the physiology and/or behavior of the bombats to result in this pattern of activity.

- temperature
- food availability
- presence of predators
- social behavior

(b) **Propose** a hypothesis regarding the effect of light on the cycle of activity in bombats. **Describe** a controlled experiment that could be performed to test this hypothesis, and the results you would expect.

3. The complexity of structure and function varies widely across the animal kingdom. Despite this variation, animals exhibit common processes. These include the following.

- transport of materials
- response to stimuli
- gas exchange
- locomotion

(a) Choose **two** of the processes above and for each, describe the relevant structures and how they function to accomplish the process in the following phyla.

Cnidaria (e.g., hydra, jellyfish)
Annelida (e.g., earthworm)
Chordata (e.g., mouse)

(b) Explain the adaptive (evolutionary) value(s) of the structural examples you described in part a.

2R,

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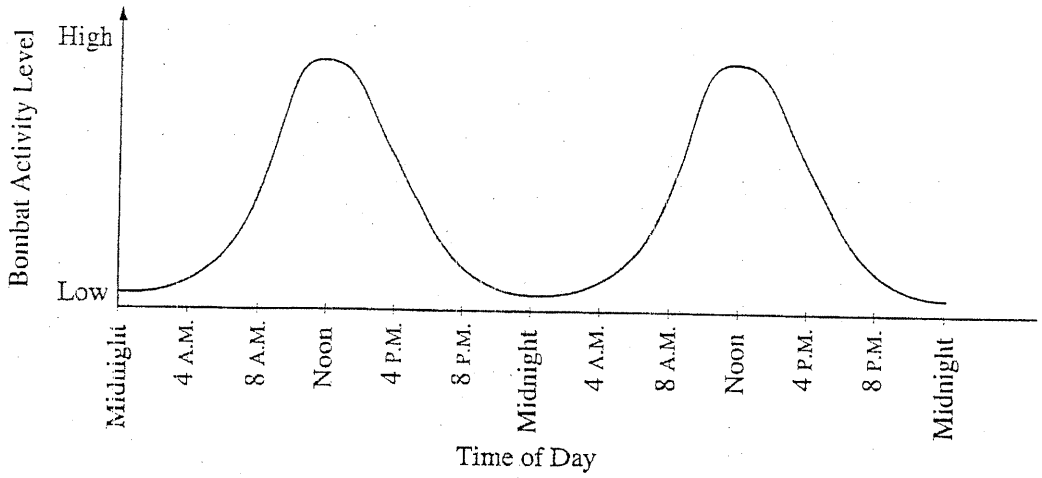

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- temperature
- food availability
- presence of predators
- social behavior

(b) **Propose** a hypothesis regarding the effect of light on the cycle of activity in bombats. **Describe** a controlled experiment that could be performed to test this hypothesis, and the results you would expect.

(A.) The lowest point of activity occur consistently at midnight. As the day progresses the activity continues to increase until it peeks around noon time. Then after that the activity begins a steady decline until its lowest point at midnight. One reason that may cause this is food availability. Since many organisms are inactive during the night the bombats wouldn't be able to acquire a lot of food and would just waste energy finding it. However, as the day wears on activity increases, which means the prey the bombat hunts also increases its activity. The high point for both is around noon, and continues to decrease after that as night begins to approach.

The second factor could be temperature. During the night it is cooler and the sun is not out. That means that organisms

GO ON TO THE NEXT PAGE.

2R2

ADDITIONAL PAGE FOR ANSWERING QUESTION 2

sleep to conserve body heat and energy. ~~[illegible]~~ As the sun begins to rise temperature goes up and more and more organisms ~~[illegible]~~ begin to move about. When the sun is at its highest point around noon the temperatures are high and ~~[illegible]~~ organisms are now everywhere. This means that it is easier for combats to find food and they expend less energy keeping warm because the sun's rays do it for them. As temperatures begin to cool down organisms begin to retreat back into their homes and activity decreases.

The last factor could be predators. The organisms that hunt combats may be nocturnal and hunt them at night. The combats activity decreases so that they have a better chance of NOT getting captured by a predator. Since most nocturnal animals sleep during the day the combats are most active then because their chances of getting killed are less. As night approaches they slow down their activity because predators will be around and more and more of them will become active during the night so again, decreasing their activity at night lowers their chances of dying.

(5) The problem is whether or not ~~[illegible]~~ light has an effect on the activity of combats. One testible hypothesis is that ~~[illegible]~~ more light helps the combats see their prey better, which would account for their increased activity during the hours around noontime.

2R3

ADDITIONAL PAGE FOR ANSWERING QUESTION 2

One way to test this is to first select an area of land where ~~the~~ combats and their prey are living. ~~For the experimental group~~. For the control group monitor how effectively the combats can capture prey during certain times of the days and record results. It will be necessary to mark about 30 individuals and monitor their progress only over a period of about 3 days.

For the experiment group, catch a new group of 30 combats and mark them. Use another plot of land with the same / near same conditions as the other one. Record results for how effectively this group of combats can capture prey for three days using the same method as before. For the variable, at night set up a large flood-light as light source. Then record the amount of prey caught by the combats for three more days. Recording the results would be displayed best on a bar graph. The x-axis would be the time of day and the y-axis would be the amount of prey caught. If the light did have an affect on improving the combats sight then the amount of prey caught in the ~~experimental~~ experimental group should be higher.

Thus, if the results proved the hypothesis, then that means that light does have an ~~affect~~ effect on combats catching their prey. This would also prove why they hunt more during the day time then at night.

Laboratory 12: Dissolved Oxygen and Aquatic Primary Productivity

*describe the physiological importance of carbon and oxygen in an ecosystem
*understand the physical and biological factors that affect the solubility of dissolved gases in aquatic ecosystems
*describe a technique for measuring dissolved oxygen
*define primary productivity
*describe the relationship between dissolved oxygen and the process of photosynthesis and respiration as they affect primary productivity in an ecosystem
*design an experiment to measure primary productivity in an aquatic ecosystem
*understand the effect of light and nutrients on photosynthesis

Oxygen is critical to the maintenance of the life processes of nearly all organisms. In the aquatic environment, it must be in solution in a free state before it is available for use by organisms. Its concentration and distribution are directly dependent on the chemical and physical factors of the water, and are greatly affected by biological processes. The atmosphere is about 20% O_2; the water contains only 0.5-1% dissolved oxygen (DO). Measurement of oxygen in an aquatic environment is a very important indicator of water quality.

Salinity and temperature have great effects on DO; both seem to decrease the available oxygen as they increase. Respiration and photosynthesis rates are also important.

The fertility of any body of water depends on the productivity of the green plants there: the primary productivity of an ecosystem is the rate at which sunlight is stored by plants in the form of organic materials (rate of photosynthesis). The rate of oxygen production in this process can be used to calculate the fixation rate [1 ml O_2 = 0.536 mg of carbon]. One method of measuring O_2 production is the light and dark bottle method. DO concentrations of samples are measured and compared after incubation in light and darkness; the difference between the bottle is the total oxygen productivity = gross productivity.

The Winkler method is often used to measure DO.

a. add alkaline iodide and manganous sulfate to water sample
b. manganous hydroxide is produced/ when acidified, is converted to tetravalent manganese compound by available O_2
c. compound reacts with iodide to release iodine, coloring the water yellow
d. titration with sodium thiosulfate will finally render solution colorless (amount = amount of dissolved O_2)

Using BOD bottles (biological oxygen demand), you can take initial DO reading: one over 24-hour period with light, one over same period with dark, and in bottles with nitrogen and phosphorus added ("enriched").

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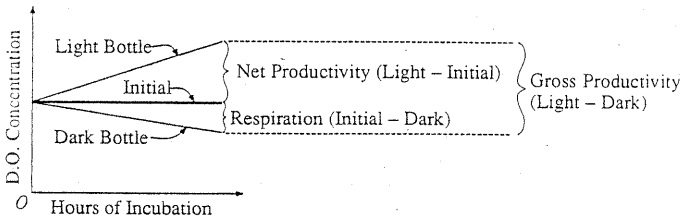


Class Averages for different Light Intensities using a *Chorella* culture

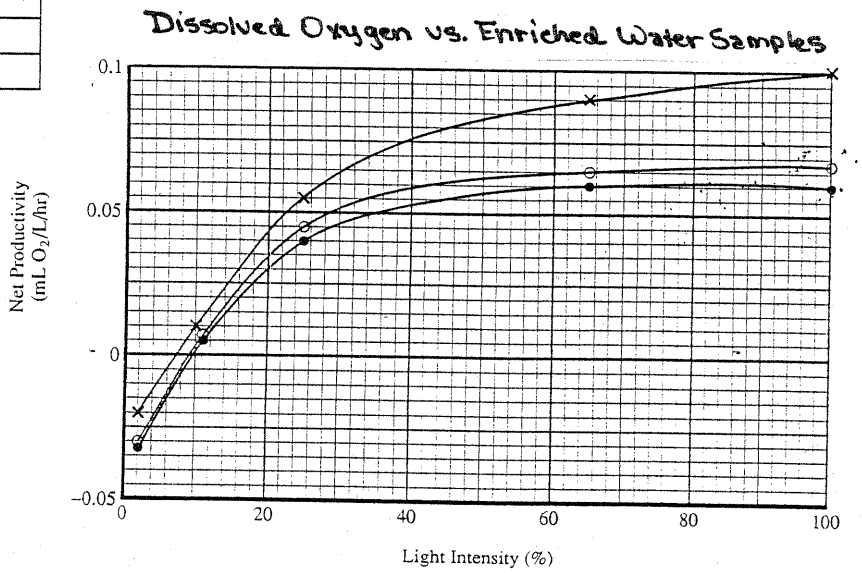
% Light	Gross*			Net*		
	N	P	—	N	P	—
100	0.19	0.16	0.15	0.10	0.07	0.06
65	0.18	0.15	0.15	0.09	0.06	0.06
25	0.14	0.13	0.13	0.06	0.04	0.04
10	0.10	0.10	0.10	0.01	0.01	0.01
2	0.07	0.06	0.06	-0.02	-0.03	-0.03

*$mL O_2/L/hr$

Respiration*
0.09

DO Concentration Data Sheet — Class Sample

Temperature	Mean DO (mg/L)	%DO Saturation
5°c	9.3	>0
20°c	8.9	95
30°c	8.5	110



Lab #12 Dissolved Oxygen

Question 3
2001

A biologist measured dissolved oxygen in the top 30 centimeters of a moderately eutrophic (mesotrophic) lake in the temperate zone. The day was bright and sunny, and the wind was calm. The results of the observations are presented below.

Hour	[O2]
6:00 A.M.	0.9 mg/L
8:00 A.M.	1.7 mg/L
10:00 A.M.	3.1 mg/L
12:00 noon	4.9 mg/L
2:00 P.M.	6.8 mg/L
4:00P.M.	8.1 mg/L
6:00 P.M.	7.9 mg/L
8:00 P.M.	6.2 mg/L
10:00 P.M.	4.0 mg/L
12:00 midnight	2.4 mg/L

(a) Using the graph paper provided, **plot** the results that were obtained. Then, using the same set of axes, draw and label an additional line/curve representing the results that you would predict had the day been heavily overcast.

(b) **Explain** the biological processes that are operating in the lake to produce the observed data. **Explain** also how these processes would account for your prediction of results for a heavily overcast day.

(c) **Describe** how the introduction of high levels of nutrients such as nitrates and phosphates into the lake would affect subsequent observations. **Explain** your prediction.

The Grading Standards for Question 3:

*(a) Using the graph paper provided, **plot** the results that were obtained. Then, using the same set of axes, draw and label an additional line/curve representing the results that you would predict had the day been heavily overcast.* (4 points possible)

(1) Proper orientation of graph (independent variable on x-axis)

(1) Graph (all must be present):
- Uniform spacing of units
- Correct labeling of axes:
  - proper numbering (a minor error acceptable)
  - X-axis label: "Time"/ "Hour"/AM, PM labels/a conversion to integers requires unit label as well
  - Y-axis: oxygen label and mg/L Unit

(1) Correct plot of data points
-no connecting line necessary
-No point if more than one data point is misplotted
-No point if there is a solid extrapolation line beyond the 6:00 AM data point to the origin or beyond the 12 midnight data point

(1) Drawing the cloudy day prediction Line/Curve (all must be present):
- Distinguish between the two curves with a legend or direct labeling of one curve
- Position completely under the bright-day curve (may touch toward the tails)
- There must be some curve to the line (no flat lines)

(b) ***Explain*** *the biological processes that are operating in the lake to produce the observed data.* ***Explain*** *also how these processes would account for your prediction of results for a heavily overcast day.* (5 points possible)

(1) Photosynthesis: production of O_2 correlated with light changes (i.e. explains changes in shape of bright-day curve). The student must link photosynthesis to increase in light to increase in O_2 production. The student must use the term "photosynthesis" or an excellent replacement such as the chemical equation for the process.

(1) Respiration: consumption of O_2. Must link respiration to decrease in O_2. The student must use the term "respiration" or an excellent replacement such as the chemical equation for the process or the name of another process such as "decomposition."

(1) Description of the interaction of the above: photosynthetic rate changes while respiration rate remains relatively constant.

(1) Overcast prediction curve explanation:
- Reduced light leads to decreased photosynthetic O_2 production, etc.
- No point given if there is no prediction line/curve on the graph.

(1) Elaboration point (1 max) for any one of the above. Examples of elaboration may include, but are not limited to
- Water split/photolysis to produce O_2 in the light phase, etc.
- Balanced equation for photosynthesis or respiration (unless used as a substitute for the term above)
- Description of "light phase" processes (photosystem II, etc)
- Gross vs. net productivity

(c) ***Describe*** *how the introduction of high levels of nutrients such as nitrates and phosphates into the lake would affect subsequent observations.* ***Explain*** *your prediction. (3 points possible)*

(1) Describe (predict) a change in lake conditions such as (must be related to the question):
- increased/decreased O_2
- increased/decreased biomass or numbers of organisms
- increased/decreased CO_2
- long-term or short-term changes
- no change

(1) Explanation of the prediction above
- may include toxic effects due to significant changes in pH, altered osmolarity, etc.

(1) Elaboration on the explanation of the prediction above or long term ecological consequences to lake.

3. A biologist measured dissolved oxygen in the top 30 centimeters of a moderately eutrophic (mesotrophic) lake in the temperate zone. The day was bright and sunny, and the wind was calm. The results of the observations are presented below.

3Q, (10 pts)

Hour	$[O_2]$
6:00 A.M.	0.9 mg/L
8:00 A.M.	1.7 mg/L
10:00 A.M.	3.1 mg/L
12:00 noon	4.9 mg/L
2:00 P.M.	6.8 mg/L
4:00 P.M.	8.1 mg/L
6:00 P.M.	7.9 mg/L
8:00 P.M.	6.2 mg/L
10:00 P.M.	4.0 mg/L
12:00 midnight	2.4 mg/L

(a) Using the graph paper provided, **plot** the results that were obtained. Then, using the same set of axes, draw and label an additional line/curve representing the results that you would predict had the day been heavily overcast.

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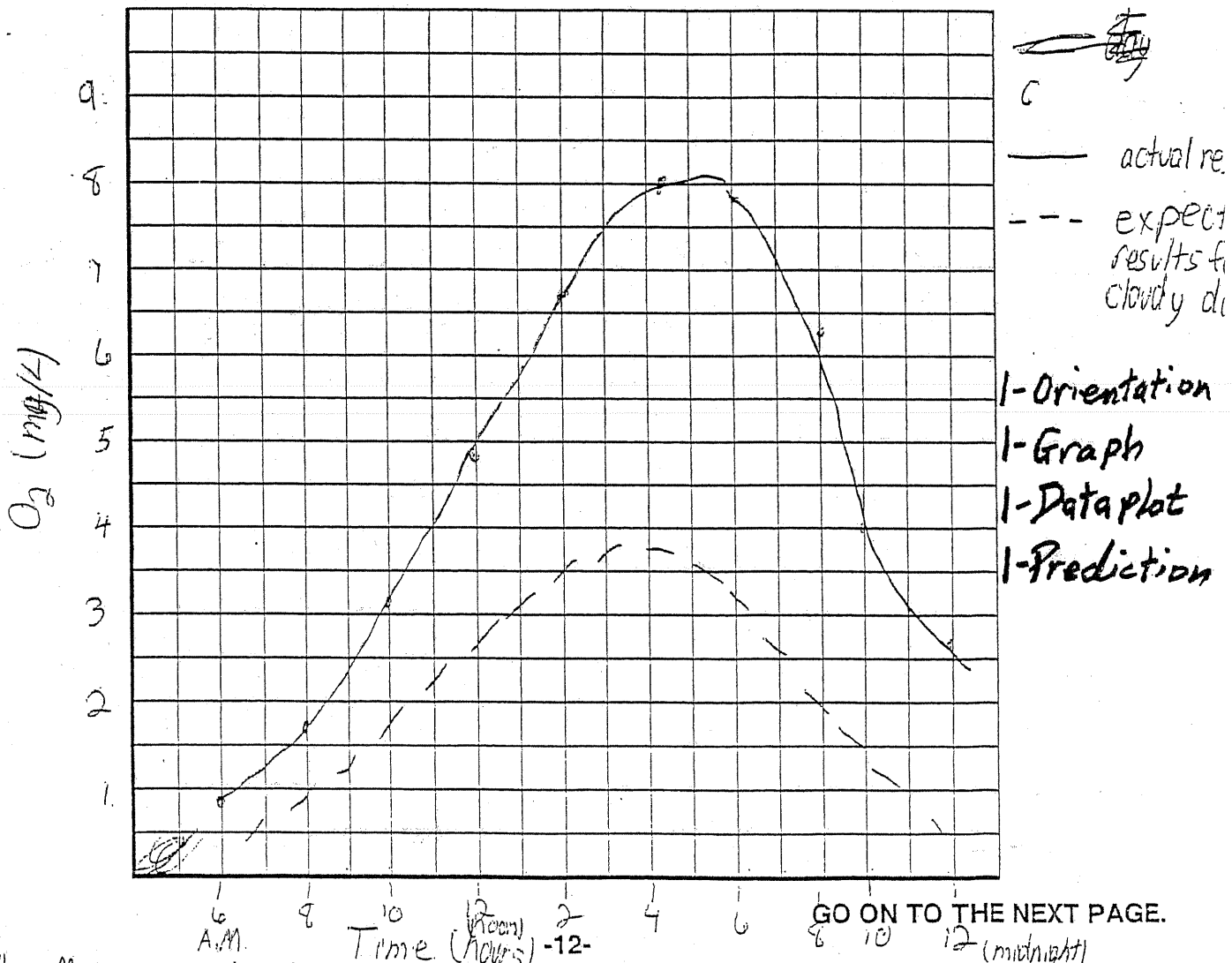

Graph 1. Measurement of Dissolved O_2

3 Q2

ADDITIONAL PAGE FOR ANSWERING QUESTION 3

B. ~~At~~ In the ~~morning~~ morning dissolved O_2 levels are low. At mid-day the levels peak, and then later in the day the levels lower. One reason for the dO_2 levels is the amount of sunlight reaching the lake. As the day continues, more sunlight reaches the plants in the lake. Because sunlight is used to initiate photosynthesis, more photosynthesis occurs with more sunlight. The sunlight helps excite the electrons in photosystems I & II which aid in the production of ATP and NADPH which are used to produce glucose and as a waste product O_2. the more ~~plan~~ sunlight the more photosynthesis, the more O_2 is given off as a waste product. The O_2 levels are also lower at night because the pl animals in the lake still have to perform cellular respiration which causes the O_2 levels to drop because O_2 is needed. ① Resp. ① photo. Photosynthesis also slows down or stops because there is no light which is needed for the process. The O_2 levels would be lower on an overcast day because there is not as much sunlight reaching the plants, thus causing less photosynthetic activity thus producing less O_2. ① Explanation of curve Also the animals ~~and~~ & plants continue cellular respiration. Since plants don't photosynthesize as much, there is not an equal production and consumption of O_2 causing O_2 levels to be lower than normal. ① Interaction

GO ON TO THE NEXT PAGE.

3Q3

ADDITIONAL PAGE FOR ANSWERING QUESTION 3

C. If high levels of nutrients such as nitrates and phosphates were added then the lake would have higher levels of O_2. (1) Prediction This is because the nitrates would become part of the nitrogen cycle. It has been shown increased levels of nitrogen increase plant fertility. This may be because the nitrogen ~~are~~ is used in the production of DNA and nucleotides such as NADPH. Since NADPH is an energy molecule, and there is more energy w/the nitrogen, the plant has more energy to make food. The added phosphate will also help because it too is part of the NADPH ~~and~~ nucleotide. Phosphate can also be used in another energy molecule ATP. Phosphate will help provide more energy. Nitrogen & phosphate are both macronutrients meaning they are required in large amounts for plants.

(1) Explanation + possible elaboration

(10)

Laboratory 12: Dissolved Oxygen and Aquatic Primary Productivity

*describe the physiological importance of carbon and oxygen in an ecosystem
*understand the physical and biological factors that affect the solubility of dissolved gases in aquatic ecosystems
*describe a technique for measuring dissolved oxygen
*define primary productivity
*describe the relationship between dissolved oxygen and the process of photosynthesis and respiration as they affect primary productivity in an ecosystem
*design an experiment to measure primary productivity in an aquatic ecosystem
*understand the effect of light and nutrients on photosynthesis

Oxygen is critical to the maintenance of the life processes of nearly all organisms. In the aquatic environment, it must be in solution in a free state before it is available for use by organisms. Its concentration and distribution are directly dependent on the chemical and physical factors of the water, and are greatly affected by biological processes. The atmosphere is about 20% O_2; the water contains only 0.5-1% dissolved oxygen (DO). Measurement of oxygen in an aquatic environment is a very important indicator of water quality.

Salinity and temperature have great effects on DO; both seem to decrease the available oxygen as they increase. Respiration and photosynthesis rates are also important.

The fertility of any body of water depends on the productivity of the green plants there: the primary productivity of an ecosystem is the rate at which sunlight is stored by plants in the form of organic materials (rate of photosynthesis). The rate of oxygen production in this process can be used to calculate the fixation rate [1 ml O_2 = 0.536 mg of carbon]. One method of measuring O_2 production is the light and dark bottle method. DO concentrations of samples are measured and compared after incubation in light and darkness; the difference between the bottle is the total oxygen productivity = gross productivity.

The Winkler method is often used to measure DO.

a. add alkaline iodide and manganous sulfate to water sample
b. manganous hydroxide is produced/ when acidified, is converted to tetravalent manganese compound by available O_2
c. compound reacts with iodide to release iodine, coloring the water yellow
d. titration with sodium thiosulfate will finally render solution colorless (amount = amount of dissolved O_2)

Using BOD bottles (biological oxygen demand), you can take initial DO reading: one over 24-hour period with light, one over same period with dark, and in bottles with nitrogen and phosphorus added ("enriched").

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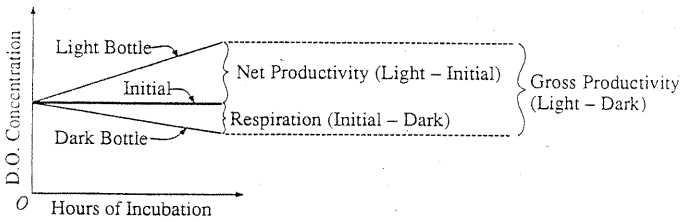

Class Averages for different Light Intensities using a *Chorella* culture

% Light	Gross*			Net*		
	N	P	—	N	P	—
100	0.19	0.16	0.15	0.10	0.07	0.06
65	0.18	0.15	0.15	0.09	0.06	0.06
25	0.14	0.13	0.13	0.06	0.04	0.04
10	0.10	0.10	0.10	0.01	0.01	0.01
2	0.07	0.06	0.06	-0.02	-0.03	-0.03

*$mL0_2/L/hr$

Respiration*
0.09

DO Concentration Data Sheet — Class Sample

Temperature	Mean DO (mg/L)	%DO Saturation
5°c	9.3	>0
20°c	8.9	95
30°c	8.5	110