Free Radicals, Antioxidants, and Coral Bleaching

1 TEMPERATURE DEPENDENCE OF CHEMICAL REACTIONS

As you observed in the preceding activity, increased sea surface temperature (SST) is associated with coral bleaching. What is the link between temperature and bleaching?

For most chemical reactions, increasing the temperature increase the rate of the reaction. Particles can only react when they collide with each other. When a substance (including a solution or a gas) is heated, its particles (atoms, ions, and molecules) move faster and therefore collide more frequently. Therefore, you should expect this to increase the rate of the reaction, and it does. However, the collision frequency has only a very small effect on the rate of the chemical reaction. A much more important factor is the energy of activation.

The energy of activation is the minimum energy that must be "invested" into a chemical reaction for it to occur. The energy of activation is supplied by the kinetic energy of the reactant particles when they collide. Consequently, when two reactants collide, they will only react with each other if they collide with sufficient kinetic energy to overcome the energy of activation. For most chemical reactions, only some particles have sufficient kinetic energy to cause a reaction in a collision. However, as the temperature increases, the kinetic energy of the particles increases, which increases the proportion of particles that collide with each other with sufficient energy to react. This is main reason why increasing the temperature of a reaction increases the rate of the reaction.

1.1 THE TEMPERATURE COEFFICIENT

Biologists and biochemists use the "Q_{10} temperature coefficient" to represent the temperature dependence of a process. Specifically, the Q_{10} it is the rate of change of a system for an increase of 10 °C:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2 - T_1)}$$

where T_1 is temperature 1, T_2 is temperature 2, R_1 is the rate at T_1, and R_2 is the rate at T_2. The Q_{10} has no units, since it is the factor by which a rate changes. If the Q_{10} for a reaction is 1, the reaction rate is independent of temperature. For most biological systems, the Q_{10} value is ~ 2 to 3. That is, the reaction rate increases by 2 to 3 times with a 10 °C increase in temperature.

But why would increasing the temperature of corals cause coral bleaching?

2 OXIDATION-REDUCTION REACTIONS

In an oxidation-reduction (redox) reaction, an electron is transferred between two chemical species. Since electrons are negatively charged, the atom, ion or molecule that gains the electron is said to be reduced, and the atom, ion or molecule that loses the electron is said to be oxidized.

When a species gains electrons, what property is "reduced"?

Perhaps the most straightforward type of redox reaction is a reaction that forms an ionic compound. For example, sodium and chlorine can combine to form the salt sodium chloride. Sodium loses electrons to form sodium ions, while chlorine gains electrons to form chloride ions.

$$2\mathrm{Na} + \mathrm{Cl_2} \rightarrow 2[\mathrm{Na^+}][\mathrm{Cl^-}]$$

Which of the above elements is reduced? Which is oxidized?

A redox reaction can be thought of as having two half-reactions: the oxidation half reaction involves the loss of electrons, and the reduction half reaction involves the gain of electrons. The two half-reactions for the above reaction are

$$2\mathrm{Na} \rightarrow 2\mathrm{Na^+} + 2\mathrm{e^-}$$

$$\mathrm{Cl_2} + 2\mathrm{e^-} \rightarrow 2\mathrm{Cl^-}$$

Determine which of these half reactions involves reduction and which oxidation. What is the net reaction of these two half-reactions? What happens to the electrons?

The terminology can then get a little confusing because there are so many terms for the same species. For example, in one half-reaction, the species that gains the electrons, (thus being reduced) is called the oxidizing agent, oxidizer, or **oxidant** and in the other half-reaction, the species which loses the electrons (and is oxidized) is called the reducing agent, reducer, or reductant.

Which reactant in the formation of sodium chloride is the oxidant? The reductant?

2.1 Free Radicals

Some redox reactions result in the creation of free radicals, which are atoms, ions, or molecules that have one or more unpaired valence electrons. Free radicals are unstable and chemically reactive because they tend to react with other atoms, ions, or molecule, and in the process to acquire an additional electron. This gives the radical an electron pair, but in some cases the species it initially reacted with becomes itself a free radical, propagating a chain reaction.

If a free radical acquires an electron from another atom, ion, or molecule, is the free radical an oxidant or a reductant? Is the other species that loses the electron oxidized or reduced?

2.2 Free Radicals and Metabolism

Aerobic respiration and photosynthesis are both complex redox reactions. In aerobic respiration, glucose ($\mathrm{C_6H_{12}O_6}$) is oxidized while oxygen is reduced to water in a complex series of electron transfers. The net reaction in aerobic respiration is

$$\mathrm{C_6H_{12}O_6} + 6\mathrm{O_2} \rightarrow 6\mathrm{CO_2} + 6\mathrm{H_2O} + \text{energy}$$

In photosynthesis, light energy is used to oxidize water and reduce carbon dioxide to glucose in a different complex series of electron transfers,

$$\text{light energy} + 6\mathrm{CO_2} + 6\mathrm{H_2O} \rightarrow \mathrm{C_6H_{12}O_6} + 6\mathrm{O_2}$$

These reactions, as well as many other biochemical reactions inside cells, can result in the formation of various free radicals as byproducts. Free radicals are not necessarily bad, but when they are present in excess, they can oxidize proteins, lipids, DNA, and other biological macromolecules. This is called oxidative damage, and it can impair cell function and lead to cell death, or even the death of the organism.

The free radicals of most importance in biological systems are derived from oxygen. Oxygen is unusual in having two unpaired electrons in separate orbitals in its outer shell, making it particularly susceptible to radical formation. The free radicals formed from oxygen are collectively called **reactive oxygen species** (ROS).

2.3 ANTIOXIDANTS

An antioxidant is a molecule that reduces the rate of oxidation reactions. Many antioxidants do this by becoming oxidized themselves. Organisms produce a variety of antioxidants in order to minimize the negative effects of the free radicals formed by metabolism. Examples of antioxidants in plants and animals are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, the enzyme superoxide dismutase, and co-enzyme Q10 (also known as ubiquinone). The total ability of an organism's tissues (or a solution) to resist oxidative damage is called the **antioxidant capacity**.

3 CORAL BLEACHING AND FREE RADICALS

View the following animation from HHMI that shows the relationship between corals, their symbiotic algae, and free radicals: http://www.youtube.com/embed/_ZfGIKiSwwQ.

As you observed in the preceding activity, increased sea surface temperature (SST) is one of several factors that appear to be capable of causing coral bleaching. We do not fully understand the causes and mechanisms of coral bleaching, but one leading theory is that it is due in part to free radicals. Zooxanthellae produce free radicals as a byproduct of photosynthesis, as is true for all photosynthetic organisms. However, because the zooxanthellae are housed within the coral polyp's tissues, some of the free radicals from the zooxanthellae can diffuse into the host, potentially causing oxidative damage to the host tissues. As is shown in the animation (referenced above), the rate at which zooxanthellae produce free radicals is partially dependent on the temperature, with temperatures that are higher than normal causing the zooxanthellae to produce more free radicals. This appears to stimulate the coral polyps to eject the zooxanthellae, presumably because the additional free radicals would otherwise cause so much oxidative damage to the coral that ejecting the zooxanthellae is worth the risk of starvation.

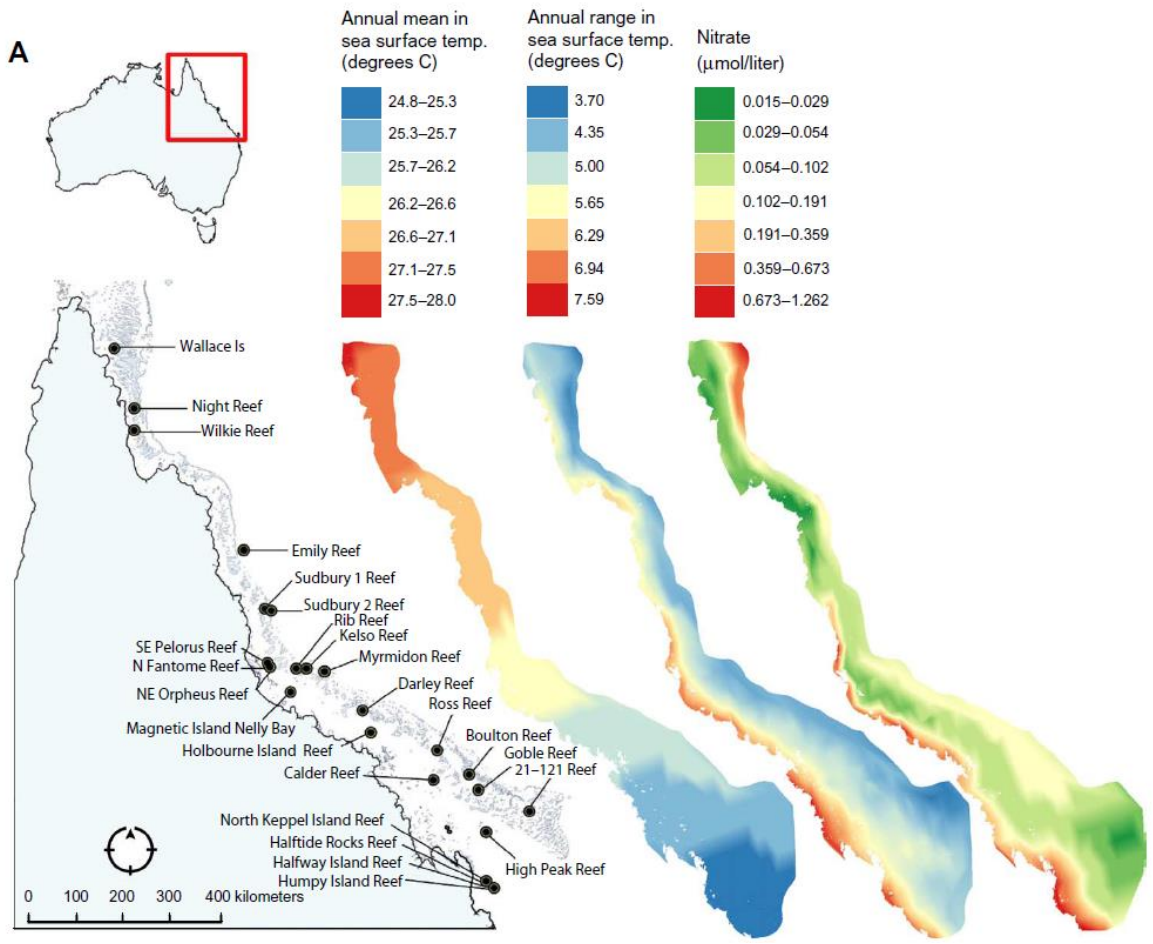
3.1 CORAL ANTIOXIDANTS

In a recent study, Jin et al. (2016)[*] investigated whether corals of the same species that differ in the temperature of their habitat have genetic differences in corals from the warmer climates that increase their antioxidant capacity and resistance to photosynthetic damage. For their study they investigated a coral species that makes up much of the Great Barrier Reef in Australia. This reef extends across 2,300 km and 14 degrees of latitude, which provided the researchers with a large north-to-south temperature gradient (see figure below).

Jin et al. collected coral polyps from different locations along a 12° north-south latitudinal gradient and examined the tissues for genetic markers of elevated antioxidant capacity and photosynthetic efficiency. They then conducted laboratory heat-stress experiments to see whether there were differences in bleaching. They found that the genetic markers for antioxidant

[*] Young K. Jin, et al. Genetic markers for antioxidant capacity in a reef-building coral. *Science Advances*, 13 May 2016.

capacity (in this case co-enzyme Q10) were correlated with warmer temperatures and increased resistance to coral bleaching during heat stress.



Map showing mean sea surface temperature (SST) and range in SST, nitrate gradients, and locations of 25 populations of coral sampled on the Great Barrier Reef.

3.2 QUESTIONS

Q1. When a copper ion Cu^{2+} is reduced by the addition of two electrons, what property is reduced?

a. The charge on the copper is reduced from +2 to 0

b. The number of electrons in the atom is reduced by two.

c. The ability of the atom to be oxidized is reduced.

d. The mass of the atom is reduced.

Q2. Choose all correct statements about the equation below.

$$2Na + Cl_2 \rightarrow 2[Na^+][Cl^-]$$

a. Sodium is reduced

b. chlorine is oxidized

c. Sodium is oxidized
d. Chlorine is reduced
e. Sodium gains an electron
f. Chlorine gains an electron
g. Sodium loses an electron
h. Chlorine loses an electron

Q3. T/F If a free radical acquires an electron from another species, the free radical is an oxidant.

Q4. T/F If a free radical acquires an electron from another species, the free radical is oxidized in the process.

Q5. What is the role of the zooxanthellae?

a. They live inside the coral polyps where they act as antioxidants for the polyps.
b. They live inside the coral polyps where they use photosynthesis to produce sugars.
c. The zooxanthellae is the solvent for the reaction mixture in this experiment.
d. The zooxanthellae is the outer solution from commercial light sticks

Q6. Students testing an antioxidant measure the following rate and temperature data:

$R_1 = 0.022$ and $T_1 = 4.0°C$

$R_2 = 0.044$ and $T_1 = 22.0°C$

What is the Q_{10} temperature coefficient?

$R_1 = 0.022$ and $T_1 = 4.0°C$

$R_2 = 0.081$ and $T_1 = 22.0°C$

What is the Q_{10} temperature coefficient?

Q7. What can you infer about the potential for antioxidant molecules to reduce the risk of oxidative damage in corals exposed to elevated temperature?

Q8. If an organism were to have a disease that decreased the antioxidant capacity of its tissues, what would you expect to happen to the level of oxidative damage?

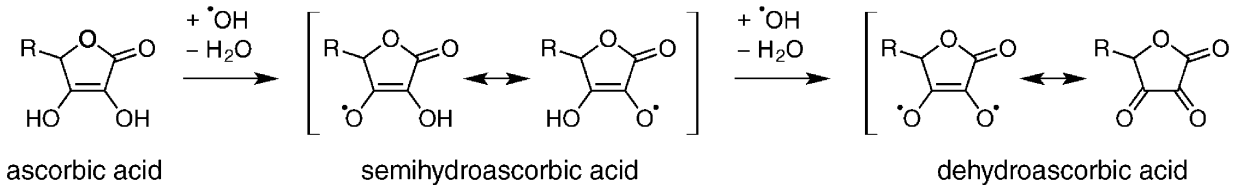
Q9. Consider two closely-related species of an animal. If species A was much more active than species B, which species would you expect to have the highest antioxidant capacity, and why?

Q10. Consider two coral reefs composed on the same coral species that also have the same species of zooxanthellae as symbionts. If coral reef A typically experiences higher temperatures than coral reef B, how would you expect the antioxidant capacity of the polyp tissues to differ between the coral hosts?

INSTRUCTOR MANUAL

BACKGROUND READING FOR INSTRUCTOR

In most cases, the antioxidative capacity of a substance can be explained by its ability to form resonance-stabilized intermediates in the presence of radical formers. Because of their resonance-stabilization, these intermediates do not lead to a propagation reaction. By their ability to bind a second radical, many antioxidants even cause the termination of the free radical reaction. This ability is shown below for ascorbic acid. Ascorbic acid forms the resonance-stabilized semihydroascorbic acid in the first step and dehydroascorbic acid in the second step when radicals are present.



From Wieczorek and Sommer, Demonstrating the Antioxidative Capacity of Substances with Lightsticks. *J. Chem. Educ*., 2011, 88(4), pp 468-469.

QUESTIONS WITH ANSWERS

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What is the Q_{10} temperature coefficient? {1.5}

$R_1 = 0.022$ and $T_1 = 4.0°C$

$R_2 = 0.081$ and $T_1 = 22.0°C$

What is the Q_{10} temperature coefficient? {2.1}

Q7. What can you infer about the potential for antioxidant molecules to reduce the risk of oxidative damage in corals exposed to elevated temperature?

This experiment demonstrated that the antioxidant ascorbic acid reduces the ability of free radicals to cause an oxidation reaction. Therefore, other naturally occurring antioxidants in animal and zooxanthellae tissues would have the potential to reduced oxidative damage that might otherwise be caused at elevated temperatures.

Q8. If an organism were to have a disease that decreased the antioxidant capacity of its tissues, what would you expect to happen to the level of oxidative damage?

The amount of oxidative damage would increase.

Q9. Consider two closely-related species of an animal. If species A was much more active than species B, which species would you expect to have the highest antioxidant capacity, and why?

The more active species would be expected to have a higher metabolic rate, and therefore produce more free radicals as byproducts. To minimize oxidative damage, the more active species would therefore be expected to have an increased antioxidant capacity.

Q10. Consider two coral reefs composed on the same coral species that also have the same species of zooxanthellae as symbionts. If coral reef A typically experiences higher temperatures than coral reef B, how would you expect the antioxidant capacity of the polyp tissues to differ between the coral hosts?

We would expect higher antioxidant capacity in the warmer-temperature species A.