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# Changes in Immunological Parameters and Disease Resistance in Juvenile Coho Salmon (*Oncorhynchus kisutch*) in Response to Dehydroabietic Acid Exposure under Varying Thermal Conditions

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**This study explored the effects of a sublethal 96-h dehydroabietic acid (DHAA) exposure on aspects of the immune system of juvenile coho salmon under varying temperature conditions. Coho were exposed to DHAA concentrations below the determined LC50 value of 0.94 mg/L (95% confidence limits of 0.81 to 1.24 mg/L) for 96 h at either their acclimation temperature (8 or 18°C), or during an acute warm-shock (8 to 18°C) or cold-shock (18 to 8°C). Acclimation temperature alone significantly affected hematocrit (Hct), neutrophil respiratory burst activity (RBA) and leucocyte proportions. With temperature-shock, leucocrit (Lct), RBA and leucocyte proportions were altered. All parameters were affected by DHAA exposure, but not always in a dose-dependent manner. Across groups, DHAA caused Hct, lysozyme, thrombocyte, neutrophil and monocyte proportions to increase, and Lct, RBA and lymphocyte proportions to decrease. DHAA-temperature interactions resulted in the exacerbation of DHAA-induced effects. Exposure temperature had the most significant effect on the susceptibility of coho to *Aeromonas salmonicida*; fish were more susceptible at cold temperatures and when subjected to a temperature-shock compared to their respective controls. DHAA exposure modulated the response of temperature-shocked fish to this pathogen.**

*Key words:* **dehydroabietic acid, immune system, disease, fish, salmon, temperature**

## Introduction

In recent years, management and technological implementations have reduced toxicant loads entering the aquatic environment from industries such as pulp and paper production. However, some constituents of mill wastewater continue to pose a potential threat to fish populations inhabiting receiving waters (Rabergh et al. 1999). Although resin acids (RAs) comprise a group of chemicals that can be found naturally in aquatic systems from the degradation of wood, they also form a major component of wood industrial effluents (Oikari et al. 1985) and are considered to contribute the greatest toxic effects of mill effluent to fish (Leach and Thakore 1977). The major consequence of RA exposure in fish appears to be a disruption of ionoregulatory and circulatory mechanisms (Kruzynski 1979; Mattsoff and Oikari 1987; Bogdanova and Nikinmaa 1998; Rabergh et al. 1999), but recent research has shown that RAs can affect stress biochemistry, resistance to disease (Kennedy et al. 1995) as well as the endocrine system (Karels et al. 1998).

Of the 10 major resin acids, dehydroabietic acid (DHAA) typically comprises 5 to 10% of the total RAs in raw pulp (Oikari et al. 1985; Mattsoff and Oikari 1987), and is considered one of the most toxic and persistent (Brownlee and Strachan 1977; Fox 1977; Oikari et al. 1983). The acute DHAA 96-h LC50 for salmonids ranges from 0.8 to 2.1 mg/L (Davis and Hoos 1975; Oikari et al. 1983). The sublethal effects of DHAA on the physiology of salmonids have been documented in numerous studies (Nikinmaa and Oikari 1982; Oikari et al. 1983; Bushnell et al. 1985) and include effects on the immune system. For example, Iwama et al. (1976) found a significant decrease in white blood cell numbers in coho salmon exposed to 0.75 mg/L DHAA for 24 h. Other studies have reported that exposure to mill effluent affected white blood cell counts (McLeay 1973; Andersson et al. 1988), an effect which may be attributable to RAs.

Schrier et al. (1991) found significant residues of pulp and paper effluent constituents in fish in the lower Fraser River, indicating that pulp mills can have potential impacts hundreds of kilometres downstream. Maturing and migrating juvenile salmonids in these situations can face innumerable chemical and physical conditions that may impact their survival. Anthropogenic challenges, such as RA exposure, can occur simultaneously with natural fluctuations in other environmental conditions. One of the most important water quality factors that can have a significant impact on the responses of fish to toxicants is water temperature. Understanding the modulating effects of temperature on the toxic effects of

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pollutants to salmonids is important for assessing and reducing the risk to this ecologically and economically important species.

The evolution of present salmonid life history has necessitated adaptation to conditions of varying water temperature. For example, in the 12 to 14 months that juvenile coho remain in freshwater, they may encounter dramatic temperature changes (e.g., 4 to 17.5°C; McLeay 1975). Physiological and biochemical adjustments allow for an expanded zone of thermal tolerance in fish through a variety of mechanisms (Hochachka and Somero 1984). Differences in physiological and biochemical systems across temperatures are also important in chemical fate and toxicity. For example, at higher temperatures, gill ventilation rate or volume in fish can increase, leading to elevated toxicant exposure (Kennedy et al. 1989a). However, since the biotransformation rates of some chemicals can increase with temperature, increased uptake may be offset by higher rates of detoxification and elimination (Kennedy et al. 1989b).

This study explores the effects of acclimation temperature and acute temperature change on the sublethal toxicity of DHAA to coho salmon (*Oncorhynchus kisutch*), which are a challenged salmonid species (Allendorf et al. 1997). Since DHAA has been implicated in affecting immunocompetence in fish, immunological parameters and disease resistance were examined after DHAA exposure under different thermal regimes. Specifically, comparisons were made utilizing cold (8°C) acclimated fish and fish acclimated to a temperature approaching a natural upper limit (18°C) (McLeay 1975).

## Materials and Methods

### Fish

Coho salmon were raised at Simon Fraser University from fertilized eggs obtained from the Department of Fisheries and Oceans Capilano Hatchery (North Vancouver, B.C.) and were approximately 10 months of age (weight 8.65 ± 0.28 g, fork length 9.1 ± 0.10 cm, condition factor 1.07 ± 0.0066) when used. Fish were held in 200-L fiberglass tanks supplied with filtered, dechlorinated municipal tap water of pH 6.7, $O_2$ saturation >95%, hardness 6.2 mg/L $CaCO_3$, at either 8 or 18 ± 1°C, and a flow rate of 6 L/min. Fish were brought from their initial acclimation temperature (~11°C) to their respective acclimation temperatures at a rate not exceeding 1°C/day, and held at their acclimation temperatures for two weeks before experimentation. Fish used in LC50 determinations were acclimated to an intermediate temperature of 13 ± 1°C. Fish were held under a 12-h light: 12-h dark (30-min dawn and dusk) photoperiod. Fish were fed commercial salmon pellets (Moore-Clark Co., La Connor, Wash.) *ad libitum* until 24 h prior to experimentation.

### Chemicals

Dehydroabietic acid (DHAA, 99% purity) was purchased from Helix Biotech Corp. (Richmond, B.C.). DHAA was dissolved in 4 mL of 100% ethanol and 4 mL of 1 N NaOH and then added to 4 L of distilled water as a stock solution to a final concentration of 326 mg/L. The exposure system used was a computer-controlled, intermittent-flow respirometer as described in Johansen and Geen (1990). For both the LC50 determination and acute sublethal studies, appropriate DHAA concentrations were maintained in the vessels by computer control of delivery pump timing (Kennedy et al. 1995). All other biochemicals were purchased from Sigma (St. Louis, Mo.).

### LC50 Determination

The 96-h LC50 for DHAA was determined as in Kennedy et al. (1995). Fish (n = 11 for each concentration) were allowed to acclimate to test conditions for 24 h before experimentation. Fish were exposed to a linear series of nominal concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 mg/L) since preliminary studies indicated that most mortality occurred over this narrow concentration range. Results from two experiments were pooled and analyzed using nonlinear interpolation as described by Stephan (1977).

### Temperature and Sublethal DHAA Exposure Regimes

Fish from either temperature acclimation group were placed into sixteen 65-L glass aquaria for at least 24 h prior to the start of an experiment (n = 10 per aquarium; loading density 0.13 g/L). Fish were exposed to DHAA concentrations below the determined LC50 value (0.0, 0.2, 0.4 and 0.8 mg/L, nominal) in the flow-through system at either their acclimation temperature or during an acute temperature-shock (warm-shock 8 to 18°C; cold-shock 18 to 8°C) for 96 h. Each hour the appropriate amount of stock solution and freshwater was delivered to each aquarium, replacing 175 L daily and maintaining the desired concentration. All exposure vessels were submersed in constant-temperature water baths to maintain appropriate temperatures ±1°C.

### Sampling and Analysis

Fish were anaesthetized with tricaine methanesulfonate (Syndel Laboratories Ltd., Vancouver, B.C.), and their length and weight were measured. Blood was collected from the caudal vasculature for hematocrit (Hct) and leucocrit (Lct) measures, as well as differential white blood cell counts. Blood was taken directly into 40-µL heparinized tubes (VWR Canlab, Mississauga, Ont.), which were then centrifuged for 3 min at 10,000 g.

Blood films were air-dried and stained with modified Wright-Giemsa (Sigma, St. Louis, Mo.). The relative leucocyte proportions were estimated by counting approximately 100 white cells for each fish sampled, with cells identified according to the guidelines of Ellis (1977). The plasma portion of the blood collected into capillary tubes was separated and stored at -20°C until further analysis for lysozyme activity. The head kidney was aseptically removed and placed in cell culture medium (Hank's Balanced Salt Solution, HBSS) on ice in a sterilized centrifugation tube.

The number of NBT-positive neutrophils present in the head kidney was measured using the nitro-blue tetrazolium (NBT) method described in Jeney and Anderson (1993). Head kidneys were homogenized by mastication using the plunger of a syringe and aliquots of HBSS/kidney homogenate (60 µL) were placed into welled microscope slides. After a 30 min incubation in a sealed container, the slides were rinsed with phosphate buffered saline and 50 µL of 0.2% NBT solution was added to the wells. The slides were left to incubate for another 30 min, after which counts of activated (stained) versus non-activated (unstained) cells were made under 500x magnification.

The lysoplate assay described in Yousif et al. (1991) was used to measure plasma lysozyme levels. Lysoagar plates were prepared using a suspension of Micrococcus lysodeikticus in a 0.5% agarose mixture containing 0.06 M phosphate buffer (pH 6.0) and 0.02% NaCl in 15-cm petri dishes. Three-millimetre holes were punched into the hardened agarose (approximately 6 holes/dish). Plasma samples were mixed 1:1 with 0.85% saline solution on Parafilm (American National Can, Neenah, Wis.). Aliquots (15 µL) were placed into the wells in the lysoagar plates. Standards were prepared from hen egg-white lysozyme (Sigma, St. Louis, Mo.). After a 20-h incubation period at room temperature, the diameters of the clearance zones were measured using precision digital calipers.

### Disease Challenge

The pathogen used in the disease challenge experiments was *Aeromonas salmonicida*, the bacterium responsible for the highly infectious disease furunculosis. An *A. salmonicida* liquid culture was prepared and administered as described in Kennedy et al. (1995). A preliminary range-finding experiment determined that the most effective bacterium concentration for disease-challenge experiments was 20,000 cells/mL.

Fish acclimated to either 8 or 18°C were randomly selected, fin clipped, and separated into eight groups (four temperature treatments, DHAA-exposed and non-exposed groups; n = 25). Fish in each group were exposed to a continuous DHAA concentration of 0.4 mg/L for 96 h since many of the immune parameters measured in initial experiments were most significantly altered at this concentration. Fish were then exposed to *A. salmonicida* and collectively placed in one of two 200-L holding tanks to a density of approximately 3 to 4 g/L. The tanks were supplied with fresh-flowing water at a mid-range temperature of 13 ± 1°C. During the course of the challenge fish were fed *ad libitum* three times a week. Mortalities were monitored daily and identified by fin clip. Furunculosis was considered the cause of death if short, Gram-negative, rod-shaped bacteria were evident in Gram-stained kidney smears, if pure cultures of a pigment-producing bacteria resembling the above bacteria were isolated onto TSA agar from kidney samples, and if clinical signs of the mortalities were consistent with those described for *A. salmonicida* infections (Bullock et al. 1983). All challenge experiments were performed in triplicate. Preceding the disease challenge experiments, unexposed fish from a stock tank were examined for the presence of the bacterium.

### Statistics

All percentage data were arcsine transformed prior to statistical analysis. Two-way analysis of variance (ANOVA) (temperatures and toxicant concentration) followed by Student-Neuman-Keuls (SNK) ($p \leq 0.05$) was used to detect differences and interactions among groups. Groups were analyzed in this fashion: cold versus warm acclimation (8°C versus 18°C), cold acclimation versus warm-shock (8°C versus 8 to 18°C), and warm acclimation versus cold-shock (18°C versus 18 to 8°C). Finally, to compare whether shocked groups resembled those acclimated to shock temperature, warm-shock was compared to warm acclimation, and cold-shock to cold acclimation. For disease challenge experiments, replicates were pooled and times to 50% mortality were compared. Mortality times were interpolated using a three-parameter non-linear sigmoidal curve fit (SigmaPlot 2001, SPSS Scientific, Chicago, Ill.).

## Results

### 96-Hour LC50 Determination

The 96-h LC50 value for coho salmon exposed to DHAA under flow-through conditions was determined to be 0.94 mg/L, with upper and lower 95% confidence limits of 1.24 and 0.81 mg/L, respectively.

### Temperature Effects

Changes in juvenile coho hematological and immunological parameters are shown in Fig. 1 and 2. In some cases, the temperature regime alone had significant effects on many of the measured parameters. Comparisons between fish acclimated to either cold or warm temperatures revealed differences including lower Hct (Fig. 1A), higher respiratory burst activity (RBA) (Fig. 1J), and a

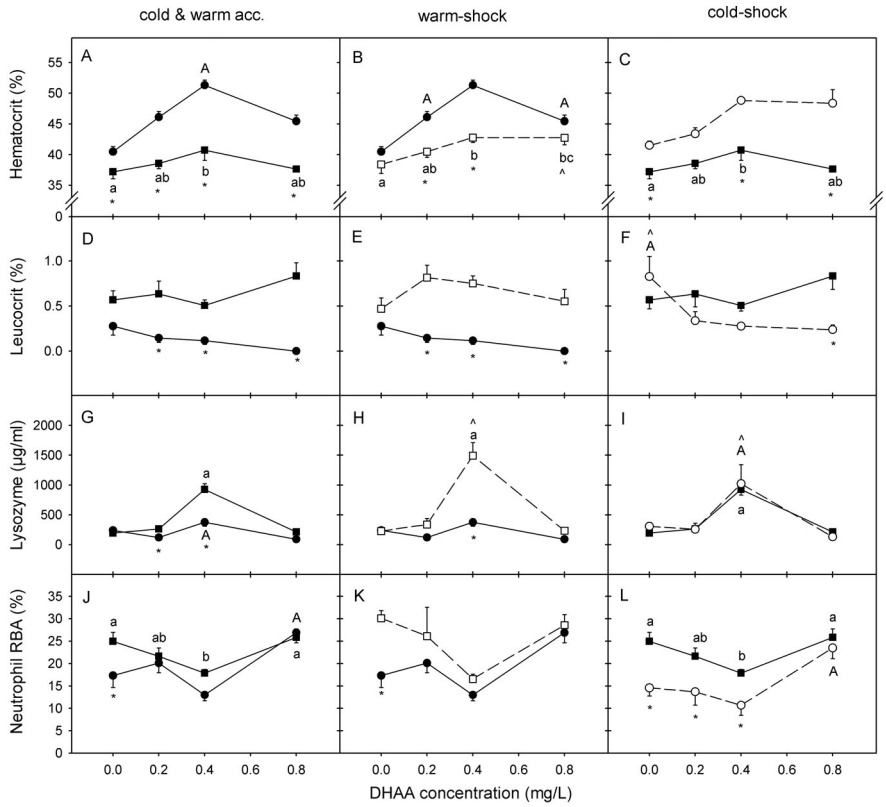


**Fig. 1.** Hct, Lct, RBA and plasma lysozyme concentration in warm (■) and cold (●) acclimated juvenile coho salmon exposed to temperature-shock (open symbols) and DHAA (values are mean ± SEM). Within temperature groups (lines), values with dissimilar alphabetical scripts are significantly different and denote DHAA effects; significant differences across temperature groups at each dose are denoted with *; significant differences between shock groups and groups acclimated to shock temperature (e.g., 18 to 8°C shock and 8°C acclimated) are denoted with hat. Significance at $p \leq 0.05$.

significantly lower proportion of monocytes (Fig. 2J) in warm acclimated fish.

RBA increased with warm-shock (Fig. 1K) to the level found in warm acclimated fish. Conversely, RBA was reduced with cold-shock (Fig. 1L), also to levels similar to cold acclimated fish. Hct showed similar trends (Fig 1A, B, C). With cold-shock, Lct, lymphocytes and thrombocyte proportions were altered (Fig. 1F, 2C, 2F).

## Sublethal DHAA Effects

The effects of DHAA on the measured parameters when fish were exposed at their acclimation temperatures were varied. In cold acclimated fish, DHAA affected Hct (Fig. 1A), lysozyme (Fig. 1G) and RBA (Fig. 1J), although not in a dose-dependent fashion. Comparable results were seen in warm acclimated fish (Fig. 1A, G, J). DHAA significantly modulated white blood cell proportions in fish acclimated to either cold or warm temperatures, however, more pronounced effects were seen in cold acclimated fish (Fig. 2A, D, G, J).

The combination of a temperature-shock and DHAA exposure resulted in significant effects on the measured parameters. Warm-shock significantly modified the impact of DHAA on Hct and lysozyme concentrations, but not Lct or RBA (Fig 1B, E, H, K), but again, no dose-dependent relationship was evident. Warm-shock also modulated the effects of DHAA on lymphocyte, neutrophil and to a lesser extent, monocyte proportions (Fig. 2B, E, H, K).

Unlike warm-shock, cold-shock only modulated the effects of DHAA on Lct (Fig. 1F). Cold-shock and DHAA tended to have a greater impact on leucocyte proportions than warm-shock; effects on lymphocytes, thrombocytes, monocytes and to a lesser extent neutrophil proportions, were modulated significantly by cold-shock (Fig. 2C, F, I, L).

## Disease Challenge

Both temperature and DHAA exposure affected the susceptibility of coho to the bacterium *A. salmonicida* (Fig. 3). The highest total mortalities were in cold acclimated fish whether unexposed or exposed to DHAA. Both groups reached 50% mortality at similar times (4.5 d control, 3.8 d exposed). In contrast, warm acclimated control and exposed groups never reached 50% mortality and had the lowest proportional mortalities by the end of the experimental period (39% control, 48% exposed). In acclimated groups, DHAA exposure did not cause a notable divergence in mortality. In contrast, DHAA affected total mortality in temperature-shock groups (Fig. 3). With warm-shock, DHAA exposure reduced the proportionate mortality (77 versus 61% control), and time to 50% mortality (6.7 versus 7.9 d control). The opposite was true of cold-shock and DHAA exposure as DHAA-exposed fish had a higher proportionate mortality (86 versus 62% control) and reached 50% mortality earlier (6.1 versus 7.9 d control).

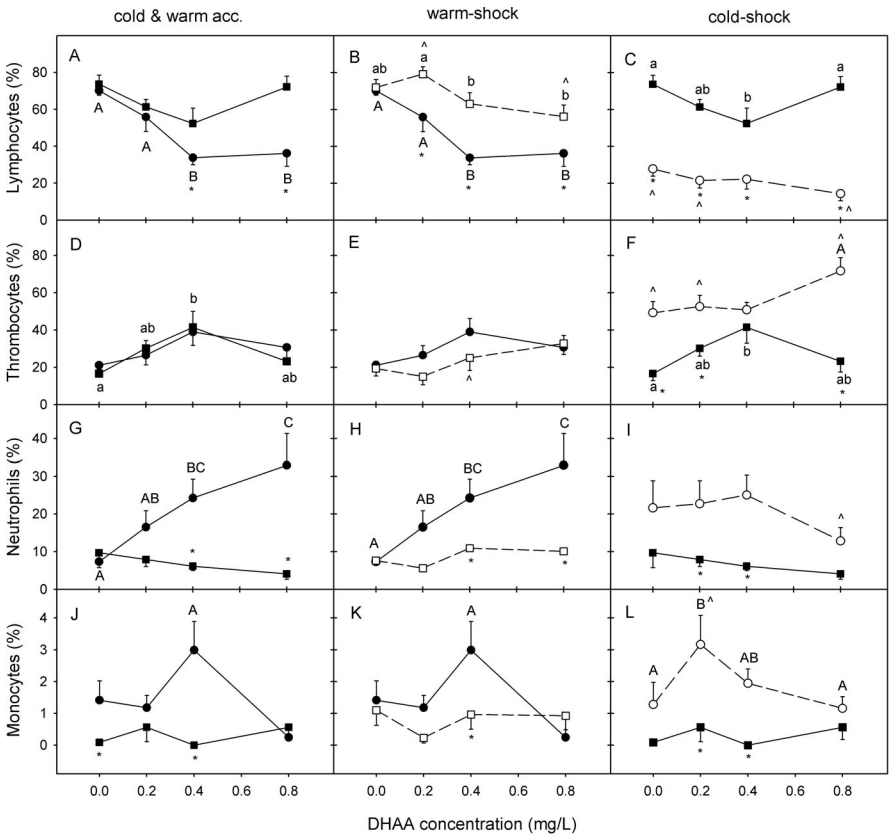


**Fig. 2.** The relative white blood cell composition of warm (■) and cold (●) acclimated juvenile coho salmon exposed to temperature-shock (open symbols) and DHAA (values are mean ± SEM). Within temperature groups (lines), values with dissimilar alphabetical scripts are significantly different and denote DHAA effects; significant differences across temperature groups at each dose are denoted with *; significant differences between shock groups and groups acclimated to shock temperature (e.g., 18 to 8°C shock and 8°C acclimated) are denoted with hat. Significance at $p \leq 0.05$.

## Discussion

In this study, a suite of selected hematological and immunological responses were used as biological indicators of acute sublethal exposure of coho salmon to dehydroabietic acid (DHAA). In addition, a disease challenge was used to determine the effects of this compound on the functioning of the immune system under various thermal regimes in order to place the results in an ecological perspective.

Most control variables (either warm or cold acclimated) were well within normal ranges for fish reported by others. Additionally, few parameters differed between warm and cold acclimated groups, likely due to the fact that acclimation serves to retain functionality across a zone of tolerance (Hochachka and Somero 1984). The lower Hct in warm acclimated fish in this study was surprising as blood oxygen carrying capacity is often increased to accommodate heightened metabolic demands caused by temperature increases. For example, Houston and Koss (1984) reported that Hct in trout was ~18% higher at 17°C than at 10°C. Neutrophil RBA, which helps kill bacteria and other pathogens by denaturing vital macromolecules (Gabig and Babior 1981), was also different between fish acclimated to different temperatures, results similar to those of other researchers. An acute temperature decrease caused a reduction in neutrophil activity of channel catfish (Ainsworth et al. 1991) and a dramatic drop in channel catfish granulocyte chemiluminescent (CL) activity (90–95%) (Dexiang and Ainsworth 1991).

In the present study, temperature-shock affected several of the measured parameters including Lct, RBA and leucocyte proportions. Lct in salmonids can be affected by stressors such as temperature change (McLeay and Gordon 1977; Wedemeyer et al. 1983). For example, warm- and cold-shock both reduced rainbow trout Lct (Wedemeyer et al. 1983). In this study, cold-shock increased Lct by approximately 45%. The fish in this study had low initial Lct, and cold-shock increased it to a value comparable to those in equally sized coho (McLeay and Gordon 1977).

RBA was also affected by temperature-shock as has been reported in the literature. Ainsworth et al. (1991) hypothesized that the enzymes required for RBA were reduced in quantity or efficiency by the lowered metabolism associated with temperature-shock. A study lending support to an enzyme efficiency change reported that CL activity in sea bass (*Dicentrarchus labrax*) head kidney leucocytes from 15°C acclimated fish incubated at 5°C was approximately 25% that of cells incubated at 20°C (Angelidis et al. 1988). In contrast with our findings, Sohnle and Chusid (1983) reported increased neutrophil activity with decreased temperature in rainbow trout.

In this and other studies, coho leucocyte proportions were also affected by temperature. McLeay (1975) found that the proportion of small lymphocytes was positively correlated with temperature. In the present study, accli-

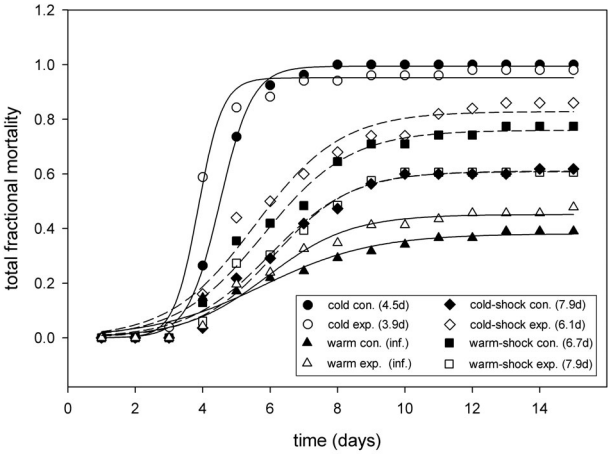


**Fig. 3.** Mortality curves for *Aeromonas salmonicida* challenged juvenile coho salmon under different temperature conditions and DHAA exposures. Three-parameter sigmoidal curves were fitted to the data and 50% mortality times were interpolated (given in brackets in symbol legend). Broken lines are used to show temperature shock groups.

mation temperature did not affect leucocyte proportions, however, they were altered by cold-shock.

DHAA affected many of the measured parameters, but a strict dose-response relationship was not evident. Moreover, both acclimation temperature and temperature-shock modified the response of fish to DHAA exposure, but not always in a consistent manner. Interestingly, maximum DHAA-induced effects generally occurred at 0.4 mg/L, the mid-range concentration used. It is possible that exposure to higher concentrations of DHAA initiated compensatory or protective responses.

Pulp mill effluent or its individual constituents are known to alter salmonid hematocrit, although the direction of change is not consistent. Increases (Oikari et al. 1983; Kennedy et al. 1995), decreases (McLeay 1973), and no changes (Iwama et al. 1976) for various salmonids have all been reported. Temperature-DHAA concentration interactions appear to determine effects on Hct. Warmer temperatures appear to mitigate the effects of high concentrations of DHAA on Hct, results similar to those of Iwama et al. (1976). Higher concentrations of DHAA increase Hct at lower temperatures (Oikari et al. 1983), results that agree with the present study.

The multiple effects of DHAA on Hct may be due to several mechanisms operating under different conditions, since the cardiorespiratory system and in particular, the erythrocyte, appear to be a major site of toxic action. DHAA is known to cause gill swelling (Tuurala and Soivio 1979), resulting in reduced oxygen uptake. This may be compensated for with splenic release of erythrocytes or increased erythrocyte production. Increases in red cell volume are not likely since Oikari et al. (1983) reported that increased Hct with DHAA was not due to an increase in red blood cell (RBC) volume. At high DHAA concentrations, RBC hemolysis can occur (Bushnell et al. 1985). It is possible that at DHAA concentrations approaching the LC50, RBCs are lysing and thus reducing Hct, possibly by decreases in erythrocyte pH (Bogdanova and Nikinmaa 1998). Similarly, McLeay (1973) noted that the decreased hematocrit in coho exposed to pulp mill effluent (PME) may be due to increased erythrocyte destruction, since an increased number of immature erythrocytes were found in circulation, indicating a possible increase in RBC death rate.

Several studies have reported decreases in Lct or total white blood cell counts with exposure to pulp mill effluent or resin acids (McLeay 1973; Iwama et al. 1976; McLeay and Gordon 1977; Andersson et al. 1988; Kennedy et al. 1995). The Lct of rainbow trout exposed to 0.82 mg/L of chlorinated DHAA was decreased approximately 30% (Kennedy et al. 1995). In this study, DHAA caused an Lct decrease of almost 60%, but only in concert with cold-shock. The significance of any alteration in Lct in fish is unknown, but potentially, a reduction may compromise disease resistance. In disease challenge tests, DHAA exposure reduced survival only with cold-shock, where Lct was significantly reduced. Conversely, DHAA increased Lct in warm-shock, which may in part explain the increase in survival of DHAA-exposed warm-shock fish in the challenge tests. Further research is warranted with respect to the relationship between Lct, temperature, and disease resistance to *A. salmonicida*.

Lysozyme functions as a nonspecific antibacterial agent and is thought to be produced by neutrophils (Ingram 1980). Mid-range DHAA concentrations caused increases in lysozyme across all temperatures; however, the effect was more pronounced with temperature-shock. Several explanations are likely; including elevated lysozyme production or blood plasma concentration. Muona and Soivio (1992) hypothesized that a twelve-fold increase in lysozyme activity in sea trout (*Salmo trutta* L.) may have been caused by increased numbers of circulating neutrophils. Oikari et al. (1983) found that the protein concentration of plasma was 44% higher in fish exposed to DHAA. It was concluded that DHAA caused an efflux of water from the circulatory system, thus concentrating plasma and possibly explaining the increased lysozyme titers found with DHAA exposure. The relationship between lysozyme levels and disease resistance to *A. salmonicida* is not clear, as DHAA increased lysozyme in all temperature groups, yet fish exposed at cold temperatures suffered higher mortalities than fish at warmer temperatures. Accordingly, either the effectiveness of lysozyme at cold temperatures was limited or lysozyme did not influence survivorship in the case of this specific pathogen.

The proportion of head kidney NBT-positive cells decreased at mid-range DHAA concentrations, but as temperature-shock did not significantly alter proportions from acclimated levels, DHAA-caused differences in RBA did not likely account for changes in *A. salmonicida* survivorship.

White blood cell proportion is known to change with exposure to RAs. McLeay (1973) found that neutrophil number increased threefold and small lymphocyte number decreased with 25-d PME exposure. In the present experiments, similar results were found: neutrophil proportion increased dramatically with DHAA exposure (7% in controls to 35% at high-dose in cold acclimated fish), and lymphocyte proportion decreased (78% in controls to 32% at high-dose in cold acclimated fish). However, while DHAA caused changes in lymphocyte and thrombocyte proportions irrespective of temperature treatment, neutrophilia only occurred with low temperature (both acclimated and shocked). The implications remain unclear, however as proportions were similar in both cold-shock and cold acclimated fish, differences in neutrophil proportion likely do not account for the differences observed in disease challenge survivorship.

Teleost immunocompetence is affected by temperature; in particular, low temperature can be immunosuppressive (Ainsworth et al. 1991). In the present study, warm acclimated fish survived an *A. salmonicida* challenge better than cold acclimated fish. In acclimated groups, survivorship was not affected by DHAA exposure, however, DHAA exposure reduced mortality with warm-shock and increased it with cold-shock. The mechanisms by which DHAA modified the susceptibility of coho to disease in this contradictory manner is unclear, however, it is possible that exposure to toxicants like DHAA can have both stimulatory and inhibitory effects on the immune system, depending on the dose received by the animal, environmental conditions and chemical fate. For example, Kennedy et al. (1995) found that *A. salmonicida* mortality in similar sized juvenile rainbow trout was significantly higher with lower concentrations of chlorinated DCAAs compared to higher concentrations.

It is uncertain whether the DHAA-mediated differences found in *A. salmonicida* survivorship with temperature-shock may impact coho in situ. For example, fish may simply avoid any rapid temperature gradients caused by heated effluent release. However, based on our findings it is reasonable to speculate that juvenile coho moving through heated mill effluents may experience transient immune system modulation.

## Conclusions

The results of this study indicate that DHAA can affect aspects of the salmonid immune system, and can modulate the susceptibility of fish to disease agents. However, the utilization of a much larger suite of immunological biomarkers is necessary to begin to identify the mechanisms behind the immunomodulatory effects of chemicals such as RAs due to the complexity of the immunological defense system. The interplay between immune system components makes it difficult to predict immunotoxicological outcomes, particularly when these interactions are overlayed with dominating environmental factors such as water temperature which can themselves significantly affect these physiological systems. Future research on the toxicological effects of RAs should continue to focus on identifying mechanisms of action for cause-effect relationships in conjunction with ecologically relevant research that includes factors such as environmental modulators.

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