DIRECT EFFECTS OF COLD SHOCK: BIOASSAYS WITH THREE COLUMBIA RIVER ORGANISMS

by C. D. Becker and M. J. Schneider

Battelle
Pacific Northwest Laboratories
Richland, Washington  99352

* This paper is based on work performed under U.S. Energy Research and Development Administration Contract No E(45-1):1830
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DIRECT EFFECTS OF COLD SHOCK: BIOASSAYS
WITH THREE COLUMBIA RIVER ORGANISMS

Authors
C. D. Becker and M. J. Schneider, Ecosystems Department

Occasion
Oral Presentation (10 min) at the 105th Annual Meeting, American Fisheries Society, Las Vegas, Nevada, Sept. 10-13, 1975. No publication.
Abstract: Cleared as BNWL-SA-5435

Presentation Text
Recent studies at the Pacific Northwest Laboratories of Battelle Northwest (PNL) have dealt with the direct effects of cold shock on three Columbia River organisms. These studies are part of a larger program involving two additional phases: (1) modeling of thermal declines following termination of heated discharges from steam electric stations, and (2) physiological changes occurring in fish exposed to sudden thermal alterations.

The studies are supported by the Energy Research and Development Administration (ERDA) through contract with Battelle Northwest.

From an ecological standpoint, aquatic species, particularly fishes, are vulnerable to sudden onset of cold. Mass mortalities in nature from temperature declines have been documented regularly from Florida and Texas, and occasionally from Georgia, North Carolina, and Mississippi.

In recent decades, expansion of the steam-electric industry has resulted in a substantial increase in the number of stations discharging heated water used in the cooling process. The water in these discharges are sometimes heated several degrees above ambient.
When a station ceases or reduces operation because of a system malfunction or scheduled maintenance, aquatic organisms living in the warmed area experience a rapid and potentially lethal temperature decline. The threat is very real, although relatively few cases resulting in mass mortality of fish populations from station shutdown have been well documented. However, attraction of fish to warmed areas during winter minimums and repulsion during summer maximums are remarkably common phenomena.

The possibility of lethal cold shock arising from man's interaction with his environment is cause for concern. In many cases, power station operations can be controlled so as to eliminate, or greatly reduce, the potential of cold shock mortalities of fish populations living in warmed discharge areas.

Therefore, studies on cold shock at PNL are based on the immediate need for predictive data on the ability of selected aquatic species, when acclimated to a given temperature, to resist both gradual and abrupt exposure to cold. At the present time, these studies are continuing.

Figure 1

Some basic features of the cold shock bioassays are listed in Figure 1.

There are two main phases to be discussed here. The first is bioassays involving gradual temperature decline. The second is bioassays involving abrupt temperature decline or instant cold shock.

Three test organisms have been used in initial phases of the study: The pumpkinseed sunfish—representing a warmwater fish, the rainbow trout—representing a coldwater fish, and the common crayfish of the Pacific Northwest—representing a decapod crustacean.
The general steps taken for the tests are in common with other bioassays. Namely: (1) collection of the species from field locations, (2) acclimation to specific temperatures at 5°C intervals, (3) conducting the test with 20 acclimated organisms per test, (4) establishing the main data base from observations on reaction of test organisms to cold shock, loss of equilibrium, and death, and (5) subsequent data analysis.

**Figure 2**

As indicated previously, two main phases of cold shock bioassays were involved: gradual and abrupt. The objectives differ slightly, and are stated in Figure 2.

For gradual temperature declines, the objective is:
- To determine the effect of various temperature decline rates on the resistance of acclimated organisms to cold.
- Resistance is measured by the point where, for a given species and acclimation level, declining temperatures result in 50% loss of equilibrium (LE) and death (D).

For abrupt temperature declines, the objective is:
- To determine the effect of abrupt cold shock on the ability of acclimated organisms to survive.
- Survival is measured by the point where, for a given species and acclimation level, abrupt exposure to cold results in 50% mortality within 4 days (96 hr) exposure.

**Figure 3**

Figure 3 is a color slide showing the experimental apparatus for conducting gradual and abrupt cold shock bioassays in actual use. Note the quiet, clustered pumpkinseed in the bottom of two glass test jars.
Figure 4

Figure 4 shows the rates of temperature declines used in studies on gradual cold shock. A decline rate of 18°C/hr is rapid, and nearly equivalent to abrupt cold shock. A decline rate of 1°C/hr is, relatively, very slow and gradual.

The decision on rates of temperature decline to use in these experiments was based, in part, on modeling of temperature decline rates. This was a separate phase of our studies.

Figure 5

Figure 5 is a systematic outline of the test sequence used in gradual cold shock bioassays.

The letters AT represent the "acclimation temperature" at which test organisms are held a minimum of two weeks before testing. Raising or lowering to the acclimation level is not more than 1°C per day. Organisms undergoing acclimation receive appropriate rations and are held in flowing water.

For conducting a typical test, 20 organisms were set in the water bath at the acclimation temperature. A carefully controlled and monitored temperature decline was then started, at a decline rate dictated by the desired test.

The time, and temperature, where half and all of the test organisms reach loss of equilibrium (LE) was recorded. The temperature was stabilized 2 hours at the 50% LE point. The temperature was then raised to a point midway between the acclimation temperature and the 50% LE point. The test organisms were then held 4 days to check immediate and delayed mortality.

Figure 6

Figure 6a shows the result of temperature decline rates on the resistance to cold of pumpkinseed sunfish, measured at the point of 50% LE.
The pumpkinseed, a warm water fish capable of being acclimated upward to 30°C, was the least resistant of the three test species to temperature decline. There are two important things to note in the figure.

First, the point where 50% LE occurs is dependent upon acclimation level. In general, the higher the acclimation level, the higher the 50% LE point. Conversely, the lower the acclimation level, the lower the 50% LE point.

Second, for a given acclimation level, the temperature of 50% LE is a function of the decline rate. There is apparently little adaptation at the most rapid rate of decline, 18°C/hr. But the next slower decline rate of 10°C/hr significantly lowers the 50% LE point. Successively slower decline rates also lower the 50% LE point, but to a lesser extent.

Figure 6b shows the results of temperature decline rates on the resistance to cold of rainbow trout, measured at the point of 50% LE.

The rainbow, a cold water fish, was more resistant to falling temperatures than the pumpkinseed. We were not successful in acclimating rainbows to 25°C. At 15°C acclimation, rainbows did not undergo LE until temperatures fell below 1°C.

Figure 6c shows the results of temperature decline rates on the resistance to cold of crayfish, measured at the point of 50% LE.

The crayfish, a decapod crustacean, was the most resistant of the three test species to falling temperatures. Healthy stocks of crayfish acclimated to 30°C were not obtained. However, at comparable 25°C acclimation temperature, the 50% LE point for crayfish was well below that of sunfish.

Stocks of crayfish acclimated at 15°C could survive rapid temperature declines ending below 0°C when ice formed in the test containers. In fact, most crayfish survived when frozen for two hours in cakes of ice just below 0°C.
Figure 7

Figure 7 is a systematic outline of the test sequence used in abrupt cold shock bioassays. It is generally similar to the sequence used in gradual cold shock tests, but the exposures from each acclimation level are abrupt.

The letters TT represent the "test temperature". The initial test temperature, to establish the resistant pattern, was selected on the basis of the 50% LE temperature in the gradual cold shock test. Subsequent tests were than above and/or below TT at 1°C intervals, as required.

For conducting a typical test, 20 organisms were plunged directly from acclimation to test temperature level. The temperature in the exposure chamber was automatically controlled and monitored at less than ± 0.2°C.

Times to 50 and 100% LE were recorded. Organisms were checked twice daily for mortality, for a maximum of 4 days exposure. At that time, temperature was allowed to rise to normal, and the survivors were observed an additional period for delayed mortality.

Figure 8

Figure 8 shows the result of abrupt thermal shock tests with pumpkinseed, rainbow and crayfish.

The plotted data points are the 4-day TL50, based on several separate tests from each acclimation level that range from the shock temperature where no mortality occurs in four days exposure, to where total mortality occurs within 24 hours.

The roughly diagonal lines for each species are not linear. Nevertheless, the space above each line represents the zone of survival where less than half the test organisms can be expected to die in 4 days exposure.
The order of resistance to abrupt cold shock is distinct. Pumpkinseed, the warm water species, are most susceptible. Rainbow, the cold water species, are less susceptible; at an acclimation 10°C, rainbow survive abrupt shock to levels slightly above freezing. Crayfish, the decapod crustacean, are most resistant; at an acclimation of 15°C, crayfish survive abrupt shock to the point just above freezing.

Figure 9

Figure 9 summarizes the salient points of our cold shock studies to date. These are fourfold:

1. Resistance to cold shock varies between species.
2. Resistance to cold shock is dependent on acclimation temperature.
3. Resistance to temperature declines is dependent on the decline rate.
4. Sever cold shock at a sublethal level is accompanied by disorientation, loss of equilibrium and immobilization.
5. Provided that the relationships between acclimation temperature and resistance to cold are known, power plant shutdowns can be controlled to avoid mortalities resulting from cold shock.
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   b. Rainbow Trout
   c. Crayfish
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8. Abrupt Cold Shock: Results
9. Conclusions
COLD SHOCK BIOASSAYS

Phases
- Gradual Temperature Decline
- Abrupt Temperature Shock

Test Organisms
- Pumpkinseed (*Lepomis gibbosus*)-Warm Water Fish
- Rainbow (*Salmo gairdneri*)-Cold Water Fish
- Crayfish (*Pacifastacus leniusculus*)-Decapod Crustacean

Steps
- Collection
- Acclimation at 5°C intervals
- Testing (20 organisms per test)
- Main Data Base
  
  Reaction of Test Organisms
  
  Loss of Equilibrium-LE
  
  Death-D
- Analysis
OBJECTIVES OF COLD SHOCK BIOASSAYS

GRADUAL TEMPERATURE DECLINE

- To determine the effect of various temperature decline rates on the resistance of acclimated organisms to cold.
- Measured by the point where, for a given species and acclimation level, declining temperatures result in 50% loss of equilibrium (LE) and death (D).

ABRUPT TEMPERATURE DECLINE

- To determine the effect of abrupt cold shock on the ability of acclimated organisms to survive.
- Measured by the point where, for a given species and acclimation level, abrupt exposure to cold results in 50% mortality within 4 days (96 hrs.).
Gradual cold shock: Rates of temperature decline
TESTING

GRADUAL COLD SHOCK: PROCEDURES

TEST ORGANISMS
(FIELD COLLECTIONS)

\[ \text{AT}_1 \]
\[ 15^\circ C \]
\[ \downarrow \]

\[ \text{AT}_2 \]
\[ 20^\circ C \]
\[ \downarrow \]

\[ \text{AT}_3 \]
\[ 25^\circ C \]
\[ \downarrow \]

\[ \text{AT}_4 \]
\[ 30^\circ C \]
\[ \downarrow \]

\[ 1^\circ \text{C/hr} \]
\[ \downarrow \]

\[ 3^\circ \text{C/hr} \]
\[ \downarrow \]

\[ 5^\circ \text{C/hr} \]
\[ \downarrow \]

\[ 10^\circ \text{C/hr} \]
\[ \downarrow \]

\[ 18^\circ \text{C/hr} \]
\[ \downarrow \]

\[ \text{a. Record Times To 50\% and 100\% LE.} \]
\[ \text{b. Hold 2 hr at 50\% LE point.} \]
\[ \text{c. Raise Temperature } \frac{1}{2} \text{way AT and 50\% LE.} \]
\[ \text{d. Hold 4 days; check immediate and delayed mortality.} \]

\[ \text{AT = Acclimation Temperature} \]
\[ \text{LE = Loss of Equilibrium} \]
PUMPKIN SEED
(Lepomis gibbosus)

RATE OF TEMPERATURE DECLINE
(°C/hr)

TEMPERATURE AT 50°C LIFE (°C)

30°C ACCLIMATIZATION

25°C

90°C

15°C

K6E 1977 TO THE CENTIMETER 48-510
IN A 350 KEUFEL & ESSER CO.
RAINBOW TROUT
(Salmo gairdneri)

0.5°C ACCLIMATION NOT POSSIBLE

TEMPERATURE AT 20% L. T. (%)

RATE OF TEMPERATURE DECLINE
(°C/hr)

18 10 5 3 1
CRAYFISH
(Pacifastacus leniusculus)

NO 30°C ACCLIMATION

35°C ACCLIMATION

RATE OF TEMPERATURE DECLINE
(°C/hr)
ABRUPT COLD SHOCK: TESTING PROCEDURES

TEST ORGANISMS
(FIELD COLLECTIONS)

AT1
15°C

AT2
20°C

AT3
25°C

AT4
30°C

Control
AT

TT+3°C

TT+1°C

TT+0°C

TT−1°C

As Needed

a. Record times to 50% and 100% LE.
b. Hold 4 days at test temperature.
c. Return temperature to normal.
d. Observe for delayed mortality.

AT = Acclimation Temperature
TT = Test Temperature
Figure 8

Abrupt shock: 4-day exposure

Zone of survival

Zone of mortality

Temperature, Temperature (°C)

Acclimation Temperature (°C)

Pumpkin seed
Rainbow
Crappie
CONCLUSIONS: COLD SHOCK STUDIES

1. Resistance to cold shock varies between species.
   - For our tests: pumpkinseed<rainbow<crayfish

2. Resistance to cold shock is dependent on acclimation temperature.
   - The higher the acclimation, the higher the lower lethal temperature.

3. Resistance to temperature declines is dependent on the decline rate.
   - There is little acclimation at a decline of 18°C/hr, but successively slower declines permit limited adaptation that lowers the 50% LE point.

4. Severe cold shock at a sublethal level is accompanied by disorientation, loss of equilibrium, and immobilization.
   - These responses are of considerable ecological importance.

5. Provided that the relationships between acclimation temperature and resistance to cold are known, power plant shutdowns can be controlled to avoid mortalities resulting from cold shock.