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ABSTRACT

Each year, Hawai‘i sees an average of more than 10 million tourists; due to beaches and other outdoor activities attracting visitors, sunscreen use in Hawai‘i is high. Active ingredients in sunscreens are examples of chemicals detected in seawater; sunscreen’s active ingredients are found to have negative effects on aquatic biota including changes in morphologies, reduced population growth, and changes in biochemical markers. The research described aims to investigate the influence of benzophenone-3 (BP-3) on the reproductive physiology of yellowfin tuna in O‘ahu seawaters. BP-3, an effective UV filter commonly used in sunscreens, washes off easily in water and infiltrates aquatic ecosystems. Due to human’s continuous interactions with coastlines, non-natural chemicals have a constant pathway into the environment. Sunscreens containing BP-3 are proven to have negative effects on the morphologies, population growth, and reproductive physiologies of various aquatic species, but local effects have yet to be identified. To investigate effects on O‘ahu, Hawai‘i, local fishermen will be employed to catch yellowfin tuna, the researcher will collect blood from the fish and collect seawater samples where fish are caught. Reproductive effects will be assessed using a vitellogenin assay with blood plasma samples from male yellowfin tuna. Based on results from previous studies, data from the research will likely show above-average levels of vitellogenin protein present in blood plasma of males. If true, results could indicate significant external exposure to BP-3; thus raising concerns for changes in reproductive physiologies of O‘ahu yellowfin tuna as well as food and economic security in Hawai‘i.

LITERATURE REVIEW

There are several reasons for concern when looking at the future of aquatic ecosystems in conjunction with the release of non-natural chemicals into the environment. Sunscreens contain numerous active chemicals, increasing the complexity of adverse effects on ecosystems; examples of active chemicals in popular sunscreens include avobenzone, octinoxate, and oxybenzone. Alternative protection such as biodegradable sunscreens may sound like a more sustainable alternative, but both biodegradable and non-biodegradable sunscreens pose a threat to ecosystems (Hernández-Pedraza et al. 2020). One study found (sunscreen's) chemical interactions in an aquatic environment reduced juvenile coral survival to almost 0% (Downs et al. 2016). An estimate of between 6,000 to 14,000 tons of sunscreen is released into oceans each year; at least 25% of applied sunscreen washes off during water activity and contamination can travel over 0.6 km from the origin of pollution. Since sunscreen chemicals can travel over half a kilometer in an aquatic environment, there is likely a threat to organisms beyond shoreline biota (Downs et al. 2016; McCoshum, Schlarb, and Baum 2016).

Benzophenone-3, also known as BP-3 or oxybenzone, is a chemical UV filter effective at limiting degradation from UV exposure. BP-3 can easily absorb light due to the molecular structure of the chemical, a conjugated ring system. Although most commonly associated with sunscreen, BP-3 is also found in packing materials, personal care products, and wastewater plant output (Sieratowicz et al. 2011; Coronado et al. 2008). BP-3 is a non-point source contaminant found in fresh water, seawater, sediments, and biota (Mao, He, and Gin 2019). BP-3 has already proven to have negative effects on coral reef ecosystems, but the travel radius of BP-3 contained in sunscreen puts all aquatic organisms at risk for contamination. In 2018, Hawai'i banned BP-3 due to the chemical's negative effects on shoreline ecosystems, but the banning did not take effect until 2021. Due to the three year time delay, BP-3 was still able to infiltrate and affect Hawaiʻi's aquatic ecosystems.

Previous researchers conducted multiple experiments to provide evidence for the changes BP-3 causes to organisms. BP-3 exists in the habitat of species as an active agent and BP-3's lingering effect can be measured by experimentation. Scholars have done experimentation on flatworms, crustaceans, alga, and coral species (Sieratowicz et al. 2011; Downs et al. 2016; McCoshum, Schlarb, and Baum 2016). Data collection from the previous experiments displayed results such as deformed shape of the organism, reduced population growth, and alterations of biochemical biomarkers (Downs et al. 2016; Chaves Lopes et al. 2020; McCoshum, Schlarb, and Baum 2016). However, there is a lack of research on organisms that dwell beyond the coral reefs, such as deep-water fish. Given the evidence, BP-3 is likely to have an effect, or multiple effects on species of deep-water fish when exposed to the chemical. How deep-water fish are affected by BP-3 contamination is significant to Oʻahu's fishermen, economy, and residents due to high-demand and consumption of deep-water species.

Of experiments done on aquatic organisms, scholars have mentioned BP-3's specific influence on the endocrine and reproductive systems of organisms. BP-3 is shown to have interactions in the reproductive system of fish mimicking estrogenic activity, and could possibly be decreasing the sperm quality in male fish (Coronado et al. 2008; Mao, He, and Gin 2019). BP-3 is shown to activate various enzymes leading to complicated endocrine disruptions difficult to understand the full scope of (Chaves Lopes et al. 2020; Hernández-Pedraza et al. 2020). The research seeks to find further answers by observing the reproductive effects of BP-3 on a deep-water species, yellowfish tuna. If BP-3 does affect reproductive and endocrine activities of yellowfin tuna, altered reproductive/endocrine activities could suggest the future decline of the yellowfin tuna population.

Research will determine the effects BP-3 contamination has on a deep-water aquatic organism, the yellowfin tuna. The geographical location chosen, Oʻahu, Hawaiʻi, could be more at risk compared to other locations due to the continuous coastlines and high tourist population. In combination with possible overfishing and steady environmental changes, realistically sustainable levels of food are of concern. Yellowfin tuna is highly desired in the seafood industry/markets; the research will look to find how BP-3 affects the yellowfin tuna's reproductive activity. Exhibiting how one chemical can affect the reproductive health of a highly consumed organism can demonstrate the importance of greater pollutant restrictions to better protect aquatic species.

Hawaiʻi is forecast to have over 5.5 million visitors in 2021. The number of tourists expected in 2021 is an increase of over 100% when compared to tourist arrival in 2020. Although the pandemic slowed the rate of tourists infiltrating the Hawaiian islands in 2020, there are little to no boundaries preventing tourists from vacationing in Hawaiʻi in 2021. The rise in tourist population implies an increased human interaction with shorelines, thus raising concern for further chemical pollutants being introduced to aquatic ecosystems. Data from the research can be applied to policy making and encourage more precautionary measures on personal care products, like sunscreen, being freely released into ecosystems.

RESEARCH DESIGN

To test the hypotheses, the principal investigator (PI) will undertake a field campaign to collect primary data in the form of blood from freshly caught male yellowfin tunas, as well as samples of seawater at the sight of each catch. The seawater will be brought back to the lab and appropriately tested for the presence of BP-3 as described below. The blood samples will be brought back to the lab and appropriately tested for the presence of vitellogenin protein as described below.

Local fishermen/fish

To obtain the fish and seawater samples, the knowledge and collaboration of local fishermen will be implemented. Fishermen will be selected using snowball sampling. Once agreed upon, the PI will accompany the fishermen throughout the duration of the study. When male yellowfin tunas are successfully caught, the PI will draw blood samples and a corresponding seawater sample will be taken. Blood samples from 20 males, along with corresponding seawater samples will be obtained in a one-month period.

The methodology integrates local knowledge from fishermen who catch yellowfin tuna to sell at the markets; local fishermen are most knowledgeable about the waters of O‘ahu. Locally caught yellowfin tunas are an accurate representation of the health of fish locals/tourists are currently consuming. Previous studies only investigate aquatic organisms reared in the lab, not wild organisms. Lab-reared organisms are typically treated with a pre-determined concentration of the chemical of interest. Although lab-reared organisms provide greater control over confounding variables, a lab organism will never be able to portray a realistic model of what wild organisms are currently facing. Therefore, implementing the described method of obtaining data is a relevant sample group to relate back to public health concerns.

Seawater

To quantify the amount of BP-3 in the seawater, the researcher will sample seawater at each of the locations where a male yellowfin tuna is caught. Methodologies from Downs et al. will be used to reinforce aseptic technique during sample collection. Sampled seawater will be analyzed using Downs et al. procedure to quantify the concentration of BP-3 in the sample, if detectable. Previous studies have found varying concentrations, ranging from non-detectable to detectable, of BP-3 in natural seawater in multiple locations. The results from previous studies showed human interaction with the seawater made a difference in the concentration of BP-3 present in the respective location. Quantifying the BP-3 concentrations in natural seawater locations far from shorelines where BP-3 is most commonly introduced to the aquatic ecosystem will provide insight as to how far BP-3 contamination has traveled.

Blood samples

To obtain plasma, blood samples will be extracted from the caudal vessel or the cardiac puncture of the yellowfin tuna using heparinized microhematocrit capillary tubes (Wheeler et al.). Upon receiving blood samples, the blood will be centrifuged, and the resultant plasma will be frozen until further analysis.

Vitellogenin

To assess the reproductive physiology of yellowfin tuna the project will use data on the vitellogenin protein located in the blood plasma of the male fish. Vitellogenin is a precursor to the egg yolk of vertebrates, including the yellowfin tuna. Males of the species showing elevated levels of vitellogenin in the blood plasma indicate endocrine disruption (Wheeler et al.). The reason for testing vitellogenin levels in males is because elevated levels indicate a non-natural estrogen stimulation. Therefore, vitellogenin data is essential because the data will work to demonstrate if external exposure to BP-3, having proven to act as an external estrogen, is influencing males of the species.

Vitellogenin assay

The project will use a vitellogenin assay to assess vitellogenin protein in the blood samples taken from male yellowfin tuna. An immunological technique will be used as the procedure to quantify vitellogenin in the blood plasma. More specifically, an enzyme-linked immunosorbent assay (ELISA) will be used for analysis. An ELISA is the most practical way of analyzing the blood plasma samples because the ELISA is specific and has simple photometric determination (Wheeler et al.). An ELISA requires little specialized equipment and can analyze many samples without increasing cost. The vitellogenin assay answers my question because the assay demonstrates an endocrine disruption (directly related to reproduction) is occurring in the males of the species.

Statistical Analyses

Statistical analyses will be carried out in the RStudio program. To assess comparisons between a baseline/control value of vitellogenin protein versus the vitellogenin protein statistic found in wild male yellowfin tuna, an ANOVA test will be used. Post-hoc (unpaired t-test) tests will be used to determine if there are statistical significances between different sites where yellowfin tunas are caught. Data will be deemed statistically significant if the p-value is less than 0.1. The reason for a higher p-value compared to the commonly used value, 0.5, is due to the conditions fish samples are being collected. Fish samples will be obtained in a non-controlled environment, therefore, there are several confounding variables in the environment influencing the fish. If time and funding allow, the spatial comparisons could be paired with long-term temporal data to assess trends over months or years.

ACADEMIC PREPARATION

Previous experience in a laboratory setting and related coursework provided an understanding of research methods and bodily systems. Past experiences with molecular biological work and participating in a statistics course will aid in analyzing the data obtained. During undergraduate studies, the student was able to access chemistry, biology, and molecular/genetics laboratories and obtain experiences within all three. The anatomy and physiology course completed also furthered the student's understanding of bodily systems and how the environment plays a significant role in the health of organisms. Taking a biostatistics course allowed the student to learn several statistical analyses; various forms of analyses will provide an understanding on the numerical data in the research. Previous lab experiences and coursework contributed to the student's motivations to show the external environmental effects on organisms. The research will expand the student's knowledge and understanding of different methods and develop the researcher's skillset while allowing the researcher to build connections with people who can collaborate on advocating for the protection and health of aquatic ecosystems.

CONCLUSION

Due to evidence from past studies, results from the current study are likely to show male yellowfin tunas having elevated levels of vitellogenin in blood plasma. Elevated levels of vitellogenin indicate endocrine disruption, leading to a cascade of effects within the affected organism. The main point of concern driving the current research is regarding the reproductivity of yellowfin tuna. If male yellowfin tunas are showing elevated levels of vitellogenin, a female characteristic, males are possibly becoming more androgynous. If more males are becoming androgynous, the affected physiological characteristics of the fish could suggest less chance for reproduction within the species. Thus, results indicating elevated levels of vitellogenin would suggest an overall threat to the population of yellowfin tuna and less/contaminated food sources for both higher trophic leveled sea organisms and humans.

Future studies related to the area of research could include extending the current research into a long-term study. Gathering data and replicating the methodologies implemented in the current research could produce a trend or reveal a pattern relating to vitellogenin protein in male yellowfin tunas. If a significant trend is observed, the trend would become valuable information to both researchers and conservationists in tackling future policy proposals aimed to protect aquatic ecosystems. Another future study could assess the effect of BP-3 on various deep-water species of fish or even bigger predators of the ocean (for example, sharks or dolphins). Physiological changes assessed on organisms in different trophic levels could provide information on how severely BP-3 bioaccumulates up the food chain. Data from a study analyzing bioaccumulation in organisms could allude to how humans are being impacted from consuming contaminated aquatic species.

Analyzing the correlation between the concentration of BP-3 in surrounding seawater and the amount of vitellogenin protein in male yellowfin tunas will reveal if external exposure to BP-3 could be affecting the reproductive physiology of yellowfin tuna. Statistical analyses comparing average concentrations of BP-3 to BP-3 measured, and normal vitellogenin levels to vitellogenin measured, will prove chemical introduction is a threat to aquatic organisms. The completed project will be submitted to be presented at a symposium. Additionally, results from the research would be submitted to conservation effort organizations to spread awareness of chemical impacts, and work to implement policies to preserve the health of aquatic ecosystems.

The current research will provide one of the first studies on a deep-water organism in relation to UV filter chemicals and reproductive physiology. Findings can provide evidence of the reality of the health of oceans and aquatic organisms humans consume daily.

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