ETIOLOGY IN ACTION:
IDENTIFYING AQUACULTURE DISEASE THREATS THROUGH DATA ANALYSIS

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Grade Level
High School

Subject Area
Biology, Oceanography, Environmental Science

The 2020-21 VA SEA project was made possible through funding from the National Estuarine Research Reserve System Margaret Davidson Fellowship Program which supports graduate students in partnership with research reserves where fieldwork, research, and community engagement come together. VA SEA is currently supported by the Chesapeake Bay National Estuarine Research Reserve, Virginia Sea Grant, and the Virginia Institute of Marine Science Marine Advisory Program.

Title: Etiology in action: identifying aquaculture disease threats through data analysis

Focus: fish health, aquatic health, disease ecology, aquaculture, graphing skills.

Grade Level: high school
Suitable for: biology, marine biology, life sciences, environmental science.

VA Science Standards
BIO.1 The student will demonstrate an understanding of scientific and engineering practices by

a) asking questions and defining problems
- ask questions that arise from careful observation of phenomena and/or organisms, from examining models and theories, and/or to seek additional information
- generate hypotheses based on research and scientific principles
- make hypotheses that specify what happens to a dependent variable when an independent variable is manipulated

c) interpreting, analyzing, and evaluating data
- construct and interpret data tables showing independent and dependent variables, repeated trials, and means
- construct, analyze, and interpret graphical displays of data
- use data in building and revising models, supporting an explanation for phenomena, or testing solutions to problems
- analyze data using tools, technologies, and/or models to make valid and reliable scientific claims or determine an optimal design solution

d) constructing and critiquing conclusions and explanations
- make quantitative and/or qualitative claims regarding the relationship between dependent and independent variables
- construct and revise explanations based on valid and reliable evidence obtained from a variety of sources including students' own investigations, models, theories, simulations, and peer review
- apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and design solutions
- construct arguments or counterarguments based on data and evidence

e) developing and using models
- develop, revise, and/or use models based on evidence to illustrate or predict relationships
- develop and/or use models to generate data to support explanations, predict phenomena, analyze systems, and/or solve problems

f) obtaining, evaluating, and communicating information
- compare, integrate, and evaluate sources of information presented in different media or formats to address a scientific question or solve a problem

BIO.4 The student will investigate and understand that bacteria and viruses have an effect on living systems. Key ideas include

a) viruses depend on a host for metabolic processes;
c) the structures and functions can be compared;
d) bacteria and viruses have a role in other organisms and the environment;

BIO.7 The student will investigate and understand that populations change through time. Key ideas include

b) genetic variation, reproductive strategies, and environmental pressures affect the survival of populations;
c) natural selection is a mechanism that leads to adaptations and may lead to the emergence of new species; and
d) biological evolution has scientific evidence and explanations.

Learning Objectives

- Students will gain an understanding of aquaculture and issues within these systems.
- Students will identify trends in data through time.
- Students will practice graphing independent and dependent variables.
- Students will assess farm conditions by integrating multiple data sources.
- Students will hypothesize which pathogen(s) are threatening fish health.
- Students will infer data from a given dataset.

Extended activity:

- Students will evaluate correctness of first hypothesis given new data.
- Students will predict future health trends of the farm.
- Students will gain familiarity with the concept of health and disease treatment both being complex and holistic.

Total length of time required for the lesson: 75 minutes, with additional optional 60-minute activity.
Teacher prep time: 15 minutes.

Key words

Abiotic: not living or from a living thing. Often used to describe a part of the environment.

Antibiotic: a treatment which deters and kills bacteria.

Aquaculture: the practice of growing aquatic species (e.g. fish, shellfish, algae) in fresh or salt water, usually for food.

Clinical signs: observable features of disease in an infected host.

CPM: cumulative percent mortality. The proportion of a population that has died, up to a certain point in time.

Culling: the selective killing of some individuals in an animal population, typically for the improved health of the remaining population in.

Disease: reduced health status of a host. Disease is often contingent on both a pathogenic infection and specific environmental conditions.

Etiology: the study of a cause or causes of disease.

Host: an organism that may be infected by a pathogen.

IHNV: infectious hematopoietic necrosis virus. A lethal virus, and the disease it causes, which affects fish in the Family Salmonidae, particularly rainbow trout and Pacific salmon species.

Infection: uptake of a pathogen by a new host organism.

Mortality: death in a population.

Pathogen: a biological agent that may cause disease.

Raceway: a long, shallow tank in a fish farm. A typical raceway has flowing water and is used to hold fish as they grow from juvenile to adult size.

Vaccine: a treatment that prevents disease from a specific virus.

Background Information

Fish farming is a growing industry. To meet the demand for fish, rainbow trout (*Oncorhynchus mykiss*) are raised in fish farms. The rainbow trout aquaculture industry produced 814 million tons of fish in 2016. The densest production region in the United States is the Hagerman Valley in Idaho, which produces 75% of domestic trout. Rainbow trout are also found naturally in the region. Farmed trout are held in long, outdoor troughs called raceways, supplied with river water.

Good living conditions are crucial for raising high quality fish and also being environmentally responsible. Trout are subject to the same health issues as other farmed animals. They need adequate abiotic conditions such as enough space, a comfortable temperature, and clean water. Water requirements are especially important for aquatic animals. Trout like cold water (10-15 degrees Celsius) with high flow rate and high dissolved oxygen content. Fish excrete ammonia as a waste product. At high levels, ammonia is toxic to fish. Bacteria, naturally present in the water, convert ammonia to nitrite and nitrate. High flow keeps all of these chemical compounds at low levels. If water flow is poor, ammonia can build up to toxic levels for the fish and cause health problems.

Disease management includes addressing abiotic health concerns on the farm, as well as biotic threats to fish health. Biotic threats include pathogens such as viruses and bacteria. Viruses and bacteria can be found everywhere in the natural world. For a given organism, only some of them are considered pathogenic. Trout cannot avoid coming into contact with all potential pathogens, but disease managers can reduce disease by planning for the most serious threats.

The biggest threat to farmed trout is a viral disease called infectious hematopoietic necrosis virus (IHNV). IHNV is native to the Pacific Northwest region of North America. IHNV causes necrosis of the kidneys and spleen and often, death. Clinical signs of IHNV include changes in swimming behavior, bulging eyes, distended belly, darkened skin, or bleeding at the fin bases. Given the right conditions, IHNV may cause 95% mortality in juvenile fish. A vaccine for IHNV exists, but it is not cost-effective for farms to administer. IHNV is not a health risk for humans but can infect most other trout and salmon species.

Clinical disease signs from bacterial infection can be difficult to tell apart from each other. While IHNV is the most important virus for farms, there are innumerable bacteria that pose low to moderate health risks. Many bacteria are a natural part of the ecosystem. Infected fish often have skin lesions, or fins and tails that look ragged. Antibiotics are commercially available, but unnecessary use is discouraged due to the possible negative outcomes. Possible consequences of antibiotic use include antibiotic resistance, downstream effects on the surrounding river system, and perceived market value.

Trout farms are businesses and must make disease management decisions not only for ethical and holistic fish health, but with their business model in mind. The profit margin for trout is small; if too many fish are lost to disease, the farm cannot turn a profit.

Materials in this Lesson Plan:
- Worksheet 1 (1 page)
- Worksheet 2 (2 pages)
- Case Study Activity – version A
- Case Study Activity – version B
- Case Study Activity – version C
- Case Study Activity – version D
- Case Study Activity – version E
- Common Clinical Signs Handout
- Worksheet 3 (2 pages, optional)
- Instructor Keys for Worksheet 1, Worksheet 2 for all Case Studies, Worksheet 3 for all Case Studies.
- Accompanying Powerpoint introduction
- Accompanying Excel Appendix (for aiding virtual/online classroom learning; not necessary for print versions)

Supplies Needed:
- Computer and projector (in person) OR computer, Internet, and screen-sharing capability (remote learning) for Powerpoint presentation.
- Pens/pencils
- Optional: graphing software (e.g. Microsoft Excel)
- Optional: calculator
- Optional: scratch paper for calculations

<u>Teacher Preparation</u>. Time = 15 minutes
Print and/or copy worksheets. Virtual classes may like to pre-assign groups and worksheets on their teaching platform of choice.

Make enough copies for:
- 1 each of **Worksheets 1, 2, 3*** per student.
  *Worksheet 3 is the Optional Extended Activity.
- 1 **Common Clinical Signs** Handout per group.
- 1 **Case Study** per group. Each group will only need 1 Case Study version, and the activity will be more informative for the class if all versions are used (groups will report their findings from each version to each other). Suggest breaking students into 6 or more groups (pairs are fine) and making a proportional number of copies of each Case Study version to randomly distribute (e.g. for 15 student pairs, make 3 copies of each Case Study version. For 5 groups of 3 students each, make 1 copy of each Case Study version).

Teachers may wish to laminate color copies of the **Common Clinical Signs** Handout and **Case Study Activity sheets A, B, C, D, E** for repeated use.

Teachers may wish to assign reading the **Background Information** section prior to class.

Classroom Set-Up: Arrange as necessary so that groups of 2-4 students may work together. Virtual classrooms may like to use 'breakout' rooms with screen sharing capability.

<u>Procedure</u>. Time = 75 minutes

Introduction: Powerpoint slides.

Individual work: Students receive Worksheet 1. Interpret and complete Worksheet 1 Example and Practice Questions.

Review: As a class, review the Worksheet 1 Example and Practice Questions. Discuss the limitations of the graph and consider what kind of farm conditions the data could represent.

Case Study set up: Sort students into small groups (2-4 people per group, and at least 2 groups so that students can report out to each other at the end of the activity). Distribute Worksheet 2 to every individual student. Each group receives one Case Study (single page labeled with A/B/C/D/or E) and one Common Clinical Signs sheet to share. If there are more groups than there are unique case studies, multiple groups may receive the same case study.

Groupwork: Case Studies. Encourage students to talk through each step of the Worksheet. Work together to complete Worksheet 2 as a team.

Class Jigsaw: Groups report their findings to the rest of the class. The class may wish to compare results visually by graphing their results on a shared board or screen. Discuss the differences in CPM, and the timing of mortality for each Case Study version examined. Brainstorm what could produce mortality for these farm situations, and list the evidence from the Health Reports that can be used to form a conclusion.

<u>**Optional Extended Activity**</u>. **Time = 60 minutes**. Second class period or home assignment.

Review Mortality Curve and/or previous Case Studies. As a class or individually, review the conclusion for each Case Study examined.

Distribute Worksheet 3.

Individual work: Individually, students will complete Worksheet 3. Using Worksheet 2 as a guide, students will repeat the data analysis steps with new data and add the data from Worksheet 2.

Class Review and Reflection: Discuss each Case Study in the Extended Activity. Reflect on what makes fish farms successful, and how fish health is assessed and treated. Revisit the differences between pathogen, disease, and health. Consider if there are parallels between fish health and human health.

<u>**Assessment**</u>

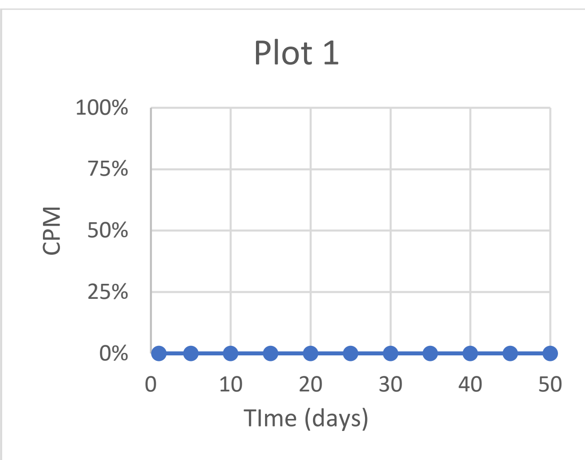
Students may be assessed by completion of worksheets 1 and 2, interpretation of graphs and results, and team participation. Optional extended activity may be assessed by worksheet 3 completion and reflection.

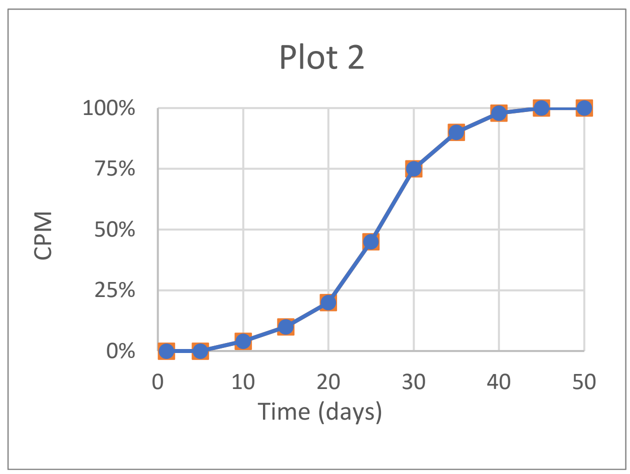
WORKSHEET 1 – PRACTICE INTERPRETING MORTALITY CURVES

INTRODUCTION
The ability to manage disease in fish farms depends on knowing the farm conditions and being able to track fish health over time. Farm conditions include water quality, temperature, and ammonia levels. The abiotic conditions can have a significant impact on fish health. It is not practical to examine individual fish for clinical disease signs, so raceways are assessed as a whole. Fish that look healthy may have undetectable infections. If conditions are good for fish, low levels of infection may never turn into disease. If conditions change to favor the pathogen, disease may progress quickly. Farm managers pay close attention to mortality rate as an indicator of disease. Some natural deaths (<5%) are expected in large populations. (But >10% mortality over time may be a problem.) If disease is suspected, a portion of fish that represents the whole population may be examined.

MORTALITY DATA
Data is often presented in the metric "cumulative percent mortality (CPM)," where each point on a graph represents the number of fish in the population that have died up to, and including, that point in time.

Example:
Below are two different examples of mortality curves. Plot 1 shows a population of fish with 0% mortality. Plot 2 shows a population with 100% mortality.





Practice Questions:

1) What are the units of the x- and y-axes?

2) Refer to Plot 1. What can you conclude about the health of the fish from this plot?

3) Refer to Plot 2. On which day do you first see the first mortality?

4) Refer to Plot 2. On which day have all the fish died (100% mortality)?

WORKSHEET 1 – PRACTICE INTERPRETING MORTALITY CURVES **INSTRUCTOR KEY**

INTRODUCTION

The ability to manage disease in fish farms depends on knowing the farm conditions and being able to track fish health over time. Farm conditions include water quality, temperature, and ammonia levels. The abiotic conditions can have a significant impact on fish health. It is not practical to examine individual fish for clinical disease signs, so raceways are assessed as a whole. Fish that look healthy may have undetectable infections. If conditions are good for fish, low levels of infection may never turn into disease. If conditions change to favor the pathogen, disease may progress quickly. Farm managers pay close attention to mortality rate as an indicator of disease. Some natural deaths (<5%) are expected in large populations. (But >10% mortality over time may be a problem.) If disease is suspected, a portion of fish that represents the whole population may be examined.

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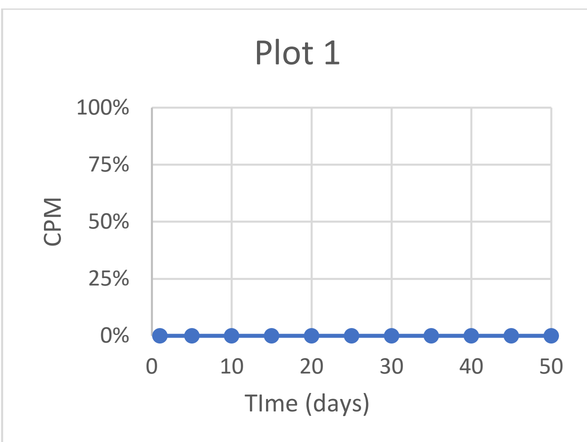
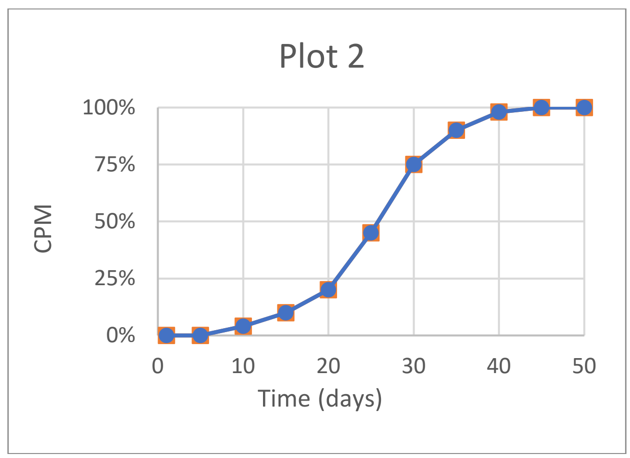




Practice Questions:

1) What are the units of the x- and y-axes?

The x-axis unit is day. The x-axis measures time.

The y-axis unit is percent. The y-axis measures Cumulative Percent Mortality.

2) Refer to Plot 1. What can you conclude about the health of the fish from this plot?

No fish have died during the 50-day period plotted. *Remind students that absence of mortality is not the same as good health; it is possible fish are experiencing sub-lethal disease.

3) Refer to Plot 2. On which day do you first see the first mortality?

Sometime between Day 6-10. *These data are only recorded every 5 days, so it is impossible to know exactly which day mortalities occurred between the datapoints on Day 5 and Day 10.

4) Refer to Plot 2. On which day have all the fish died (100% mortality)?

Sometime between Day 40-45.

WORKSHEET 2 – CASE STUDY ACTIVITY **Page 1/2**

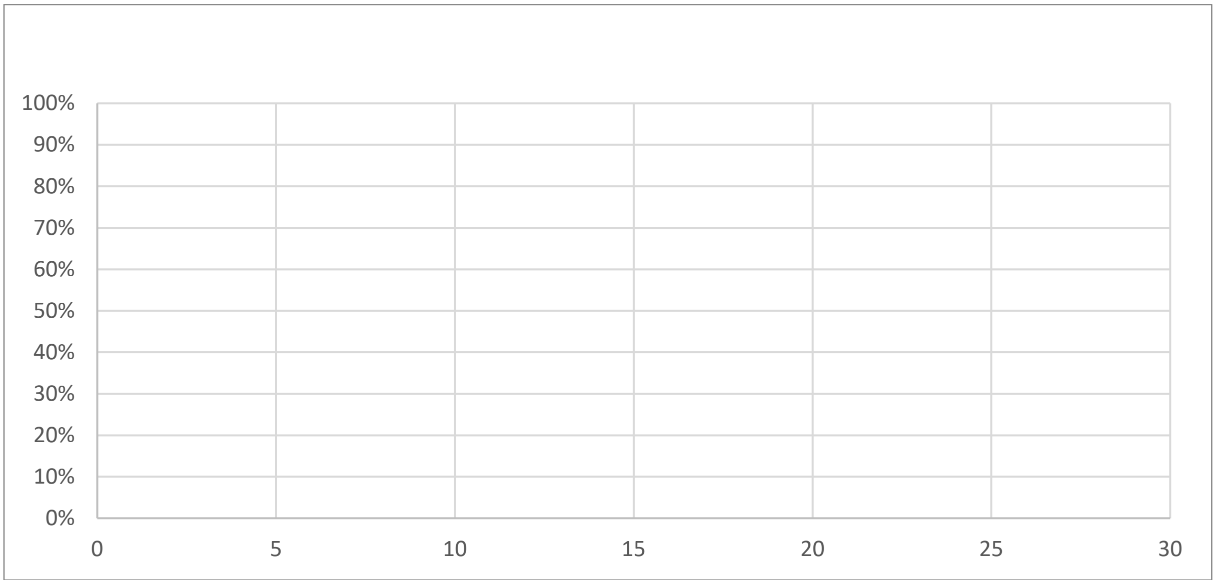
CASE STUDY OVERVIEW
A series of trout farms in Idaho needs someone to review their health reports. You are the disease management team for the region, tasked with assessing the health of trout farms, and responding to disease concerns. In this activity, your team will interpret a farm's health report, consider which, if any, health concerns may be affecting the farm's trout population, and offer a recommendation to the farm based on your findings.

Review the attached health report and answer the following questions. All team members are expected to participate equally.

1. You want to examine the Cumulative Percent Mortality (CPM) curve, but the Farm has only given you raw data. You'll need to do some calculations to convert number of mortalities to CPM; use the table below as a guide.

Day	**Number of Dead Fish**	**Cumulative Number of Dead Fish**	**Starting Population**	**Cumulative Mortality**	**CPM**
	(new mortalities observed on Day # from Health Report)	(for each row, add the **Number of Dead Fish** to the previous **Cumulative Number of Fish Dead.)**	Original number of Fish in the Raceway	(for each row, divide the **Cumulative Number of Dead Fish** by the **Starting Population**)	Multiply the **Cumulative Mortality** by 100% and round the nearest percentage point.
1			1000		%
5			1000		%
7			1000		%
10			1000		%
12			1000		%
15			1000		%
17			1000		%
20			1000		%
25			1000		%
30			1000		%

2. Graph the CPM over time. Day will be on the x-axis; CPM will be on the y-axis. Remember to label your plot with a title and axes units.



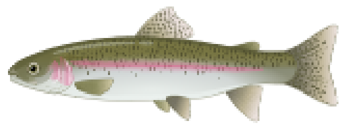
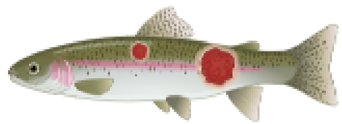
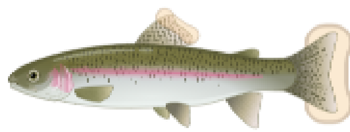
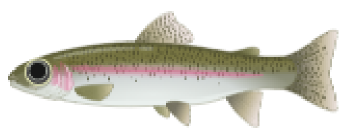
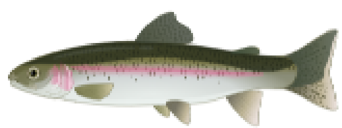
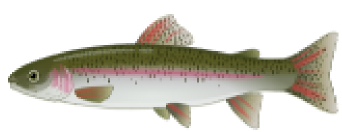
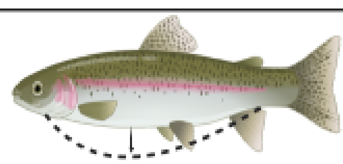
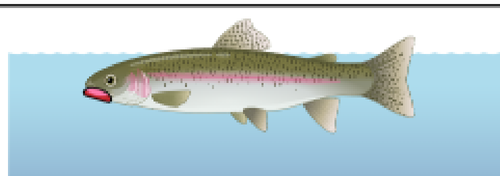
3. Is the level of mortality unusual?

4. Over what time span is your dataset describing?

5. If you have determined there to be a concerning level of mortality, what might be responsible for the fish mortality?

6. Offer a recommendation for how to treat fish/help farm (3-5 sentences or bullet points). Include a rationalization for your recommendation (e.g. what specific evidence has informed your decision?).

Common Clinical Signs in Rainbow Trout (*Oncorhynchus mykiss*)

Example Rainbow Trout	Description	Possible Health Threat:
	Healthy, normal.	none
	Skin lesions. (Lesions may occur anywhere on fish skin, not just locations shown on example.)	Bacterial infection
	Bacterial growths. (May occur on fins or in conjunction with skin lesions. Frequently white-yellow, "fuzzy" appearance.)	Bacterial infection
	Exophthalmia. (Enlarged, often bulging eyes.)	IHNV
	Discolored skin. (Typically darker than normal, especially on dorsal surfaces.)	IHNV
	Bleeding in fin rays. (May occur in any or all fins and tail, especially at base of rays where they connect to the body.)	IHNV
	Distended belly. (Fish ventral surface will appear swollen, mostly visible from the side. May also appear lumpy.)	IHNV
	Gasping - behavioral change. (Fish struggling to breathe may spend more time at water surface, rapidly opening and closing mouth.)	IHNV low dissolved oxygen

Created in BioRender.com

A

CASE STUDY ACTIVITY
HEALTH REPORT FOR FARM A

Farm Name: “Aberdeen Aquaculture”
Farm Location: Hagerman Valley, ID

Mortality Data
These data are averages across all farm raceways from routine checks throughout the month. Each raceway has 1000 fish at the start of data collection. Each raceway appeared to experience the same conditions; variation between raceways was negligible.

Day	Number of Dead Fish
	(new mortalities observed)
1	5
5	5
7	0
10	1
12	5
15	2
17	5
20	2
25	0
30	5

Photos of the Farm’s Fish

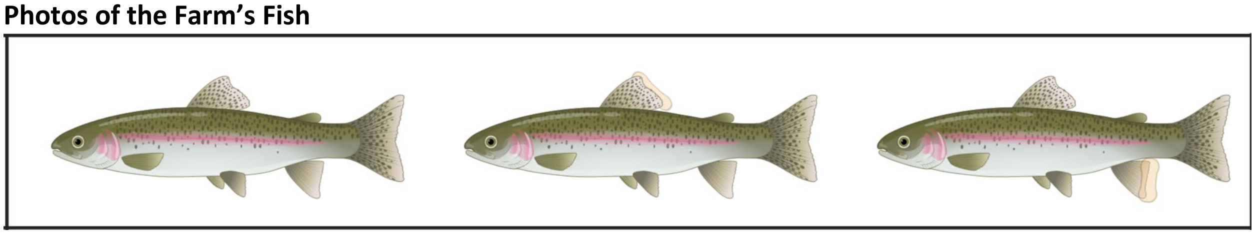


Image Credit: created in Biorender.com

Notes from the farm’s technician:
- High flow this year from the glacial melt
- Colder temps than normal
- Some issues with ammonia levels

B

CASE STUDY ACTIVITY
HEALTH REPORT FOR FARM B

Farm Name: "Brown Bear Farm"
Farm Location: Hagerman Valley, ID

Mortality Data
These data are averages across all farm raceways from routine checks throughout the month. Each raceway has 1000 fish at the start of data collection. Each raceway appeared to experience the same conditions; variation between raceways was negligible.

Day	Number of Dead Fish
	(new mortalities observed)
1	0
5	10
7	5
10	12
12	10
15	12
17	4
20	23
25	20
30	18

Photos of the Farm's Fish

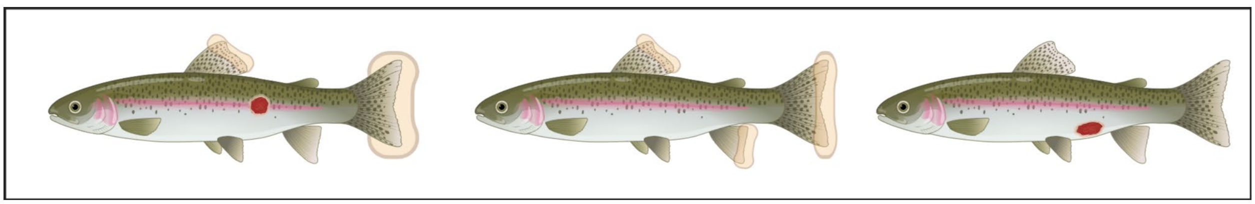


Image Credit: created in Biorender.com

Notes from the farm's technician:
- fuzzy growths on fish fins
- ragged tails
- some dead fish show skin lesions on side, back

C

CASE STUDY ACTIVITY
HEALTH REPORT FOR FARM C

Farm Name: "Council Creek Fish"
Farm Location: Hagerman Valley, ID

Mortality Data
These data are averages across all farm raceways from routine checks throughout the month. Each raceway has 1000 fish at the start of data collection. Each raceway appeared to experience the same conditions; variation between raceways was negligible.

Day	Number of Dead Fish
	(new mortalities observed)
1	5
5	180
7	165
10	96
12	74
15	25
17	32
20	5
25	12
30	10

Photos of the Farm's Fish

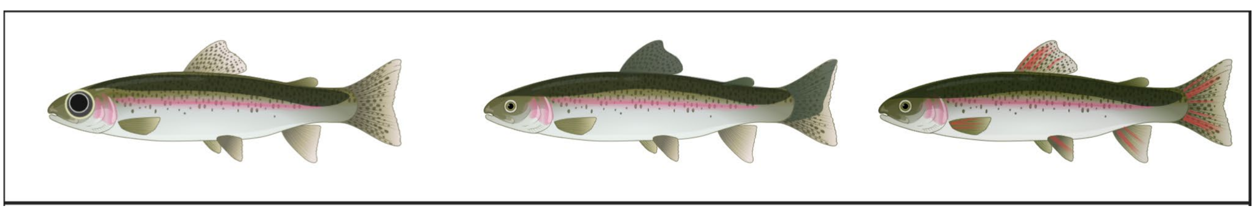


Image Credit: created in Biorender.com

Notes from the farm's technician:

- irregular swimming behavior, especially erratic swimming at surface
- occasional gasping
- dark skin
- some blood in the fins
- exophthalmia if some morts
- concerning number of fish deaths

D

CASE STUDY ACTIVITY
HEALTH REPORT FOR FARM D

Farm Name: "Dubois Dam Trout"
Farm Location: Hagerman Valley, ID

Mortality Data
These data are averages across all farm raceways from routine checks throughout the month. Each raceway has 1000 fish at the start of data collection. Each raceway appeared to experience the same conditions; variation between raceways was negligible.

Day	Number of Dead Fish
	(new mortalities observed)
1	10
5	65
7	65
10	30
12	25
15	10
17	5
20	25
25	20
30	40

Photos of the Farm's Fish

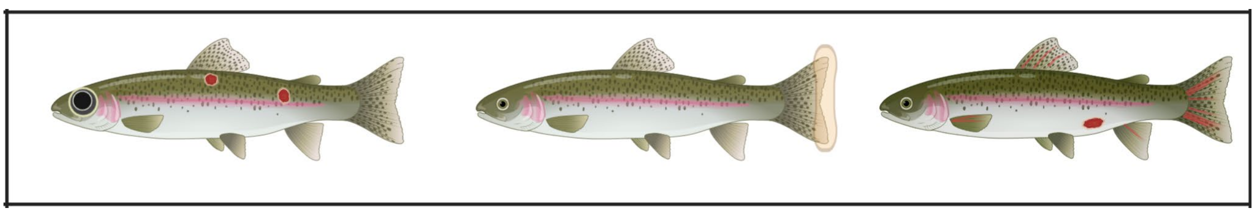


Image Credit: created in Biorender.com

Notes from the farm's technician:
- Warmer water than usual
- Some small lesions on fish skin
- Occasional mort with blood in the fins
- A few furry-looking growths on tails
- Fish are lethargic overall

E

CASE STUDY ACTIVITY
HEALTH REPORT FOR FARM E

Farm Name: "Eagle Eco-foods"
Farm Location: Hagerman Valley, ID

Mortality Data
These data are averages across all farm raceways from routine checks throughout the month. Each raceway has 1000 fish at the start of data collection. Each raceway appeared to experience the same conditions; variation between raceways was negligible.

Day	Number of Dead Fish
	(new mortalities observed)
1	10
5	310
7	304
10	125
12	22
15	10
17	35
20	28
25	10
30	28

Photos of the Farm's Fish

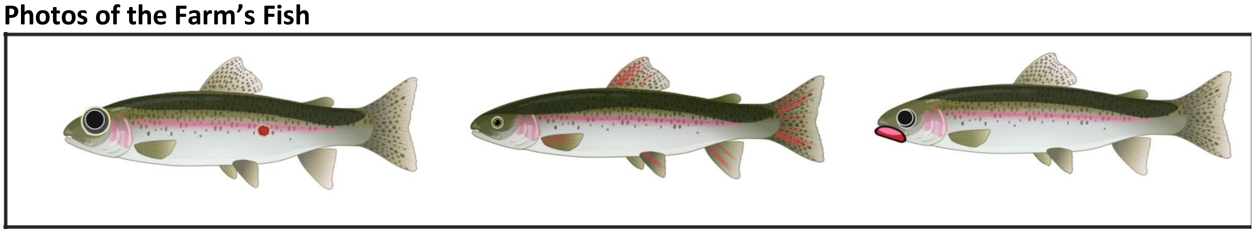


Image Credit: created in Biorender.com

Notes from the farm's technician:
- erratic swimming
- fish will sometimes come up to the surface, opening mouths repeatedly like they're talking
- lots of fish with swollen eyes and bellies
- some skin lesions and blood in fins in the morts

WORKSHEET 2 – CASE STUDY ACTIVITY **INSTRUCTOR KEY**

CASE STUDY OVERVIEW

A series of trout farms in Idaho needs someone to review their annual health. You are the disease management team for the region, tasked with assessing the health of trout farms, and responding to disease concerns. In this activity, your team will interpret a farm's health report, consider which, if any, health concerns may be affecting the farm's trout population, and offer a recommendation to the farm based on your findings.

Review the attached health report and answer the following questions. All team members are expected to participate equally.

1. You want to examine the Cumulative Percent Mortality (CPM) curve, but the Farm has only given you raw data. You'll need to do some calculations to convert number of mortalities to CPM; use the table below as a guide.

Farm A:

Day	Number of Dead Fish	Cumulative Number of Dead Fish	Starting Populatio n	Cumulative Mortality	CPM
	(new mortalities observed)	(for each row, add the Number of Fish Dead to the previous Cumulative Number of Fish Dead)	Original number of Fish in the Raceway	(for each row, divide the Cumulative Number of Dead Fish by the Starting Population)	Multiply the Cumulative Mortality by 100% and round the nearest percentage point.
1	5	0	1000	0	0%
5	5	5	1000	0.005	1%
7	0	5	1000	0.005	1%
10	1	6	1000	0.006	1%
12	5	11	1000	0.011	1%
15	2	13	1000	0.013	1%
17	5	18	1000	0.018	2%
20	2	20	1000	0.02	2%
25	0	20	1000	0.02	2%
30	5	25	1000	0.025	3%

Farm B:

Day	Number of Dead Fish	Cumulative Number of Dead Fish	Starting Populatio n	Cumulative Mortality	CPM
	(new mortalities observed)	(for each row, add the Number of Fish Dead to the previous Cumulative Number of Fish Dead)	Original number of Fish in the Raceway	(for each row, divide the Cumulative Number of Dead Fish by the	Multiply the Cumulative Mortality by 100% and round the nearest percentage point.

				Starting Population)	
1	0	0	1000	0	0%
5	10	10	1000	0.01	1%
7	5	15	1000	0.015	2%
10	12	27	1000	0.027	3%
12	10	37	1000	0.037	4%
15	12	49	1000	0.049	5%
17	4	53	1000	0.053	5%
20	23	76	1000	0.076	8%
25	20	96	1000	0.096	10%
30	18	114	1000	0.114	11%

Farm C:

Day	Number of Dead Fish	Cumulative Number of Dead Fish	Starting Population	Cumulative Mortality	CPM
	(new mortalities observed)	(for each row, add the Number of Fish Dead to the previous Cumulative Number of Fish Dead)	Original number of Fish in the Raceway	(for each row, divide the Cumulative Number of Dead Fish by the Starting Population)	Multiply the Cumulative Mortality by 100% and round the nearest percentage point.
1	5	5	1000	0.005	1%
5	180	185	1000	0.185	19%
7	165	350	1000	0.35	35%
10	96	446	1000	0.446	45%
12	74	520	1000	0.52	52%
15	25	545	1000	0.545	55%
17	32	577	1000	0.577	58%
20	5	582	1000	0.582	58%
25	12	594	1000	0.594	59%
30	10	604	1000	0.604	60%

Farm D:

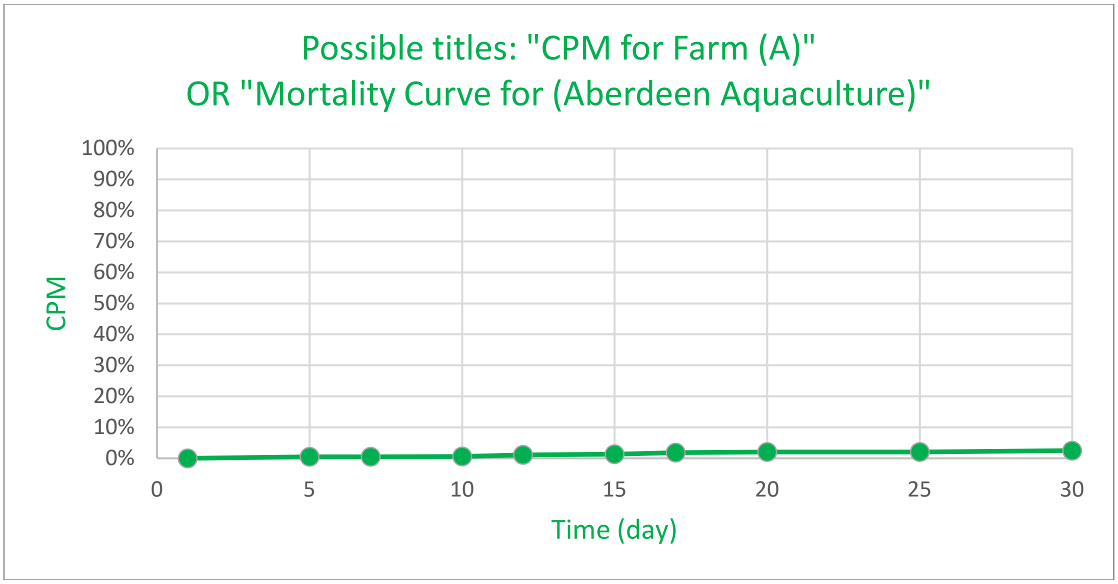
Day	Number of Dead Fish	Cumulative Number of Dead Fish	Starting Population	Cumulative Mortality	CPM

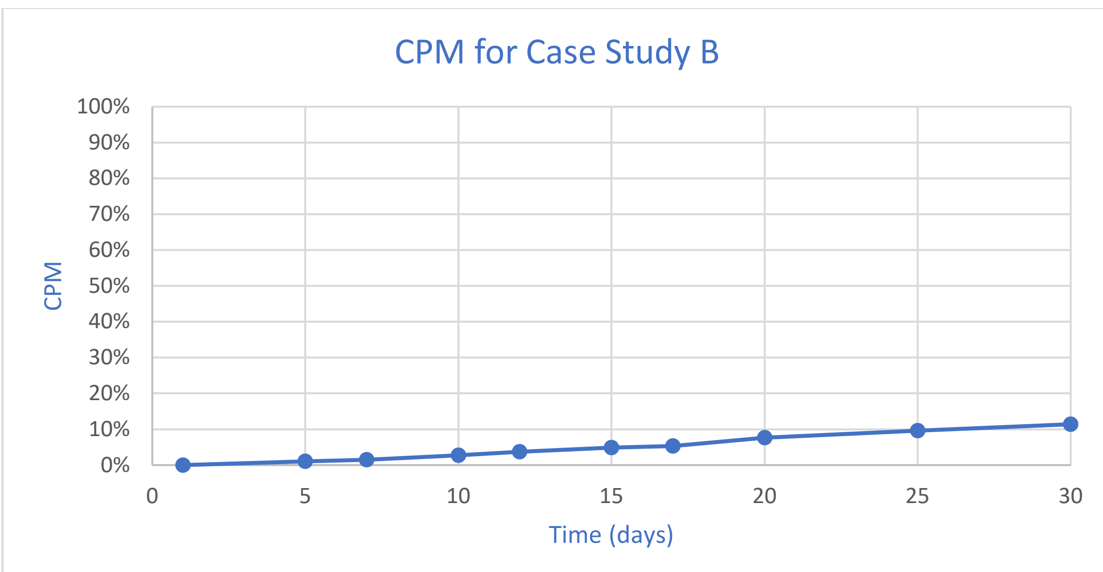
	(new mortalities observed)	(for each row, add the Number of Fish Dead to the previous Cumulative Number of Fish Dead)	Original number of Fish in the Raceway	(for each row, divide the Cumulative Number of Dead Fish by the Starting Population)	Multiply the Cumulative Mortality by 100% and round the nearest percentage point.
1	10	10	1000	0.01	1%
5	65	75	1000	0.075	8%
7	65	140	1000	0.14	14%
10	30	170	1000	0.17	17%
12	25	195	1000	0.195	20%
15	10	205	1000	0.205	21%
17	5	210	1000	0.21	21%
20	25	235	1000	0.235	24%
25	20	255	1000	0.255	26%
30	40	295	1000	0.295	30%

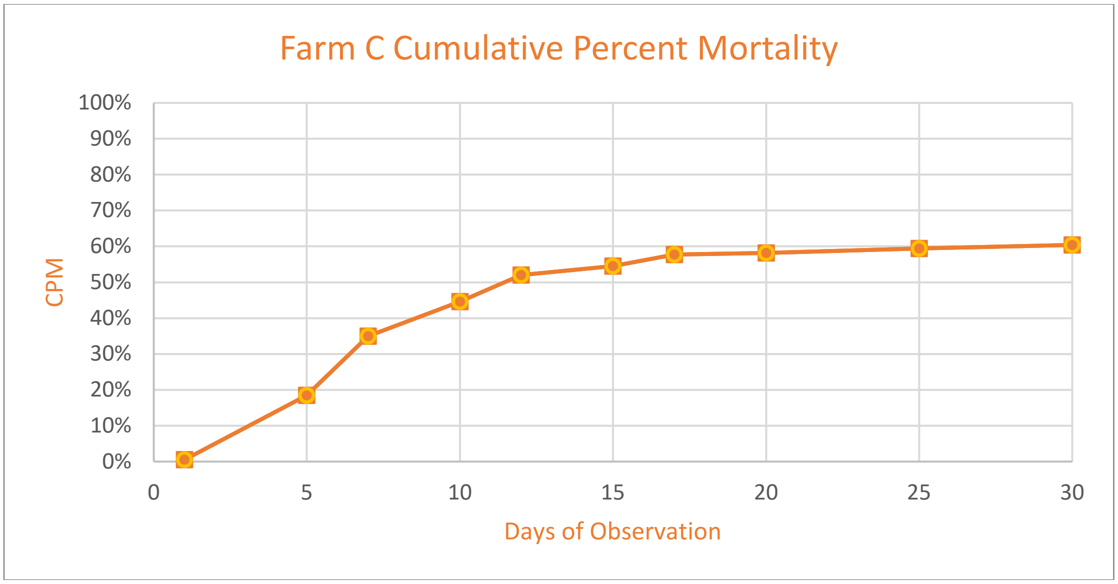
Farm E:

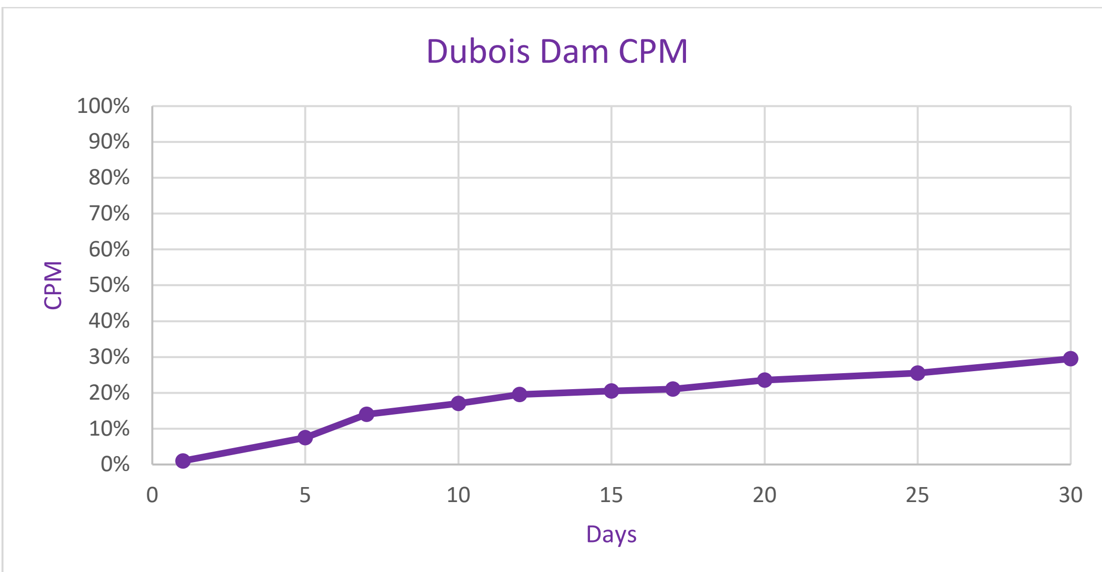
Day	Number of Dead Fish	Cumulative Number of Dead Fish	Starting Populatio n	Cumulative Mortality	CPM
	(new mortalities observed)	(for each row, add the Number of Fish Dead to the previous Cumulative Number of Fish Dead)	Original number of Fish in the Raceway	(for each row, divide the Cumulative Number of Dead Fish by the Starting Population)	Multiply the Cumulative Mortality by 100% and round the nearest percentage point.
1	10	10	1000	0.01	1%
5	310	320	1000	0.32	32%
7	304	624	1000	0.624	62%
10	125	749	1000	0.749	75%
12	22	771	1000	0.771	77%
15	10	781	1000	0.781	78%
17	35	816	1000	0.816	82%
20	28	844	1000	0.844	84%
25	10	854	1000	0.854	85%
30	28	882	1000	0.882	88%

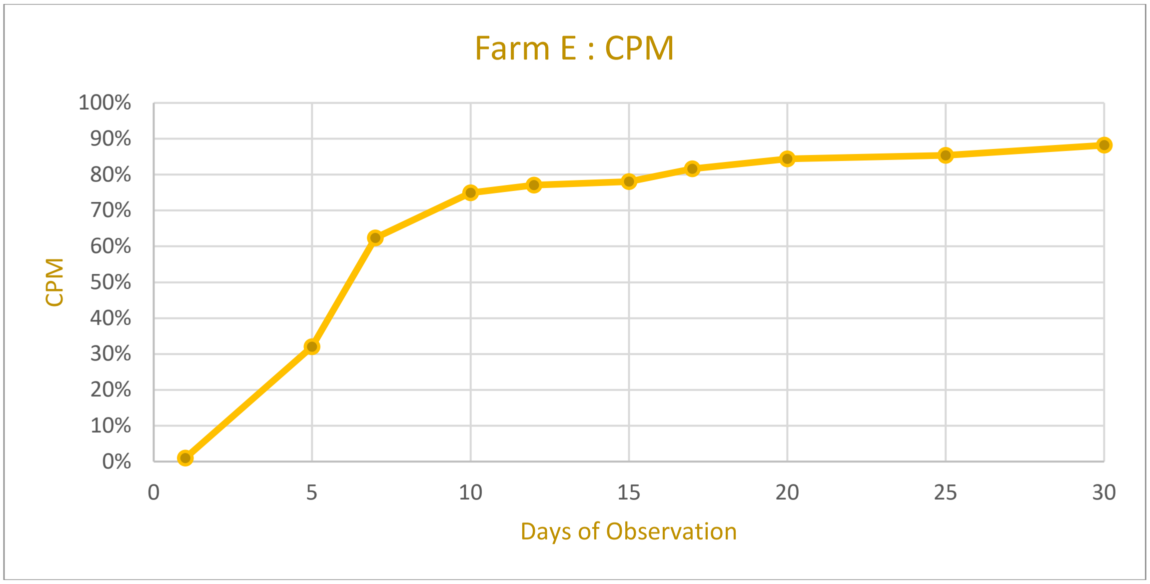
2. Graph the CPM over time. Remember to label your plot with a title and axes units.


Possible titles: "CPM for Farm (A)"
OR "Mortality Curve for (Aberdeen Aquaculture)"
CPM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
30
Time (day)


CPM for Case Study B
CPM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
30
Time (days)


Farm C Cumulative Percent Mortality
CPM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
30
Days of Observation


Dubois Dam CPM
CPM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
5
10
15
20
25
30
Days



3. Is the level of mortality unusual?

Farm A: No. CPM < 10%, which could be considered natural loss.
Farm B: Somewhat, yes. CPM = 11% could be indicative of a pathogenic health problem, but could also be the result of hard environmental conditions killing the weakest fish.
Farm C: Yes. it is concerningly high.
Farm D: Yes. A majority of fish are living, but 31% CPM indicates a health problem.
Farm E: Yes. Over 80% of the fish are dead. The farm is probably operating at a loss.

4. Over what time span is your dataset describing?

Farm A: 30 days
Farm B: 30 days
Farm C: 30 days
Farm D: 30 days
Farm E: 30 days

5. If you have determined there to be a concerning level of mortality, what might be responsible for the fish mortality?

Farm A: No cause for concern. However, some bacterial growths depicted could indicate low level bacterial infection.
Farm B: In this case, the technician noted growths, ragged fins, and lesions, which are all consistent with bacterial infection.
Farm C: Clinical signs are all consistent with IHNV. It is likely a viral infection.
Farm D: Some generic and viral clinical signs point to either low level viral infection or both viral and bacterial infections. Severe water quality issue is possible, but the technician only noted sporadic water temp issue, so it is likely pathogenic.
Farm E: Clinical signs are consistent with IHNV. It is likely a severe viral infection.

6. Offer a recommendation for how to treat fish/help farm (3-5 sentences or bullet points). Include a rationalization for your recommendation (e.g. what specific evidence has informed your decision?).

Farm A: The farm CPM is within normal mortality rate for a healthy population. Minor presence of bacterial growths on the fish suggest that fish may be susceptible to problematic levels of bacterial infection if conditions change for the worse, but for now the farm can continue to operate without change. Bacterial infection may be due to suppressed immune system function from cold temperatures. Fish health overall appears good.

Farm B: If possible, improve holding conditions for fish. If health issues persist, an antibiotic may be necessary to control the bacterial infections. Health of the fish population is generally good, but 11% CPM is beginning to look like a problem since mortality rate is consistent through time and not going away.

Farm C: The CPM is very concerning for the farm. A variety of clinical signs, evident in the images (darkened skin, enlarged eyes, frantic swimming behavior, bleeding in fin rays) and matching description from the technician notes point to IHNV as the likely culprit. The farm may wish to look into vaccination, or raising a resistant line of fish in the future. If fish continue to die, it is likely more economical to cull the remaining fish and start anew; however, the CPM has leveled off so the farm looks like it will be able to retain the remaining 40% of fish from the starting population.

Farm D: A variety of clinical signs are present in fish (lesions, exophthalmia, bacterial growths, bleeding fins, lethargy), and indicate a widespread health issue, likely a combined result of IHNV and subsequent bacterial infection. Maintain cold water temps to alleviate environmental pressure on fish. Administer antibiotics if conditions worsen. Mortality rate does not appear to be slowing, so action is needed to improve fish health.

Farm E: The CPM is high and although the mortality rate has slowed, it looks as if 100% CPM will occur in 10-15 days if the rate continues. This case study demonstrates classic clinical signs of IHNV. The farm should consider ways to curb viral mortality, such as vaccination, or raising a resistant line of fish in the future. It is probably most economical to cull the remaining fish, clean the raceways, and start with a new line of fish.

Ask each group to share their answer to #6, to learn from each other and be able to compare different datasets and interpretations.

WORKSHEET 3 **Page 1/2**

OPTIONAL EXTENDED ACTIVITY: CASE STUDY PART 2

After hearing your recommendation, the farm director decides to proceed by administering a common antibiotic, oxytetracyline, mixed into regular trout food. The director provides you with new data for the following season.

FARM A

Day	Number of Dead Fish
	(new mortalities observed)
35	10
40	8
45	12
50	4
60	0
70	2
80	0
90	0

FARM B

Day	Number of Dead Fish
	(new mortalities observed)
35	5
40	8
45	10
50	7
60	0
70	3
80	2
90	0

FARM C

Day	Number of Dead Fish
	(new mortalities observed)
35	8
40	10
45	5
50	2
60	5
70	5
80	5
90	5

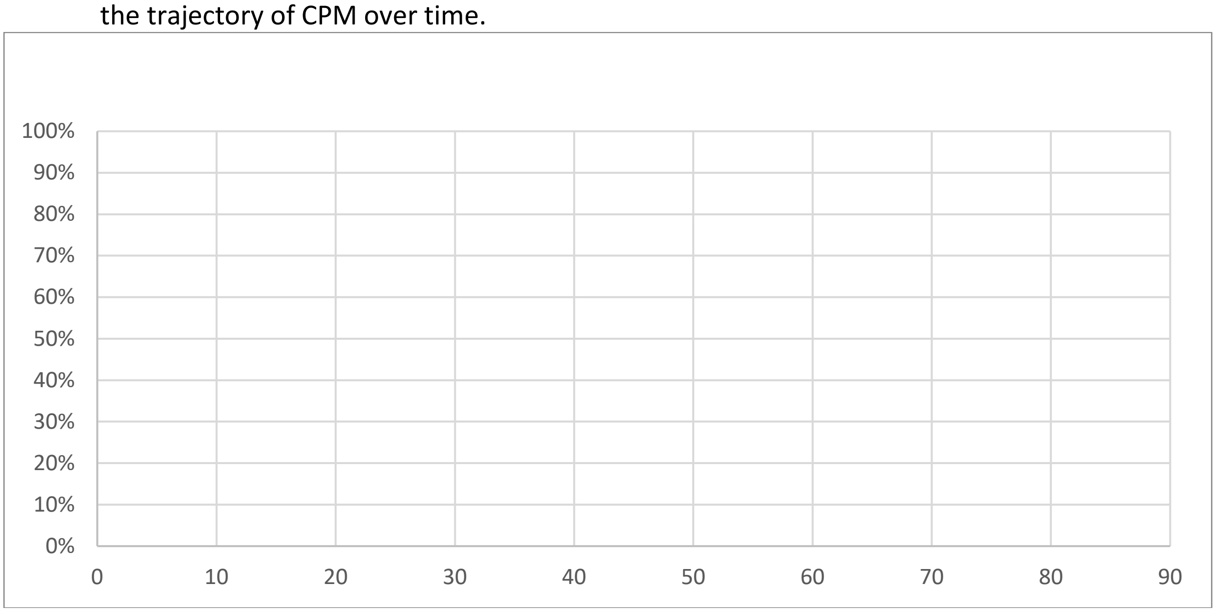
FARM D

Day	Number of Dead Fish
	(new mortalities observed)
35	50
40	30
45	25
50	15
60	5
70	15
80	10
90	10

FARM E

Day	Number of Dead Fish
	(new mortalities observed)
35	45
40	28
45	10
50	27
60	10
70	8
80	0
90	0

1. Graph the new data pertaining to the Farm you studied previously in the first part of the Case Study. Include datapoints from your previous CPM curve from Worksheet 2, so you can examine the trajectory of CPM over time.



2. What happened to the fish population over time? (interpret the graph)

3. Did the treatment appear to be effective for the fish? Do you think this was a good choice for the farm?

4. Do you still agree with your initial recommendation for treatment?

Questions for discussion:

5. What causes disease?

6. What can mitigate disease?

7. What does “good health” mean, and how can a fish farm facilitate healthful conditions for their fish?

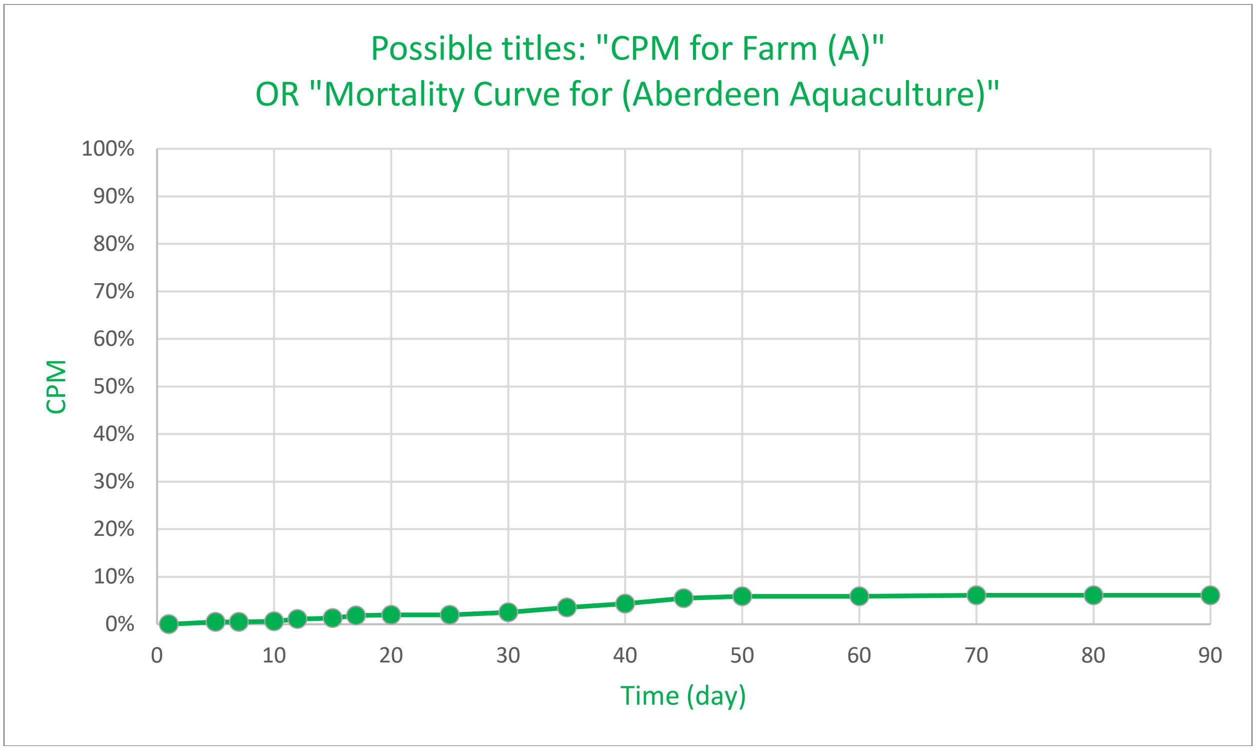
8. A trout farmer may only earn 10 cents per fish by the time it is sold from the farm. Consider how this small profit margin may influence their decisions regarding large-scale farm health.

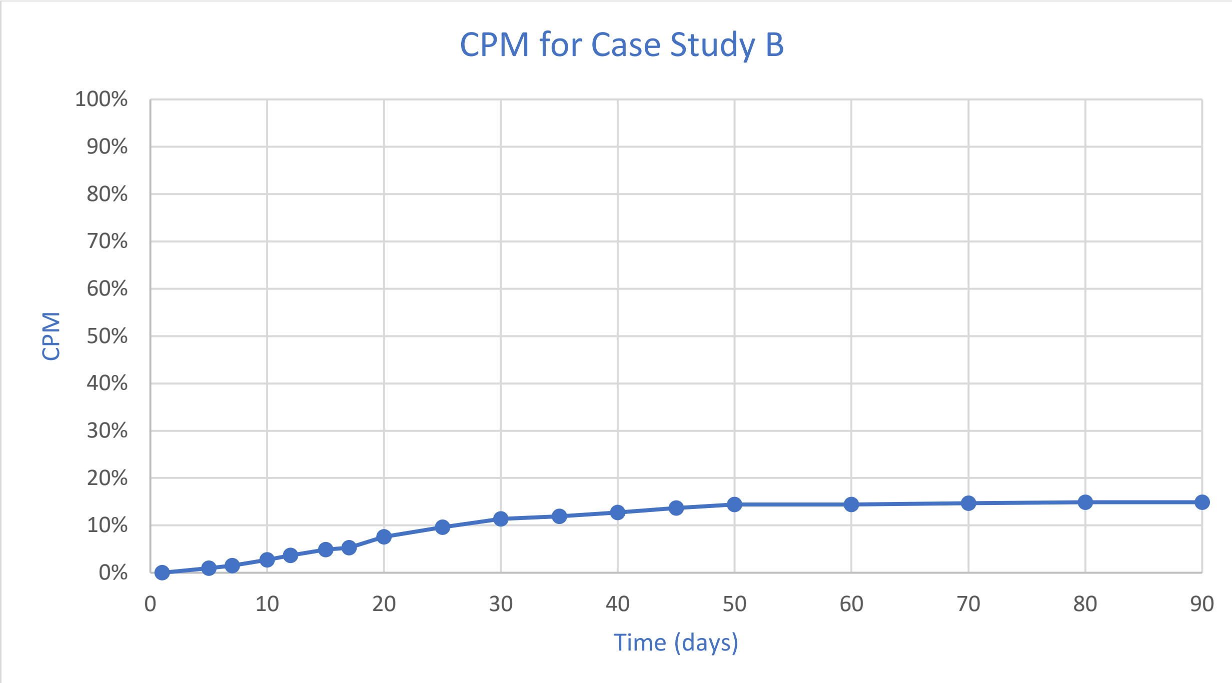
WORKSHEET 3 **INSTRUCTOR KEY**

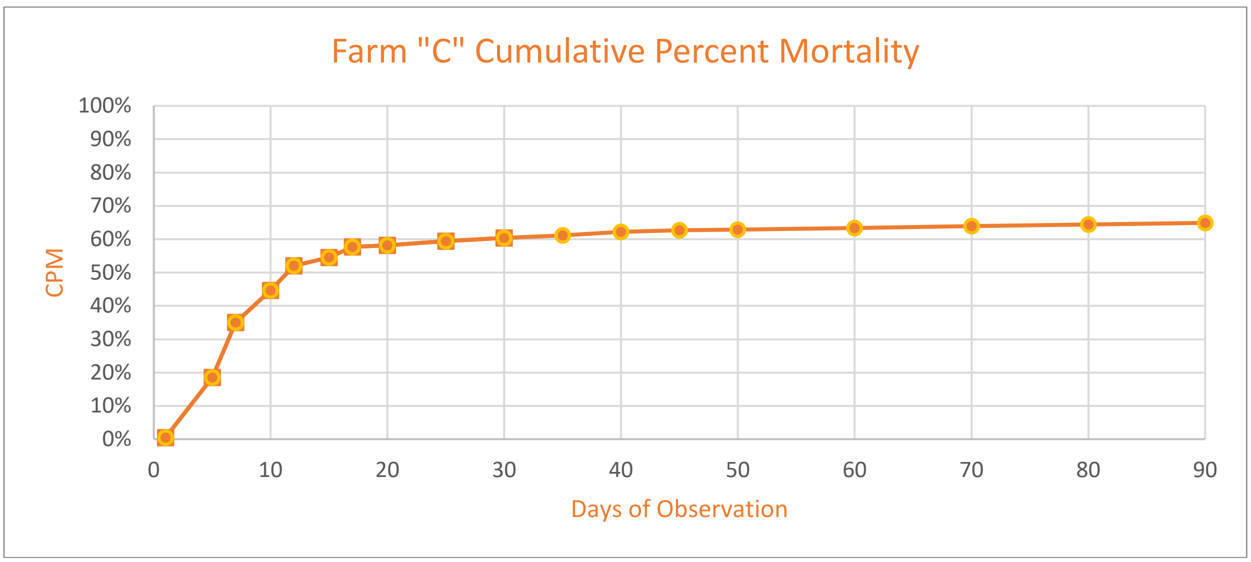
OPTIONAL EXTENDED ACTIVITY: CASE STUDY PART 2

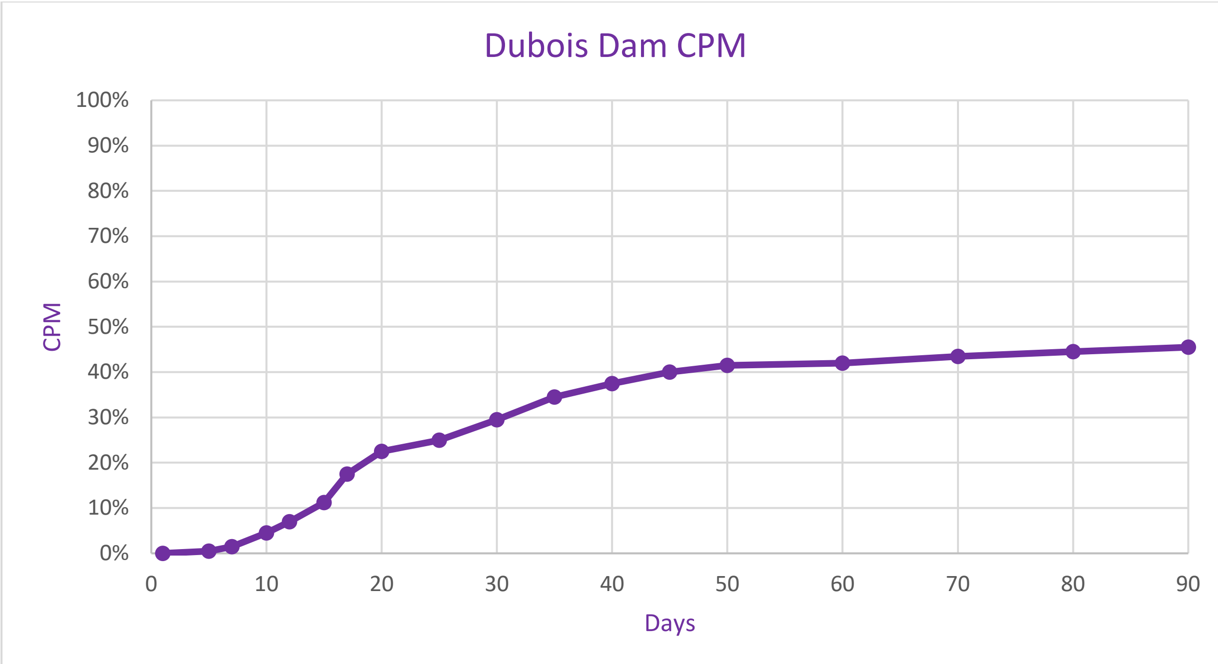
After hearing your recommendation, the farm director decides to proceed by administering a common antibiotic, oxytetracyline, mixed into regular trout food. The director provides you with new data for the following season.

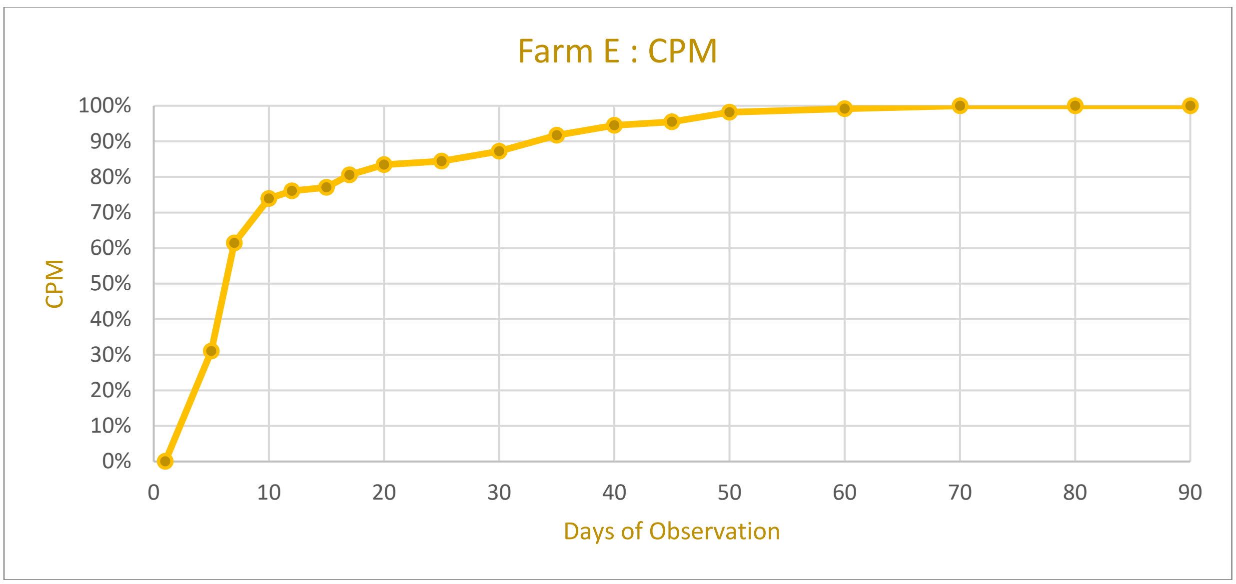
9. Graph the new data. Include datapoints from your previous CPM curve, so you can examine the trajectory of CPM over time. *Students need not graph all 10 datapoints from previous Worksheet as long as the same shape of CPM curve is represented, with correct beginning and end datapoints.




CPM for Case Study B
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CPM
0
10
20
30
40
50
60
70
80
90
Time (days)


Farm "C" Cumulative Percent Mortality
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
CPM
0
10
20
30
40
50
60
70
80
90
Days of Observation





10. What happened to the fish population over time? (interpret the graph)

Farms A: Very low mortality persisted over time.

Farms B,D: slowed mortality rate in days 30-90. Fish continued to die, but at a much slower rate. The majority of fish were alive at day 90.

Farms C,E: the rate of mortality did not change/did not slow. Fish continued to die at a rate similar to the end of the first month. In Farm E, all fish were dead by day 90.

11. Did the treatment appear to be effective for the fish? Do you think this was a good choice for the farm?

Farm A: Probably not effective. CPM was 3% at Day 30, and doubled to 6% by Day 45. Losing 3% of fish in 14 days is not good, but the low CPM overall (6% at Day 90) indicates that antibiotics were probably not necessary to administer. The fish are in good health overall and no pathogenic infection is evident.

Farm B: yes it was effective. Farm B saw a reduced rate of mortality (plateau line) in days 30-90. The antibiotic appears to have been effective in reducing mortality. This is likely a bacterial infection, helped by poor water quality. This appears to be a good choice for the farm.
*Students are not expected to know this, but if your class is curious: the antibiotic oxytetracycline is extremely common and relatively cheap compared to other therapeutics. If effective against an infection, it is probably an economical choice for disease management. However, farms should still consider how an antibiotic may affect future infections at the farm (antibiotic resistance) and whether the antibiotic is entering the environment, which could impact wild fish or other organisms.

Farm C: Not effective. It is reasonable to say the mortality rate is lower in days 30-90 than in days 0-30, but the CPM had already plateaued by Day 30. This is likely a deadly virus; the fish have developed some resistance by Day 30 so the reduced mortality is due to improved host function, not reduced pathogen inhibited by the antibiotic. This was not a good use of farm resources.

Farm D saw continued mortality in days 30-90. The antibiotic was not effective against the pathogen. This could be a mild viral infection (mortality due to virus tends to be rapid), a bacterial infection that is not treatable by oxytetracycline, or a severe water quality issue. This was not a helpful treatment to spend the farm's resources on.
*This is a difficult curve to interpret. Grade at teacher's discretion.

Farm E saw continued mortality in days 30-90. The graph appears to plateau because 99% of fish were dead by Day 60. The antibiotic was not effective against the pathogen. This was not a helpful treatment to spend the farm's resources on. Most of the fish were already dead at Day 30. This is almost certainly a viral infection. It would be reasonable for a farm to cull the entire raceway at Day 30 and not bother with antibiotics at all.

12. Do you still agree with your initial recommendation for treatment? List your initial diagnosed pathogen, and the pathogen you now think is the culprit.

(Answers will vary. If student initially identified bacteria as part of the health issue and saw a decline in mortality rate, they will agree. If initial answer was virus, and they saw a decline, they will disagree; virus was incorrect. If the initial answer was virus and the mortality rate did not slow, they will agree; virus was correct.)

Questions for discussion:

13. What causes disease?
    Possible answer: any stressors or combination of stressors including but not limited to bacterial pathogens, viral pathogens, poor water quality. Both abiotic and biotic factors can contribute to disease manifestation.
14. What can mitigate disease?
    Possible answer:
    Some or all of: improved holding conditions such as good water quality, low/no pollutants, reduced density, antibiotics (for bacterial pathogens only), vaccines (for viral pathogens only), choosing special bred lines of pathogen-resistant fish (difficult and only with variable success).

15. What does “good health” mean, and how can a fish farm facilitate healthful conditions for their fish?
    Holistically appropriate conditions including: clean habitat (no pollutants, effective removal of waste such as high waterflow rate), access to nutritious food, abiotic factors within their natural ranges (high dissolved oxygen level, appropriate cool temperatures), pathogen control (both through good husbandry methods or resistance treatments i.e. antibiotics, vaccines). Emphasis on environmental health and proactive management of wellbeing > reactive application of therapeutics.

References

Behringer, D. C., Wood, C. L., Krkošek, M., Bushek, D. (2020). Disease in fisheries and aquaculture. In *Marine Disease Ecology* (pp. 183–210). https://doi.org/10.1093/oso/9780198821632.003.0010

Brüssow H. (2009). The not so universal tree of life or the place of viruses in the living world. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *364*(1527), 2263–2274. https://doi.org/10.1098/rstb.2009.0036

Cultured Aquatic Species Information Programme. Oncorhynchus mykiss. Cultured Aquatic Species Information Programme . Text by Cowx, I. G. In: *FAO Fisheries Division* [online]. Rome. Updated 15 June 2005. [Cited 7 December 2020]. http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en

FAO. (2018). *The State of World Fisheries and Aquaculture 2018*. https://doi.org/10.1093/japr/3.1.101

Kennedy, D. A., Kurath, G., Brito, I. L., Purcell, M. K., Read, A. F., Winton, J. R., & Wargo, A. R. (2016). Potential drivers of virulence evolution in aquaculture. *Evolutionary Applications*, *9*(2), 344–354. https://doi.org/10.1111/eva.12342

Troyer, R. R. M., & Kurath, G. (2003). Molecular epidemiology of infectious hematopoietic necrosis virus reveals complex virus traffic and evolution within southern Idaho aquaculture. *Diseases of Aquatic Organisms*, *55*(3), 175–185. https://doi.org/10.3354/dao055175

Additional Resources for Using Graphing Software

Resources for Microsoft Excel:

Microsoft Support: Create a Chart in Excel (with video)
https://support.microsoft.com/en-us/office/video-create-a-chart-4d95c6a5-42d2-4cfc-aede-0ebf01d409a8

Excel Easy: Create a Line Chart
https://www.excel-easy.com/examples/line-chart.html

Microsoft Support: Overview of Formulas in Excel
https://support.microsoft.com/en-us/office/overview-of-formulas-in-excel-ecfdc708-9162-49e8-b993-c311f47ca173

Resources for Google Sheets:

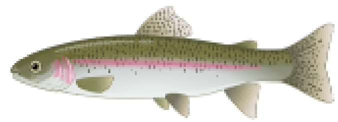
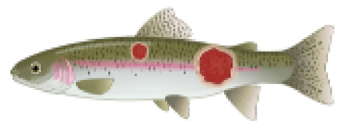
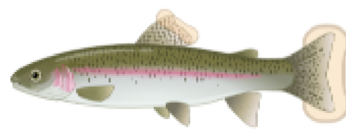
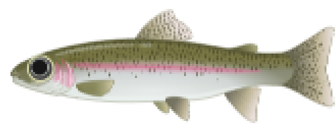
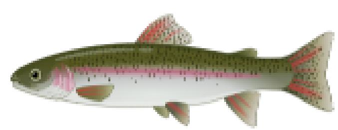
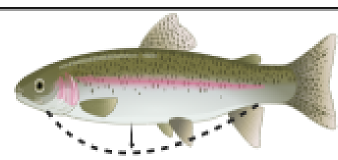
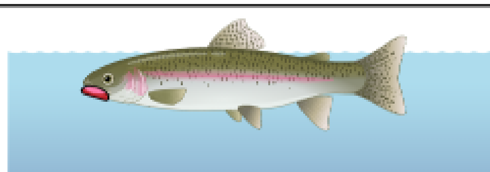
Google Support: Create a Line Chart in Google Sheets
https://support.google.com/docs/answer/9142593?hl=en

Productivity Spot: How to Make a Line Chart in Google Sheets
https://productivityspot.com/line-chart-google-sheets/

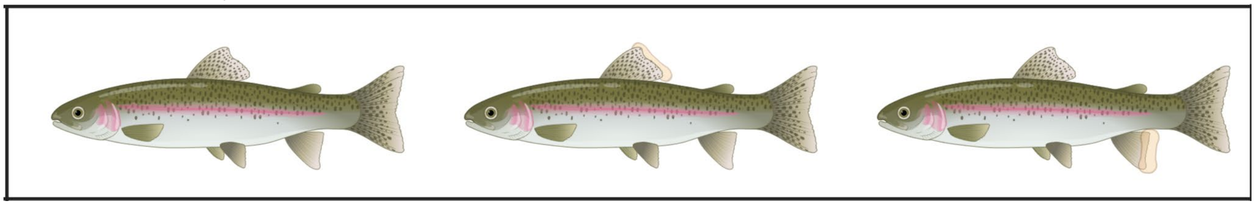
Creating a Line Graph in Google Sheets (Youtube video)
https://www.youtube.com/watch?v=QbWNatcnT8w&ab_channel=ChadEllis

Creating Simple Formulas in Google Sheets
https://edu.gcfglobal.org/en/googlespreadsheets/creating-simple-formulas/1/

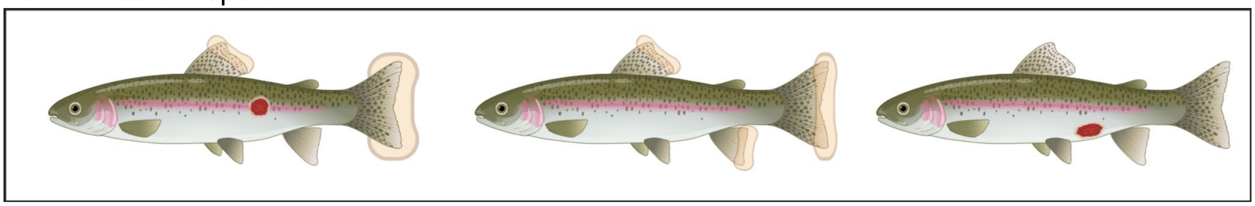
Common Clinical Signs in Rainbow Trout (*Oncorhynchus mykiss*)

Example Rainbow Trout	Description	Possible Health Threat:
	Healthy, normal.	none
	Skin lesions. (Lesions may occur anywhere on fish skin, not just locations shown on example.)	Bacterial infection
	Bacterial growths. (May occur on fins or in conjunction with skin lesions. Frequently white-yellow, "fuzzy" appearance.)	Bacterial infection
	Exophthalmia. (Enlarged, often bulging eyes.)	IHNV
	Discolored skin. (Typically darker than normal, especially on dorsal surfaces.)	IHNV
	Bleeding in fin rays. (May occur in any or all fins and tail, especially at base of rays where they connect to the body.)	IHNV
	Distended belly. (Fish ventral surface will appear swollen, mostly visible from the side. May also appear lumpy.)	IHNV
	Gasping - behavioral change. (Fish struggling to breathe may spend more time at water surface, rapidly opening and closing mouth.)	IHNV low dissolved oxygen

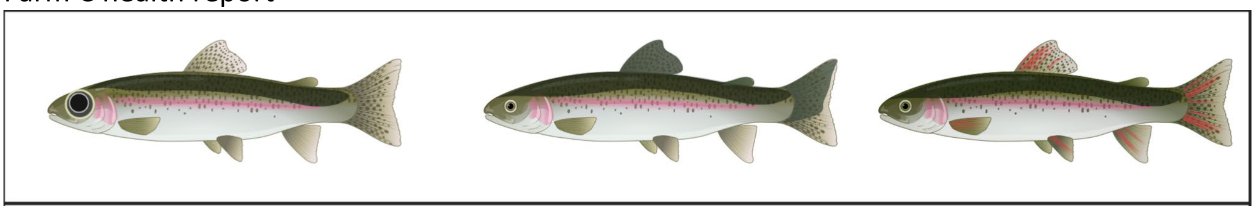
Farm A health report



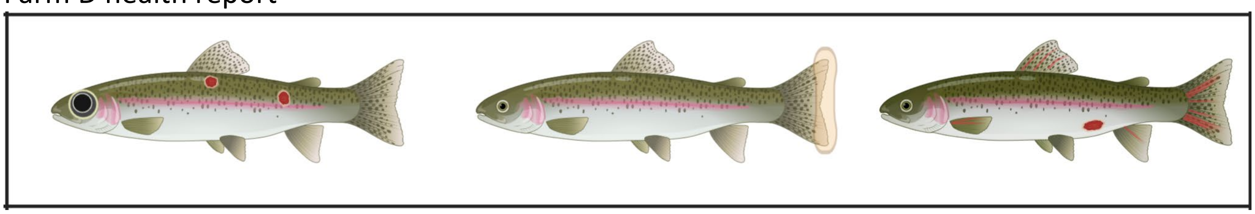
Farm B health report



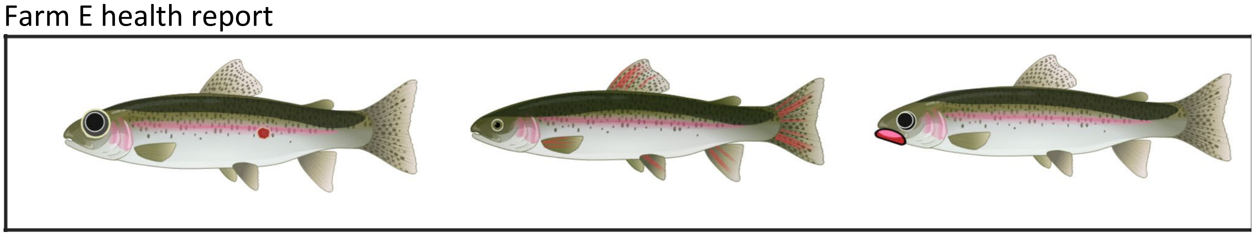
Farm C health report



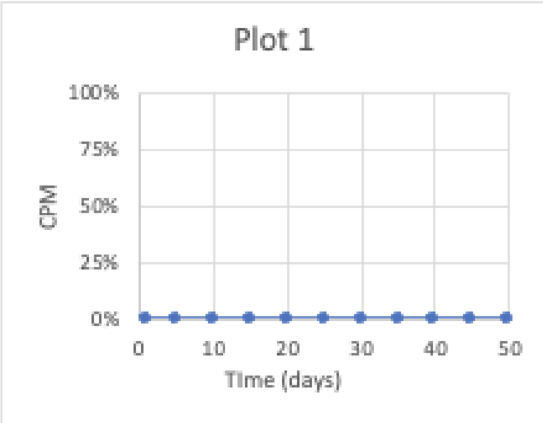
Farm D health report

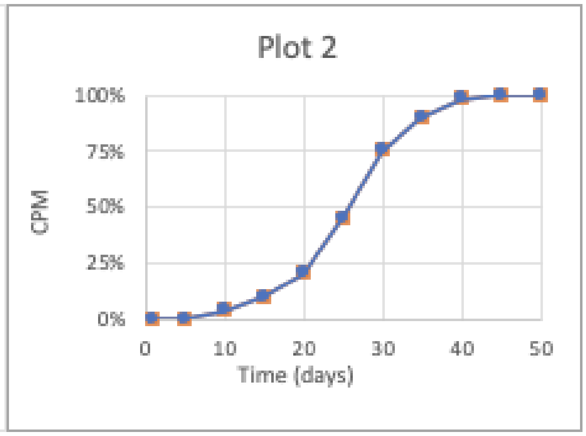


Farm E health report

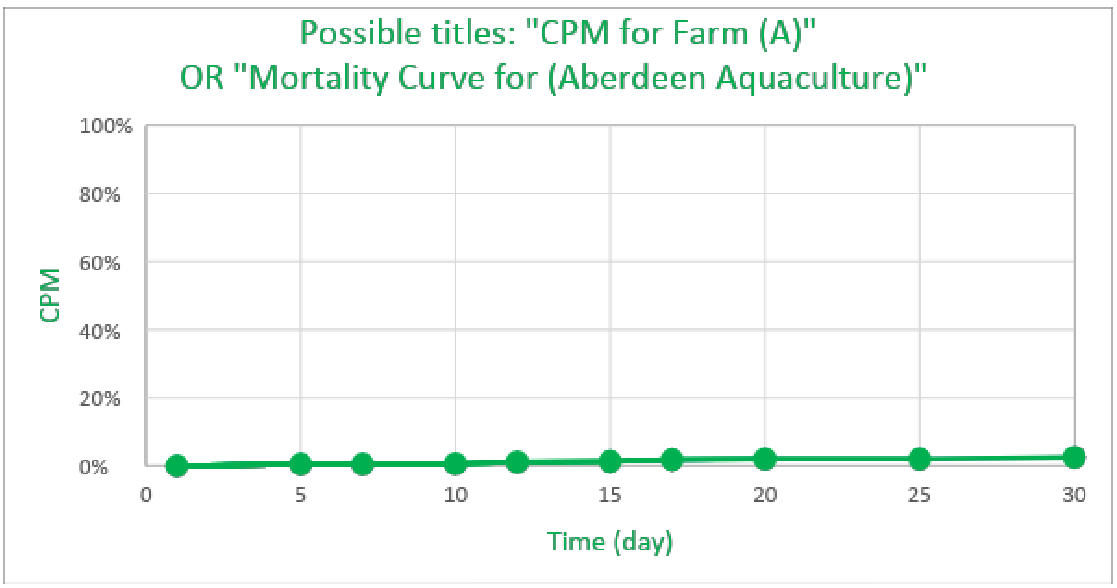


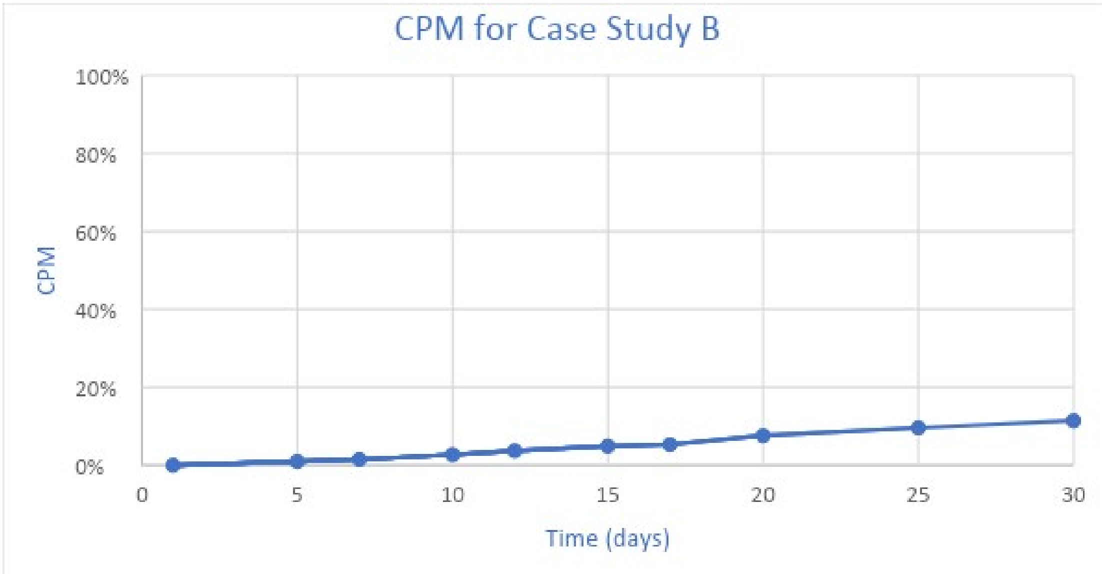
Example Plots for Worksheet 1



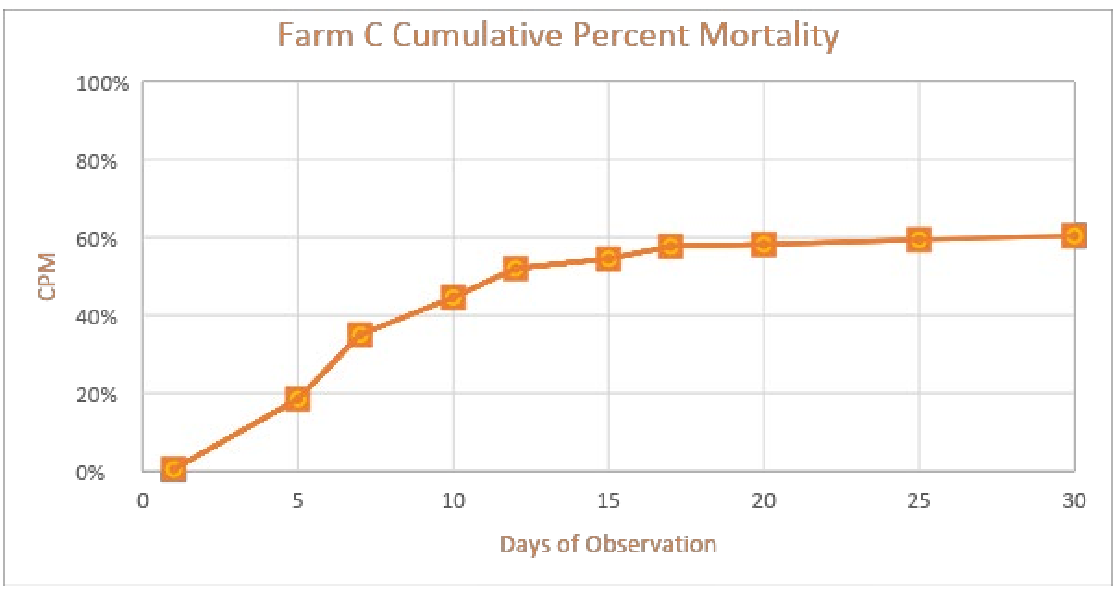


Answer Key Plots for Worksheet 2: CPM Curves, Farms A-E

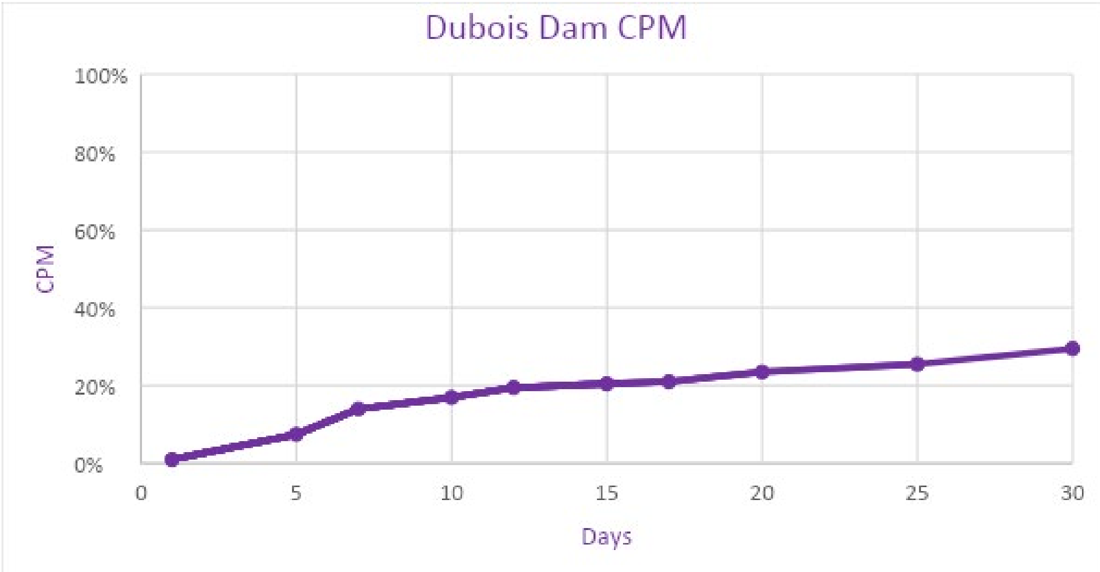


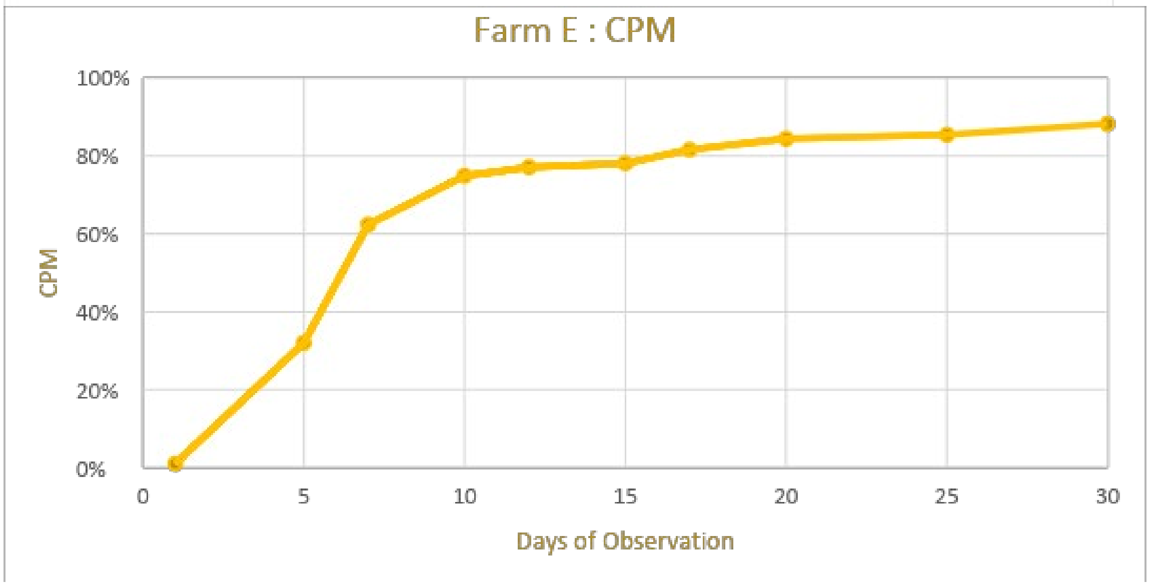


CPM for Case Study B
100%
80%
60%
40%
20%
0%
CPM
0
5
10
15
20
25
30
Time (days)

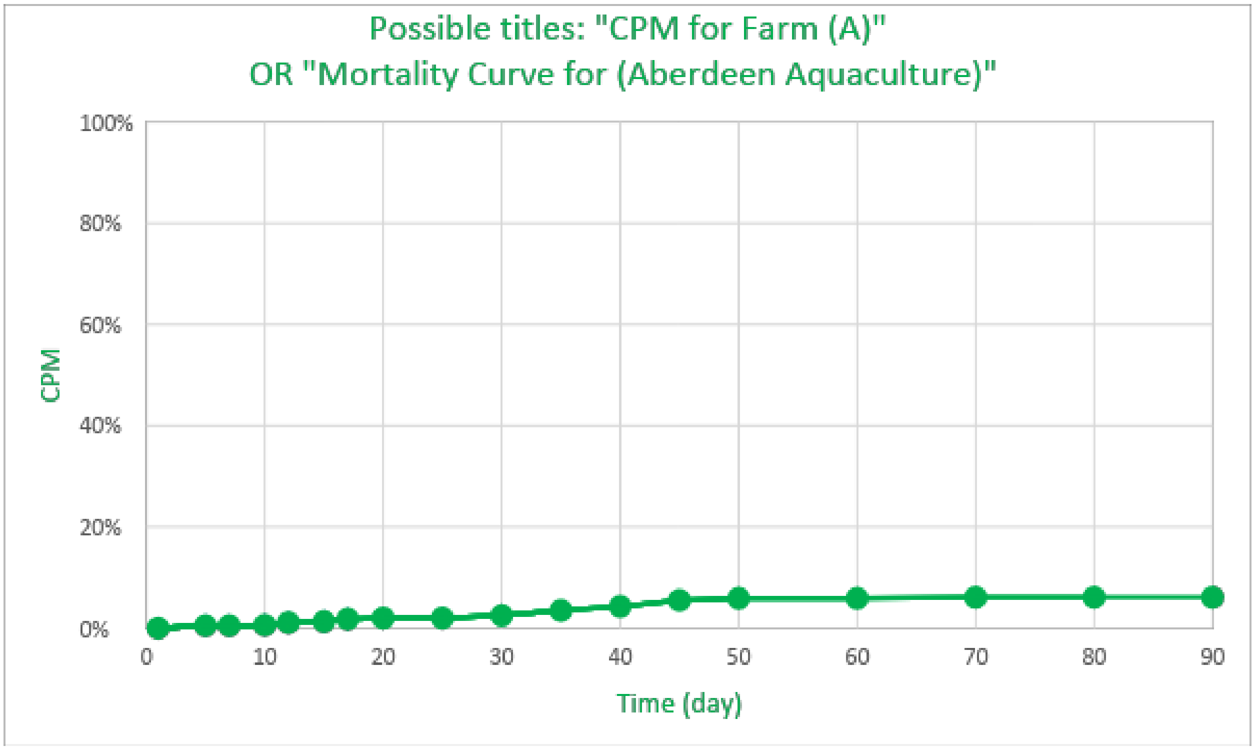


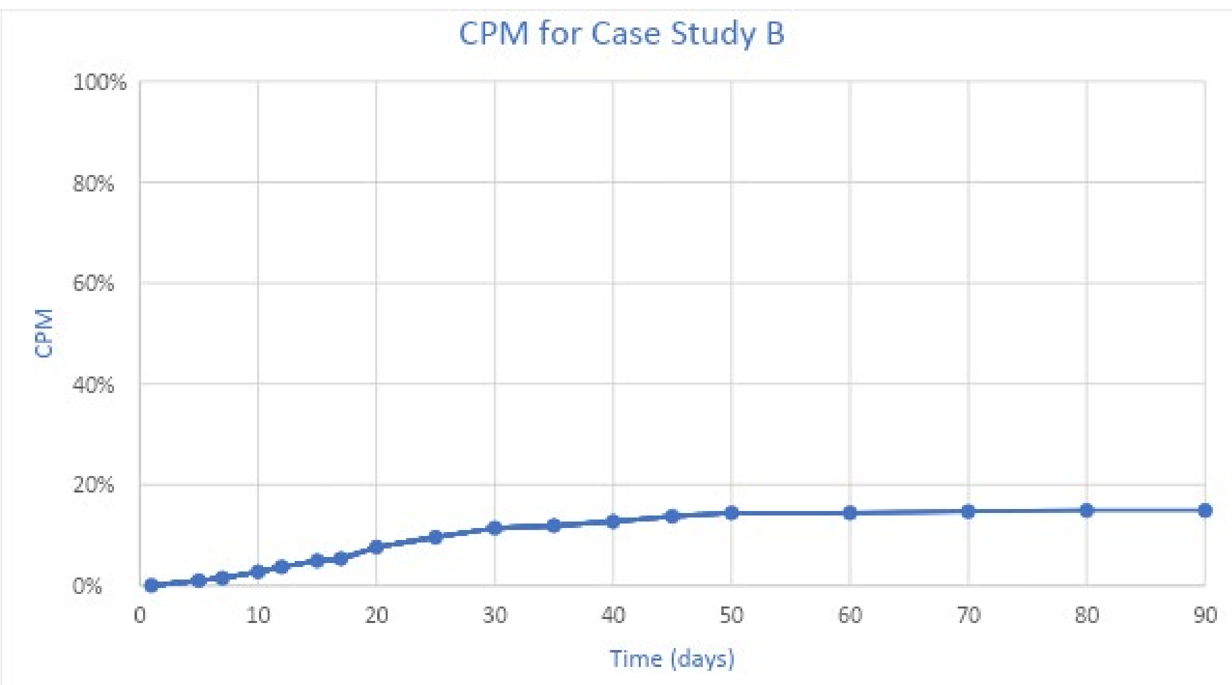
Farm C Cumulative Percent Mortality
100%
80%
60%
40%
20%
0%
CPM
0
5
10
15
20
25
30
Days of Observation

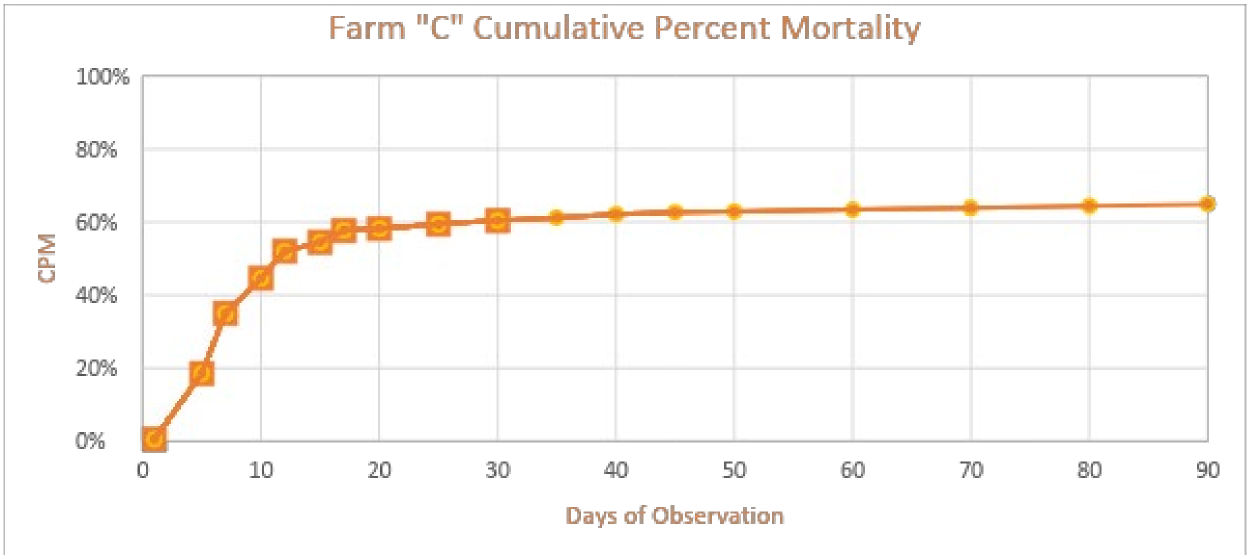

Dubois Dam CPM
100%
80%
60%
40%
20%
0%
CPM
0
5
10
15
20
25
30
Days

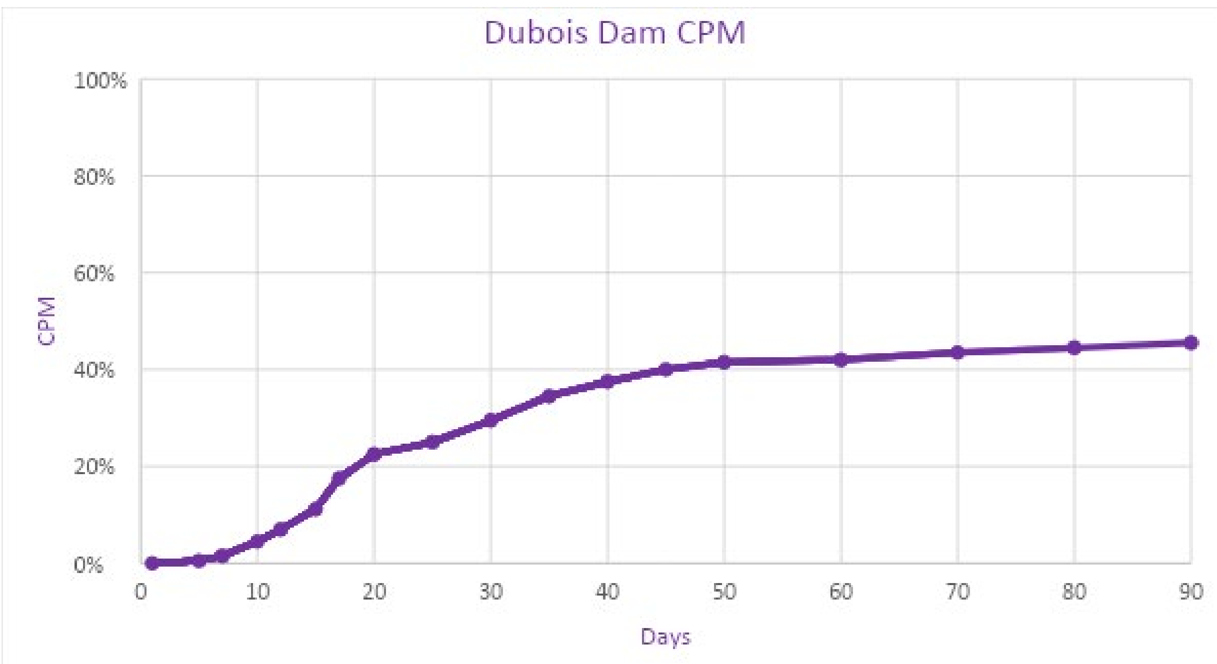

Farm E : CPM
100%
80%
60%
40%
20%
0%
CPM
0
5
10
15
20
25
30
Days of Observation

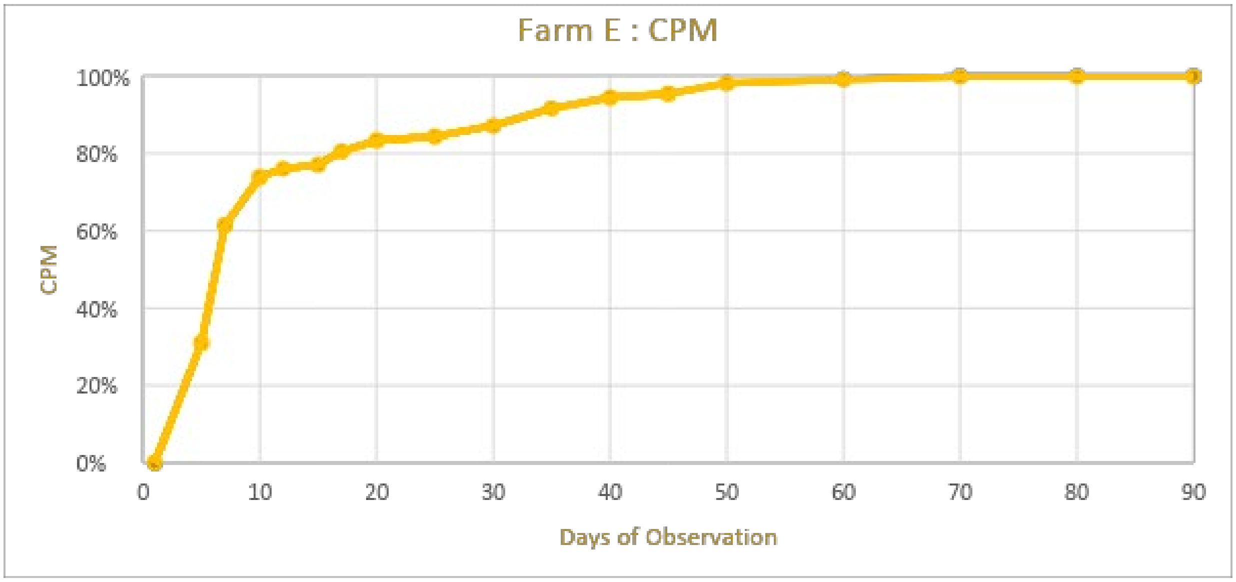
Answer Key Plots for Worksheet 3: CPM Curves, Farms A-E






Farm "C" Cumulative Percent Mortality
100%
80%
60%
40%
20%
0%
CPM
0
10
20
30
40
50
60
70
80
90
Days of Observation


Dubois Dam CPM
100%
80%
60%
40%
20%
0%
CPM
0
10
20
30
40
50
60
70
80
90
Days


Farm E : CPM
100%
80%
60%
40%
20%
0%
CPM
0
10
20
30
40
50
60
70
80
90
Days of Observation