Testing for Grazer Adaptation to Toxic Algae

by
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Part I – Introduction and Background

Phytoplankton, microscopic single-celled algae, are natural components of aquatic ecosystems and are responsible for half of the carbon and oxygen produced by plants in the world. Some phytoplankton species, however, produce toxins. These toxin producing algae are becoming more common and showing up in more places worldwide. Blooms of the toxic dinoflagellate genus *Alexandrium* spp. (many species are known by the misnomer "red tide"; Figure 2, inset) are common from Long Island Sound in the United States, to the Bay of Fundy, Canada. *Alexandrium* produces neurotoxins that prevent nerve transmission signals, resulting in negative effects (including paralysis and eventual death) in animals (grazers) that consume it. Toxins from phytoplankton can be transferred through the food web (Figure 1) and accumulate in higher trophic levels, causing mortality in shellfish, fish, sea birds, and humans. Humans that eat shellfish (e.g., clams) contaminated by toxic phytoplankton, such as *Alexandrium* spp., can become sick and die, an illness known as paralytic shellfish poisoning (PSP). A major challenge for aquatic scientists and environmental managers is to predict the response of animal populations to the proliferation of toxic phytoplankton. A great deal of scientific effort is spent trying to understand the population dynamics of toxic algal blooms (e.g., when and where they will occur) and their effects on the food web.

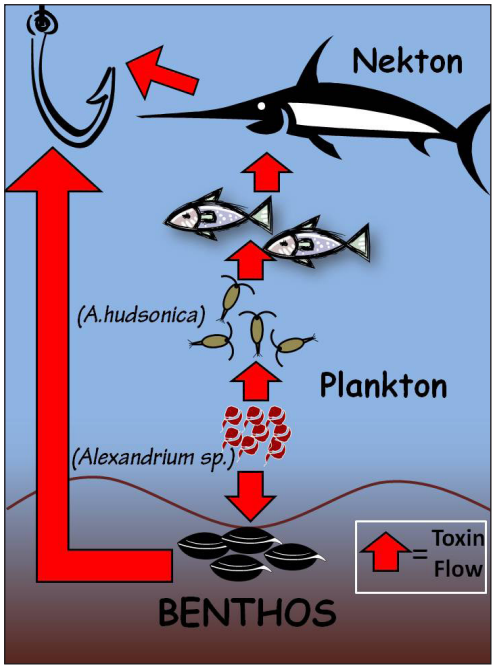

Figure 1: Simplified marine food web, including toxic phytoplankton *Alexandrium* spp.

A vital link between phytoplankton and higher trophic levels (e.g., fish) is provided by copepods, a type of zooplankton. Zooplankton are animal-like plankton that need to ingest other organisms to survive. Copepods are estimated to be the most abundant animals on the planet and are the main food source for many larval fish species. This leads to the refrain: no copepods, no fish. When copepods eat toxic algae they generally experience reduced growth, lower fecundity, and increased mortality. However, not all copepods suffer negative consequences. Some seem to cope well with the toxic algae. Scientists believe that exposure of copepods to toxic food is related to their tolerance. Some populations of the copepod *Acartia hudsonica* (Figure 2, main image) live in areas that commonly experience blooms of highly toxic *Alexandrium*

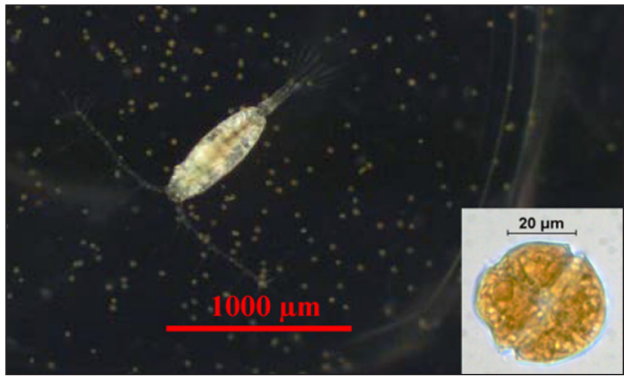

Figure 2: The copepod *Acartia hudsonica* (main) and a toxic phytoplankton cell, *Alexandrium fundyense* (inset).

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spp., whereas other populations live in areas free of *Alexandrium*. How would the growth, fecundity and mortality differ in copepod populations often exposed to toxic *Alexandrium* versus those free of *Alexandrium*? That is, which of the copepod populations are better able to cope with (be more adapted to) toxic *Alexandrium*? This case study will illustrate how to answer this question and test for adaptation of animals to toxic prey (phytoplankton in this case). You will use the model system of the ubiquitous coastal copepod species *Acartia hudsonica* and its toxic phytoplankton prey *Alexandrium* spp. Specifically, you will formulate testable hypotheses, design an experiment to test your hypotheses, and interpret the results.

Questions

1. What negative effects can toxic algae produce in aquatic ecosystems?

2. Why would environmental managers care about the interaction between toxic algae and their grazers?

3. Why do scientists think that some copepods have adapted to toxic phytoplankton?

Part II – Problem and Hypothesis Formulation

An adaptation is a heritable feature in an organism that provides a fitness advantage (i.e., an advantage increasing an organism's ability to leave offspring to replace itself) in a given environment. In our case, grazer tolerance to phytoplankton toxins is the fitness advantage; tolerance allows copepods to eat more, produce more eggs, and live longer in the presence of toxic algae, resulting in greater reproductive output. Organisms without the adaptation are culled from the population, leaving mostly individuals with the adaptation in the population. If we can relate variation in an organismal trait to a fitness advantage then we infer that the variation is adaptive. The challenge is to design an experiment that can show adaptation.

The geographic range of toxic *Alexandrium* is from the Bay of Fundy, Canada, to about Long Island Sound, New York and Connecticut. There is a strong north to south gradient in the frequency (how often blooms occur) and toxicity (toxin per cell) of *Alexandrium* blooms (Figure 3). Toxic *Alexandrium* is not found south of Long Island Sound. The copepod *Acartia hudsonica* is found from the Bay of Fundy to the Delaware Bay. One could then logically infer that the variations in exposure to toxins among copepod populations could lead to different degrees of adaptation, if adaptation is present.

In science, observations of natural phenomena often lead to interesting questions. A hypothesis is formulated as a well-reasoned explanation to these questions. This case study presents a clear example of the natural progress of observation to hypothesis to analysis and conclusion.

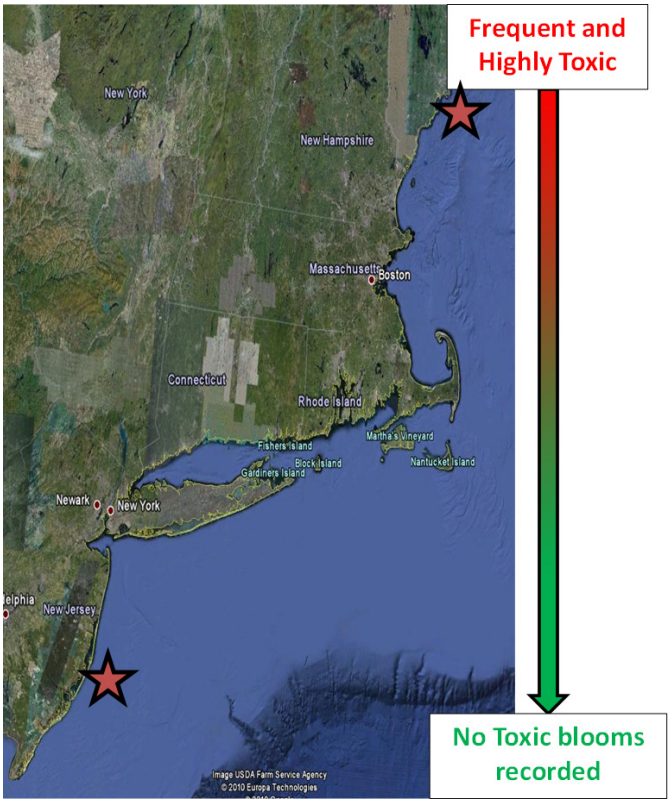


Figure 3: Geographical distribution of toxic *Alexandrium* spp. The frequency and toxicity of *Alexandrium* decreases from north to south. The copepod *Acartia hudsonica*, a main grazer of toxic *Alexandrium*, is found throughout the entire range shown. Stars correspond to population origins from experiments described in Parts III–V of this case study. Northern star indicates Maine, southern star indicates New Jersey sampling sites.

Questions

4. Identify the observation that led scientists to pose the question: "Can copepods be adapted to toxic phytoplankton?"

5. Develop a testable hypothesis regarding adaptation of *A. hudsonica* to toxic *Alexandrium* spp.

6. What measurements would you make to test your hypothesis?

Part III – Experimental Design

Genes, Environment, and the Phenotype

Expression of phenotypic traits (e.g., ability to grow or reproduce) is a complex interaction between genetic makeup and environmental variables. Phenotypic variation can be broken down into three main components:

Phenotypic variation = Genes + Environment + (Genes × Environment) (Equation 1)

Consider for example the height of individuals. Identical twins have the same genetic makeup; however, if one is raised on a poor diet compared to the other (two different environments) then the second twin may not grow as tall relative to the twin raised on a highly nutritious diet. Because both twins are genetically identical, we infer that the environment (diet) and the interaction of the environment and genes determined the differences in size and growth between the twins. Alternatively, if we raise individuals from different parents in the same environment for several generations, we eliminate the environmental effect on phenotypic variation. Any differences among these individuals are inferred to be genetic. Since natural selection requires genetically heritable variation, a challenge to test for population adaptation is to show that differences in traits among populations are genetic, not just due to the effect of environmental variation.

In our model system, copepods from the north (e.g., Maine) live in a different environment than copepods from the south (e.g., New Jersey). Thus, testing for adaptation to toxic *Alexandrium* requires we account for or eliminate environmental differences.

Questions

7. What are some of the differences in the marine environment between Maine and New Jersey? How could these affect copepods and the outcome of any experiment comparing the two populations?

8. Using the equation above, brainstorm ways to reduce the influence of the environment on a copepod's phenotypic traits. (*Hint:* eliminate the variables you don't want to influence the outcome.)

Food Choice

The type of food you feed your copepods is also important. Undoubtedly, you are thinking of feeding copepods a diet that contains some amount of toxic *Alexandrium*. This is a good start; however, only testing ingestion on a toxic diet does not answer the question completely. Let's say you observe that copepods from Maine grow better on a toxic diet than those from New Jersey. Is the difference due to adaptation of the Maine population, or is it due to something else? For example, maybe copepods from Maine always grow better on any type of food than copepods from New Jersey. Feeding both populations a non-toxic food is also important for this reason.

Questions

9. If the response between populations is the same on non-toxic food, but different on toxic food, then what does that suggest regarding copepod adaptation to toxic food? What does it mean if the response between New Jersey and Maine is the same on both toxic and non-toxic food?

Part IV – Results and Data Analysis

Scientists wanted to determine if a history of exposure led to adaptation of copepods to toxic *Alexandrium*. They sampled copepods from Maine (ME), which are often exposed to toxic *Alexandrium*, and from New Jersey (NJ), which are not exposed to it. Copepods from these two locations were raised under similar environmental conditions (e.g., light, temperature, food, etc.) for several generations. This "common garden" approach reduces the influence of the environment on the phenotype. That is, in Equation 1 above, since the environment is the same between the populations, the "Environment" component is removed from the equation, leaving any differences observed during experimentation attributable to genetic factors.

Scientists measured life-history traits of copepods that are important to fitness on both toxic and non-toxic diets for the New Jersey and Maine populations. These are the two metrics of fitness the scientists used and how they are measured:

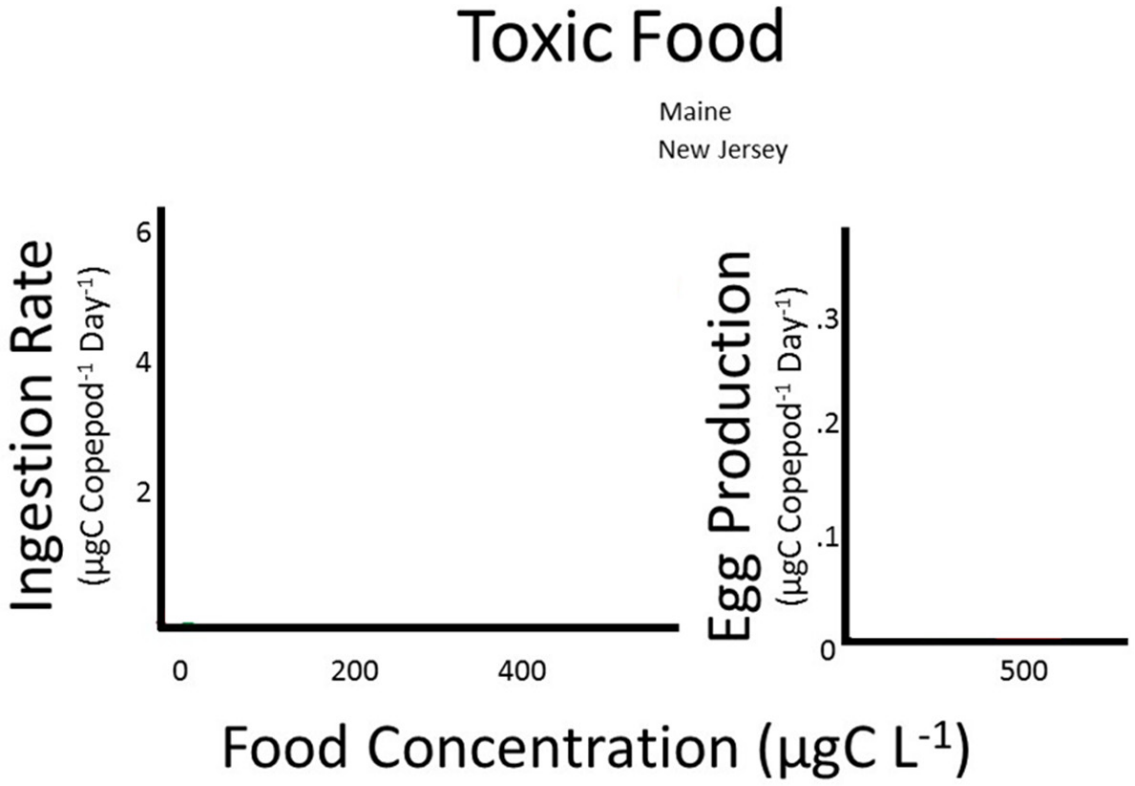
Ingestion:

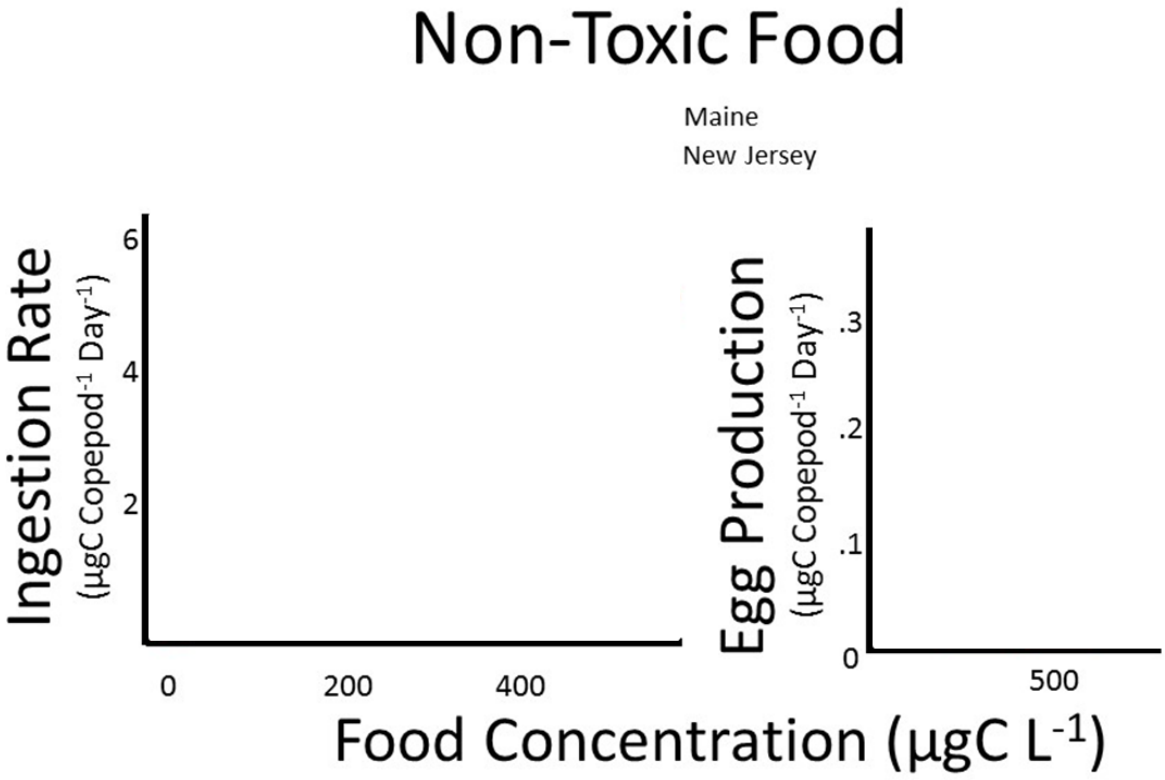
- Ingestion rate is a measure of feeding for copepods.
- It is commonly represented by an asymptotic curve. That is, feeding is saturated beyond a certain food concentration; copepods cannot physically eat any more food beyond this food concentration. This is known as a functional response.
- To measure this, copepods are placed in separate sealed containers containing various concentrations of the algae. Replication is typically done in triplicate (three bottles per treatment). To account for algal growth during incubation, control bottles of no copepods are also included.
- Units are given in μgCL^{-1} to account for differences in cell size among algal strains

Egg Production:

- Egg production rate is a measure of reproductive output—an important metric in fitness. Copepods cease somatic growth upon maturation so all excess energy in females goes to eggs.
- This curve is also asymptotic in nature (females can only physiologically lay a given number of eggs) and is known as a numerical response. For this exercise we are only focusing on the highest food concentration. Thus, a histogram is used.
- Eggs can be counted from the same bottles for ingestion, above, but are more commonly conducted separately using the same methodological approach. If groups of copepods are used the total eggs are divided by the total number of copepods; the goal is to have a rate of growth per copepod. This type of copepod is known as a broadcast spawner. This means that the females lay their eggs into the environment as they are created. Copepod egg production is closely tied to their food environment at the moment; in other words, the more they eat the more eggs they should produce. Eggs are negatively buoyant and a fraction of the size of adult copepods and can be easily separated through filtration and viewed at the bottom of a Petri dish.

Below, you will find two sets of blank axes. The units and treatments have been supplied for you. Based on your hypotheses draw the expected functional and numerical responses for both toxic and non-toxic diets. Do not look ahead. Discuss your predictions with your instructor.


Toxic Food
Maine
New Jersey
Ingestion Rate
(µgC Copepod-1 Day-1)
6
4
2
0
200
400
Egg Production
(µgC Copepod-1 Day-1)
.3
.2
.1
0
500
Food Concentration (µgC L-1)


Non-Toxic Food
Maine
New Jersey
Ingestion Rate
(µgC Copepod-1 Day-1)
6
4
2
0
200
400
Egg Production
(µgC Copepod-1 Day-1)
.3
.2
.1
0
500
Food Concentration (µgC L-1)

Revealed!

Figures 4 and 5 provide a summary of the results. Error bars were omitted from the functional responses for clarity. Compare and contrast these results with what you drew above. What is similar? What is different? After a thorough discussion proceed to the questions following the figures.

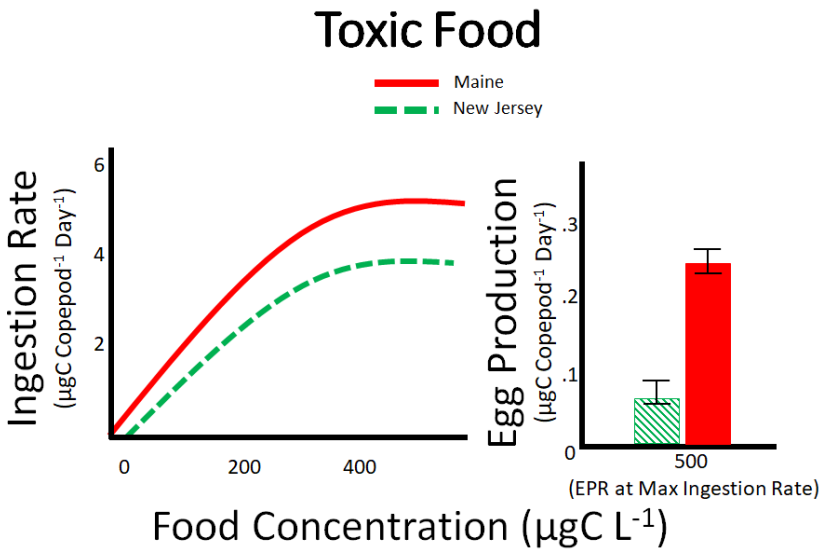


Figure 4. Ingestion rate (feeding; left) and egg production rate (reproduction; right) for copepods feeding on a diet containing toxic *Alexandrium* spp. Ingestion rates were measured over a range of food concentrations while egg production rates correspond to a single concentration. Food saturation occurs around 500 µgCL^{-1}; this means that this concentration represents maximum ingestion and egg production. Scientists chose this concentration for egg production because this is where the greatest difference, if any, in ingestion rate occurred. The Maine population (red) had statistically higher ingestion and egg production rate compared to copepods from New Jersey (green). Units for the dependent variable are in micrograms of carbon (µgC; food) and per copepod per day (ingestion and egg production). Error bars represent standard deviation among replicates in egg production; they are omitted for clarity from ingestion rates. Data adapted from Colin and Dam (2007, 2004).

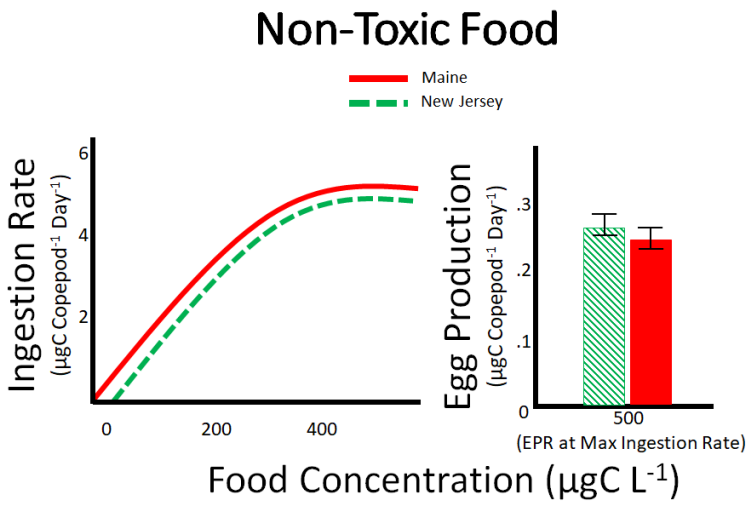


Figure 5. Ingestion rate (feeding; left) and egg production rate (reproduction; right) for copepods feeding on a non-toxic diet. Ingestion rates were measured over a range of food concentrations while egg production rates correspond to a single concentration. Food saturation occurs around 500 µgCL^{-1}; this means that this concentration represents maximum ingestion and egg production. There were no differences between the Maine (red) and New Jersey (green) populations for both ingestion and egg production rate compared. That is, the two populations fed and reproduced at the same rate. Units and error bars are the same as Figure 1. Data adapted from Colin and Dam (2007, 2004).

Before answering the questions, first re-write your hypothesis.

Hypothesis: _____

_____

Questions

10. Does geographic origin appear to influence the ingestion and EPR of copepods feeding on a toxic diet? On a non-toxic diet?

11. Do populations of copepods naïve to toxic *Alexandrium* suffer a decrease in fitness when fed a toxic diet?

12. Do these data support your hypothesis above? Explain.

Evolutionary Change

Evolutionary change can occur through several mechanisms. Notably, either random (e.g., genetic drift) or non-random (i.e., natural selection) means. Adaptations only result from natural selection. The three requirements for natural selection are 1) variations in traits, 2) advantage of certain traits under specific environments 3) heritability of advantageous traits.

Questions

13. Were the above evolutionary changes most likely a result of random or non-random mechanisms? What additional experiment would further help support your claim?

14. If natural selection, indicate how the three requirements have been fulfilled.

Part V – Ecological Significance

Adaptation and the Food Web

You have now identified that there is clear evidence of local evolutionary adaptation in copepod populations that have a history of exposure to toxic *Alexandrium*. The consequences of adaptation are different for the control of toxic phytoplankton blooms and for toxin transfer up the food web. Adaptation can keep toxic blooms under control as adapted animals can feed on the toxic food with fewer negative consequences. Conversely, the adapted animals can accumulate more toxins without becoming poisoned. This may result in higher toxin transfer up the food web.

Using the data from Part IV discuss the following questions.

Questions

15. How can adaptation in copepods lead to increased toxin levels in upper trophic levels such as fish and whales?

16. How can adaptation in copepods be used to predict bloom dynamics?

References

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