# *Appendix D: Working Group B — ECOLOGICAL INVESTIGATION*

| | |
|---|---|
| *Leader:* | *Frances Cassirer* |
| *Facilitator:* | *Esther Rubin* |
| *Recorder:* | *Kelly Buckle* |
| *Participants:* | *Mark Atkinson* |
| | *Vern Bleich* |
| | *Sue Fairbanks* |
| | *Kristin Mansfield* |
| | *David Miller* |
| | *Sri Srikumaran* |
| | *Cynthia Tate* |

## FLIPCHARTS:

Objectives:

- Identify (1) and prioritize (2) field experiments to test hypotheses about causes of pneumonia in free-ranging bighorn sheep.

Outcomes:

- A matrix that prioritizes factors associated with pneumonia that are high priorities for future study.
- A list of potential experiments in free-ranging, captive and laboratory conditions that would address top-priority factors.

## Overview:

The expected outcomes of the working group were reviewed, as well as the scheduled topics and time frame.

## STEP 1: Assessment of Matrix Factors

### *Factor 1—Introduction of novel pathogens:*

Questions:

- Is shipping fever in cattle a result of pathogen mixing or actual introductions of novel pathogens, or both?
- Can techniques such as microarrays be used to detect existing and novel pathogens?

Discussion Points:

- Pasteurellosis has not been experimentally recreated in cattle by challenge with *Pasteurella* bacteria (unless put directly into the lower respiratory tract) except when preceded by infection with one of several respiratory viruses.
- There is documentation of transmission of pathogens between domestic and wild sheep, but not consistently as a precursor to epizootics in wild sheep. This has been used by opponents of the disease-transfer from domestic sheep theory.

**Suggested Final Wording of What is Not Known**—"Which pathogens are of most importance in causing pneumonia in free-ranging bighorn sheep? How does transmission occur? How much and what kind of contact is needed? What physiological, behavioral, or ecological factors might be conducive to transmission and causing disease? Do we have the methods/tools to detect and link pathogens to disease?"

### *Factor 2—Weather*

Questions:

- Have die-offs been associated with changes in weather?
- Is there evidence for bluetongue in die-offs?

Discussion Points:

- Experimental evidence in livestock has been mixed on the importance of weather.
- Sometimes only a single bighorn sheep population within a weather regime is affected, so maybe weather is a factor but not the primary factor.
- In California, the desert sheep population had 2 seasons of monsoon-like weather which was followed by an increase in documented deaths and at least one death associated with bluetongue– speculation was that water availability created increased parasitism (culicoides)
- Titers to bluetongue and EHV across the California desert – no chronic lesions, only serological data. Bluetongue and EHV are both vectored by gnats. In California, 1 bighorn male died, tested positive for bluetongue during a season with tons of gnats, 2005.
- Weather may be related to disease for the following reasons: snow may cause increased/decreased grouping patterns or decreased nutrition, snow may change spatial use of environment; cold weather may decrease mucocilliary clearance,

pathogens may require certain weather conditions to survive in the environment or to persist on fomites, disease vectors (culicoides, other gnats) respond to weather conditions.

**Final wording of What is Not Known**—"How would weather affect pathogen transmission and immune function in bighorn sheep? Weather may have different effects in different situations and in different ecosystems. Mechanisms differ among ecosystems."

### *Factor 3—Overcrowding creating conditions conducive to disease transmission*

*(renamed to: Density-related disease transmission.)*

Questions:

- Do we see disease outbreaks in big populations because the population is big, or are populations big because they haven't had a recent disease outbreak?
- Is density related to population size in bighorns, or does behavior cause high density regardless of population size?

Discussion Points:

- Not all high density populations experience pneumonia outbreaks, but it is logical to think that crowding increases the risk of disease transmission.
- Due to behavioral differences, major problems exist with taking human and domestic animal studies and trying to apply them to natural populations. Ewes are in tight groups year-round, except for during lambing.
- The density of some populations studied are estimated at 0.2-0.3 sheep/$km^2$ up to 2 sheep/$km^2$. We only have point estimates (no confidence intervals). Good data are not available that we know of. (Multiple participants agree with this general observation of density).
- One high-density bighorn sheep population occurs on Wildhorse Island in Flathead Lake, Montana.
- In general, increased density leads to greater potential for aerosol and contact transmission. It is hard to distinguish whether overcrowding comes with behavioral and nutritional stress factors. Is it simply a density factor with increased opportunity for transmission? How do we distinguish between density-dependent factors and other factors (such as size, nutritional issues) that come with increased density?

**Decision made to rename factor to read**—Density-related disease transmission.

### *Factor 4—Bottom-up regulation: density dependence through nutritional constraints.*

Questions:

- Does evidence exist for a link between poor condition and die-offs?
- Is nutrition linked to poor recruitment (decreased lambing)?
- Do we see links between nutrition and poor recruitment/disease in captive animals?

Discussion Points:

- Usually bighorn populations that are affected by pneumonia epizootics are in good condition at the beginning of the die-off.
- Some bighorn sheep populations in the Sonoran desert are in very poor body condition due to environmental factors, and are not affected with pneumonia like some groups in better conditions.
- No all-age die-offs have been seen in the desert within the last 30 years. The all-age die-offs have all been in more mesic areas.
- Nutrition provided ad lib in captivity, so we never see poor condition animals. No evidence for Pasteurellosis link to poor nutrition in domestic animals.
- Medicine texts talk about poor body conditions, micronutrient deficiencies being linked to disease. We don't really have the methods for assessing bighorn body condition, micronutrient deficiency, and other factors.
- No standardization of bighorn body condition. We need something to be able to compare between species, regions.

**Final wording of What is Not Known**—"Can pneumonia outbreaks be induced in bighorn sheep through poor nutrition alone? Can nutrition be an important contributing factor? We don't have standard methods/tools for assessing body condition. We also don't have standard methods of assessing/comparing forage quality."

### *Factor 5—Trace element or other environmental deficiencies*

Questions:

- Is there any indication from other studies that disease is more prevalent in populations with low micronutrient levels?

Discussion Points:

- Theoretically, bighorn may have adapted to native environment, but new infectious pathogens may challenge that adaptation. Ability to resist disease may be increased by supplementation.
- Supplementation has been done in Hell's Canyon. Just putting supplement blocks out doesn't mean they'll use them – must use them a lot to actually cause increased selenium concentrations. It appears that populations with low selenium sometimes have no disease and vice versa.
- There are no known normal levels for micronutrients in bighorn sheep.

**Final wording of What is Not Known**—"Can pneumonia outbreaks be induced in bighorn sheep through trace element deficiency alone? Can trace element levels be an important contributing factor, or can supplementation reduce the severity or frequency of disease out breaks?"

### *Factor 6—Anthropogenic stressors such as human disturbance, being held in captivity, or natural stressor such as the rut (the former was renamed to Anthropogenic [such as stress from handling, recreation, capture])*

Questions:

- At what point does a stressor become part of an animal's environment? Bighorn in California sometimes coexist with freeways and low-altitude planes. At some point when the "stressor" is benign, these animals very clearly get used to it.

- Should we differ between anthropogenic and physiological stressors?

Discussion Points:

- In cattle, shipping fever is linked to stress.
- In Montana, large-scale captures of bighorn sheep followed by transport and hard-release are probably very stressful events for the sheep. Most of these animals have been monitored post-release with radiotelemetry. Over the past number of years, capture operations have never been followed by a pneumonia outbreak.
- After a major capture with hard-release, 2 animals died after release, but no herd outbreak. But animals also scattered (so maybe they just couldn't spread disease to each other).
- Distinction needs to be made between chronic versus acute stressors.

Discussion follows about the appropriateness of distinguishing between natural and anthropogenic stressors and creating new categories for it. Different physiological stressors, hormones, can affect immune function and cause switching of energy allotments among different components of the immune system within the animal.

**Final wording of What is Not Known**—"May play a secondary role? Stress is difficult to define and/or measure. There may be difference between "acute" and "chronic" stress (stress levels can change over time)."

Another factor, Natural Stressors, is added.

### *Factor 7—Natural stressors (stress caused by rut, lactation, migration)*

Questions:

- What type of immune response is helpful for these pneumonias?
- We don't known how stress relates to other factors impacting immune functions.
- How do factors 1–6 interact with immune function to increase the risk of disease?

Discussion Points:

- Different functions of the immune system are useful for targeting different types of disease agents (e.g. viruses versus parasites). TH1 versus TH2 immune function. Exposure to one type of parasite can decrease immune function for fighting off other disease.

## STEP 2: What are the important criteria to use in prioritizing research factors? (FLIPCHART)

Questions:

- Are we more interested in assessing a factors influence on mortality or on fecundity?
- How do we predict a priori a factor's importance to bighorn disease?
- Are we more interested in assessing causes of chronic or acute disease?

- Is low recruitment always preceded by large-scale population die-offs?
- Can disease, either acute or chronic, be helpful in predicting the carrying capacity of an environment?
- Is it helpful to look at factors that have potential local applicability, but that do not necessarily have universal application?

Discussion Points:

- Group agrees to use both mortality and fecundity effects of a factor, and to combine them into the general question, "Does this factor have a demographic consequence?"
- Group agrees that low recruitment is sometimes, but not always, preceded by large-scale population die-offs.
- In California, as carrying capacity is approached, recruitment appears to decrease, and the population levels off.
- If money is being committed to a project, we should rate factors higher that have wider applicability, not just local applicability.

## Criteria for rating factors (FLIPCHART):

A. Wide application across ecosystems/situations

B. Scientific merit – does it build on existing knowledge?

C. Does it directly affect management decisions?

D. Does the factor have potentially important population-level effects via either mortality and/or reproduction?

E. Could this provide the most parsimonious explanation?

Ranking sheets were handed out, and participants asked to anonymously rank each of the 7 factors 1-3 (1 lowest, 3 highest) for each of the 5 ranking criteria described above. Participants are asked to add up the total points received for each factor.

Results of ranking

| Factor | Total points received | Rank |
|---|---|---|
| **Introduction of novel pathogens** | **109** | 1 |
| **Density-dependent nutritional constraints** | **87** | 2 |
| **Density-related disease transmission** | **82** | 3 |
| **Natural stressors** | **77** | 4 |
| **Anthropogenic stressors** | **71** | 5 |
| **Trace elements/other env. deficiencies** | **69** | 6 |
| **Weather** | **64** | 7 |

## STEP 3: Development of Hypotheses

**Ranking**—Factor 1 was ranked #1. It was decided that the initial primary focus for hypothesis development would be on factor 1. Other factors were ranked as indicated above in parentheses.

### Hypothesis development for Factor 1:

Hypothesis 1: Domestic sheep transmit novel pathogens to bighorn sheep that cause respiratory disease.

Discussion Points:

- The use of “agents” instead of “pathogens” is decided upon, since the use of non-pathogenic agents could be useful for assessing disease transmission.
- It would be useful to break the hypothesis into several parts that are more easily addressed, such as: is there contact, are agents transferred, are agents pathogenic?
- Would it be sufficient to prove that transmission of benign agents occurs, or is it necessary to prove that transmission causes disease?
- Definition of “exposure” is discussed. Does exposure refer to inhalation/ingestion of pathogens, or the contacting of infected individuals? Group agrees to use the latter definition (contact with infected individuals).
- How could you demonstrate that a bighorn population was naïve with regard to contact with domestic sheep? Would hand-raising of lambs be necessary to produce domestic-pathogen-free bighorns?
- Hypotheses are contingent on domestic sheep being apparently healthy at time of agent transmission to bighorn sheep.

**Final hypotheses (FLIPCHARTS)—**

General hypothesis: Organisms that are carried by apparently healthy domestic sheep, but which are lethal to bighorn sheep, are transmitted from domestic sheep to bighorn sheep when these two species co-mingle.

Sub-hypotheses:

1. When bighorn sheep are exposed to domestic sheep, transmission of novel organisms occurs from domestic sheep to bighorn sheep.
2. After transmission, respiratory disease develops in the bighorn sheep.
3. The organism is transmitted among the bighorn sheep population and causes disease.
4. This causes a significant mortality in the population.

**Experimental Designs (brainstorming):**

Points of Discussion:

- The wisdom of building on experimental, captive trials in order to assess the approximate types and lengths of contact necessary for transmission prior to embarking on more expensive/risky/limited field experiments.

- Use of captive bighorns would be superior to use of purely domestic sheep in these captive trials, due to differences in disease presentation and behavioral differences in the different species (e.g. bighorns are more profoundly affected and might transmit disease more quickly within the flock than predicted if using domestic sheep).
- Experiments using marked mutant strains might be possible, and would allow differentiation between the experimental agent and enzootic agents.
- Marking is superior to genetic fingerprinting due to the absolute uniqueness of marked organisms.
- It is important for studies to be replicated in different populations of bighorn and in different states due to presumed genetic differences between populations.
- Hypothesis: If we take the domestic sheep away, the bighorn sheep will recover. Is this hypothesis important to our work? If we don't care what happens when domestic sheep are removed, why not leave domestic sheep living near bighorn sheep populations, since the bighorns have already been exposed to pathogens?

Experimental Design Ideas:

1. Replicated experiment as at Starkey with tame bighorn sheep using marked organisms (looking for conversion of individuals – before and after) to assess transmission of the agent between bighorn sheep.
2. Replicated experiment in free-ranging bighorn sheep (with 10 ecologically-similar populations) using marked organisms and introduction of domestic sheep into 5 randomly-selected bighorn populations.

## STEP 4: Presentation

It was decided that of particular interest for presentation were:

- Factor definition process
- Factor ranking process
- Hypotheses
- Ideal experimental designs

## Factor Identification and Prioritization

*Preliminary Assessment for Mountain Sheep Respiratory Disease Workshop 2007*

| Factor | Evidence for and against | What is Not Known? | Research Priority (H, M, L) |
|---|---|---|---|
| **Introduction of novel pathogens** | **For:** Documented experimentally in captive bighorn sheep and in livestock. Epidemiological support in free-ranging bighorn sheep and livestock. **Against:** Introduction of novel pathogens into free-ranging bighorn sheep populations has not been well documented. | Which pathogens are of most importance in causing pneumonia in free-ranging bighorn sheep? How does transmission occur? How much and what kind of contact is needed? What physiological, behavioral, or ecological factors might be conducive to transmission and causing disease? | |
| | | | |
| **Weather** | **For:** Postulated in livestock and bighorn sheep. Epidemiological support in livestock and in some bighorn sheep populations. **Against:** No experimental evidence? Many pneumonia outbreaks are confined to a single population within a geographic region, suggesting that if a landscape-level factor such as climate is involved, it likely plays a secondary role. | How would weather affect pathogen transmission and immune function in bighorn sheep? | |
| | | | |
| **Overcrowding creating conditions conducive to disease transmission** | **For:** Identified as a factor in livestock pneumonia from epidemiological investigations. Most pneumonia die-offs in bighorn sheep are detected when populations are relatively high. Many die-offs occur during winter when animals may be more concentrated. **Against:** No experimental evidence? Some large bighorn sheep populations never experience pneumonia outbreaks, and some small populations are affected periodically by pneumonia die-offs. Increased detection in larger populations may be a function of visibility rather than prevalence? | Are disease outbreaks in bighorn sheep a function of population size or vice-versa? If there is relationship, is this due to animal density or to increase in potential for introduction of novel pathogens, or other factors? | |
| | | | |
| **Bottom-up regulation: density-dependence through nutritional constraints** | **For:** Evidence in other wildlife populations and in livestock that poor nutrition can predispose animals to disease. Infection by macroparasites, such as lungworms, is often correlated to population density and animal condition. **Against:** Same as for overcrowding factor. | Can pneumonia outbreaks be induced in bighorn sheep through poor nutrition alone? Can nutrition be an important contributing factor? | |
| | | | |

### Factor Identification and Prioritization

*Preliminary Assessment for Mountain Sheep Respiratory Disease Workshop 2007*

| Factor | Evidence for and against | What is Not Known? | Research Priority (H, M, L) |
|---|---|---|---|
| **Trace element or other environmental deficiencies** | **For:** Selenium, copper, and zinc are known to play a role in immune function. Trace element levels in forage in some areas used by bighorn sheep are considered marginal or deficient for domestic sheep. There is some experimental evidence that selenium deficiency may reduce immunocompetence in domestic lambs. **Against:** No experimental evidence in free-ranging or captive bighorn sheep. | Can pneumonia outbreaks be induced in bighorn sheep through trace element deficiency alone? Can trace element levels be an important contributing factor, or can supplementation reduce the severity or frequency of disease outbreaks? | |
| | | | |
| **Anthropogenic stressors such as human disturbance, being held in captivity, or natural stressors such as the rut.** | **For:** Thought to be important in shipping fever complex in livestock. Increased cortisol levels have been shown to be associated with reduced immune function at the cellular level in bighorn sheep. **Against:** No experimental evidence in animals. Pneumonia outbreaks can occur in the apparent absence of these stressors. | May play a secondary role? | |
| | | | |
| | | | |
| | | | |
| | | | |