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Marine Biotoxins: Invisible, Odorless, and Lethal

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Introduction

Seafood provides a source of protein, essential vitamins and minerals, and omega-3 fatty acids. Americans are encouraged to include seafood as a part of a healthy diet. Adult Americans are recommended to eat at least 8 ounces of seafood each week, while pregnant and breastfeeding women are recommended to eat between 8-ounces and 12-ounces of certain types of seafood.[1] In 2018, the average American ate approximately 16.1 pounds of seafood, including fish and shellfish, and the amount of seafood Americans are eating each year is gradually rising.[2]

Changes in environmental conditions in our waterways and oceans attributed to human actions contribute to an increasing prevalence of naturally occurring biotoxin producing algae. Marine life including clams, mussels, crabs, and sardines show few to no negative impacts from consuming these biotoxins. However, they can become vectors of toxins when larger predators such as birds, otters, sea lions, whales, and humans consume contaminated organisms. Biotoxin poisoning can cause minor to significant negative health effects, including death. Humans can potentially lose the ability to secure safe consumable seafood from our oceans. Communities and economies dependent on fishing and harvesting of seafood are also vulnerable. Even more at risk are marine species that do not have alternative food sources.

Learning Objectives

In our high school statistics textbook, statistics is defined as the science and art of collecting, analyzing, and drawing conclusions from data.[3] Understanding statistics "will help you make good decisions based on data in your life."[4] A challenge I face when communicating the mathematical and statistical content and concepts to my students is the frequent disconnect between textbook *real-life* scenarios with the realities and knowledge my students possess from their lived experiences.

This curriculum unit introduces students to the broader environmental issue of marine biotoxins and their impacts on the human seafood supply. Biotoxins found along the California coastline will be a focus of this unit, with examples from the San Francisco Bay and Monterey Bay areas included due to their local proximity to East Side San Jose. Throughout this unit, students will learn the history of California's Marine Biotoxin Monitoring Program, examine existing data presented in research and reports from local, state, and federal government agencies, and understand the sampling techniques and methods used to determine health policies and advisories. Students will look at existing evidence of human contributions to conditions that are enabling increased proliferation of toxic algae. My students may also participate in the processes to monitor marine biotoxins. Students will develop materials to inform their community of ways to minimize risks associated with certain seafood consumption practices.

Statistics is classified as a mathematics course, yet the content has applications across the biological, physical, and social sciences. This curriculum unit is written for students taking Advanced Placement (AP) Statistics and includes activities that examine data collection techniques, experimental design, and statistical inference where data are used as a basis for making predictions, decisions, and conclusions on populations of study.

Students will examine data from existing studies and government agencies to make predictions regarding the effects of domoic acid among humans and other sea creatures. Existing studies report confidence intervals and confidence levels regarding rates and number of expected instances of domoic acid poisoning. Students will interpret the meaning of the confidence intervals and whether year-to-year trends are expected versus outliers. There will also be interpretations of the conclusions of studies with considerations to societal, corporate, and ecosystem impacts. Furthermore, students will be able to inform and educate the Overfelt community how to minimize risk of toxin exposures through seafood preparation and consumption.

Components of this unit can be adapted and used to teach the statistics content found in our Integrated Math 1, 2, and 3 courses. The content background provides an overview of two categories of common seafood illnesses caused by marine biotoxins and may be of interest to life science, biology, and chemistry teachers. Teachers from areas outside of California can utilize the examples in this unit and compare their local relevant city, county, and state agency algae and biotoxin monitoring programs that occur at oceans, lakes, rivers, and other bodies of water.

Background

William C. Overfelt High School is in the heart of the East Side of San Jose, California. In the East Side of San Jose, the population demographic data indicated about 55% Hispanic/Latinx, 33% Asian, 5% White, and 3% Black or African American. This contrasts with the entire City of San Jose, which is a population of approximately 1 million people, which is about 28% White, 32% Hispanic/Latinx, 34% Asian, and 3% Black or African American. Our school serves approximately 1400 students which are composed of approximately 80% Hispanic/Latinx, 10% Asian, 8% Black or African American. Over 80% of our students qualify for free or reduced lunch.

A challenge at Overfelt is to encourage more students to enroll and be successful in Advanced Placement (AP) courses. Historically, students from lower socioeconomic and underrepresented minority backgrounds have difficulties in achieving a passing score on the AP exams. Overfelt established a team of teachers, counselors, and administrators working to increase access, create supports, and motivate students to take on rigorous coursework. I am involved with increasing the number of opportunities Overfelt students have in experiencing science, technology, engineering, mathematics (STEM) within multiple content areas beyond the classroom. As part of our initiative, I was able to work with one of our counselors to bring students to the 2019-2020 San Jose Speaker Series where they heard informal talks from Dr. Sanjay Gupta, Bill Nye, Captain Scott Kelly, and Paul Nicklen. A common trend among all of the talks was the need for the generation of scientists, leaders, and thinkers to create and implement solutions to address the impacts humans have had on Earth's environment and climate and to create sustainable ways to allow our planet to thrive.

Rationale

Seafood plays a large role in the cuisine and food traditions for many of my students' cultural backgrounds. The community Overfelt serves is predominately Hispanic/Latinx and Asian. At many Mexican restaurants, octopus, raw oysters, and ceviche made of a variety of fish and seafood are common on the menus. Chinese restaurants will use tanks of live fish, king crabs, Australian crabs, shrimp, and other seafood as décor. Deep fried Dungeness crabs, ginger scallion lobsters and fresh prawns are staples for dinners celebrating special occasions. Living in Northern California in the greater San Francisco Bay Area region, the iconic Dungeness crab season that traditionally starts in mid-December is timed perfectly to coincide with holiday and New Year's gatherings.

Figure 1: Fried whole smelt fish being portioned at a Chinese dim sum restaurant. Large quantities of clams in black bean sauce are also available.[5]

Overfelt is located within blocks of Asian and Mexican shopping centers, restaurants, and businesses. Of note are the local supermarkets. We have the usual major grocery chains available in our area. However, what makes the East Side unique and vibrant is the wide variety of large Mexican, Vietnamese, Chinese, and Filipino grocery stores offering ingredients and items that meet the local demands. One of the key differences in these grocery stores is the seafood counter. American run chains typically have very clean, sleek refrigerated cases displaying filets of fish and bags of shellfish sitting on ice, and maybe a lobster tank with a few lobsters. In our Asian and Mexican markets, customers can choose from varieties of fish, squids, octopi, oysters in their shells sold by the dozens, tanks of Dungeness and blue crabs, lobsters, clams, shrimp, geoducks, sea snails, sea cucumbers, abalone and frogs so fresh that many options are still alive.

What goes unnoticed or ignored are government mandated warning signs of potential foodborne illnesses from undercooked or raw seafood, California Proposition 65 warning signs indicating that seafood may contain chemicals that are known to cause cancer, birth defects, or other reproductive harm. Notably absent or not as apparent are signs indicating the potential for ingesting seafood containing biotoxins that are not removable through cooking, nor are there signs clearly visible mentioning ways to minimize exposure. Methods to minimize exposure, which are discussed below, often contradict and conflict with cooking methods and cultural practices used by my students and their families when preparing and consuming seafood, potentially putting the health and lives of many at risk.

Furthermore, beaches on the Pacific are less than an hour away from San Jose. Just over an hour to the south is Monterey Bay, where you can find many restaurants offering their version of clam chowder and fresh seafood. An hour north of San Jose is San Francisco, where Fisherman's Wharf is a major tourist attraction and the annual Dungeness crab season beginning in the Fall is a highlight. One may easily be mistaken to believe that the restaurants are serving seafood caught off the local coastline and wild mussels, clams and other bivalves can be found along the coast on beaches. However, the seafood most likely came from other sources as the wild populations found off the Bay Area county coastlines have elevated levels of biotoxins that can sicken and potentially kill humans that consume contaminated seafood.

Content Background

Phytoplankton and algae form the base of the ocean's food web. Phytoplankton and algae are primary producers that synthesize their own food through photosynthesis or chemosynthesis and are necessary to support marine ecosystems.[6] Estimates from the United States Food and Drug Administration (FDA) show that less than one percent of algae is toxic to humans, but thrive in conditions close to coastlines where many commercial fishing and aquaculture takes place.

Concerns regarding sustainability and safety with both wild caught and agriculture farm raised seafood are increasing. Organizations such as the Monterey Bay Aquarium Seafood Watch increases awareness and provides education to consumers to make seafood choices that are sustainable and better protect the wild fishery habitats. Another major concern in recent years is the increasing prevalence and types of toxic phytoplankton appearing in fishing habitats around the world. Many indications show that human influences have created favorable environmental conditions that allow for the proliferation of such toxic algae and has significant consequences for many species connected to the ocean food web.

Seafood, as with all other types of food, is regulated by various local, state, and federal agencies. As demands for seafood, and food in general, rises with increasing populations of people and shifts in dietary trends among people across the world, one consequence is an increasing prevalence of marine phytoplankton that produce toxins that may be harmful to larger animals in the food web. Human contributions include climate change, unsustainable aquaculture practices, and pollution of water. This curriculum unit will look at the studies investigating Amnesic Shellfish Poisoning (ASP) caused by ingestion of domoic acid produced by *Pseudo-nitzschia*, due to its increasing prevalence and impact along the California coastline.

Historical Background

In 1921, the State of California Fish and Game Commission released a report titled *The Edible Clams, Mussels and Scallops of California*. At the time, approximately 500 different species of clams, mussels, and scallops were found along the United States Pacific coastline. Over a century ago, the report notes that bivalves along the California coastline were "a source of food now distinctly neglected.[7] " A list of 43 species found along California coasts deemed to be edible by humans includes a variety of clams, mussels, geoduck, cockles and oysters (Figure 2). Goals of the report included a survey of the variety and abundance of edible species, to be a comprehensive guide for citizens to identify types of bivalves, and also to promote “the possibilities of food in the clams, mussels and other bivalves of the California coast, for these animals are at their best when freshly taken.[8] ” People were encouraged to educate themselves about the varieties of bivalves such that “the attention of the camper to the interesting adaptations and beauties of these little known animals, his pleasure in the great out of doors will be correspondingly increased.”[9] The guide also includes recipes for clam and tomato bisque, fried clams, fried croquettes, clam sauce, steamed mussels (or clams), savory clams en casserole, and scalloped clams.

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LIST OF THE EDIBLE BIVALVES OF CALIFORNIA

	Scientific name	Common name	Other names
1.	*Ostrea lurida* Carpenter	Native oyster	
2.	*Pecten circularis* Sowerby	Scallop	Fan shell
3.	*Hinnites giganteus* Gray	Rock scallop	Rock pecten
4.	*Anomia peruviana* d'Orbigny	Rock oyster	
5.	*Monia macroschisma* Deshayes	Rock oyster	
6.	*Mytilus californianus* Conrad	Sea mussel	
7.	*Mytilus edulis* Linnaeus	Bay mussel	
8.	*Modiolus rectus* Conrad	Horse mussel	
9.	*Cardium quadragenarium* Conrad	Spiny cockle	
10.	*Cardium corbis* Martyn	Cockle	
11.	*Cardium elatum* Sowerby	Giant cockle	
12.	*Tivela stultorum* Mawe	Pismo clam	
13.	*Amiantis callosa* Conrad	Sea cockle	
14.	*Saxidomus nuttalli* Conrad	Washington clam	Butter clam Money-shell
15.	*Saxidomus giganteus* Deshayes	Washington clam	Same as above
16.	*Chione fluctifraga* Sowerby	Hard-shell cockle	
17.	*Chione undatella* Sowerby	Hard-shell cockle	
18.	*Chione succincta* Valenciennes	Hard-shell cockle	
19.	*Paphia tenerrima* Carpenter	Thin-shelled cockle	
20.	*Paphia staminea* Conrad	Rock cockle	Little-neck Hard-shell Tomales Bay cockle Rock clam
21.	*Tellina bodegensis* Hinds	Tellen	
22.	*Metis alta* Conrad	Metis	
23.	*Macoma nasuta* Conrad	Bent-nosed clam	Mud clam
24.	*Macoma secta* Conrad	White sand clam	
25.	*Semele decisa* Conrad	Flat Clam	
26.	*Psammobia californica* Conrad	Sunset shell	
27.	*Psammobia edentula* Gabb	Sunset shell	
28.	*Sanguinolaria nuttalli* Conrad	Purple clam	
29.	*Donax californica* Conrad	Wedge shell	
30.	*Donax laevigata* Deshayes	Common wedge shell	Bean clam
31.	*Tagelus californianus* Conrad	Jackknife clam	Razor clam
32.	*Solen sicarius* Gould	Jackknife clam	Razor clam
33.	*Solen rosaceus* Carpenter	Jackknife clam	Razor clam
34.	*Siliqua lucida* Conrad	Razor clam	Razor shell Sea clam
35.	*Siliqua patula* Dixon	Razor clam	Same as above
36.	*Spisula sp.*	Dish shell	
37.	*Schizothaerus nuttalli* Conrad	Gaper	Summer clam Horse clam Otter-shell
38.	*Mya arenaria* Linnaeus	Soft-shell	Soft clam Long clam Mud clam
39.	*Platyodon cancellatus* Conrad		
40.	*Panope generosa* Gould	Geoduck	
41.	*Zirfaea gabbi* Tryon	Piddock	Borer
42.	*Parapholas californica* Conrad	Borer	Piddock
43.	*Pholadidea penita* Conrad	Rock clam	Borer Piddock

Figure 2: List of Edible Bivalves of California published in 1921.[10]

California's Marine Biotoxin Monitoring Program began in 1927 as a response to a large paralytic shellfish poisoning (PSP) event. Over a hundred people became ill and several died after eating contaminated wild mussels. The California Department of Public Health established an annual mussel quarantine prohibiting the collection and resale of mussels from California coastlines in 1942 from June 1 through September 30. The current annual mussel quarantine now includes the period from May 1 through October 31, and dates can be extended if necessary, based on detected levels of marine biotoxins. The quarantine period is determined by examining historical records which show this time frame typically has higher levels of biotoxins and 99 percent of all PSP illnesses in California since 1927 have occurred during these months.[11]

Nearly a century after the California report encouraging the public to consume more bivalves, on July 1, 2020, the California Department of Public Health issued a warning advising consumers to not eat sport-harvested mussels, clams, or whole scallops from the waters in San Francisco, San Mateo, Santa Cruz, and Monterey counties in Northern California. Naturally occurring biotoxins along the California coastline, and in waters and coastlines across the United States and world have increased in prevalence. Changes in climate and increased water temperatures have enabled biotoxin producing phytoplankton, algae, to expand their territory and proliferate. Bivalves and small fish consume these phytoplankton; although many species are unknown to be harmed, they become reservoirs for biotoxins. Larger species in the food web such as crustaceans, sea lions, otters, and humans can consume contaminated sea creatures and suffer seafood borne illnesses.

Algal Blooms

Algal blooms occur when overgrowth of colonies of algae occur in a region of water. Not all algal blooms are toxic, as only a small percentage of algae produce biotoxins. Conditions needed for algal blooms are sunlight, slow-moving water, and nutrients such as nitrogen and phosphorus. The United States Environmental Protection Agency (EPA) notes that sources of nitrogen and phosphorus nutrient pollution in waterways can be attributed to agriculture water runoff, stormwater, wastewater, fossil fuel burning, fertilizers, pet waste, and certain soaps and detergents.[12]

Harmful Algal Blooms

Harmful algal blooms (HABs) are algae blooms that contain species of algae that produce biotoxins or harmfully affect other living organisms such as people, shellfish, fish, marine mammals, and birds. Every coastal state in the United States has reported HABs. HABs can occur in fresh and saltwater environments and are commonly referred to as "red tides," as some HABs can turn waters red brown in color (Figure 3). However, water discoloration can appear in a variety of colors, or not at all, based on the species of phytoplankton, cyanobacteria, algae, dinoflagellates, and diatoms present in a colony. The National Oceanic and Atmospheric Administration (NOAA) works with regional agencies to monitor, track, and forecast HABs across the United States.[13]

Figure 3: *Karenia brevis* “Red Tide”, La Jolla, California.[14]

Climate change is implicated as a major contributor for the proliferation of a variety of harmful toxic algae blooms along the California Coast and internationally. Increased water temperatures allow toxic algae to thrive across a wider range of area. Shipping has inadvertently spread toxic algae and phytoplankton to new ecosystems on a global scale. Azaspiracid Shellfish Poisoning (AZP) originated in Europe in 1995, is now found in Asia, and is beginning to emerge in the United States.[15] Overfishing of populations has thrown off the balance in some ecosystems which allowed other species to thrive. Increased aquaculture to meet the global demand for seafood has given opportunities for some parasites and toxic algae to proliferate and contaminate water sources.[16]

Seafood Borne Illnesses

The Food and Drug Administration identifies five types of toxic algae that are known to impact humans. The types include Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), Diarrhetic Shellfish Poisoning (NSP), Diarrhetic Shellfish Poisoning (DSP), and Azaspiracid Shellfish Poisoning (AZP). Ciguatera Fish Poisoning (CFP) is also a concern to humans. Each type of seafood poisoning is categorized by the genus type of algae that produces a specific type of toxin resulting in the type of seafood poisoning. This curriculum unit will look at PSP and ASP as they are the most common types of seafood poisoning that occur from consuming seafood sourced from California coastal waters.

Amnesic Shellfish Poisoning (ASP)

Amnesic Shellfish Poisoning (ASP) is caused by the ingestion of seafood that has the neurotoxin domoic acid produced by the algae *Pseudo-nitzschiaaustralis*. Domoic acid is not harmful to certain sea animals that feed on *Pseudo-nitzschiaaustralis*, including small fish such as sardines and anchovies. Larger predators including larger species of fish and crabs that feed on sardines and anchovies can become reservoirs for domoic acid. Filter feeders such as clams, mussels, and oysters can also accumulate domoic acid. Levels of domoic acid in an organism can decrease but the rate and concentration levels vary dramatically by species. For example, domoic acid levels in some species of mussels can decrease to safe levels within days, while levels of domoic acid in razor clams can remain elevated for weeks to months. The Food and Drug Administration safety threshold for domoic acid is 30 parts-per-million action level.

Repeated exposure to domoic acid or consuming a large quantity of contaminated seafood results in ASP. Symptoms include lethargy, disorientation, seizures, coma, and possible death. As a neurotoxin, domoic acid targets the brain, particularly the hippocampus which is responsible for memory. There are no medications to treat domoic acid poisoning directly. Treatments for ASP include administering fluids to help remove domoic acid from an infected animal or person. Long-term impacts of ASP include irreversible memory loss and the inability to form short term memories.

In November and December 1987, the first large scale outbreak of domoic acid poisoning through consuming contaminated mussels was recorded in Canada.[17] In 1998, the first mammal exhibiting domoic acid poisoning was recorded at the Marina Mammal Center in Sausalito, California, just across the Golden Gate Bridge from San Francisco where over 400 sea lions suffered significant neurological symptoms or death.[18] In 2015, an unprecedented postponement of the California commercial Dungeness crab fishing season was enacted due to dangerous levels of domoic acid as the result of a large scale algae bloom attributed to changing weather patterns.[19]

California Sea Lions

Sea lions feed on a variety of small fishes, such as anchovies and sardines, crabs, and clams. In San Francisco, they are a tourist attraction and can be seen lounging on the docks near Fisherman's Wharf. As animals higher up the food chain, regular consumption of domoic acid contaminated sea creatures can lead to buildup of domoic acid in sea lion brains. Affected sea lions may exhibit symptoms of dizziness, vomiting, seizures, and loss of pregnancy. Observations of erratic behaviors of sea lions, characterized by head weaving, are used as indicators of localized algae blooms. It is not practical nor possible to determine when and where toxic algae blooms will form. These sea lions are essentially canary birds in a coalmine, as they serve as warning signals for humans to avoid certain regions of coastline for fishing when increased numbers of sea lions behaving erratically, a symptom of domoic acid poisoning, are reported.

Figure 4: Marine Mammal Center volunteers work to rescue a California sea lion.[20]

The Marine Mammal Center maintains records of the number of sea lions with domoic acid poisoning along the Central California Coastline. Confidence intervals based on past data are used to make predictions on the expected number of live sea lion stranded on beaches, exhibiting symptoms, and requiring treatment at the center for suspected domoic acid poisoning. In 2015, projections expected between 43 and 86 sea lions to be stranded on beaches while exhibiting symptoms. Due to a large algae bloom, there were 229 stranded sea lions in 2015 in the same region.[21] In 2020, reports of California sea lions exhibiting domoic acid poisoning in mid-May were earlier in the year than expected.[22]

Paralytic Shellfish Poisoning (PSP)

Gonyaulacoid dinoflagellates are unicellular organisms that can develop algal blooms and dinotoxins. Saxitoxin is an example of a heat stable neurotoxin associated with PSP. Onset of symptoms can occur within minutes and may take up to a couple of hours after consuming contaminated seafood. Symptoms include tingling sensations in fingers, lips, mouth, and toes, followed by loss of muscle control in legs and arms, difficulty breathing, nausea, paralysis of muscles, including the lungs which can lead to death through suffocation. Death can occur in less than 30 minutes.[23] California has recorded 543 illnesses and 39 deaths attributed to PSP since 1903.[24] As with ASP, there are no medications that treat PSP directly. Treatments address symptoms and may include placing affected individuals on ventilators.

Seafood Safety

Commercial seafood in California and the United States is generally considered to be safe. Populations of wild mussels and other shellfish are not harvested for commercial sale. Shellfish growers of mussels, oysters and clams occur in hatcheries where toxin levels are regularly monitored and tested. Commercial producers are required to submit samples to the CDPH laboratories at least once a week and this accounts for two thirds of all samples tested.

Minimizing Risks

Common misconceptions regarding food safety are that sensory perceptions such as look, smell, and taste will indicate whether seafood is toxic. The belief is that cooking seafood to a certain temperature may prevent some food borne illnesses, but unfortunately this has no effect on removing certain toxins from food as many of the toxins are heat-stable and do not break down. Many of the toxins are odorless. Many species of clams, mussels, abalone, geoduck, crabs, lobsters, fish, and other organisms are relatively unaffected by biotoxins.

The California Department of Health advises wild mussels should never be assumed safe for consumption. This includes mussels harvested during and outside of the annual mussel quarantine period. Commercially harvested and distributed seafood are considered safe, but consumers are advised to follow certain preparation methods to lower risks as biotoxins may still be present in levels below the FDA safety thresholds.

What goes relatively unnoticed or ignored are required signs stating the potential dangers of eating certain types of seafood. Certain preparations and types of seafood have active health advisories and warnings to consumers in California. For example, deep frying is discouraged in favor of steaming and boiling. Discarding cooking liquids is recommended as some of the biotoxins can be released from the internal organs of clams, mussels, crabs, lobsters, and other seafood. It is strongly advised that the viscera of Dungeness crabs be discarded and completely removed prior to consumption (Figure 5). Digestive tracts of clams and scallops, which typically appear as dark brown and black regions, where domoic acid is most concentrated are advised to be removed prior to cooking and eating.

Figure 5: Cooked Dungeness crab with viscera, yellow-brown goo and liquid located in the shell. Viscera should be removed and discarded.[25]

The broths made from shellfish, a common ingredient valued for its flavor, to make soups and sauces are advised to be tossed to help reduce toxin exposure. Traditional preparation of cioppino, an Italian seafood stew originating in San Francisco, and commonly prepared by boiling in clams, mussels, and crabs all in their shells, in the soup broth is no longer advised. Dungeness crabs are considered safe to eat, but viscera, the internal organs where higher levels of toxins concentrate, are advised to be thrown out. However, viscera are considered a delicacy among some Asian cuisines. Most Chinese restaurants will serve deep fried Dungeness crab and include the shell body with viscera. Also, we have many local popular restaurants specializing in Cajun seafood boils and Chinese hot pot restaurants that serve whole Dungeness crabs, clams, mussels, and a variety of seafood cooked in their shells. Rarely, if ever, will you find a warning or be advised to discard the viscera or avoid consuming the flavored broths.

California Sampling and Testing Methods

Sampling of wild populations of shellfish along the California coast occurs and varies. A patchwork of local, state, federal, and Tribal agencies, researchers, private educational organizations, and volunteers collect samples for the CDPH laboratory to test. Volunteers are able to sign up, receive training, and are provided with nets for collecting seawater samples.[26] For Dungeness crab, testing occurs primarily between September and January,[27] but the number of samples per region depends on the quantity submitted and the results.

The California Department of Public Health lab is in Richmond, California, about an hour north of San Jose and across the bay from San Francisco. In a December 2019 article, the San Francisco Chronicle sought to provide some insight to the testing and sampling methods used by the laboratory and its five laboratory workers to explain how decisions regarding the start, and postponements of fishing seasons are determined.

The procedures for testing levels of domoic acid in Dungeness crab used by the CDPH laboratories are meant to emulate how a consumer would prepare and cook crab in their homes. With each sample, a batch of six crabs are selected and steamed for about 50 minutes. After cooking, the viscera of each crab are separated, and 4 grams are collected. The sample of viscera is then processed, and liquid chromatography machine identifies markers for domoic acid and other toxins.[28]

If samples indicate levels of domoic acid higher than the FDA designated safety thresholds, the CDPH issues advisories against consumption of Dungeness crab, and possibly other shellfish and seafood in areas where the sample was collected. This can lead to delays or cancellations of commercial fishing seasons. For advisories to be lifted, two consecutive subsequent tests in samples taken at least a week apart in affected areas must show levels of domoic acid and other toxins below the safety threshold.[29]

Statistics Concepts

The four main themes in a high school Advanced Placement (AP) Statistics course are exploring data, sampling, and experimentation, anticipating patterns, and statistical inference. Statistics is classified as a mathematics course and a common misunderstanding is that students spend most of their time computing data sets. However, most computations are done using graphing calculators or statistical software and only make up a small part of the curriculum. Many of the concepts and learning objectives in statistics require interpreting contexts and making data-based decisions regarding characteristics of populations. The following section provides an overview of key concepts addressed in the lesson activities.

Sampling

Samples from the same population can yield different results due to variability among the individuals of a population. *Variability*, the amount of variation or differences, of statistics is a central concept essential for understanding statistics. *Controlling* for variability when sampling a population is necessary for the sample data to provide statistically significant information that can be used to make inferences on the general population. Often it is not feasible or practical to take a *census*, collecting data from every individual in a population, sampling allows for insight regrading specific *parameters*, a characteristic of interest in a population. A *simple random sample* (SRS), a sample taken where every individual in a population has the same chance of being selected for the sample, is ideal as it also can help minimize bias.

Inference

Inference is when sample data are analyzed, and the results are used to make broader generalizations regarding a population. Practicing inference requires understanding the context of a problem, the parameters, and population of interest. Inference employs a variety of techniques including inference tests which utilize sample data to compute probabilities that are used to test assumptions. Sample data can also be used to construct *confidence intervals*, a range of expected amounts or observations of parameters within a population.

Hypotheses

Inference tests begin with a *hypothesis*, a claim that can be assessed. A *null hypothesis*, is the claim that there is no difference between specified populations. An *alternative hypothesis*, , the claim that there is a difference between specified populations. Inference tests have two possible outcomes. One outcome is that we reject the null hypothesis in support of the alternative hypothesis, meaning the sample data provided convincing statistical evidence that there is a difference between specified populations. The second outcome is that we fail to reject the null hypothesis, meaning the sample data does not provide convincing statistical evidence that there is a difference between specified populations. A challenge of statistics is that inference does not allow us to use samples of populations to prove there are no differences between populations.

Type I and Type II Errors

Samples provide insight regarding a specific parameter of a population. However, samples are not perfect data may be misleading. Errors do occur and have consequences. There are two types of errors that can occur when conducting statistical inference tests. *Type I Error* occurs if a statistical inference test fails to reject a null hypothesis when the null hypothesis is true. This means there is convincing evidence that the alternative hypothesis is true when it is in fact false. *Type II Error* is an error that occurs if a statistical inference test fails to reject a null hypothesis when the alternative is true. Here, the inference test concludes that there is not enough convincing evidence that the alternative hypothesis is true when it is in fact true.

Teaching Activities and Strategies

Statistics quantifies the world. I want my students to understand and apply the key statistical concepts outlined by the College Board in the AP Statistics course and exam description. I also want my students to experience as many aspects of the statistical processes, from experimental design, sampling, data analysis, inference, and communicating results. Many of the examples and practice problems used in statistics classes are based on published journal articles and studies. Student understanding of statistical concepts in relation to the context of a given situation is emphasized throughout the course. These sample activities will have students practice interpreting the methods and results based on data in existing studies regarding domoic acid along the California coastline. A culminating project I have planned include having students investigate seafood eating habits among their peers and families in our community and developing media to raise awareness of seafood toxicity in the local community. Potential field trips to local coastline to conduct sampling would depend on funding for transportation and coordination with local and state agencies.

Activity: Interpreting Correlation

California sea lions are regularly exposed to domoic acid through their diet which poses exposure risks to developing fetuses of pregnant sea lions. In the 2018 study “Domoic acid in California Sea lion fetal fluids indicates continuous exposure to a neuroteratogen poses risks to mammals” Lefebvre et al. measured domoic acid levels in fetal samples of California sea lions.

Domoic acid levels are quantified using enzyme-linked immunosorbent assay (ELISA) kits and using Liquid

Chromatography Mass Spectrometry (LC/MS-MS) methods. ELISA tests are more sensitive to detection than LC/MS-MS methods, but using multiple testing types can help confirm presence of domoic acid in samples.

A total of ten fetal fluid samples are analyzed to detect levels of domoic acid using both ELISA and LC/MS-MS methods. Linear regression of domoic acid levels quantified via ELISA and LC/MS-MS on the ten samples results in a p-value of and an R-square value of 0.69.

Questions

1. Is there a significant relationship between the quantified values of domoic acid using ELISA versus LC/MS-MS? Explain how you know.
2. Compute the correlation coefficient and interpret the value in the context of the problem.
3. Interpret the R-square value in the context of the problem.

Activity: Interpreting Confidence Levels

The Marine Mammal Center in Sausalito, California, works to rescue California sea lions stranded on central California beaches suspected of domoic acid poisoning. Using the data collected from 2010 to 2013, a 95% confidence interval of 64 21 sea lions per year admitted to the Marine Mammal Center is constructed to provide insight on the number of expected stranded sea lions due to domoic acid poisoning each year.

Questions

1. What is the mean number of California sea lions stranded on beaches between 2010 and 2013?
2. Identify the margin of error. What is the range of expected California sea lions each year?
3. In 2014, there 229 California sea lions were treated for domoic acid poisoning at the Marine Mammal Center. Was this number expected based on the confidence interval? What does this tell you about the amount of domoic acid in along the central California coastline in 2014?

Activity: Errors in Sampling – Dungeness Crabs

Using the example of domoic acid testing in Dungeness crab at the California Department of Public Health Students will examine the sampling techniques currently used in California to measure levels of toxins in seafood supplies and the surveys given to patients of suspected seafood poisoning used to determine whether government actions are needed to maintain a safe food supply.

Prompt: Health officials are testing domoic acid levels in Dungeness crabs from San Francisco Bay. Domoic acid is a neurotoxin that causes Amnesic Seafood Poisoning (ASP), which can cause memory loss and death If levels of domoic acid are above 20 mg/kg in the Dungeness Crab, the health officials issue a warning and crab fishing in San Francisco Bay must stop and sales of crab is banned. If levels are below the accepted amount, crab fishing can continue.

Questions

1. Identify the parameter and population.
2. Write the null hypothesis and alternative hypothesis.
3. What implications are there if there is a no warning, but levels of domoic acid are above the safety threshold? What type of error is this?
4. What implications are there if there is a warning, but levels of domoic acid are below the safety threshold? What type of error is this?

Project: Seafood Safety in the Local Community

Based on the responses to the questions from the previous activities, students will take their understanding of sampling and address the issue of seafood safety in the local community. Students will work in groups of 3 to 4 students to investigate the community's awareness of seafood toxicity. Each group will be assigned a specific species of seafood currently monitored for domoic acid, such as Dungeness crab, clams, mussels and conduct a survey to gather information regarding the proportion of students aware of seafood toxicity and how many have recently eaten that particular type of seafood. Using this information, students will use the data to estimate what proportion of students at our school are aware of seafood toxicity and the proportion of students and their families that eat the species of seafood. They will then create visual media in the form of a poster, image, or video, that communicates the danger of domoic acid and how to minimize risks through safer seafood preparation and consumption methods. Groups will communicate their findings in writing, present their work in class to their peers and potentially other faculty members and community members.

Appendix on Implementing District Standards

This curriculum unit incorporates the following course skills as outlined in the Advanced Placement Statistics Course and Exam Description:

1.B Identify key and relevant information to answer a question or solve a problem.

1.C Describe an appropriate method for gathering and representing data.

4.A Make an appropriate claim or draw an appropriate conclusion.

4.B Interpret statistical calculations and findings to assign meaning or assess a claim.

4.D Justify a claim based on a confidence interval.

The course skills are practiced throughout the various activities and project while addressing the primary individual learning objectives from the Advanced Placement Statistics Course and Exam Description emphasize the course skills.

Students analyze the correlation coefficient and coefficient of determination in the *Interpreting Correlation* activity. The two objectives addressed are: DAT-1.C Interpret the correlation for a linear relationship [Skill 4.B] and DAT-2.B Identify appropriate generalizations and determinations based on observational studies [Skill 4.A]

The *Interpreting Confidence Levels* activity has students interpret the confidence interval while understanding the implications when observations exceed the upper bound. The learning objectives addressed are: DAT-2.B Identify appropriate generalizations and determinations based on observational studies [Skill 4.A], UNC-4.S Interpret a confidence interval for a population mean, including the mean difference between values in matched pairs. [Skill 4.B], and UNC-4.T Justify a claim based on a confidence interval for a population mean, including the mean difference between values in matched pairs. [Skill 4.D].

Understanding sampling and errors in the inference process incorporates several skills in the *Errors in*

Sampling – Dungeness Crabs activity. The learning objectives addressed include DAT 2.C Identify a sampling method, given a description of a study. [Skill 1.C], DAT-2.E Identify potential sources of bias in sampling methods [Skill 1.C], VAR-3.A Identify the components of an experiment. [Skill 1.C], VAR-3.C Compare experimental designs and methods. [Skill 1.C], VAR-3.D Explain why an experimental design is appropriate. [Skill 1.C], UNC-5.A Identify Type I and Type II errors. [Skill 1.B], UNC-5.C Identify factors that affect the probability of errors in significance testing. [Skill 4.A] and UNC-5.D Interpret Type I and Type II errors. [Skill 4.B].

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Notes

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[3] Starnes

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