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The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

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ABSTRACT

This study investigates the effects of cold smoke exposure on the behavior and lung health of twelve wild-caught and acclimated Anna's hummingbirds. Half of the group was placed inside an environmental chamber and exposed to cold wood smoke at a $1,419 \mu\text{g}/\text{m}^3$ concentration for 4 hours (smoke group). The remaining six birds were placed in a separate environmental chamber without smoke (control group). At the end of the exposure, half of each group was euthanized immediately in order to study short-term effects, and the other half were euthanized two weeks later in order to study long-term effects. A necropsy was performed on all the birds, and their lungs were processed for histology and analyzed after hematoxylin and eosin (H&E) staining. Each bird's lung histology was graded on the amount of anthracosis present to determine if exposed birds had a greater amount of anthracosis in their lungs compared to unexposed birds. We also investigated if the later-euthanized group had more anthracosis compared to the immediately euthanized group. No significant differences in anthracosis were observed between exposed and control groups or across euthanasia time points. Observational results showed no differences in behavior between the two groups during the experiment. Statistical analyses (t-tests) showed no significant differences in food consumption between the smoke and control groups or in body mass change after the experiment. T-tests did show that the males ate more than the females. Overall, this research aims to advance our understanding of avian respiratory physiology and behavior as well as to enhance our knowledge of the size and density of particulates that affect hummingbird lungs after wildfires.

KEYWORDS: hummingbird, lungs, wildfires, smoke, Anna's hummingbird, anthracosis, hummingbird behavior

FACULTY MENTOR - Dr. Christopher J. Clark; Department of Evolution, Ecology, and Organismal Biology



Dr. Christopher J. Clark has been a professor at UC Riverside since 2013. An expert on hummingbirds, his research focuses on sounds produced by hummingbird and owl flight. In 2001 he received an undergraduate degree in Zoology at Washington State University, a Ph.D. in Integrative Biology at UC Berkeley in 2009.



Isabelle Rose Loaisiga

Isabelle Loaisiga graduated *cum laude* from the University of California, Riverside with a Bachelor's of Science in Biology in 2025, where she was the president of the pre-veterinary club, part of the Honors program, and worked as a necropsy assistant at the California Animal Health and Food Safety Laboratory. She is currently in her second year at the University of Pennsylvania School of Veterinary Medicine, where she is working toward her future goal of improving animal health and welfare while advancing animal science through research. She is particularly interested in pathology, surgery, and translational medicine, but is open to the variety of paths veterinary school has to offer.

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

INTRODUCTION

Wildfires have recently become a severe crisis for California because of their abnormal frequency. Long periods of drought, in addition to the Santa Ana Winds, have led to California facing three times the annual average burned area in 2020-2023 (1.8 million acres) compared to the 2010s (0.6 million acres) (Yu, 2025). Depending on the winds and other climate conditions, wildfire smoke can travel hundreds of miles (LA County DPH, 2025). Humans can escape the harmful smoke from wildfires by staying indoors. However, wild animals have fewer ways of escaping this smoke, making them more vulnerable to wildfires.

When a wildfire starts, it first emits hot smoke. This smoke is hazardous because it contains gaseous pollutants, hazardous air pollutants (HAPs), particulate matter (PM), and numerous other volatile organic compounds (VOCs) (Jaffe et al., 2020). The primary concern with hot smoke is that it immediately kills animals because carbon monoxide binds tightly to hemoglobin, starving the body of oxygen (Varon et al., 1999).

As smoke physically cools, it undergoes a series of chemical reactions and transformations, creating secondary pollutants, including ozone (O₃) and secondary organic aerosols (SOA) (Jaffe et al., 2020), while VOCs largely dissipate, leaving the PM behind. This “cold smoke” remains a hazard. Depending on its size, the PM can penetrate through an animal’s respiratory tract and reach the lungs, posing a serious threat to the animal’s health. Particles measuring 2.5-10 micrometers in diameter may be inhaled and penetrate the lungs (Stone et al., 2019). PM 2.5 micrometers in diameter or less are known as fine inhalable particles and are more dangerous since they have more significant health risks (Stone et al., 2019).

Scientists already understand the effects of hot smoke leading to death because of CO, but cold smoke’s physiological and behavioral effects have not been as clear. There is very little research about how different species, specifically birds, behaviorally respond to cold smoke from the wildfires around them. Additionally, there is limited research on how harmful, if at all, cold smoke could be to bird lung health. Birds are crucial to the ecosystem and directly impact our health, economy, and food production

through pollination, spreading seeds, keeping coral reefs alive, controlling pests, and more. The animal model used in this experiment was Anna’s hummingbirds (*Calypte anna*). Their smaller body size makes them more sensitive to pollutants and respiratory damage compared to larger birds, allowing a more sensitive test of a bird’s threshold to cold smoke. Additionally, they are abundant in Southern California, where our lab is located, and which specializes in hummingbird research.

Birds may be more vulnerable to the effects of cold smoke than other animals because of their physiology. Avian metabolism is very high due to the high energy demands of flight, leading to high rates of gas exchange, making them susceptible to inhaling more PMs (Munshi-South et al., 2010). Unlike mammals, birds’ respiratory system includes densely packed, narrow capillaries with unidirectional airflow through their lungs and air sacs (Brown et al., 1997). This contributes to their highly efficient gas exchange but can be problematic for trapping particles. Their trachea is relatively longer than those of mammals and contains mucus-secreting cells and cilia that work together to remove harmful particles (Jacob and Pescatore, 2013). A recent study comparing the lung health of different zoo animals found that avian species have a higher occurrence of anthracosis than do mammals (Leya et al., 2023). Anthracosis is a condition that is caused by the “accumulation of carbon in the lungs due to...exposure to air pollution or inhalation of smoke” (Mirsadraee, 2014). Anthracosis poses a serious threat to human and avian respiratory health because it causes chronic lung inflammation and immunodeficiency and invites potential parasitic conditions such as lophomoniasis (Tajik Jalayeri et al., 2024).

This experiment studied hummingbird behavioral response to cold smoke particulates through observational assessment. We compared normal day-to-day hummingbird behavior in the lab to their behavior while exposed to smoke in the smoke exposure chamber, noting signs of stress (e.g., decreased activity, fluffed feathers, mouth opening, slow blinking, decreased food consumption) and normal health (e.g., flying around occasionally, perching, regular food consumption). We hypothesized that birds exposed to smoke will show behavioral signs of stress compared to the control group, which will show signs of normal health.

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

This experiment also studied the physiological effects of cold smoke on avian lungs by comparing the amount of anthracosis between the control and exposed groups on histopathology. Anthracotic granules (black pigment) may be located inside or outside of alveolar macrophages, in the perivascular, or in the peribronchiolar area, along with inflammatory infiltrates and accompanied by edema (Leya et al., 2023). We hypothesized that the smoke group will have more anthracosis from smoke irritation in the lungs compared to the control group. Furthermore, we also examined the effects of smoke on recovery time after exposure. Half of the birds from each group, smoke and control, were euthanized two weeks after smoke exposure to study long-term effects, and the other half were euthanized immediately to study short-term effects. We predicted that the birds euthanized two weeks after smoke exposure will show long-term effects, such as an increased amount of anthracosis due to impaired respiratory recovery from smoke damage. Birds euthanized immediately were expected to show acute physiological effects in lung histology, such as lower levels of anthracosis.

MATERIALS AND METHODS

(a) Bird Care

Twelve Anna's Hummingbirds (*Calypte anna*), eight females and four males, were captured in the wild on the campus of the University of California, Riverside (UCR). All components of the experiment and treatment of birds followed the approved California Department of Fish and Wildlife collecting permit (#S-183160004-19092), U.S. Fish and Wildlife Service (USFWS) collecting permit (MBPER3871689), and UCR IACUC protocol (#30063). The birds were acclimated to individual cages and housed in captivity. Birds were also weighed and monitored daily.

(b) Chamber and Smoke Details

This experiment used two environmental chambers (one for the smoke-exposure group and the other for the control group) constructed from transparent acrylic sidings that were 0.635 cm thick. The dimension of each chamber was 101.6 x 81.3 x 63.6 cm.

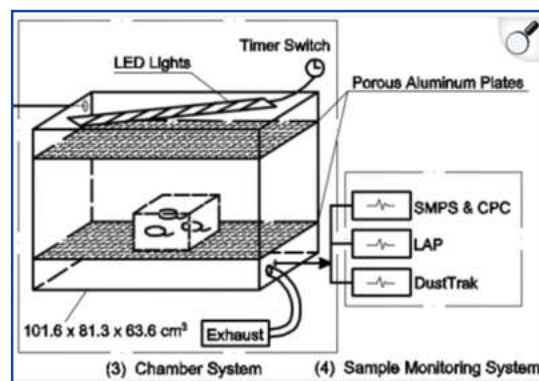


Figure 1: (3) Chamber system used during the experiment along with the SMPS in (4) sample monitoring system (Image from Peng et al, 2019).

Each chamber had controlled airflow, with approximately one air change per hour. According to The Guide For the Care of and Use of Laboratory Animals, by the National Research Council, this is below the 10-15 air changes per hour recommendation. However, a hummingbird's metabolic rate is approximately 40 mL O₂/g/hr; therefore, six flying hummingbirds are estimated to consume about 1 L of O₂ per hour, or approximately 1% of the chamber volume (Clark and Dudley, 2010).

Smoke simulating wildfire conditions was generated using a food smoker inside a fume hood. The device heated wood chips on a metal plate, with charcoal and fatwood used as the heat source beneath it. Once a target temperature was reached, the chips released smoke that was then drawn into the tubing toward the environmental chamber. Before reaching the chamber, the smoke was filtered with activated charcoal to remove harmful pollutants, such as carbon monoxide (CO). However, some CO could still reach the smoke chamber, so a CO monitor was used to track levels the birds were exposed to and ensure the amount of CO did not impact the hummingbirds' welfare. If the CO monitor inside the environmental chamber exceeded 50 ppm, we reduced the flame.

Particles of 2 nm to 1000 nm were measured and characterized using a Scanning Mobility Particle Sizer (SMPS) (Model 3080 Electrostatic Classifier using Kr85, TSI, Minnesota, USA) system. Every 130 seconds the SMPS

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

captured the concentration of smoke being exposed to the birds (27 scans/hour).

(c) Acclimation

We acclimated the birds to the insect cages (30.5 x 30.5 x 30.5 cm) and the environment they would be in during the experiment to decrease their stress on experiment day. On Days 1 and 2, birds were placed in individual mesh insect cages for 5 hours daily. The insect cages were meant to separate each bird and allow for PM to be inhaled during the experiment. On Day 3, a test run of the experiment was conducted to ensure minimal behavioral stress for the birds on the experiment day. The birds were placed in their designated mesh insect cages and separated into two groups: smoke-exposed (N=6) and control (N=6). Each group had 4 females and 2 males. Birds were placed into their designated environmental chambers for 3 hours without any exposure to smoke.

(d) Smoke Exposure

On Day 4, the experiment was conducted. All birds were weighed at 07:30 h and transported to the experimental facility. The birds were placed in their individual mesh insect cages with a perch and 30 mL of nectar solution. Then, at 08:30 h, six birds inside the insect cages were placed in the smoke environmental chamber, while the remaining 6 were placed in the control environmental chamber. Between 11:00 h and 16:00 h, the smoke-exposed group was exposed to filtered smoke particles that were 0.2 μm - 1 μm at an average concentration of 1,419 $\mu\text{g}/\text{m}^3$. There were approximately 108 scans for smoke particle concentration during the entire experiment. The maximum concentration was 3574.73 $\mu\text{g}/\text{m}^3$, and the minimum was 715.62 $\mu\text{g}/\text{m}^3$. Fluctuations in concentration were attributed to changes in airflow, fuel addition, and carbon monoxide control (i.e., increasing airflow into the chamber following rises in CO levels), while maintaining the flame.

(e) After the Experiment

Immediately after the experiment, each bird's weight was recorded. Three birds (one male and two females) from each group were euthanized using isoflurane. The six birds then received a post-mortem necropsy with a prosection from the sternum to the cloaca and then soaked in 10% neutral

buffered formalin for 24 hours. The lungs were extracted and sent to University of California, Irvine (UCI) Health for tissue processing and paraffin embedding.

After exposure, the remaining six birds were returned to their original housing cages. They were observed four times daily, for one hour each throughout the day for respiratory distress, behavioral changes, and health concerns such as lethargy, weight loss, nasal discharge, lack of grooming, hunched posture, mouth breathing and opening, decreased flight, and fluffed feathers. After 15 days, the remaining six birds were euthanized following the same protocol as above.

(f) Hematoxylin and Eosin (H&E) Staining

Once all the tissue sections were returned from processing, all 12 slides were stained with hematoxylin and eosin (H&E) for histopathological analysis. First, the slides were deparaffinized. Each slide was placed in xylene twice for 5 min, followed by 100% ethanol (EtOH) twice for 5 min each. The slides were then rehydrated through a graded ethanol series of 95%, 70%, 50%, and 30% EtOH for 2 min each, and subsequently immersed in phosphate-buffered saline (PBS) for 10 min. After deparaffinization, slides were immersed in hematoxylin for 4 min, rinsed in tap water (10 dips), and briefly immersed in phosphate-buffered saline (PBS) for 1 min. The slides were then stained with eosin for 5 min and rinsed again in tap water (10 dips). Following staining, the slides were dehydrated through a graded ethanol series: 50% EtOH (3 dips), 70% EtOH (3 dips), 95% EtOH (1 dip), and 100% EtOH (3 dips), followed by clearing in xylene twice for 5 min each. Finally, the slides were mounted and allowed to dry for 24 hours prior to examination.

(g) Analysis

Following the histological staining, slides were sent to a veterinary anatomical pathologist for blind analysis and evaluation. Each lung slide was graded for anthracosis from 0 to 3 (none, mild, moderate, severe).

(h) Statistics

We used JMP statistical software for the analysis. In JMP, an ordinal logistic fit was performed for anthracosis (on a scale from 0 to 3) found in the hummingbird lungs. Anthracosis was the dependent variable, and the independent

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

variables were treatment (smoke exposure), sex, and time of euthanasia (immediately or two weeks post-exposure).

JMP was used to perform t-tests to compare food consumption and body mass change before and after the experiment. Smoke exposure and sex were used as grouping variables in separate analyses. One female hummingbird in the control group was excluded from these two T-tests because she did not eat at all and was an outlier.

RESULTS

(a) Engineering particles/density results

Scans of the smoke inside the chamber were conducted from 09:00 to 16:00 h. The first 2 hours were excluded due to clogging-related errors in the tubing system delivering smoke to the chamber. Starting at 11:00 h, SMPS scanned the chamber 108 times during 4 hours, showing fluctuations in the concentration of smoke levels. The average concentration of smoke particles was $1,419 \mu\text{g}/\text{m}^3$. The maximum concentration was $3574.73 \mu\text{g}/\text{m}^3$, and the minimum was $715.62 \mu\text{g}/\text{m}^3$.

(b) Behavioral analysis

Hummingbird behavior was assessed by observation. Both groups showed no significant differences before, during, or after the experiment. Both control and exposed groups flew around occasionally, perched most of the time, drank their nectar solution, and showed no signs of stress (e.g. slow blinking eyes, opening their mouth, fluffed feathers, and decreased movement).

(c) Food consumption and weight gained

For the analysis of food consumed and body mass change, one bird was dropped as an outlier because it had consumed no food. For the remaining 11 birds, there was also no effect of smoke on body mass ($t(9) = 0.615$, $p = 0.554$). However, there was a significant effect of sex, with male hummingbirds gaining more weight than females ($t(9) = 2.613$, $p = 0.028$).

There was no effect of smoke on food consumed during the experiment ($t(10) = 0.882$, $p = 0.399$) or of sex ($t(10) = 2.103$, $p = 0.062$).

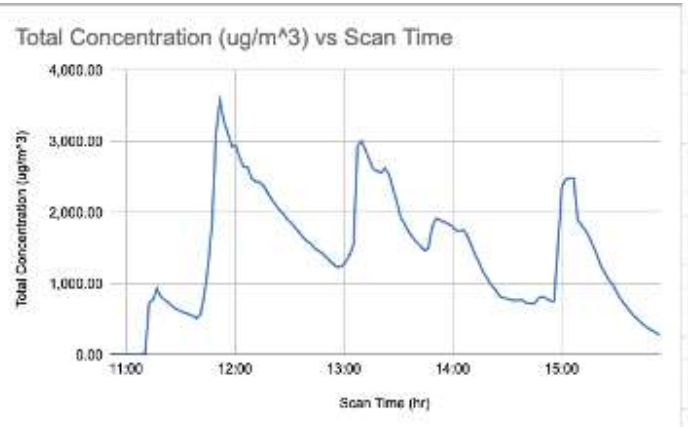


Figure 2: Total concentration of smoke particles ($\mu\text{g}/\text{m}^3$) fluctuated over the course of 4 hours. The concentration ranges from $3574.73 \mu\text{g}/\text{m}^3$ to $715.62 \mu\text{g}/\text{m}^3$.

(d) Histology evaluation

Hummingbird lung histology showed variations of anthracosis between all groups studied and no patterns when comparing groups. There was no significant effect of cold smoke on anthracosis (Ordinal Logistic Fit, $p = 0.336$, $df = 10$), nor were there significant effects of time after smoke exposure ($p = 0.552$) or sex ($p = 0.618$).

Scans of the smoke inside the chamber were conducted from 09:00 to 16:00 h. The first 2 hours were excluded due to clogging-related errors in the tubing system delivering smoke to the chamber. Starting at 11:00 h, SMPS scanned the chamber 108 times during 4 hours, showing fluctuations in the concentration of smoke levels. The average concentration of smoke particles was $1,419 \mu\text{g}/\text{m}^3$. The maximum concentration was $3574.73 \mu\text{g}/\text{m}^3$, and the minimum was $715.62 \mu\text{g}/\text{m}^3$.

DISCUSSION

This study showed that exposure to an average of $1,419 \mu\text{g}/\text{m}^3$ smoke particles for four hours did not affect any of the measured variables.

The degree of anthracosis in the hummingbirds' lungs did not show any significant differences between smoke and control groups or between euthanasia time points. This does not support our hypothesis that the smoke group

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

would show more pathogenesis in histology compared to the control group. It also contradicts our hypothesis that there would be long-term effects of smoke exposure on the later-euthanized group versus short-term effects on the immediately-euthanized group. Considering that both groups had some degree of anthracosis, we can infer that ambient air in the study environment contains harmful pollutants capable of irritating their respiratory systems.

Exposure to smoke also showed no effect on the amount of food consumed and body mass change between the groups. Our hypothesis that the smoke group would have a larger body mass change and would eat less than the control group was not supported. This implies that the smoke did not cause stress.

During the experiment, the smoke and control groups showed no signs of stress and behaved as if in good health (e.g., flying around occasionally, perching, and regular food consumption). This does not support our hypothesis that birds exposed to smoke would show behavioral signs of stress compared to the control group.

The unexpected results of this study may be due to the small sample size and limited duration of the experiment. One day, for four hours may have been too short an exposure time for the smoke to affect the birds' behavior and physiological processes in the lungs. This short duration and length of exposure could allow the birds to recover more easily and not be as affected by the smoke. Having longer exposures, such as every day for a month and for more hours in the day, such as 8 hours, would more realistically mimic the effects of a true wildfire. This type of experiment would focus on the physiological processes to overcome constant exposure (which would be faced in a real wildfire) and test the bird's immune system.

Additionally, the smoke concentration might have constrained our results by being too weak to affect the birds' behavior and lung health. Based on the Air Quality Index (AQI), the smoke particle concentrations used in this experiment fell within the upper end of the "Hazardous" category (purple range), corresponding to $PM_{2.5}$ concentrations of $\geq 222.5 \mu g/m^3$ (IQAir). Although this is a large amount of smoke inhaled by the birds, since it was

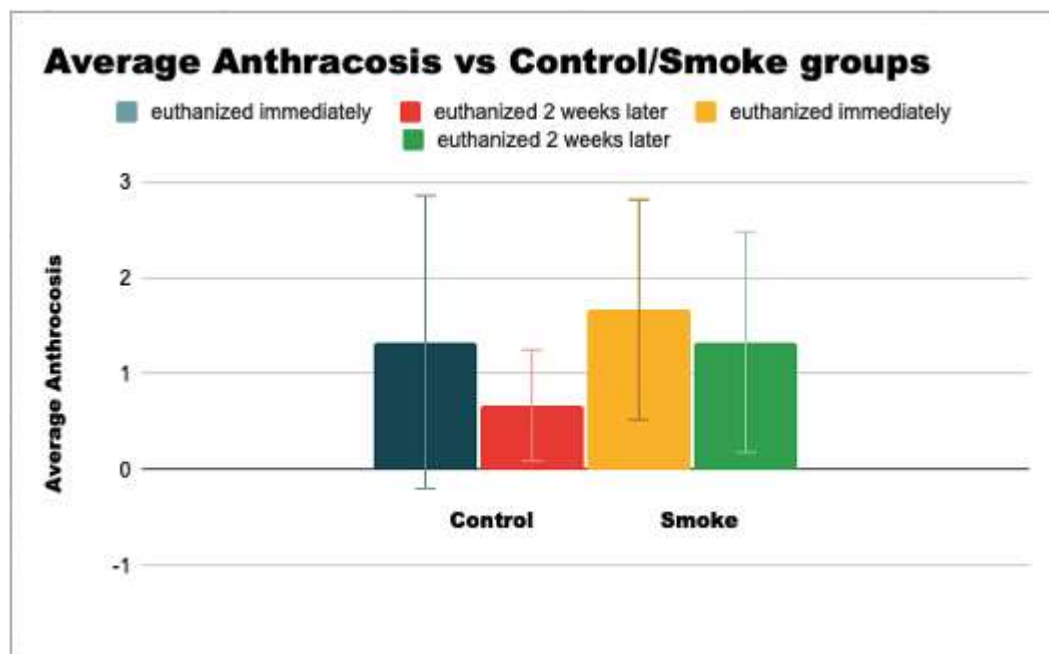


Figure 2: Anthracosis scaling (0-3) for all control groups (euthanized immediately and euthanized 2 weeks later) and smoke groups (euthanized immediately and euthanized 2 weeks later). There are no significant differences between groups. Error bars capture ± 1 SD.

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

for only four hours in one day, this type of exposure may be recoverable. By increasing the concentration of smoke and duration of exposure, we can better test how resilient the birds are.

Finally, the mechanism for creating smoke was limiting because of the need to continuously maintain a flame, add fuel, change air flows, and control CO. For instance, once the CO monitor inside the environmental chamber reached >50 ppm, we reduced the flame. However, reducing the flame also led to a lower concentration of PM to which the birds were exposed. We had to find the right balance between feeding the flame to increase particulate concentration and reducing the flame to not expose the birds to overwhelming amounts of CO. This caused some fluctuations in the PM concentration over time. This led to the birds not inhaling the expected higher, consistent amounts of PM for an extended period and, therefore, not affecting their respiratory systems as intended.

For future projects on this topic, we suggest modifying some factors, including more frequent exposures, no CO emissions, and pure PM to create a more realistic scenario that reflects actual exposure to cold smoke in the wild. Periodically exposing birds to smoke, such as every day for a month, or exposing them to higher acute concentrations, such as $10,000 \mu\text{g}/\text{m}^3$, would ensure the birds are inhaling enough PM to test their resistance and tolerance to extreme cases of smoke. Researchers could then study the birds' responses and histological pathologies (both long- and short-term) and identify physiological resilience or effects and behavioral changes over time. Also, using a different metric of lung pathology would give greater insight into the damage occurring in the respiratory tract. This could include measuring inflammatory cells, edema, and necrosis. Additionally, gathering histology on the air sacs, other organs, and lymph nodes could provide more insight into the physiological effects, since they play a vital role in hummingbird breathing and contribute to the unidirectional flow through the lungs.

Although this project did not show significant differences between the behavior and amounts of anthracosis in the lungs of cold smoke-exposure compared to the control, further research is important to better understand bird

respiratory physiology and immunology that contributes to them possibly being resilient creatures. Ultimately, it is important to better understand how cold smoke affects bird lung health and behavior as they play a crucial role in our ecosystem and wildfire cold smoke may pose a major threat to their health and survival.

The Effects of Cold Smoke on Hummingbird Behavior and Lung Physiology

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