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Crayfish and Climate Change: How the Growing Acidity of Freshwater Lakes, Streams, And Ponds Negatively Impact the Nervous System of Crayfish

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Table of Contents

Abbreviations:

Acetylcholine: ACh

Adenosine Triphosphate: ATP

Calcite: $CaCO_3$

Calcium: Ca^{2+}

Central Nervous System: CNS

Dihydropyridine Receptors: DHPR

End Plate Potential: EPP

Excitation-Coupling: EC

Excitatory Post Synaptic Membrane Potential: EPSP

Gamma Aminobutyric Acid: GABA

Inhibitory Post Synaptic Membrane Potential: IPSP

Neuromuscular Junction: NMJ

Peripheral Nervous System: PNS

Potassium: K^+

Sodium: Na^+

Sulfur dioxide: SO_2

Transverse Tubules: T-tubules

Abstract

The nervous system of crayfish, a freshwater crustacean, is largely regulated by the flow of calcium (Ca^{2+}) ions in a cell across a concentration gradient that produces electrical impulses and controls movement (Krause et al., 1998). Crayfish obtain Ca^{2+} from their external aquatic environment. Therefore, rapid changes in the concentration of Ca^{2+} in freshwater environments may cause negative impacts to the neural system of crayfish and subsequently the freshwater food chain. These impact of altered Ca^{2+} levels may create hyperactive or hypoactive movement due to over-, under-stimulation, respectfully in the skeletal muscle of the crayfish, which may alter the of growth and survival of these animals. As these unique freshwater environments are dependent upon climate change in the surrounding atmosphere, the Ca^{2+} present in freshwater environments is also regulated by external factors such as temperature and exposure to sunlight (Betini et al., 2016; Hammond et al., 2005). The aim of this literature review is to compare how climate change, a global crisis, can disrupt regular crayfish neural functions and interrupt the freshwater food chain. Varying temperatures, acid rain, and heavy metals are considered to influence aquatic Ca^{2+} levels to the greatest extent. Other considerations scientists have proposed includes the idea of temperature being the limiting factor in extinction rates of crayfish and other freshwater crustaceans rather than Ca^{2+} levels (Hammond et al., 2005). Here, I will discuss the effects of altered of Ca^{2+} levels in different freshwater environments on the crayfish neural system and a durational effect of rising pollution levels as predictions of the food chain repercussion. As climate change adversely impacts the nervous system of crayfish, it also contributes to extinction rates and lack of thermal tolerance in crustaceans (Kelly et al., 2011). Not only do humans rely on crayfish as a food source, but also as a model organism to help develop medications and vaccines for new diseases.

PART I: Crayfish Nervous System

Impact of Ca^{2+} on Muscle Contraction Physiology

Freshwater crayfish are invertebrate animals that are affected internally by the amount of available Ca^{2+} in the environment. Muscles of crayfish are important in both movement and posture. Fast-twitch muscles control the rapid movement of the tail to propel the crayfish forward, while slow-twitch muscles maintain proper posture (Leise & Mulloney, 1986). The skeletal muscle in crayfish requires extracellular Ca^{2+} in order to produce a potential to stimulate excitation-contraction (E-C) coupling (Gyorke & Palade, 1991). The E-C coupling model is considered a quantitative evaluation of the somatic nervous systems regulation of Ca^{2+}. As Ca^{2+} stimulates the release of neurotransmitters from synaptic vesicles across the synaptic cleft of the neuromuscular junction (NMJ), neurotransmitters bind to their appropriate receptors on the motor end plate generating an end plate potential (EPP), a specialized form of an excitatory post synaptic potential. As the EPP travels from the motor end plate, an action potential is produced that propagates along the sarcoplasmic reticulum. The large depolarization of the sarcoplasmic reticulum from the action potential travels into transverse-tubules (T-tubules) embedded between the A and I bands of the sarcomere. These T-tubules, surrounded by the sarcoplasmic reticulum, stimulates the release of Ca^{2+} ions into the muscle fiber by voltage gated calcium channels called dihydropyridine receptors (DHPR). As the DHPR's become activated, they are then able to subsequently active other receptors located on the distal end of the sarcoplasmic reticulum to release Ca^{2+} ions into the cytosol (Hudecove & Krizanova, 1991). As Ca^{2+} ions are released into the cytosol, they then bind to troponin, a protein involved in muscle contraction, changing the confirmation of tropomyosin (a secondary protein), leaving the actin binding sites available for myosin binding. Requiring adenosine triphosphate (ATP), myosin binds to actin and stimulates contraction. In order for contraction to stop, a Ca^{2+}ATPase actively pumps calcium against its concentration gradient and back into the sarcoplasmic reticulum. As this occurs, the tropomyosin relaxes to its original state, covering the actin binding sites needed for muscle contraction (Brini & Carafoli, 2011; Stern et al., 1997).

As crayfish have a simplified somatic nervous system, the action potentials generated by contraction of the skeletal muscle are often generated through only one motor axon, although a select few may have two. The somatic nervous system is a division of the peripheral nervous system (PNS) responsible for motor control over voluntary muscles. Muscles aiding in digestion, contraction, relaxation, release of hormones, or heart rate are not included in this classification as they are not voluntary. In humans, the division of the PNS includes afferent neurons, responsible for directing signals from the sensory stimuli to the central nervous system (CNS), and efferent neurons, that carry information from the CNS direct to the effector organ. Often, these pathways include multiple synapses and ganglions in order to produce the appropriate effector response. (Akinrodoye & Lui, 2020). However, in crayfish, the case of more than one motor axon innervating a muscle and originating from the ganglion is uncommon. This means that there one point of innervation to control the area of muscle contraction. Although only having one motor axon, crayfish are still able to sense the environment in the PNS, transmit an action potential to the CNS, integrate the response, then produce an action potential to influence the response in an effector organ. As with humans, integration within the CNS involves a large density of neuropils. As neuropils are responsible for integrating sensory information, they contain a significant concentration of dendrites and axons that overlap with each other (Kagaya, n.d.) In the case that a muscle is innervated by more than one motor neuron, the skeletal muscle is able to control the speed of the contraction leading to fast and slow-twitch contractions that are dependent on the muscle fibers of the particular muscle (Atwood, 1967).

The Ca^{2+} threshold to generate an action potential in slow-twitch muscle fibers is lower than that of fast-twitch muscle fibers. Fast-twitch fibers are used as quick reflexes that require a significant amount of energy and power. Due to the significant amount of energy required in the short duration of time, fast-twitch fibers are often fatigued easily. Therefore, fast-twitch muscle fibers predominate in exercise that is performed in short-moderate durations of time. Anatomically, fast-twitch fibers can operate anaerobically on the basis of glycolysis or aerobically using oxidative phosphorylation. Fast-twitch fibers are located in the head, abdomen, legs, and tail of the crayfish, as they are responsible for movement. On the other hand, slow-twitch fibers are more resistant to the fatigue but generate a lower threshold of force to perform work on an object. These fibers only undergo oxidative phosphorylation as its energy source. This differentiation allows for the crayfish to preserve ATP when possible but also expend energy as needed. However, due to this differentiation, intracellular Ca^{2+} levels must be within an exceedingly small range in order to exert the correct effects. In essence, the greater degree of complexity in crayfish muscle fibers, the greater the chances are of unpredictable crayfish skeletal muscle fiber contractions in times of aberrant Ca^{2+} levels. (Stephenson & Williams, 1981; Moody, 1978; Lievens et al., 2020). Slow-twitch fibers are mostly located in positions responsible for maintain posture. This would be the torso and tail of the crayfish, both of which are involves in posture. This anatomical innervation and the potentials produced from these fibers are quantitatively recorded and compared to control groups. Therefore, crayfish are able to serve as a model organism for medical research.

Crayfish have been an important scientific research model for the past 70 years. In addition to their relative abundance, these invertebrate animals are relatively inexpensive to buy and maintain. Furthermore, crayfish have a long life-span, and share similar neural structures to humans including a similar role of Ca^{2+} at the NMJ, as indicated earlier. In sharing common neural features, neurological studies, histology, and other physiological responses, crayfish are able to be analyzed at length without human contamination. This could mean a potential role for further research in the areas of various cancers of the nervous system to be easily studied in crayfish to help produce viable treatment options in the future. Using crayfish as a model organism is growing exponentially as the study of xenobiotics (foreign molecules within the environment) continues to rise, also benefiting the future for human health (Belanger et al., 2017). As the crayfish nervous system had been studied extensively since the 1950’s, new changes in average Ca^{2+} levels now pose the question of how crayfish will be able to adapt and survive in a new environment.

Generation of Action Potentials in Skeletal Muscle is Dependent on Ca^{2+}

All crayfish muscles require Ca^{2+} for muscle stimulation. Two of the largest muscles, the extensor and flexor muscles which are located superior and inferior respectively, to the ganglion in the tail. These muscles are responsible for tail movement and posture along with being neurogenic. The extensor and flexor muscles each have five excitatory motor neurons while only one peripheral inhibitor. In order to induce movement, depolarization in the one of the five excitatory motor neurons in the extensor muscle and activation of the inhibitory motor neuron in the flexor muscle are both needed. Reciprocal innervation of antagonist muscles assure movement in the correct direction for the tail. If both muscles were to contract, no movement would succeed contraction. As one muscle contracts and the other extends, E-C coupling allows the binding of myosin to actin to elicit the appropriate response. In essence, the importance of the extensor and flexor muscles is much greater than evading prey, it is also postural to assure flexibility while acting as a predator (McCarthy & Macmillion, 1999).

In order for these muscles to perform their appropriate function, there must first be an uptake of Ca^{2+} from the surrounding environment. Branchial epithelium, which is largely responsible for the uptake of Ca^{2+}, is located primarily in the gills. As water containing Ca^{2+} is passed across the gills, Ca^{2+} is able to be transported into the circulatory system of the crayfish where it is then transported to appropriate locations within in the internal environment of the crayfish (Wood & Part, 1997). In order to enter the cell, Ca^{2+} goes through a Ca^{2+}-ATPase to cross the basolateral membrane. This assures a steady influx of Ca^{2+} into the cell, while still maintaining appropriate homeostatic internal gradients (Gao & Wheatly, 2004). In addition to brachial epithelium, Ca^{2+} can also be absorbed through digestive, hypodermal, and renal epithelia located on anatomical structures such as the antennal gland in the renal system, the cuticle layer of the hypodermis, and exposed digestive organs. Most importantly, these anatomical structures are located externally on the body of the crayfish in order to acquire extracellular Ca^{2+}. Although these structures are designed to uptake Ca^{2+}, there becomes a threshold that when reached, membrane and proteins are unable to support proper regulatory measures in adverse conditions (Wheatly, 1999).

Once absorbed through the environment and into cells, Ca^{2+} must first pass through a voltage-gated Ca^{2+} channel in order to pass into the presynaptic terminal of the nerve cell forming the NMJ (Wojtowicz & Atwood, 1988). Different types of voltage gated Ca^{2+} channels stimulate the release of different neurotransmitters into the synaptic cleft to stimulate contraction in the post synaptic cell in order to stimulate contraction. Acetylcholine (ACh), epinephrine, and norepinephrine were found to be the excitatory neurotransmitters while gamma aminobutyric acid (GABA) is inhibitory in crayfish (Cooper et al., 2011). As higher levels of Ca^{2+} pass into the presynaptic cell, more neurotransmitters are released to stimulate contraction (Bernardo et al., 2006). Depending on the anatomic features of the muscle fibers, the release of neurotransmitters can cause slow, prolonged muscle contractions or can cause rapid, short muscle contractions. These are called slow-twitch and fast-twitch muscles, respectively. In regard to fast and slow twitch muscle fibers, the amount of Ca^{2+} required to stimulate the release of ACh and cause muscle contraction varies (Stephenson & Williams, 1981). Therefore as the environment changes around crayfish, their ability for muscle contraction in the extensor and flexor muscles located in the tail that aid in movement are subject to inhibition or overstimulation. However, it may be predicted if motor neuron damage occurs, crayfish may regenerate the damaged neurons themselves.

In an important deviation from most organisms' nervous system, crayfish have the ability to regenerate motor neurons. An additional model organism, Zebrafish, also has adapted this ability. Yet, these select organisms are exceptions to the rule, not the majority. Motor neurons inside the muscle of crayfish are the main target of crayfish neuron regeneration, rather than the muscle fibers themselves (Krause et al., 1998). Neurons either create inhibitory postsynaptic membrane potentials (IPSPs), which lowers the chance of an action potential occurring in the postsynaptic membrane, or exhibitory postsynaptic membrane potentials (EPSPs), which increase the chance of an action potential. Research concluded that single impulse, large EPSP's were commonly generated in the NMJ of regenerated motor neurons. Knowing that neuron regeneration causes electrical impulses to rapidly travel through the neuron, Ca^{2+} ions will readily open to disperse Na^{+} ions into the affected cell. Even if neurons regenerate, they still become Ca^{2+} dependent. If levels of Ca^{2+} vary in the environment initiating damage to nerves, no significant change will be seen in motor function. The concentration of Ca^{2+} ions is important because if deviated, it could lead to significant hypomobility or hypermobility in crayfish muscles. As motor neurons are responsible for voluntary muscle contraction in skeletal muscle, the lack of neurons able to generate action potentials could cause a decrease in function in both extensor and flexor muscles. Additionally, if only the inhibitory neurons were impacted, hypermobility may be a result due to loose regulation of the excitatory potentials. Lastly, the least obvious repercussion is the possibility that reproduction and advanced stimulation in crayfish may diminish as well. (Krause et al., 1998).

In knowing that Ca^{2+} is required for the stimulation and contraction of flexor and extensor muscles located in the crayfish's tail, it is important for further research to investigate whether the influx of Ca^{2+} from the environment and efflux of Ca^{2+} into the environment directly impact the stimulation at the NMJ. In reviewing the literature, three possible outcomes are feasible. First, the increase in Ca^{2+} levels in freshwater environments directly impact stimulation at the NMJ leading to the hypermobility of crayfish. Second, the increase in Ca^{2+} levels in freshwater environments have no impact stimulation at the NMJ. Lastly, the results may be undetermined, and variability could be due to a separate limiting factor. This literature review will report multiple studies to help provide evidence that Ca^{2+} levels in the crayfish's surrounding environment directly impacts the ability to contract and move throughout the water.

EPSP's Dependence on Ca^{2+} Concentrations

A study was performed to determine the cooperativity of Ca^{2+} dependence and extensor muscle contraction by evaluating the strength of contraction between first and second EPSP's. This was evaluated at different levels of extracellular Ca^{2+} levels to determine the level of facilitation of the muscle. EPSP's are able to be measured through the amplitude of the wave in which they emit while producing a signal. The larger the difference from resting potential to complete depolarization, the greater the contraction. Additionally, a second EPSP is able to be generated in summation with the first EPSP to elicit a stronger response. In order for this to occur, greater levels of Ca^{2+} are needed to contribute to longer contraction (Bernardo et al., 2006). The muscle evaluated in the study was the extensor muscle which is responsible for tail movement and posture. The extensor muscle is located on the superior/posterior region of the crayfish extending into the tail. Resting potentials were recorded through microelectrodes placed into the extensor muscle while 15mV-150mV of current was given through one microelectrode and 10V-100V was delivered through the other microelectrode into the muscle. This was performed in order to assure an EPSP would be generated in a rhythmic state. Saline solution (0.9% NaCl) was provided externally to the crayfish throughout the experiment. The saline was mixed with different Ca^{2+} levels to see how EPSP responses may vary. The levels of Ca^{2+} being evaluated were 3.38 mM Ca^{2+}, 6.75mM Ca^{2+}, 10.1mM Ca^{2+}, and 13.5mM Ca^{2+} (Bernardo et al., 2006). The average internal Ca^{2+} concentration in crayfish is approximately 13.5mM Ca^{2+}. It was found that the EPSP with the greatest amplitude, meaning the fastest to reaching threshold potential, was generated as crayfish were placed in a solution with a below normal calcium level whereas the weakest EPSP was found in above average calcium level environments. The difference in hypoosmotic and hyperosmotic Ca^{2+}/saline solution EPSP's compared to the "medium" grade EPSP generated at 13.5mM Ca^{2+}. This indicates that crayfish have regulatory processes to selectively regulate Ca^{2+} entry. In cases of environmental stress, regulatory processes may shut down and cause variability within the nervous system (Bernardo et al., 2006).

To discuss these findings further, an important conclusion was able to be drawn from the experiment. Greater facilitation was seen between 3.38mM Ca^{2+} - 6.75mM Ca^{2+} compared to the other concentrations. This is suggestive of Ca^{2+} concentration contributing to variability in synapses. As this is the lowest Ca^{2+} concentration, it indicated that as climate change continues to contribute to elevated Ca^{2+} levels in freshwater environments, contractions may become more scarce and crayfish may be unfit to evade predators. Another insight to be drawn is the occurrence of overstimulation. If a rise in Ca^{2+} concentration levels in freshwater environments makes a sudden decline either due to natural or manmade occurrences, the result of overstimulation may become a greater problem if adaptation to the Ca^{2+} levels had already occurred. In other circumstances, environmental changes may call for drastically lower Ca^{2+} concentrations. Overstimulation and hyperactive movement is likely in this instance leading to the higher probability of being prey, rather than a predator. Lastly, scientists suggest the higher Ca^{2+} concentration in the internal environment, the plasma membranes and associated enzymes could act as a regulatory control mechanism to prevent overstimulation if Ca^{2+} levels drop to below-ideal levels. It is inferred that as internal Ca^{2+} levels continue to rise, less stimulation will present contributing to death and hypomobility of the crayfish (Bernardo et al., 2006).

PART II: Ca2+ Levels and Climate Change

Ca^{2+} Levels Dependence on Environment

Ca^{2+} levels in the crayfish nervous system are directly altered by the freshwater environment in which they reside. Crayfish exist in freshwater environments that often contain silica-rich rocks that are release calcite ($CaCO_3$) into the surrounding environment. Higher rates of calcite released into the environment are observed in extreme temperatures. When the calcite deposit dissociates, free Ca^{2+} is released into the surrounding environment where it is able to be absorbed by nearby crayfish. In addition to the temperature of the water, a separate factor influencing the erosion of the silica-rich rocks is a decrease in pH of the water. This decreased pH results most significantly from acid rain. Other more subtle mechanical forces such as heavy rains, landslides, duration of snow/ice, and temperatures around freezing point are the main contributors to calcium release as well. As these mechanical forces have become more prevalent world-wide, Ca^{2+} levels are predicted to rise to previously unseen levels and impact all surrounding wildlife (Kopacek et al., 2017).

Mechanical forces produce highly acidic or extreme temperatures in surrounding environments. Not only can this dangerously affect the internal regulation of homeostasis within many organisms, it also directly impacts Ca^{2+} levels within in ecosystem. Besides generating action potentials, Ca^{2+} also is involved in exoskeleton maintenance and intracellular communication (Dicks, 2004). As motor neuron function is important in evading predators, food consumption, shelter, and many other processes will be negatively influenced as well. Referring back to acidity, as crayfish are exposed to a more acidic environment, the Ca^{2+} -ATPase responsible for the influx of Ca^{2+} into appropriate tissues faces a significant reduction in activity. As this reduction of activity stems from the acidic pH of the environment, this concludes that an acidic pH environment causes both a reduction in Ca^{2+} levels in the freshwater environment and a significant reduction in the rate the available Ca^{2+} is up taken into the body (Parker et al., 1985; Rodeau, 1984).

Harsh Mechanical Forces Alter pH in Freshwater Environments

Harsh mechanical forces are a major cause for the variability of Ca^{2+} in freshwater environments. The mechanical forces that will be referred to are precipitation amounts, landslides, type of precipitation (rain/snow/sleet), and temperature. As these become more prevalent in abnormal events, environmental ecosystems may get destroyed. The IPCC has found that pH of large bodies of water, such as oceans, have been declining by a rate of 0.017-0.027 pH units per decade starting in approximately 1980. As salt does not have a variable effect on the acidity of water, the IPCC also concluded that this pH variability is likely caused by calcium carbonate becoming unstable and degrading in the increase of severe weather environments (IPCC). While visible changes such as the melting of glaciers, harsh winters, and landsides are easily noticeable, what transpires at the molecular level of living organisms may also affect future generations of both saltwater crustaceans and freshwater crustaceans. Knowing that crayfish are directly impacted by Ca^{2+} levels and Ca^{2+} directly correlates with neural function, it is theorized that the increase in mechanical forces interfering with molecular Ca^{2+} will lead to a downfall in the crayfish species. Due to some freshwater ecosystems being located nearby highly industrialized cities (i.e. Chicago, New York, etc.), it is possible for Ca^{2+} concentrations to increase. Yet, Ca^{2+} levels are expected to decrease in more rural ecosystems (IPCC).

Temperature Fluctuations Will Alter Ca^{2+} Regulation

Beginning with temperature, data collected over a 50-year periods suggests that global temperatures are rising. Scientists predict that there is a greater than 90% chance that heat-waves worldwide will become more prevalent along with an increase in heavier precipitation event. These ecologically devastating events suggest that human pollution is the leading cause of climate change due to greater than 50% of the population now living in city areas, an increase of 20% from 50 years ago (Luber & Prudent, 2007). As this has occurred over nearly half a century, researchers continued to monitor Ca^{2+} absorption levels as mean temperatures increased. Research from the 1980's first indicated that as water temperature became elevated in an enclosed environment, protoplasts and cells suddenly began to acquire higher levels of Ca^{2+}. This was accounted for by having a greater degree of cell membrane permeability in higher temperature environments. (Klein & Ferguson, 1987).

As proteins have an optimal temperature in which they perform their cellular function at, there is a risk of denaturation with increasing global temperature levels. This is of concern for the Ca^{2+} ATPase responsible for maintaining intracellular Ca^{2+} levels and ultimately skeletal muscle function. Additionally, with an increase in intracellular temperature, there may be plasma membrane disruption of molecules that normally require active transport or facilitated to diffusion to get into the cell to readily diffuse through. Example of these molecules would include amino acids that act as hormones to stimulate erratic responses or ions such as potassium (K^{+}) or sodium (Na^{+}) that would create a disequilibrium in membrane potential. If the membrane potential is not within the normal limits for the cell, over-contraction or under stimulation may result. If these scenarios occur, the cell is no longer able to normally function and can lead to harmful consequences including cell and animal death. (Quinn, 1988). As temperature may not have a significant amount of the total concentration of Ca^{2+} present in the freshwater lakes, it can have a direct effect on absorption rates.

As a poikilothermic organism, evolutionary processes have naturally selected crayfish to be adaptive to its environment. Being a poikilotherm means that the internal temperature in which the crayfish are considered to reside vary slightly, although still within normal limits (Stephens & Atwood, 1982). In contrast, humans are only able to deviate slightly from 37 degrees Celsius before serious health consequences occur. As the plasma membrane becomes more permeable to ions, K^+ and Na^+ are expected to diffuse into the cell at a greater rate. If a triggering event occurs at this time, Na^+ influx into the cell will occur at a greater rate allowing threshold to be reached at a faster rate. As this happens, Ca^{2+} is endocytosed into the synaptic knob stimulating neurotransmitters to complete the propagation of the action potential. If more rapid events as described occur, Ca^{2+} levels will begin to increase and linger in the cell results in skeletal muscle hypermobility.

Acid Rain Decreases Ca^{2+} Levels

As many mechanical forces account for an increase Ca^{2+} concentrations in freshwater environments or intracellularly, it is important to describe how acid rain works in the opposite effect and decreases Ca^{2+} concentrations. Increased events of acid rain are likely to be most common in Europe, the eastern region of North America, Southeast Asia, and Southern and Central China (Wang et al., 2006). Acid rain occurs when sulfur dioxide (SO_2) and nitric oxides are emitted into the air by millions of particles each day. It is noted that the increase in acid rain events correlates directly with the burning of fossil fuels, automobiles, and other forms of waste. The harmful effects of acid rain on the environment include the deposits of acid that turn into salts in different environments, even if the source of the pollution is over 600 miles away from the deposition (Singh & Agrawal, 2008). As the acid rain falls into the freshwater lakes, its acidity dissolves the Ca^{2+} deposits in freshwater lakes making Ca^{2+} unavailable to crayfish ("What is Acid Rain?" n.d.). The average amount of Ca^{2+} in a lake located in North America is 2.00 mg/L. At this level, nothing in the environment or any of the living organisms will be in any danger. Deviating from this fine concentration is considered to be extremely dangerous for crayfish. If drastically deviated from hormone, neurological, reproductive concerns may appear and ultimately influence extinction ("Calcium Concentration in Lakes," n.d.).

As acid rain stems directly from pollution, again, highly industrialized areas are expected to warrant the greatest effects. Rather than determining exact locations of acid rain in a highly variable world climate, 440,559 freshwater samples were taken from 57 unique countries equating to 43,180 different freshwater ecosystems. In addition to the water samples being acquired, acid rain trends were also analyzed in each area. From this study, it was found that areas where acid rain was frequent, Ca^{2+} levels decreased to $\leq$ 1.5mg/L, a critical level. Additionally, researchers were also able to contribute the fluctuating Ca^{2+} concentrations with carbonate alkalinity (concentrations of carbonate and bicarbonate) suggesting acid deposition was the root determinant. From this 40-year-long study, the data is suggestive of decreasing Ca^{2+} levels in a large amount of freshwater ecosystems. In addition to the Ca^{2+} levels changing, carbonate and bicarbonate concentrations that are now changing are indicative of acid rain being a major contribution to the critically low Ca^{2+} levels. If this trend continues, many crayfish will receive insufficient amount of Ca^{2+} and therefore see detrimental effects lowering the species lifespan (Weyhenmeyer, 2019).

Heavy Metals

A secondary action of Ca^{2+} being present in freshwater ecosystems is aiding in the elimination of heavy metals. Heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr) are readily released into the environment through the recent increase in rates of pollution. Heavy metals are known carcinogens. As these become free in the environment, these systemic toxins are able to cause systemic organ failure and death in animals. Most recently studied in humans, heavy metals are able to be transported throughout the blood stream, enter epithelial cells, and target specific organelles such as lysosomes and the endoplasmic reticulum. In addition to targeting organelles that aid in protein production, heavy metals also bind with genetic contents located within the nucleus and are able to arrest the cell cycle. This leads to the death of many cells, and exaggerated immune response, and in severe cases, systemic organ failure (Tchounwou et al., 2014). These same immune responses would be of normal occurrence if not for the excess Ca^{2+} located within the freshwater ecosystem. However, as described previously with acid rain, decreasing levels of Ca^{2+} are becoming more prevalent world-wide. As this is happening and crayfish aim to maintain a proper intracellular homeostatic environment, less Ca^{2+} is available to bind to heavy metals, therefore increase heavy metal concentration in the freshwater ecosystem. Not only does Ca^{2+} limit the amount of free heavy metals within the environment, it also has been found to ultimately decrease the genotoxicity of the present heavy metals. When Ca^{2+} is present, it was found that when Ca^{2+} concentrations increase by as little as 1mM/L (from 3mM/L to mM/L), there was a 54% decrease in Pb uptake. This was additionally followed by Cd and Cr rates for fish heavy metal uptake being decreased to 58% and 53% respectively. As heavy metals are produced from the environment through pollution, it is important to both limit the amount of pollution in the environment while also assuring positive steps are being taken to conserve Ca^{2+} concentration at its normal level (Ghosh & Adhikari, 2012).

PART III: Crayfish in the Food Chain

Food Chain Importance

As crayfish begin to experience abnormal and unpredictable movement due to over-, under stimulation of skeletal muscles, death becomes a high probability. The death of crayfish seems minor in the realm of the world’s ecosystem, but its higher mortality rate affects the life of numerous other species. Crayfish are responsible for population control of multiple animal and algal species. As crayfish have been found to eat bluegill eggs, bullfrog tadpoles, *Chara* macroalgae, and *Cladophora*. Overgrowth of one of these organisms could subsequently limit the nutrients and resources needed for other organisms to grow and prosper. As this occurs, multiple other food webs are indirectly impacted. Furthermore, when crayfish are present, zooplankton and phytoplankton biomass increase. These marine algae take in acidic CO_2 present in the ocean or other water environments and convert it into usable products while respiring oxygen. Therefore, as crayfish produce CO_2 through respiration, they are feeding benefical algae who in turn purify their ecosystem and produce oxygen (“Phytoplankton,” n.d.). If the average number of crayfish decline, less zooplankton and phytoplankton biomass would be available as resources for other organisms. Lastly, peak dissolved oxygen ratios decrease in freshwater environments as crayfish population increases (Dorn & Wojdak, 2004). As crayfish are integral members of the aquatic freshwater environment, it is predicted that if climate changes alters the course of the crayfish lifespan, many other organisms will be impacted as well.

Crayfish as Environment Indicators

In understanding how the environment may be affected by a decline in crayfish population, it is important to highlight the diverse environments crayfish reside in. First, crayfish can survive both on land and in aquatic environments. As discussed previously, crayfish use gills to exchange oxygen with the surrounding water. However, crayfish must also have a specialized organ to allow for oxygen exchange in terrestrial environments. In terrestrial environments, the gills are specialized to function when moist. The gills of crayfish are protected by an outer covering called a carapace. When this structure is moist from either the surrounding air or is still wet upon exiting the water, sufficient oxygen is exchanged allowing for respiration. Expanding on their ability to live on land, many crayfish are known to burrow into land features in order to assure moist air. Although crayfish have the extensive ability to survive on land, crayfish prefer ecosystems that include cool or warm lakes, streams, terrestrial swamps, temporary wetlands, or caves. Due to their diverse habitat, crayfish have many other regulatory functions within their ecosystem besides being a predator or being ate as prey. The roles include indicating the quality of water, controlling of trophic webs, generation of unique structures for shelter, while also being referred to as "bioindicators" to help regulate and signal community needs. If crayfish populations decrease, these regulatory roles will also decrease affecting the other species occupying the ecosystem.

As most crayfish reside in the habitat to which they were born (homotopic), slight variations in the reproducibility of crayfish can lead to a quick, step decline in environmental regulatory roles. When crayfish populations decrease, many species will become less aware of the water quality often leading to poisoning or death. The presence of different species of crayfish in a certain water quality environment allows other freshwater organisms to recognize the quality of water. An example of this is with the species *Austropotamobius torrenium* and a white-clawed crayfish *A. pallipes* in Europe. These two species are present when the quality of water (lacking pollutants) is extremely high. This means that the water lacks pollutants. Furthermore, a different subspecies of the white-clawed crayfish is able to recognize poorer quality of aquatic habitats in England. As these crayfish populations began to dwindle, they will become less detectable by other organisms in need of information about the environment (Reynolds et al., 2013).

In addition to their role as indicators of water quality, crayfish also provide structural and material advantages to the environment. By regulating the environment by their normal feeding, movement, and reproduction patterns. As sediment present in the freshwater environment becomes dislodged due to increased movement, habitat quality changes along with resources being freed into accessible areas for other organisms to obtain. As hypermobility and hypomobility may result from changes in Ca^{2+} levels, there are two outcomes possible in relation to their structural role in the ecosystem. First, if hypermobility occurs, an increased amount of sediment may become dislodged into the environment. This could create a structurally weak environment, in turn decreasing the quality of water for the other organisms in that same ecosystem. Secondly if hypomobility occurs, less movement is produced, and no particles are moved in that aquatic environment. This would decrease the amount of nutrients available for other organisms. This therefore can lead to a slow decline of other organisms until they evolve to adapt to this new function (Reynolds et al., 2013).

Predator and Prey Roles

Crayfish are not particular about what they consume, noting they are mostly omnivores. Depending on availability of species, crayfish have been found to eat snails, insect larvae, tadpoles, vegetation, or even worms. In acting as a predator, their main environmental role is to prevent overpopulation of the species they are consuming while not allowing the species to go extinct. In an instance where hypo- or hypermobility could occur in crayfish, overgrowth of many of the subsequent organisms may be likely. The main consequence in elimination of crayfish in a predator capacity indicates possible destruction to the local ecosystem. If crayfish are not able to effectively control the population of snails (as an example), then there will be a larger population of snails. As the number of snails then increases, there is now a competition for resources. As snails are known for eating aquatic plants and algae, these food sources become underpopulated and can lead to extinction ("What do…," n.d.). Additionally in a large population of one species completing for a food source, other species may also not receive adequate nutrients for survival. This subsequently affects following organisms and creates a large disequilibrium within the environment (Loureiro et al., 2015).

While effects that crayfish cause as acting as a predator are pronounced, the detrimental effects indicated by the organism that consumes crayfish may be more widely predicted. Large fish, insects, pathogens, birds, and otters have all been known to eat crayfish as a primary food source. Opposing the effects of crayfish as a predatory species, crayfish as prey leads to a reduced population for the organism consuming it as a food source, and likely extinction of some organisms. As a food source becomes scare, there is a higher likelihood of inter-organism competition for food along with competition from similar species or even a complete diet change based on the lack of food. An example of this may include populations such as those of otters and large fish begin to diminish. While this change is not rapid, if climate change continues with the current trends of increasing temperature and more frequent acid rainfall, this may be the new reality for some species (Loureiro et al., 2015). As majority of the decline stems from the regulatory roles of crayfish, changes in population due to the food web is still expected. Currently, there is little data present about crayfish populations over the last 20 years yet a high need as crayfish are intricately designed in current freshwater ecosystems.

PART IV: Human Comparison

Importance of Linking Crayfish to Humans

While it may seem hard to find the correlation between the lifespan and nervous system of crayfish and humans, there are considerable similarities that could indicate the same negative fate of human life due to climate change. The anatomy of vertebrate and invertebrate neurons retain different nomenclature, but the physiology of the neuron is comparable. As the dendrite is the acceptor in vertebrates, receiving the signal through an action potential due to neurotransmitters moving across the gradient; invertebrates in the simplest form contain multiple different forms and combinations of presynaptic neurons form together to create a dendrite like structure. Additionally, membrane potentials are created by the same ions; Cl^-, K^+, and Ca^{2+}. With the simplest form of a neuron comparable in both invertebrates and vertebrates, a valid claim is able to be made between the effects of increase Ca^{2+} on the nervous system of Crayfish and human life (Watson, 1992).

Humans rely heavily on crayfish, although most associations are indirect. The most obvious contribution crayfish provide to humans is the ability to sell them for profit to use at bait of for food. The catching, cleaning, and selling of crayfish provides a steady income for many individuals. As the population of crayfish may decrease, not only will people who fish for crayfish lose their job, other fisherman may as well. A subsequent impact in the role crayfish have with marine plants. Marine plants are responsible for almost 70% of the world’s oxygen. If these plants begin to decrease because of negative environmental factors, oxygen levels will also decrease. Lastly, crayfish are a good model organism to be able to test medical advancements on. Because they share common neurological features, physiological responses, and anatomical histology with humans, crayfish are strong predictors of human neuronal function if mutations, cancers, or diseases arise. Studying the negative effects Ca^{2+} can have on specific motor neurons may help lead to a solution that could one day cure a disease or cancer that acts by varying intracellular Ca^{2+} levels.

Conclusion

In conclusion, if environmental Ca^{2+} levels continue to vary, crayfish may be the first to experience adverse side effects which would largely damage the freshwater plants and animals that cohabitate in these environments. Humans must be aware of the negative effects pollution is causing within both the terrestrial and aquatic environment and how it is affecting all species. If temperature levels and acid rain precipitation begin to rise due to human influence, crayfish populations will begin to decrease. Not only will this directly impact the food and retail market sales industry, but it will impact the organisms in the same environment as crayfish as well, and possibly worse. When populations of several organisms and species begin to dwindle, large scale ecosystem changes will be made to adjust to the new environment, often limiting the number of species present and possibly changing the landscape irreversibly forever.

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