The 7.014 Final Exam

*YOU MUST BRING YOUR ID.

This will be a cumulative, closed book exam. Please bring a non- programmable calculator.

Your ongoing interest in bioluminescent organisms has lead you to study the Hawaiian squid, *Euprymna scolopes*. Most nocturnal creatures cast shadows under the bright Hawaiian moonlight, and are easy prey to predatory fish. This type of squid can shine light downwards to match the moonlight and avoid casting a shadow thereby decreasing predation.

To provide squid for your studies, you maintain a large, self-supporting squid tank. The food web of the tank is shown below.



When your tank is at steady state, you can harvest 10 kg of squid carbon/week and all the other biomasses (phytoplankton, fish, squid, and detritivores) remain constant.

Given the following values:

- NPP of phytoplankton = 2.0 kilograms of organic carbon per gram of phytoplankton per day
- plant production efficiency = 60%
- fish exploitation efficiency = 50%
- fish assimilation efficiency = 20%
- fish net production efficiency = 5%
- squid exploitation efficiency = 50%
- squid assimilation efficiency = 80%
- squid net production efficiency = 40%

a) What NPP (in kilograms of carbon) is required to support a harvest of 10 kg of squid carbon per week at steady state? Show your work.

Question 1, continued

b) Month after month, your squid harvest is 10 kg/week and all the biomasses remain constant. One month, you find that you can only harvest 5 kg of squid carbon/week and maintain steady state.

You suspect that something has contaminated your tank and reduced your yield. You propose two models to account for the reduction in yield. To test these models, you measure the respiration rate of the detritivores in the tank. You find that detritivore respiration has increased by 5 kg of carbon per week.

<u>Model 1</u>: Phytoplankton photosynthesis is inhibited so that they now only produce 1.0 kilogram of organic carbon per gram of phytoplankton per day, but at this new steady state, all other efficiencies remain constant.

i) Is this model consistent with your measurement? Explain.

<u>Model 2</u>: 5 kg of fish per week are dying with all other efficiencies remaining constant.

ii) Is this model consistent with your measurement? Explain.

c) In their native ocean environment, the squid feed on a wide variety of small fish and invertebrates. You suspect that the squid is the keystone species in this ecosystem. What experiment could you do to test this theory? What result do you predict?

Question 2

a) You want to build a larger squid facility. To make better use of your resources, you plan to construct a life table to determine the mortality rate, fecundity, and the net replacement value of your population. You follow 1000 squid eggs and collect the data below.

stage	number
unfertilized eggs	1000
eggs hatched into juveniles	840
juveniles at 2 month	210
juveniles at 4 months	100
juveniles at 6 months	33
juveniles at 8 months = adult	24
adult at 10 months	14
adults at 1 year	5
adults at 2 year	0

i) If you were to make a cohort life table using this data, how many squid would you initially have in your cohort?

ii) If the fecundity of squid at 8 months = 10, and the fecundity of squid at 10 months = 16, during which 2 month time period are more offspring produced?

Question 2, continued

b) Surprisingly, your tank-raised squid are not luminous. In fact, the glow seen in free living squid is due to a species of bacteria, *Vibrio fischeri* that live in a specialized light organ within the squid. When juvenile squid hatch, ambient water enters the light organ. *V. fischeri* and the many other bacterial species in this ambient water also enter the light organ and colonize it. All the bacteria initially flourish in this nutrient rich environment, but after 10 hours, only *V. fischeri* remain in the light organ.

i) The interaction between *V. fischeri* and the other bacterial species initially colonizing the light organ can best be described as ______.

ii) The interaction between the squid and *V. fischeri* can best be described as ______

iii) Is the light organ of the squid the fundamental niche of *V. fischeri*. Why?

iv) Is the light organ of the squid the realized niche of *V. fischeri*. Why?

c) The interaction between the squid and *V. fischeri* is dynamic. *V. fischeri* are only luminescent when the density of cells is near carrying capacity, yet when nutrients become limiting, luminescence ceases. To overcome this problem, the squid expel 90-95% of the *V. fischeri* from the light organ as each new day begins. By the following evening, the bacteria that remained have repopulated the light organ and it is once again fully functional.

Using this information, graph the *V*. *fischeri* population within the light organ of a squid over several days. Indicate carrying capacity on the above graph.



a) Match each of the following structures to the type of molecule it represents:



Question 3, continued

b) Fill in the blanks in this representation of the central dogma.

Choose from:

nucleus, translation, RNA, membrane, DNA, cytoplasm, polysaccharide, transcription, or protein



Question 4

In analyzing differences between star squash players and armchair warriors, you have discovered a protein that exists only in the squash players. You design substrates that will bind to the "star protein".



Question 4, continued

a) Give the name for the strongest intermolecular interaction between the substrate and the following amino acids on the star protein. Choose from ionic bond, covalent bond, hydrogen bond, and van der Waals forces.

	Amino Acid	Strongest interaction
i)	Val	
ii)	Glu	
iii)	Asp	
iv)	Ala	

b) You make the following additional substrates



c) Which substrate would you expect binds the most tightly to the star protein?

substrate 1 substrate 2 substrate 3

Why?

STRUCTURES OF AMINO ACIDS at pH 7.0



To investigate the yeast metabolic pathway for serine biosynthesis, you screen for serine auxotrophs (mutants which are unable to grow without serine supplied in their growth medium). You isolate four such mutants, and test them for growth on medium supplemented with several intermediates (A, B and C) known to be part of the pathway. The results are shown below ("+" represents growth, "-" represents no growth).

	minimal	minimal	minimal	minimal	minimal
Strain	medium	+ A	+ B	+ C	+ serine
wild type	+	+	+	+	+
m1	-	+	+	-	+
m2	-	-	-	-	+
m3	-	+	-	-	+
m4	-	+	+	-	+

You then mate the haploid m1 strain with the haploid m4 strain to create a diploid yeast carrying both the m1 and the m4 mutations. You test the diploid for growth on the same conditions as above and observe that the diploid exhibits the same growth requirements as m1 or m4 haploid.

a) Are the m1 and m4 mutations in the same gene or different genes? Briefly explain your reasoning.

b) Draw the metabolic pathway for the synthesis of serine, consistent with the data given above. Include the intermediates (A, B, and C) and serine, and indicate which mutants (m1, m2, m3, m4) are defective at each step in the pathway.

c) You create a haploid strain that has both the m1 and m3 mutations.

i) This haploid mutant will grow on media supplemented with which of the following intermediate(s) (A, B and/or C)?

ii) Which of the following intermediate(s) (A, B and/or C) will accumulate when this haploid mutant is grown on minimal medium?

a) Indicate whether the following statements are true or false. If false, correct the statement or provide a brief explanation for why the statement is false.

i) DNA replication is initiated at promoter sequences in the DNA.

ii) RNA polymerase requires primers to initiate RNA synthesis.

iii) Okazaki fragments are the short fragments of DNA that are produced on the leading strand at the DNA replication fork.

iv) The 5' to 3' direction of DNA synthesis implies that deoxyribonucleotides are added to the 5' OH group on the growing strand.

v) Transcription terminates at stop codons in the mRNA sequence.

b) Shown below is the DNA sequence of a gene from a virus that encodes a short viral peptide, and also the sequence of the mRNA synthesized from this gene.

genomic DNA sequence:

5 ' - AGCTCATGTGCGAGTCCTGACGCTGACGTAGG-3 ' 3 ' - TCGAGTACACGCTCAGGACTGCGACTGCATCC-5 '

mature mRNA sequence:

5 ' - UCAUGUGCGAACGCUGACGUAGG - 3 '

i) In the genomic DNA sequence shown above, draw boxes around the exons.

ii) Write the sequence of the peptide encoded by this gene. Indicate the NH_3^+ and the COO⁻ ends of the peptide.

	U	С	А	G	
U	UUU phe (F)	UCU ser (S)	UAU tyr (Y)	UGU cys (C)	U
	UUC phe	UCC ser	UAC tyr	UGC cys	C
	UUA leu (L)	UCA ser	UAA STOP	UGA STOP	Α
	UUG leu	UCG ser	UAG STOP	UGG trp (W)	G
C	CUU leu	CCU pro (P)	CAU his (H)	CGU arg (R)	U
	CUC leu	CCC pro	CAC his	CGC arg	C
	CUA leu	CCA pro	CAA gln (Q)	CGA arg	Α
	CUG leu	CCG pro	CAG gln	CGG arg	G
A	AUU ile (L)	ACU thr (T)	AAU asn (N)	AGU ser (S)	U
	AUC ile	ACC thr	AAC asn	AGC ser	C
	AUA ile	ACA thr	AAA lys (K)	AGA arg	Α
	AUG met (M)	ACG thr	AAG lys	AGG arg	G
G	GUU val (V)	GCU ala (A)	GAU asp (D)	GGU gly (G)	U
	GUC val	GCC ala	GAC asp	GGC gly	C
	GUA val	GCA ala	GAA glu (E)	GGA gly	A
	GUG val	GCG ala	GAG glu	GGG gly	G

a) Indicate whether the following statements are true or false. And if false, correct the statement or provide a brief explanation for why the statement is false.

i) Plasma B cells secrete antibody into the bloodstream.

ii) T cells produce antibodies that neutralize antigen.

iii) Each B cell can make many different kinds of antibodies.

b) When a rabbit protein is injected into rabbits, no antibodies against this protein are generated. If, however, the same rabbit protein is injected into guinea pigs, the guinea pigs generate antibodies against the rabbit protein. Briefly (in one or two sentences) explain this observation.

c) The genomes contained in almost all of the somatic cells in an adult human are identical. Name one (diploid) cell type that is an exception to this and name the primary mechanism by which this cell type arose.

You are studying a common genetic condition. The mutant allele differs from the wild-type allele by a single base-pair (bp) substitution. This substitution eliminates a NheI restriction site that is present in the wild-type allele. (The mutant allele is not cut by NheI.) A pedigree of a family exhibiting this condition is shown below:



You isolate DNA from four individuals in the pedigree. Using PCR techniques, you amplify a 1000 bp portion of their DNA which includes the site affected by the mutation. You digest the PCR products with NheI and analyze the resulting DNA fragments on a gel:



a) Based on these data, is this gene located on an autosome or the X-chromosome? Briefly justify your reasoning.

b) Based on these data, is the mutant phenotype dominant or recessive to wild-type and why?

c) If individuals 3 and 4 have a daughter, what is the probability that she will be affected? Justify your reasoning.

Question 8, continued

d) You sequence the region around the NheI site in the wild-type PCR product. You then sequence the corresponding region in the mutant PCR product and discover that not only did the mutation eliminate the NheI site in the mutant allele but it has created a new PvuII restriction site. The recognition sites for the two enzymes are indicated below.

NheI c	uts: 5	' GCTAGC 3	' PvuII	cuts:	5'	CAGCTG 3'
	3 '	CGATCG 5'			3'	GTCGAC 5'

A portion of one strand of the wild-type DNA sequence is shown below: 5'....GCTAGCTG...3'

What is the sequence of this same region in the mutant allele? Indicate the 5' and the 3' ends of the DNA sequence.

e) Individuals 1 and 2 have another child, 9, who is affected by the genetic condition.



You PCR amplify the 1000 bp region affected by the mutation from individuals 1, 2, and 9, digest the PCR products with NheI or PvuII, and analyze the restriction fragments on a gel:



What event occurred and how does this explain the data shown above?

Consider the following hypothetical chromosomal region containing two genes, *fadA* and *fadB*, necessary for the breakdown of oleic acid in a bacterium.



The FadX protein, which is continuously produced, binds to the operator in the presence of oleic acid.

a) Is the FadX protein a repressor or an activator of the *fadA* and the *fadB* genes? Briefly justify your reasoning.

b) For each of the following mutants (m1 - m4), predict the level of FadA in the presence of oleic acid. Circle either "Low" or "High".

		Level of FadA with oleic acid present		
m1	O is deleted	Low	High	
m2	Loss-of-function mutation in <i>fadX</i>	Low	High	
m3	P is deleted	Low	High	
m4	FadX is always bound to O	Low	High	

Solutions:

Question 1:

a) 10kg/(.4)(.8)(.5)(.05)(.2)(.5) = 12,500 kg/week

i) This model would predict that 1/2 as much carbon would be fixed by the phytoplankton. Since all efficiencies remain the same, this would cause all flows to decrease to 50% of their non-poisoned levels. This would therefore reduce the detritivore respiration by 50%. This is not consistent with the observed increase.

ii) This model would predict that 5 kg carbon per week of fish are dying and therefore becoming detritus. Since all efficiencies are the same and the tank remains at steady state, this additional input to the detritus must be made up for by an equal increase in output from detritus. Since the only output from detritus is respiration (refractory carbon cannot increase at steady state), detritivore respiration must increase by 5 kg carbon per day. This is consistent with your measurement.

Question 1, continued

c) You would need to exclude the squid from an area of the ocean. If squid are the keystone species, you would expect to see a decrease in the species diversity of the small fish and invertebrates.

Question 2:

a) i) *840*

ii) More offspring are produce during the period 8-10 months, where 24(10) = 240 (as compare to the period 10-12 months, 14(16) = 224)

b) i) The interaction between *V. fischeri* and the other bacterial species initially colonizing the light organ can best be described as <u>competition</u>

ii) The interaction between the squid and *V. fischeri* can best be described as <u>symbiotic or mutualism or mutualistic</u> The squid are less visible to predators, and the bacteria are provided a safe, nutrient rich environment

iii) No, The fundamental niche is anywhere that the V. fischeri can exist. You know that V. fischeri can also live in the ambient water, and it is likely that they could exist in a wide variety of places.

iv) No, The realized niche is where you actually find the V. fischeri . You know that V. fischeri can also live in the ambient water, so the light organ can not be the realized niche



a) In order, they are DNA, RNA, and protein



Question 4

a) The m1 and m4 mutations are in the <u>same gene</u>. This is a <u>complementation test</u>. The diploid strain has the same growth phenotype as the haploid single mutants; the two mutations <u>fail to complement</u> (fail to produce the wild-type phenotype) in the double heterozygote. The m1 and m4 mutations must both inactivate the same gene (which codes for an enzyme essential for serine biosynthesis) so that the diploid double mutant has two mutant alleles of the same gene.



c) i) This haploid mutant will grow on media supplemented with intermediate A.

ii) Intermediate C will accumulate when this haploid mutant is grown on minimal medium.

Question 5

- a) i) van der Waals ii) hydrogen iii) ionic iv) van der Waals
- *b)* strongest interaction with substrate 2 is van der Waals strongest interaction with substrate 3 is ionic

c) substrate 3 would bind the most tightly. In substrate 3, you have two ionic bonds and two van der Waals. In substrates 1 and 2, you have one ionic bond, one hydrogen bond and two van der Waals.

Question 6

a i) FALSE. DNA replication is initiated at the origin of replication. RNA polymerases bind to promoter sequences to initiate transcription.

ii) FALSE. DNA polymerase requires primers to initiate DNA replication.

iii) FALSE. Okazaki fragments are made on the lagging strand of the replication fork.

iv) FALSE. DNA synthesis occurs by addition of dNTPs to the 3' OH growing strand.

v) FALSE. Transcription terminates at the termination stop sites in the DNA. Translation stops at stop codons in the mRNA.

Question 6, continued

b)

ii) NH3⁺-met-cys-glu-arg-COO⁻

Question 7

a i) True.

ii) False. B cells produce antibodies.

iii) False. Each B cell makes one distinct kind of antibody.

b) The rabbit protein is recognized as foreign (non-self) by the guinea pig.

c) B cells, by gene rearrangement of Ab genes (VDJ rearrangement). Also, *T* cells (by rearrangement of *T* cell receptor genes).

Question 8

a) An autosome, because individual 6, a male, has 2 alleles.

b) The mutant phenotype is recessive, because individuals 5 and 6 each have one copy of the mutant allele, m, and are both phenotypically normal.

c) 1/4. *Since individuals* 3 *and* 4 *already have an affected child, then they must both be heterozygotes.*

d) 5'...GCCAGCTG...3'

e) A mutation occurred which led to the production of a new mutant allele, m*. This mutant allele has a recessive phenotype and its PCR product is cut by neither NheI nor PvuII. Individual 9 has the genotype m/m*.

Question 9

a) The FadX protein is an activator, since FadA and FadB need to be synthesized in the presence of oleic acid.

b)

- *m1:* If the activator FadX cannot bind to the operator, transcription of fadA will be low.
- *m2:* Without a functional FadX activator transcription of fadAwill be low.
- *m3:* Without the promoter, RNA polymerase cannot transcribe the fadA gene, leading to a low level of FadA.
- *m4:* If FadX is always bound, it will lead to the constitutive expression of fadA, leading to a high level of FadA.