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DEPARTMENT OF
WATER RESOURCES

REBUTTAL

Expert Report

In Matter of Distribution of Water to Rangen, Inc's Water Right Nos. 36-02551 and 36-07694

Prepared for Rangen, Inc

by

Charlie E. Smith

REBUTTAL

In December, 2012 I received and reviewed expert reports of Gregory Sullivan, John S. Church & Tom Rogers. The following rebuttals to these reports are as follows:

Water quality used by the Rangen Research Hatchery has an optimum temperature for growth of rainbow trout of 59 – 60 degrees F, a pH of 7.8 – 8.1 & hardness of 130 ppm as calcium carbonate and, is saturated with dissolved oxygen, which is typical for most of the hatcheries along the Northern rim of the Snake River in the Thousand Springs area. Along with the large volume of water these attributes provide the optimum environmental conditions for maximal rainbow trout production using flow-through fish culture management. Pristine, first use spring water contains the maximum possible useful dissolved oxygen so it is the preferred water source

Gravity flow is much more desirable than pumped water or reused water. Pumping water is costly and reused water is subject to unpredictable failure due to loss of power. Hence, there is increased uncertainty and risk to the water supply. Backup systems are necessary. Recirculating reused water provides water with a low dissolved oxygen content which must be aerated by some means, and removal of waste products before being reused. On some occasions ground water and pumped water may have reduced oxygen and/or increased nitrogen which results in reduced growth and gas bubble disease. Recirculation of waste water (recycle) may significantly increase fish morbidity and mortality.

Rogers talks about certain hatcheries in Idaho (see list Table 2.4 p. 18 of report) using pumped water and recycled water. However, all are either federal or state hatcheries who receive monies to operate – they don't have to make a profit to operate, but are guaranteed funding to build, operate and maintain these hatcheries. Private growers in the Twin Falls, Buhl & Hageman area who raise trout for processing need to make a profit, and most of the time profits margins are low for processed fish. While pumped water is used in some public, conservation hatcheries, or in some small scale commercial farms it is too unreliable for large commercial ventures.

Recirculation hatcheries, which clean (remove waste products), aerate and reuse water often operate on 90% reuse, 10% new water and 10% waste water and use the process of biological nitrification for ammonia removal. They are, however, subject to catastrophic losses of fish be they due to failure of pumping systems, backup systems, nitrite toxicity due to failure or imbalance in the nitrification process, and/or disease outbreaks where pathogens are constantly being recirculated and mortality is extremely high until the disease is brought under control and the bacterial nitrification system is brought back in equilibrium. The potential for catastrophic losses due to Infectious hematopoietic necrosis (IHN) virus disease and bacterial cold water disease in hatcheries using water reuse systems is great. This is especially true of IHN in the Hagerman, Thousand Springs area where infectious hematopoietic necrosis (IHN) virus disease is endemic and occasionally becomes epidemic.

I have also read and reviewed an expert report submitted by John R. Macmillan in a previous proceeding. A copy of that report is attached hereto as Exhibit 4. Dr. Macmillan's report elaborates on many of the points I have raised above and I am in general agreement with what his report says.

Tom Rogers' report appears to include some minor mathematical errors, including the following:

- Expert Report of Tom Rogers, at p. 24: Under Small Raceways at bottom for Flow Index Calculation, 0.3 Flow Index Calculation Tom uses 6508 for water flow, but the number is really cubic feet. Flow should be 4,888. Weight would be 6,785 lbs rather than 9,840 lbs

- Expert Report of Tom Rogers, at p. 25: Large Raceways left column. Flow = 9,645 GPM or 12.5 CFS. 12,5 CFS = 5610 GPM not 9,645.CTR Raceways Right Column says Flow = 12.5 CFS or 9,649 GPM when it should say 5,610 GPM. He uses 9649 in all his calculations for Flow Index calculation at bottom. Values for 0.5, 0.8 & 1.5 FI should be 28,050 lbs, 45,880 lbs & 84,150 lbs, respectively, not what he has listed (48,245, 77,192 & 144,735, respectively).

Rangen uses its hatchery for reasons different than other hatcheries in the area which are mostly used for production of large fish for slaughter. These include raising fish for planting in reservoirs and rivers such as for Idaho Power Company, raising fish for research projects both in house and contract as research studies, as well as raising production fish. Different Flow Indices and Density Indices are appropriate for different purposes. While it is true that fish can be raised with different flow indices and density indices, the flow indices and density indices used by Rangen for each of the purposes are reasonable. Rangen is currently beneficially using all available water and is not wasting water. After reviewing the above reports it doesn't change my opinion that the Rangen Research Hatchery could use more water to raise fish if it was available.

Prepared and Submitted by Charlie E. Smith

Charlie E. Smith

Date 2/8/13

EXPERT REPORT OF
JOHN R. MACMILLAN, PH.D.

I. QUALIFICATIONS AND BACKGROUND

1. I make this statement as an employee of Clear Springs Foods, Inc. in which I serve as Vice President of Research and Environmental Affairs. I am an expert in aquaculture science, fish pathology and health management, minor animal species drug approval, environmental regulation, seafood quality assurance, and aquaculture public policy. I hold Bachelor of Science (1973, University of Maryland), Master of Science (1976, Michigan State University), and Doctor of Philosophy (1980, University of Washington) degrees. I was a Senior Research Fellow at the Medical School, University of Washington (1980 -1982) conducting research in radiobiology and developing models of cellular senescence. A list of my professional memberships, professional activities, research activities, publications and other scholarly activities, offices held in professional and scientific organizations and service positions, honors and awards, and civic and community activities is contained in a copy of my most recent curriculum vitae, which is attached hereto as Exhibit "1."

2. My experience related to aquaculture, fish health management, water quality management, physiologic requirements of fish, and seafood quality assurance spans over 28 years. I am currently Vice President of Research and Environmental Affairs at Clear Springs Foods. Clear Springs Foods was founded in 1966 and I joined the company as Director of Research and Development in 1990. As Research Director and Vice President of Research I direct a staff conducting research and development on a variety of issues related to aquaculture of rainbow trout including their environmental requirements, fish health management (vaccine and stress responses), fish culture, brood stock improvement, and environmental science. In 1991 I conducted an evaluation of the water quality in the Snake River. Prior to accepting a position with Clear Springs Foods I was an Associate Professor of Veterinary and Aquatic Animal Medicine at Mississippi State University, College of Veterinary Medicine. During that time I conducted fish disease research including physiologic responses of fish to various stressors, pathogens and

toxins. I also taught veterinary students and served as major professor for both Master's Degree and Doctor of Philosophy degree students.

3. From 1982 until 1985 I served as an Area Extension Fisheries Specialist for Mississippi State University. In this position I primarily provided fish disease diagnostic and fish health management services to the Mississippi catfish industry. I conducted research on various fish diseases including impact of various environmental conditions on fish production capacity and fish survival.

4. I served as a Senior Research Fellow in the Department of Pathology, School of Medicine at the University of Washington from 1980 until 1982. I conducted basic cell biology and pathology research looking at potential mechanisms of cellular senescence. Cellular kinetics and modeling were my primary research focus using bone marrow transplant and radiobiological techniques.

5. I currently serve on the Idaho Board of Environmental Quality. I have served on this board since its inception in 2000. From 2004-2006 I served as the Board Chairman. I was initially appointed to the Board by Governor Dirk Kempthorne who reappointed me in 2003. I was recently re-appointed by Governor Butch Otter (2007) with term expiration in 2011. The Board of Environmental Quality has decision making responsibilities over proposed environmental rules and has quasi-judicial responsibilities during contested cases in which parties object to departmental decisions.

II. Purpose of Report

The North Snake and Magic Valley Ground Water Districts propose to mitigate for their injury to Clear Springs Foods Snake River Farm complex based on the 2005 water delivery order, by:

1. Pumping Snake River Farm effluent waste water or Clear Lake water into the Snake River Farm complex head ditch.

2. Pumping spring water from their Spring No. 1, or Spring 1 and 2, to the Snake River Farm complex head ditch.

3. Drilling a well at about the location of their Spring No. 1 down to a depth of 200 feet and pumping water to the Snake River Farm complex head ditch.

What seems clear from these mitigation proposals is that the parties have little understanding about rainbow trout, their sensitivity to stress, basic fish health management and the science of intensive, flow-through aquaculture. Yet informed decision as to the suitability and utility of these mitigation water's *in lieu* of gravity fed spring water from existing springs requires just such understanding of fish biology including physiological impacts of various factors constituting water quality and the impact of fish pathogens, chemicals and drugs and interrupted water supply on intensively reared fish. It also requires knowledge about the farming of rainbow trout in flowing water systems. This report describes the general practice of flowing water fish culture and provides analysis of impacts from each water quality factor individually and collectively on fish production capacity. Peer reviewed scientific literature provides the basis for this analysis.

Opinions expressed in this matter

- Rainbow trout are complex, physiologically integrated vertebrate aquatic animals.
- Commercially reared rainbow trout are farmed intensively and fed a nutrient dense, high energy diet.
- Rainbow trout are farmed in flow-through, serial reuse systems.
- Water quality suitable for intensive rainbow trout husbandry is diminished with each water use necessitating progressive change in husbandry with each use. The usefulness of Snake River Farm food fish production facility effluent water for intensive rainbow trout production is zero.
- Intensive fish husbandry causes significant physiologic stress.
- Intensive aquaculture requires more stringent water quality conditions than that required for extensive aquaculture or wild fish.

- The purposes for which commercially raised rainbow trout are raised are different than for public conservation production. This creates different environmental requirements.
- Physiologic stress reduces fish adaptability and ability to withstand additional stress, increases susceptibility to disease (infectious and non-infectious), and reduces fish performance capacity.
- Stress shifts the bioenergetic flow of feed resources (energy and protein) away from somatic growth toward maintenance of homeostasis thus negatively impacting fish production.
- Existent research reports demonstrate the impact of individual stresses and stressors on homeostasis but stress most frequently occurs simultaneously from a variety of factors. The impact of multiple stress factors on fish is real but poorly researched.
- Eggs, fry and fingerlings are the most sensitive life stages of rainbow trout.
- Early life stages of rainbow trout are immunologically naïve and more sensitive to most diseases compared to later life stages.
- Recirculation of effluent water will increase fish stress, will diminish carrying capacity, will increase disease prevalence, severity and fish loss, and will create food safety problems.
- Pumping water from other springs, from a well or from effluent will decrease water delivery certainty, increase physiologic stress when delivery fails, create food safety issues and would diminish utility of existing water rights.

III. Foundation of Opinion

The basis of the opinions expressed in this report arise directly from the rainbow trout, it's biology, it's farming under intensive flowing water commercial conditions, it's response to water quality diminishment, bioenergetics, and various food safety and quality issues. These factors significantly impact the profitability of commercial operations and the competitive position of Clear Springs Foods.

A. Rainbow Trout Biology

General Characteristics

Rainbow trout are native to both shores of the North Pacific Ocean (Pennell et al. 2001) but now has a global distribution due to its relative temperature adaptability and human instituted transplantation (Wolf and Rumsey 1985; Laird and Needham 1988). Most fish biologists classify the rainbow trout as a spring spawner (February to June) although selective breeding in hatcheries and photoperiod control enables trout to breed throughout the year (Gall and Crandell 1992). Clear Springs Foods uses photoperiod control at the Snake River Farm Complex brood facility to manipulate timing of ovulation and ensure year round supply of selectively bred rainbow trout. Feral populations tolerate temperatures of 0 to 25° C while excellent growth under conditions of good water quality occurs at 15 to 20° C (Gall and Crandell 1992). Fornshell (2002) reports the optimal rainbow trout growth temperature for intensively farmed trout is 15°C. Male fish mature at 9 to 12 months of age under hatchery conditions while feral fish mature at 2-3 years depending on water temperature and food availability. The species is amenable to genetic modification via selective breeding (Gall and Crandell 1992). Stocks do show wide variation of spawning times, growth and carcass quality, taste, and swimming performance (summarized by Gall and Crandell 1992). The rainbow trout while adaptable, maintains a relatively narrow environmental scope due to high oxygen demands, sensitivity to xenobiotics, carnivory, sensitivity to water quality perturbations and physiologic complexity. This feature has enabled aquaculture of rainbow trout through-out the world but not on the same scale as occurs at Clear Springs Foods. **Optimal growth conditions for the rainbow trout occur where the water temperature is at the rainbow trout optimum (15° C), where water temperature does not fluctuate during grow-out (10-16 months), where water is saturated with dissolved oxygen, where metabolites of fish are absent, where there are no drug or pesticide contaminants in the water, infectious disease agents are absent and water flow does not fluctuate over a 0-72 hour time frame.** The 1000 Springs area of Idaho, where spring water comes from the Eastern Snake Plain Aquifer, has historically provided such optimal conditions. This area provides a large volume of essentially pristine water with optimal constant water temperature and other water quality characteristics (Fornshell 2002).

The rainbow trout (*Oncorhynchus mykiss*) is a physiologically sophisticated, complex, aquatic vertebrate animal whose organ systems are physiologically integrated and interdependent. In contrast to plants but similar to terrestrial vertebrate animals, the trout is composed of ten primary organ systems (skeletal, muscular, digestive, excretory, respiratory, circulatory, nervous, endocrine, immune and reproductive) specially adapted to function together in a particular environment, in this case water. The similarities and differences are noteworthy.

Aquatic animals must expend considerable energy to obtain sufficient oxygen from water because of oxygen's low solubility in water. In contrast, terrestrial animals have a ready supply of oxygen with which to drive metabolism. Trout are able to obtain dissolved oxygen using a very unique and sophisticated but also very fragile gill architecture which when combined with multiple types of hemoglobin molecules in erythrocytes enables trout to survive in reduced (compared to air) oxygen environments. Even with these anatomically sophisticated organs, the trout is only able to extract 30-40% of the available dissolved oxygen (Jones and Randall 1978). The trout gill is covered by a complex but also very thin epithelium that is the site of a multitude of vital functions including gas exchange, regulation of ion movements between fish and water, acid-base regulation, and excretion of waste nitrogen. **Unlike the terrestrial animal's lungs, this fragile gill epithelium is constantly exposed to the water the fish live in (i.e. their own wastes). It is constantly under challenge by water quality conditions that are less than optimum. Flowing-water aquaculture continually renews the environment minimizing accumulation of metabolic wastes.**

In contrast to humans (and most mammals) that have a single main hemoglobin component in erythrocytes, fishes commonly exhibit hemoglobin multiplicity (different "iso-hemoglobins" that occur in the same individual at the same or different stages of its development) and hemoglobin polymorphism (different "allo-hemoglobins" in genetically different strains of the same species) (Jensen et al. 1998). Each hemoglobin type responds differently to oxygen availability or acid-base conditions with different oxygen binding capacity. The rainbow trout has at least nine types of hemoglobin (Fago et al. 2002). These different hemoglobin's may occur all at once but in different abundance within individual fish (Tun and Houston 1986; Marinsky et al. 1990). Rainbow trout subjected to variable conditions of dissolved oxygen or photo-period over a 2-3 week period can shift the relative abundance of hemoglobin isomorphs. The bioenergetic impacts of this shift have not been well researched but low and fluctuating dissolved oxygen concentrations have been associated with diminished fish growth (Smart 1981).

Rainbow trout may compensate for less than optimal dissolved oxygen in the water by increasing the number of oxygen carrying red blood cells but there is a consequent additional physiological burden that is incurred which deprives the fish of energy or protein that would otherwise be put to growth. This is similar to humans living at high altitudes where increased numbers of erythrocytes are produced over time to help in delivery of oxygen. In trout, an acute response to low oxygen occurs with release of effete (aged) red blood cells from the spleen and appears related to adrenergic stimulation of the spleen (Wells and Weber 1990). While primarily a lymphomeyeloid organ, the spleen also removes aging erythrocytes that are nevertheless functional when released back into the blood stream. The increased number of circulating red blood cells is assumed to be a physiological adjustment to maintain oxygen transfer to tissues. It is suspected that trout actually increase production of erythrocytes in response to chronically low dissolved oxygen. This does come at a physiological price- too many red blood cells may be detrimental (Erselv and Gabuzda 1974). Experimental evidence indicates elevated hematocrit (polycythemia) of 55 % (compared to a normal of 30%) may be detrimental to rainbow trout by compromising oxygen transfer to tissue (Val et al. 2002). The impact of long-term polycythemia in trout has not been examined but a rise in hematocrit is associated with an exponential rise in blood viscosity, and consequently in the work that has to be performed by the heart (Wells and Weber 1991).

Many terrestrial vertebrate animals are homeothermic (maintain thermal homeostasis primarily by regulating their metabolic rate) while fish (but also reptiles and amphibians) are poikilothermic or ectotherms in which their body temperature is primarily controlled by the external environment. The consequence of ectothermy is that metabolism varies with temperature. Ectotherms also have more complex metabolisms than homeotherms in that important chemical reactions in ectotherms may have 4-10 enzyme systems that operate at different temperatures. In contrast, a homeotherm may have just one enzyme system. The bioenergetic consequence of multiple enzyme system is unclear since little research has been devoted to this area.

The differences between homeothermy and ectothermy are significant, and there are advantages and disadvantages. Because metabolism is so variable in ectotherms they do not easily support complex, high energy organ systems such as complex brains. But, for the same body weight, fish need only 1/3 to 1/10 of the energy of homeotherms. This has certain advantages in that all other factors being equivalent, feed conversion to flesh is more efficient in fish than in mammals and other homeotherms.

A significant disadvantage to ectothermy is that animals may be more sensitive to environmental disturbances than homeothermic animals. Rainbow trout appear to be far more sensitive to such disturbances than homeothermic animals or plants. Terrestrial homeotherms have lungs that while delicate, are maintained in a well protected body cavity and receive relatively constant air supply with constant content of oxygen gas. In contrast the breathing apparatus of rainbow trout (mouth, buccal pump, gills and hemoglobin) is fragile and easily damaged by pathogens, toxins and suspended solids in the water. The re-circulation of waste water or water from Clear Lake proper to the Snake River Farm would cause damage to the respiratory structures of rainbow trout and would be detrimental to fish production. Most importantly oxygen is often in short supply in the aquatic environment compared to a terrestrial environment. The immune system of trout while sophisticated is more primitive than and not as responsive as the immune system of humans, cows or mice. For example, the trout immune system appears to be temperature sensitive (Yamaguchi et al. 1980). Feral rainbow trout appear very sensitive to stress which adversely affects their growth and survival. When rainbow trout are raised under intensive commercial aquaculture conditions their sensitivity to even minor environmental or management changes is magnified. The rainbow trout is also very sensitive to various carcinogens and metal toxicities (Kotsanis and Illopoulou-Georgudaki 1999; Daglish and Nowak 2002). For example, chronic exposure to arsenic can cause liver hyperplasia and kidney fibrosis in rainbow trout. **Illustrative of the exquisite sensitivity of rainbow trout is that the US Environmental Protection Agency (EPA) uses the rainbow trout as one of their required test organisms in whole effluent toxicity (WET) tests.**

As introduced above, rainbow trout have all of the basic organ systems that other vertebrate animals have albeit theirs evolved to accommodate the challenges of an aquatic environment. These organ systems and their functional interfaces would likely be damaged by the re-circulation of waste water or Clear Lake water, or the use of any contaminated water as proposed by the Ground Water Districts.

Circulatory system- The circulatory system of trout is designed to transport nutrients and gases, hormones and wastes through the body. It consists of a four chambered heart (sinus venosus, atrium, ventricle and bulbous arteriosus), extensive vasculature and a blood flow path that takes de-oxygenated blood directly from the heart to the gills where blood is re-oxygenated and waste CO_2 and ammonia removed.

Nervous system- While trout do have a primitive brain their nervous system seeks to accomplish the same basic things a nervous system in human's does- to relay electrical signals through the body. The nervous system directs behavior and movement and along with the endocrine system, controls physiological processes such as digestion and circulation. The brain of trout is divided into three basic sections but does not have a neo-cortex thus fish cannot feel pain.

Digestive system- The digestive system consists of the mouth, esophagus, stomach, and the intestine. Trout do not chew their food although they do have teeth for holding and grasping prey. They also have a liver and pancreas that functions very similarly to that of terrestrial vertebrates. In trout there are also pyloric ceca that function in digestion and probably osmoregulation (Veillette et al. 2005). Taste buds are located in the mouth and esophagus and may also occur on the skin.

Excretory system- A significant challenge for fish, as for all animals whether aquatic or terrestrial, is to maintain water and dissolved solutes in the body in balance. This process is called osmoregulation and it is an active, energy dependent process. Rainbow trout are hyper-osmoregulators (Marshall and Grosell 2006) because they live in a dilute environment where there is diffusive ion loss and osmotic water gain across the large surface area of the gill epithelium. The sodium chloride (NaCl) content of their body fluids is approximately 40% that of sea water (Evans 1987). The trout produces a large volume of extremely dilute urine in a kidney specialized for electrolyte absorption. The kidney of trout has numerous glomeruli and extensive renal tubules. The urine is not as dilute as fresh water so some salt is lost. Salt lost must continually be replaced and this is accomplished partly by food and partly by active, energy dependent, absorption of chloride and sodium from the water by special cells on the gills variously called chloride or mitochondria-rich cells (Marshall and Grosell 2006).

Reproductive system- The reproductive system consists of either female or male gonads. At Clear Springs Foods only female rainbow trout are raised in production. The female trout becomes sexually mature at a later age (2 years) than many males (1 yr) under intensive culture. All female populations allow for greater uniformity in growth and ensure consistent flesh quality at harvest, and are generally more bioenergetically efficient because they are able to direct feed resources into somatic tissue development rather than reproductive tissue.

Immune system- The immune system of trout functions fundamentally like that in evolutionarily advanced vertebrates although there are some key differences. There is both innate and acquired immunity. Innate immunity derives from all those elements with which an individual is born and that are always present and available at very short notice to protect the individual from foreign challenges. These elements include the skin, mucous membranes and the cough reflex. In addition to these physical barriers there are chemical influences such as pH, secreted fatty acids, the enzyme lysozyme, and serum proteins such as β-lysin, various polyamines, kinins and complement. Granulocytes and macrophages are also part of innate immunity. Acquired immunity, which only occurs in vertebrate animals, is specific to a particular foreign challenge and it is only acquired following an initial contact with the foreign challenge or immunogen. Initial contact leads to the activation of lymphocytes and the synthesis of antibodies with specificity against the foreign agent. By this process the individual acquires the immunity to withstand and resist a subsequent attack by, or exposure to, the same offending agent.

The immune system of trout does have some additional important differences. For example, blood forming tissue (erythrocytes and leukocytes) is principally located in the interstium of the kidney and spleen rather than in bone marrow. The trout immune system is also temperature sensitive and impacted by season (Yamaguchi et al. 1980). Rainbow trout held at constant temperature are subject to seasonal suppression of antibody responses suggesting that photoperiod still affects immunity. Similar to higher vertebrate animals, the immune system is sensitive to the impacts of stress becoming depressed if stress is acute or chronic. Stress causes many changes in the physiologic systems of the body including those associated with tissue repair, phagocytosis (engulfment), inflammation and the immune system (Ellis 1981). Stress modulates many of the defense mechanisms in trout and higher vertebrates. Unfortunately this modulation is most often injurious. In its most significant form stress (discussed further below) induces the General Adaptation Syndrome (Selye 1950) that modulates the neuron-endocrine system which in turn modulates the immune system. The impact of hormones on lymphocyte function can be profound and adverse. For example stress appears to depress phagocytosis by macrophages in rainbow trout (Narnaware et al. 1994). Stress then sets the stage for enhanced susceptibility to disease and diminished growth. **In recognition of the immune system depression associated with stress, fish health managers strive to minimize stress and anticipate disease outbreaks within about**

two weeks of stressful events. They also strive to prevent the introduction of pathogens into rearing systems in hopes of preventing infectious disease.

Development of the immune system appears to occur gradually as the trout matures. While fry are capable of mounting a humoral immune response very early in ontogeny (within 1 month), the ability to respond to different antigens develops incrementally over time (Tatner 1986). Response to thymus dependent antigens appears to develop later than to particulate (thymus independent) antigens. Part of the delay may arise because processing of antigens may not be as efficient at the early life stages. By 3-4 months of age the immune response to both thymus dependent and independent antigens appears fully mature.

Respiratory system- The respiratory system consists of gills by which the fish breathes water. When first hatched, the respiratory system appears rather rudimentary so much of gas exchange may occur through the entire body. A swim bladder is also present in rainbow trout and is used to maintain neutral buoyancy in water. Functioning similar to gills, there is a counter current blood-gas exchange process (see below). Neutral buoyancy helps reduce the amount of energy needed to swim.

Endocrine system- The endocrine system of trout, together with the nervous system controls the physiology of the fish. They function to ensure homeostasis (maintenance of a stable internal state) in response to many factors (both internal and external). The endocrine system is generally organized like those in higher vertebrates although the location, form or function may at times differ. For example *in lieu* of parathyroid glands trout have Stannius corpuscles which are the source of hypocalcin involved in the regulation of calcium and a urophysis which is important in controlling osmoregulation (Matty 1985). Interrenal cells (generally located in the anterior part of the kidney in association with the post-cardinal vein; Donaldson 1981) suffice for part of an adrenal gland (adrenal cortex in mammals) that secretes the corticosteroids glucocorticoid (cortisol) and mineralcorticoid (aldosterone) hormones. Glucocorticoids help maintain blood pressure and respond to stresses. In excess glucocorticoids inhibit inflammation and the immune system. Mineralcorticoid hormones impact the use of fats and protein. The other equivalent part of an adrenal gland (adrenal medulla) in trout is the chromaffin cells which line the walls of the posterior cardinal vein (Reid et al. 1998). Chromaffin cells produce catecholamines such as dopamine, norepinephrine (noradrenaline) and epinephrine (adrenalin). Cortisol and epinephrine are important hormones in response to stress. Both exocrine and endocrine pancreatic tissues are present in trout but not as a discrete organ. Exocrine pancreas is diffused throughout the adipose tissue that surrounds the pyloric caeca. The exocrine pancreas produces digestive enzymes that flow into the ascending intestine. Endocrine tissue (i.e. the islets of Langerhans) are present in Brockman bodies located on the periphery of the kidney and at times near the pyloric caeca. The pituitary is believed to be a significant controller of hormone activity, just as in terrestrial vertebrates, but significant hormonal control may also occur through the gills (Olson 1998). The gill itself may even be an endocrine organ.

While all organ systems of the trout are essential, functionally interdependent and affected by stress, an understanding of some basics about gills may be most helpful for ultimately appreciating the negative impact all of the mitigation proposals at issue would have on rainbow trout aquaculture as conducted at Clear Springs Foods. The gills are particularly important because of their intimate exposure to the aquatic environment and their fragility and sensitivity to changes in that environment.

Water Breathing: Gill Physiology and Function

The gills of rainbow trout play a central role in gas exchange, ion regulation, acid-base balance and nitrogenous waste excretion (Sardella and Branner 2007). They are the "primary corridor for molecular exchange between the internal milieu of a fish and its environment" (Olson 1996). Ammonia excretion occurs through the gills via a diffusion gradient as does oxygen and CO_2. The gills also provide an internal regulatory function through their ability to modify plasma hormones. The anatomy of the gills has been well described by Olson (1996) so the anatomy will only be summarized here. The rainbow trout has four pairs of gill arches (four arches per side) that are covered by a gill flap or operculum. There are two rows of gill filaments on each gill arch. The number of filaments in rainbow trout is not well documented but is approximately 100-140 per arch (Shewmaker, personal communication). Each filament is covered by hundreds to perhaps thousands of lamellae. Water is pumped by the fish during breathing from the buccal cavity (mouth) to the opercular (gill) chambers. On each side or each gill filament are rows of shelf-like respiratory lamellae where gas exchange and exchange of other molecules takes place. The greater the number of filaments and the greater the number of lamella, the greater is the surface area available for gas exchange. The lamellae, where gas exchange via diffusion occurs, are very thin, only about 2 cells thick (mean of 6.37 µm; Hughes 1984) and easily damaged.

What makes the exchange via diffusion work most efficiently is a countercurrent system. The direction of blood flow in fish gills is opposite (countercurrent) to that of water flow. The gradient of oxygen tension (difference in oxygen tension between the fish blood and the surrounding water) is the driving force governing oxygen uptake (Jobling 1994) but the oxygen concentration is sensed by the fish to help regulate how much water must be pumped over the gills in order for the fish to obtain sufficient oxygen. Thus water with relatively high oxygen tension is exposed to blood with relatively low oxygen tension. The greater the differential, the more efficient is the diffusion. The more efficient the diffusion, the more efficient is the trout. Even still, only 30-45% of the total available oxygen is removed from water passing over the gill due to constraints of diffusion, gill disease and other conditions (Jones and Randall 1978).

The vascular anatomy of the fish gill has also been well characterized (Olson 2002a; Olson 2002b). There are three blood flow pathways in the lamella, an outer and inner marginal channel and the lamellar sinusoid. The highly vascularized gills, extensive surface area (filaments and lamellae) and very thin lamella optimize opportunity for gas to diffuse in or out. Any factor, such as would be present in re-circulated waste water, which diminishes these respiratory elements individually or collectively adversely affects trout ultimately reducing production. For example, acid-base disturbances cause fish to readjust transport mechanisms in the gills perhaps by manipulating the surface area of branchial chloride cell surface area (Goss et al. 1994). The readjustment is energy dependent.

The flux of oxygen from the water to the sites of consumption in fish tissue has been well described by Nikinmaa and Salama (1998) and consists of the follows steps: **(1) breathing movement continuously brings new oxygen molecules in contact with lamella. (2) Oxygen diffuses down its tension gradient from the ambient water into the capillaries of the gills. (3) Oxygen binds to hemoglobin in erythrocytes. The amount of oxygen bound per unit volume of blood depends on the number of erythrocytes, the prevailing oxygen tension, and the oxygen-binding properties of the hemoglobin molecule. (4) Oxygen is transported in the bloodstream from the gills to the sites of consumption. (5) In tissue capillaries, the tension of oxygen decreases and consequently, oxygen dissociates from hemoglobin. (6) Oxygen diffuses from the capillaries to the oxygen-requiring sites, mainly mitochondria, within the cells.** Since the mitochondrial oxygen tension is very close to zero, the rate of oxygen diffusion per unit area in a given tissue (with a unique diffusion coefficient for oxygen) is the function of the diffusion distance between the capillaries and the mitochondria and the oxygen tension of capillary blood. Factors that increase diffusion distance or create barriers to diffusion, such as is associated with bacterial gill disease, will be detrimental to the animal. Use of re-circulated waste water, Clear Lake water, or any other contaminated water would likely lead to bacterial gill disease which create barriers to gas diffusion which if extensive, would be catastrophic to individual fish or a population of intensively reared fish.

Hemoglobin, present in erythrocytes, greatly increases the carrying capacity of oxygen, carbon dioxide (CO_2), and hydrogen (H^+) in blood (Jenesen et al. 1998). Various molecular and cellular control mechanisms account for hemoglobin's remarkable properties and these have been reviewed in a variety of texts (e.g. Perry and Tufts 1998). These mechanisms include various allosteric interactions between binding sites, conformational changes and multiple functions of hemoglobin. Hemoglobin is for example subject to Bohr, Root and Haldane effects which result in shifts in oxygen binding properties enabling oxygen to be unloaded at appropriate times and CO_2 to be bound for release into the water by the gills. What is critical is that these essential molecular control features are subject to disturbances that adversely impact oxygen carrying capacity and oxygen delivery to tissues. **Where these disturbances are significant, as would occur with re-circulated waste water, less oxygen is present to nourish tissues resulting in diminished fish growth and injury.**

Environmental and internal factors can cause the oxygen dissociation curves to shift to the right or left (lowering or raising the oxygen affinity, respectively; Heath 1987). For example as pH is lowered, or the CO_2 is raised, the oxygen dissociation curve shifts to the right causing the hemoglobin oxygen-carrying capacity to be decreased. **This has broad implications for the survival of fish, bioenergetics and ultimately over-all fish production.** It is common to see blood acidosis (lower blood pH) in fish exposed to hypoxia. Decreased delivery of dissolved oxygen in flow-through aquaculture facilities occurs whenever water is used, re-circulated or an interrupted supply occurs. This causes injury to fish production capacity. While we cannot be certain of the extent of disturbance, the catastrophic consequences could be great and do not justify the risk associated with re-circulation of waste water or use of other contaminated water sources.

The epithelium of the gill filament is composed of five major cell types including squamous pavement cells, mucous cells, heavily innervated neuroepithelial cells, accessory cells and chloride or mitochondrial-rich cells (Evans 1987). The epithelium of the lamellae (sometimes referred to as secondary lamellae) consists of two major cell types- the superficial cells and the basal cells that replace the superficial cells over time. **It is crucial to fish survival and fish production that these complex and vital cell systems be kept intact and function efficiently**. Research now indicates that while reserve capacity exists in fish for gas exchange, detrimental effects occur to other gill functions (e.g. osmoregulation or ammonia nitrogen excretion) if the gills are compromised by disease or exposure to toxic elements. For example, damage to the gill from bacterial gill disease or other agents may adversely impact blood pressure regulation in trout (Hoagland et al. 2000). Exposure of rainbow trout to copper (e.g. copper sulfate) appears to adversely affect osmoregulation and ammonia excretion (Lauren and McDonald 1985).

Early Life Stages

Early life stages are generally most sensitive to stress and disease. These stages include the egg, larvae or alevin, fry, fingerling and sub-adult.

Egg- Building an animal with distinct organs requires the coordination of cell identity and cell behavior during embryogenesis. This makes the fish egg highly sensitive to all kinds of low-level environmental changes to which it might be exposed (von Westernhagen 1988). It is the earliest embryonic stages (before gastrulation) that are more vulnerable than embryos that have completed gastrulation. Salmonid eggs are relatively large and well known to require high concentration of dissolved oxygen (Pennell et al. 2001). This is attributed to a demanding surface to volume relationship. Dissolved oxygen must enter the egg via simple diffusion. The greatest demand for high concentrations of dissolved oxygen occurs just before hatching. **Rainbow trout eggs are most sensitive to nitrate toxicity compared to later life stages** (Kincheloe et al. 1979). A variety of environmental and stress factors are also known to impact teleost reproduction (Billard et al. 1981). Adverse brood stock feeding, temperature and water quality can also cause low fertilization and hatching rates.

Yolk-sac or alevin- the single most sensitive life stage in the fish life-cycle is the yolk-sac or alevin stage (von Westernhagen 1988). At this stage organs are developed but just barely. The alevin gut is present but not functioning. Nutrition occurs via absorption of the nutrient rich yolk. When the yolk is nearly used, the alevin's behavior changes from negative geotropism and avoidance of light to that of swimming upward into the water column. It is at this time that the swim bladder is filled by swallowing air (Tait 1960), learn to feed and deal with conspecific rivals. The gills of alevin are only poorly developed and hence very susceptible to challenge from toxins and bacteria that may cause gill disease. At this stage of development oxygen diffuses in via both the gills and the skin. The lateral line, important for mechanoreception, develops sometime after hatching (Blaxter 1988). If these sensitive life stages are exposed to any of a number of possible stressors, development would be imperiled and massive death occur.

Fry- once the yolk sac has been absorbed the fish is called a fry. At this stage the trout is able to take feed. The majority of biological traits, including physiological rates, are size dependent (Jobling 1994). While large fish generally consume more oxygen than small fish, small fish on a unit-weight basis, consume more oxygen. This occurs because the metabolic rate of the smaller fish is greater than the larger fish. Once the fry stage is complete fish are referred to as fingerlings and then sub-adult.

At hatching through to the next 3 months of life (fry through fingerling) the young fish are immunologically less developed. They are consequently immunologically naïve and have not developed significant immunity to common pathogens. It is at this stage that trout are most susceptible to both infectious and non-infectious diseases. Indeed it is at this stage that trout are most susceptible to IHNv, one of the most problematic diseases affecting rainbow trout (Groff and LaPatra 2000).

Bioenergetics

How fish utilize high energy- nutrient dense food fed in today's commercial fish farms is critical to the financial success of the fish farm. Feed costs typically account for 60-80 % of the total cost of production (Westers 2001). **The primary aims of fish farming are to maximize fish survival and growth at minimal cost** (Knights 1985). **Unfortunately, all of the mitigation proposals from the Ground Water Districts will decrease fish survival, decrease growth and production capacity, and increase production costs.**

Fishes, like all organisms, use ingested food resources (C) as building blocks in the synthesis of tissues (production, P) and as fuel in the metabolic processes that power this synthesis and other physicochemical work (R) (Calow 1985). Physicochemical work is commonly referred to as metabolism. Some of the resources introduced in feed are lost as waste products (E). All biological systems obey the laws of thermodynamics so the flow of food resources can be summarized as follows:

$$C = P + R + E$$

Metabolism can be dissected into a number of subcomponents: standard metabolism (R_S) which is the metabolism of an animal at rest; routine metabolism (R_R) which is the animals metabolism when routinely active; feeding metabolism (R_F) which is the metabolism of the animal just after feeding (sometimes known as specific dynamic action or effect), and active metabolism (R_A), which is the metabolism of the animal undergoing sustained activity (e.g. swimming). Thus

$$R = R_S + aR_{R\text{-}S} + bR_{F\text{-}S} + cR_{A\text{-}S}$$

where *a, b* and *c* are constants expressing the fraction of time that each type of metabolism is used. P represents both somatic (P_g) and/or reproductive components (P_r). Both somatic growth and development of reproductive components are important to Clear Springs Foods, but at Snake River Farm complex the focus is on maximizing somatic growth. That is why only female fish are raised at our farms. Female fish are used because of flesh quality concerns associated with sexual maturity and the need to focus feed resources on somatic growth (i.e. flesh) rather than reproductive products. Sexual maturity of rainbow trout generally occurs at 2 yrs of age in female rainbow trout while male trout may become sexually mature at 1 yr of age. Clear Springs Foods harvests fish generally from 10-16 months of age. By using female fish and harvesting by about 16 months of age we minimize food resource diversion by individual trout into reproduction. Finally E consists of feces (F), urea and ammonia (U), and miscellaneous secretions such as mucus (Muc) (Calow 1985). Thus, the equation representing the disposition of ingested food resources becomes:

$$C = (R_S + aR_{R\text{-}S} + bR_{F\text{-}S} + cR_{A\text{-}S}) + (P_g + P_r) + (F + U + Muc)$$

Since food resources are finite (there is only so much food a fish can eat) and the resources available for allocation in the fish are finite, resources used in one aspect of metabolism will not be available for use in others. For the intensive production of rainbow trout, it is essential that somatic growth be emphasized since that means more muscle mass for sale. Factors that shift resources away from somatic growth are detrimental to commercial food fish production. Such detrimental factors include, but are not limited to, stress, physiological shifts needed to maintain homeostasis, adaptation to new environmental or inconstant environmental conditions, pathogen challenge, and chronic infections. All of these factors would likely be present with the mitigation proposed by the Ground Water Districts.

Since fish are dynamic systems involving inputs and outputs which are constantly changing, none of the symbols in the bioenergetic equations above are constants. As conditions deviate from optimum, fish must expend more energy to compensate and maintain homeostasis which diminishes the energy that can be devoted to growth. Many abiotic factors that affect rates of food consumption and metabolism may be expected to have a profound influence on the growth of fish. The fish farmer's task is to enable fish to display rates of growth that approach the full physiological potential of the fish. Thus, it is essential that stresses and stressors are minimized. At Clear Springs Foods, selective breeding of rainbow trout has focused genetic resources on somatic growth resulting in about a 50% improvement in growth rate of our trout over time. **The physiological potential of Clear Springs Foods selectively bred rainbow trout has been significantly improved compared to wild or other domestically raised rainbow trout. All of the proposed mitigation projects would be detrimental to the expression of these selected characteristics and would be unacceptable.**

The metabolic rate of fed fish are higher than those of fish deprived of food, and regular provision of food can lead to rates of metabolism being maintained well above those recorded for unfed fish (Jobling 1994). There is a well established post-prandial increase in the rate of oxygen consumption. The increase in oxygen consumption is readily evident in 24 hr measured dissolved oxygen concentrations in raceways. The increase is generally 2-3 times the pre-feeding level. The peak usually occurs within a few hours after the end of a meal, and the metabolic rate then gradually declines to the pre-feeding level. The post-prandial increase in metabolic rate results from the energy requirement for the digestion, absorption and storage of nutrients, for the deamination of amino acids and synthesis of excretory products, and for the biosynthesis, turnover and deposition of tissue components (Jobling 1994). While feeding is going on, the homeostatic work of the fish must continue. It is at feeding and during post-prandial elevation in metabolism that fish are probably most sensitive to stress.

Much of this report identifies factors or processes that injure the production capacity of the Snake River Farm. The mitigation efforts proposed by the Ground Water Districts each, or collectively, will cause decreased fish production capacity. In addition, re-circulating waste water will significantly increase fish morbidity and mortality. All the mitigation proposals will be detrimental to the realization of the physiological potential of Clear Springs Foods selectively bred rainbow trout.

<u>Stress</u>

All species of fish are designed by natural selection to live in a certain optimum environment (Priede 1981). Indeed, organisms live within a limited range of conditions due to evolutionarily optimized structural and kinetic coordination of molecular, cellular, and system processes. While individual cells or molecules may function well under a variety of physiologic conditions, the increasing complexity of organs, organ systems and their interdependence to form the animal create functional constraints (Pörtner and A. P. Farrell. 2008).What is evolutionarily optimum for one species may not be optimum for another. For example channel catfish have a wider temperature and dissolved oxygen tolerance than do rainbow trout. Presumably the environment in which channel catfish evolved was more variable than where rainbow trout evolved.

Yet, fish even under the best of conditions, live under dynamic chemical and physical conditions (Wedemeyer and McLeay 1981). Fish are continually impacted by the normal demands of the aquatic environment. Fish must physiologically work to maintain homeostasis (i.e. physiologic stability) to accommodate this environmental dynamism and are successful up to a point- as long as the limits of accommodation are not exceeded. **As the fish works to maintain homeostasis, there is an energy drain (Lugo 1978), loss of adaptability and a reduction of performance capacity** (Schreck 1981). Superimposed on the routine stress of living in the normal chemical and physically dynamic aquatic environment, fish in intensive aquaculture are exposed to even more adverse conditions from operating procedures such as handling, crowding, grading, competing at high population density, consumption of high energy-nutrient dense feeds, high concentrations of pathogens and disease treatments, and decline in water quality associated with high population density and water use (Wedemeyer and McLeay 1981). **The challenge for fish producers is to control and/or minimize the stresses that can be controlled and seek to accommodate stress that cannot be readily controlled. Water quality, management practices, feed, fish pathogens and disease, and disease treatments are examples of stresses that can be controlled.**

Environmental conditions which can debilitate but are not necessarily lethal when they occur singly, include exposure to low concentrations of aquatic contaminants, unionized ammonia, nitrite, carbon dioxide (especially in recirculating systems), unfavorable temperatures, hypoxia, atypical light levels, total suspended solids, physical trauma, and population densities in hatcheries dictated more by production goals than by biological considerations (Wedemeyer and McLeay 1981; Piper et al. 1982). All of these, singly or together, can impose a considerable load, or stress, on homeostatic mechanisms of the fish. While fish farmers most often focus on the whole animal in fact, not only can the fish as a whole organism be stressed, but the diverse complement of individual cells in the fish as well. As the internal milieu changes, a cell must continually adapt to maintain their viability (Kedersha and Anderson 2002). In response to stress cells continually modify the repertoire of proteins they synthesize. These proteins affect individual cells and ultimately the entire animal.

Stress that exceeds physiological tolerance limits will be lethal. **Use of re-circulated waste water will be lethal to early life stages of fish raised at Snake River Farm. Use of re-circulated waste water could be lethal but will most assuredly damage and impair the growth and quality of later life stages. Use of other spring or ground water of inferior quality to what is currently used would also impair growth.** Less severe stress, that is acute or chronic, will load or limit physiological systems thereby reducing growth and predisposing the fish to infectious diseases if pathogens (the cause of infectious disease) are present (Wedemeyer and McLeay 1981: Pickering 1981). Stress also reduces the capacity of a fish to accommodate additional stress (Schreck 1981). The recovery time from stress depends upon the severity and duration of the initial stress (Schreck 1981). As discussed previously in the bioenergetics section, stress shifts the distribution of energy and food ingredients (e.g. protein and energy) resources away from somatic growth into self-preservation.

At some physiological point, sufficient stress can occur to induce a distinct stress response in which hormones are released that cause a coordinated cascade of physiologic changes. This is commonly known as the General Adaptation Syndrome (GAS) first proposed by Hans Selye (1950). The GAS is also known as the "stress response." Wedemeyer and McLeay (1981) define primary, secondary and tertiary alterations associated with the "stress response."

- Primary alteration
    - Release of adrenocorticotropic hormones from the adenohypophysis (part of the pituitary gland)
    - Release of "stress hormones" (catecholamines and corticosteroids) from the interrenal tissues
- Secondary alterations
    - Blood chemistry and hematological changes such as hyperglycemia, hyperlactemia, hypochloremia, leucopenia, and reduced blood clotting time.
    - Tissue changes, such as depletion of liver glycogen and interrenal ascorbic acid
    - Metabolic changes such as a negative nitrogen balance, and oxygen dept.
    - Diuresis with resultant blood electrolyte loss
- Tertiary alterations
    - Impaired growth and spawning success
    - Increased disease incidence (infectious and non-infectious)
    - Death several weeks after the stress with no apparent warning (Mazeaud and Mazeaud 1981).

The sensitivity of a trout to stress is dependent on a variety of factors. Fundamentally the primary response, i.e. the adrenergic response, is simply very sensitive. Stress thus results in an increase in the plasma concentration of catecholamines very fast (Mazeaud and Mazeaud 1981). In the rainbow trout the concentration of catcholamines (adrenaline) can rise several hundredfold within a few minutes (Matty 1985). Following an acute stress in salmonids it may take several days before the concentration of adrenalin goes back down to "normal" levels. The secondary alteration associated most often with the adrenaline rush following acute stress is an alteration in osmoregulation. Chronic stress, if of sufficient magnitude, can induce long-term elevation in plasma adrenaline with long term implications to growth and fish performance. Some stresses may not be of sufficient magnitude to induce a "stress response" characterized by the release of catecholamines and corticosteroids. For example respiratory dissolved oxygen stress or CO_2 stress responses may be primarily mediated by a neural pathway, without elicitation of hormones (Hughes 1981). These types of stress can be more readily accommodated by the fish by increased ventilation or breathing rate. **Various aspects of accommodation have been reviewed by Fontaine (1993). While stress that induces the need for accommodation may be tolerable, it causes increased expenditure of energy with subsequent diminishment in protein accrual and hence decreased growth of somatic tissue.**

The intensification of salmonid culture and the consequent rearing of fish in man-made environments have resulted in the fish being exposed to a number of stresses which they do not experience at all in the natural environment or do not experience to the same degree. One category of stress occurs as a consequence of the artificial environment itself and can include such factors as temperature, rate of change of temperature, salinity change, stocking density, abrasion, oxygen concentration, free ammonic concentration, pH and water velocity (Donaldson 1981). Donaldson (1981) states further that salmonids in particular are sensitive and responsive to those stresses mediated by the hypothalamic-pituitary-interrenal (HPI) axis.

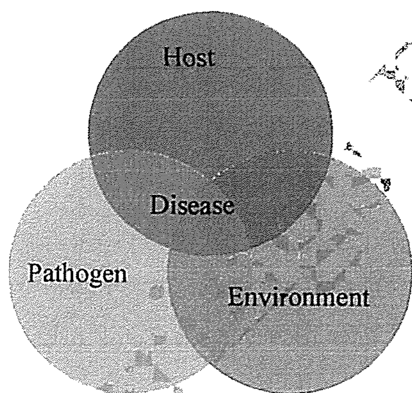
Wedemeyer and McLeay (1981) identify additional environmental stressors that are debilitating to both warm and cold water fishes. Among the stressors they identify are dissolved oxygen less than 6 mg/L at temperatures of 10-15° C, crowding, chronic low oxygen (4 mg/L), particulate matter in water and unionized ammonia concentrations of 0.02 mg/L or greater.

There appear to be a number of consequences of stress in addition to the consequences of stress mentioned above. Lesel (1981) reports that stress causes a change in the digestive tract microflora of fish and accelerates the passage of material through the entire digestive tract. This may decrease the availability of nutrients to the fish from otherwise good feed. van de Salm et al. (2002) provide evidence that exposure to stressors leads to multiple changes in the skin epithelium which are mediated by cortisol. In addition stress of upstream rainbow trout may impact downstream fish. Tao et al. (2004) demonstrated that conspecifics exposed to stressed trout skin or trout skin extract caused elevated cortisol levels in non-stressed fish.

Biology of Disease

The rainbow trout's tolerance of adverse influences is limited by the ability of its cells, organs, organ systems, and the whole fish to sustain injury. Whenever the cells, organs, organ-organ systems, and whole fish adaptive capability is overtaxed, damage results. Such damage may be sublethal and permit recovery, or more intense or prolonged, resulting in death. The adapted, the sub-lethally injured, and the dying animal are merely stages in a continuum of progressive encroachment on the animals homeostasis. For the trout farmer, all challenges to homeostasis are important because they impact fish performance and ultimately profitability. Adaptation is an energy demanding process that diminishes feed conversion efficiency and reduces overall production capacity. Sublethal injury further diminishes fish performance and profitability. Management efforts by the fish farmer to prevent mortality of compromised fish, including use of drugs, can be very costly. Prematurely dead trout (i.e. death not occurring at the processing plant) reduces overall profitability and may ultimately result in loss of customers dependent on a reliable supply. The closer to optimum the farmer can manage the production environment, the close to maximum profitability.

While disease is part of a continuum of cellular and whole organism physiologic efforts to maintain homeostasis, it is also the result of an interaction between the host, pathogen and environment (Sneiszko 1974). A convenient way to envision that interaction is with the venn diagram below.



A susceptible host is of course essential for disease. A fish at its physiologic optimum is far less likely to suffer disease than a fish physiologically compromised. Considerable research (e.g. Wedemeyer and McLeary 1981; Ellis 1981) indicates that fish subjected to sufficient stress that activates the General Adaptation Syndrome (above) are likely to succumb to diseases such as Motile Aeromonas Septicemia or develop disease from various fungi. Even stress that does not invoke the GAS may compromise the host making it more susceptible to pathogenic bacteria or the sequelae associated with mild viral infections. **The environment then becomes an important element in disease because the environment often determines the extent of physiologic work the host must exert to accommodate.** The environment can be the direct source of toxins (e.g. arsenic, copper, zinc, $KMnO_4$, un-ionized ammonia), of physical factors such as temperature, gas bubbles or suspended solids, or of factors that compromise normal physiologic function (e.g. nitrite that causes methemoglobinemia). **Finally, a suitable pathogen is required. Not all pathogens are equally as virulent or able to cause disease.**

While disease can occur without a pathogen, of particular challenge in intensive aquaculture is the prevalence of a variety of potential pathogenic organisms. The pathogens may be opportunistic (e.g. *Aeromonas hydrophila, Flavobacterium psychrophilum* and *Trichodina spp.*) or obligate (e.g. infectious hematopoietic necrosis virus- IHN or *A. salmonicida*, the cause of furunculosis). Obligate parasites may also infest rainbow trout such as *Ichthyopthirius multifiliis* and *Myxobolus cerebralis*. The bacteria associated with bacterial gill disease may be obligate (*F. columnare*) or opportunistic (various yellow pigmented bacteria and *A. hydrophila*). There are also pathogens of indeterminate nature. These include *Ichthyobodo* and *Ichthyophonus*. With the exception of *M. cerebralis*, all of these pathogens, as well as others are endemic in the 1000 Springs aquaculture industry and at Snake River Farm. What varies is that the abundance and concentration of pathogens increases with serial use. Fortunately, the mere presence of a pathogen in or on a fish does not necessarily lead to disease (Groff and LaPatra 2000). The host must be susceptible (e.g. stressed) and an environment suitable for pathogen survival and inimical to the host may need to be present.

<u>Biosecurity</u>

Stress (such as from pathogens) to the extent it is introduced by man is unacceptable. For example, the movement of fish pathogens within rearing units or farms is controllable if proper precautions are taken. Use of re-circulated waste water or mitigation water from whatever the source containing pathogens is unacceptable. Infectious diseases inevitably occur in aquaculture but the risk of pathogen transfer can be minimized through an Integrated Pest Management Plan (IPM) (LaPatra and MacMillan 2008). An IPM includes various biosecurity measures, surveillance for early detection of pathogens or disease, timely treatment, and the use of various nonchemical methods of reducing disease incidence. Non-chemical methods include proper selection of fish, use of vaccines, pathogen vector control, site fallowing and stock management to break pathogen life-cycles (LaPatra and MacMillan 2008).

Because fish pathogens can significantly impact intensive aquaculture profitability and there are few effective treatments for most diseases affecting rainbow trout, considerable management effort is directed at biosecurity at Clear Springs Foods. Much of this management effort is focused on avoiding the introduction and the movement of pathogens. At Clear Springs Foods Snake River Farm an IPM has been implemented. Fundamentally only spring water is used. Spring water is largely free of most potential fish pathogens because, depending on where the springs originate, there are no fish that might harbor pathogens present. Water is not re-circulated or inter-mingled. Water is used (see diagram in flow-though aquaculture section) in a linear series of raceways to ensure immuno-competent fish are the ones most likely exposed to pathogens. **At subsequent use raceways fish loading and feeding rates are also reduced in recognition of the additional stress from diminished water quality those fish are subject to**. Water is maintained in a single raceway series, it is not intermingled thus limiting opportunity for farm-wide epizootics. A bird exclusion cage completely surrounds Snake River Farm. Predator control measures are in place. Nets and hauling equipment are disinfected. Visitors are excluded from most sensitive rearing areas such as the Hatch House. Hand washing and foot bath disinfections prior to nursery (hatch house) entry is mandatory. Water used in the hatch house is only first-use spring water. Any eggs used in production are inspected and certified pathogen free. The spring water source is covered so there is less opportunity for contamination. Early life stages are reared in the best, most pathogen free water available (i.e. first use). Feed is manufactured using heat and pressure to eliminate potential fish pathogens.

Not only are pathogens a focus of biosecurity concern but chemicals and toxins as well. There are many chemicals that can be inadvertently or purposefully introduced into water supply or feed that could harm fish or cause them to be unsafe for human consumption.

Some chemicals are acutely toxic causing death within a very short time while others are more chronic adversely impacting fish health over a long time period. Some chemicals could bio-accumulate over time if there were periodic exposure. These issues are very similar to those of concern to public drinking water suppliers albeit the rainbow trout may be even more sensitive than humans depending on the particular poison.

Because of these biosecurity concerns Clear Springs Foods instituted a concerted effort to improve security and reduce potential access to spring water feeding the Snake River and Clear Lakes Farms. Clear Springs Foods now owns and controls much of the access to the talus slopes (up to the canyon rim on the north side of Clear Lake Road) from which the springs are believed to emanate. Additionally Snake River Farm complex spring water collection is covered to further limit access to spring water by unauthorized individuals. **Clear Springs Foods would oppose any delivery of water that is not similarly secure from unauthorized access.**

Selective Breeding at Clear Springs Foods

Rainbow trout used in aquaculture have been domesticated for over 100 years (Gall and Crandall 1992). Clear Springs Foods has been selectively breeding its strain of rainbow trout for over 23 years (since 1985). Selective pressure has focused on growth rate with co-selection for disease resistance (IHN and cold water disease). The selection and grow-out of each generation has occurred at the Snake River Brood Operation housed at the Research and Development Station (part of the Snake River Farm Complex). Water supplied to this facility is first use spring water. All hatching and rearing has occurred in this water since the breeding program began. It is essential that that continue. Water quantity available to the Snake River Farm Complex has steadily declined over time necessitating continued diminishment of water flow to the brood stock and research programs located at the Research Division facility. This decline causes more stress on sensitive life stages. It is known that an animal's robustness, adaptability, and resilience arise within the context of environmental, genetic, biochemical and morphological elements of the animal and environment. Selection for fast growth under these conditions gives rise to an integrated organism response. **It is essential for the success of this program that water flows do not diminish further and that use of pristine spring water is maintained.**

<u>Vaccination at Clear Springs Foods</u>

Clear Springs Foods relies heavily on the use of various vaccines as it attempts to manage aquatic animal health. Vaccines have potential to prevent infectious disease as long as other factors do not compromise their efficacy. As discussed above and below, various environmental conditions, environmental constancy, stress factors (physiologic and operational), and immunologic competency all impact vaccine efficacy. These vaccines are developed by Clear Springs Foods scientists and are manufactured at the Clear Springs Foods Research and Development Center. Clear Springs Foods produces autogenous vaccines that are only used at their own farm operations. Vaccines of variable effectiveness have been developed for enteric redmouth disease, IHN disease, and coldwater disease. Of the three, the most effective is for ERM disease. **Recirculation of waste water will diminish vaccine efficacy. Interrupted water delivery will stress early life stages and impair vaccine efficacy. Decreases in water quantity will also impair vaccine efficacy by causing additional stress. Decreased or intermittent water supply will adversely impact existent research programs devoted to development of effective vaccines and selective breeding.**

IV. Determinant's of Fish Production Capacity and Negative Consequences of Mitigation as Proposed

A. Why 1000 Springs for production of rainbow trout?

The foundation for rainbow trout production in the US was well established by about 1870 (Stickney 2001) but such production was directed at fishery conservation. Fish production for fishery conservation purposes (stocking in public waters) is less intensive than commercial food fish production such that physiologic demands on the fish are generally less. Additionally conservation fishery fish culture typically produces a batch or single cohort of fish while commercial production typically relies on a continuous, sequential, multiple cohort production program (Westers 2001). Commercial rainbow trout production, particularly in the 1000 Spring's area did not begin until about 1928 when C.S. (Jack) Tingey (reported as either a former Secretary of State for Utah, a Conservation Officer, or both) started the Snake River Trout Company (currently owned and operated by Clear Springs Foods, Inc.). The reason the Snake River Trout Company was started is conjecture but IDWR (1975) suggests it was because of an abundant water supply, water quality, and proximity to market. An earlier attempt at commercial rainbow trout production occurred in 1910 when a farm was started at Devil's Corral Spring near Shoshone Falls but this venture did not last long due to poor market conditions. **An aquaculture development time-line is attached (Exhibit "2").**

Application of the scientific method to unravel the limits and factors enabling intensive fish farming is ongoing but did not begin until about 1930-1940 when joint research conducted at New York State Laboratory and the Hagerman Tunison Laboratory (now the University of Idaho Hagerman Aquaculture Experiment Station) developed a dry feed formulation that replaced those originally made from animal carcasses. In the early 1940's dry diets were first tested at Tupper's Trout Farm in Hagerman. In the 1950's David Haskell with the New York Fish Conservation Department first applied analytical investigation to the art of flowing water fish culture (Soderberg 1995). He provided a quantitative approach to the definition of chemical and biological parameters affecting fish in confinement and allowed fish culture to progress from art to science.

The early Idaho trout aquaculture pioneers had fortuitously established rainbow trout aquaculture in the 1000 Springs area not knowing that it provided the ideal location for commercially intensive rainbow trout production. Today Idaho in the 1000 Springs area produces approximately 70% of all the rainbow trout produced in the US for human consumption (Fornshell 2002). The large volume of gravity fed, essentially pristine spring water at nearly constant water temperature (58° F) 24 hours per day, seven days a week with little short-term fluctuation in flow and saturated with dissolved oxygen is now known to provide the optimum environmental conditions for maximal rainbow trout production using flow-through fish culture techniques. That and the continued development of scientific understanding about rainbow trout and trout aquaculture allow aquaculture to prosper in Idaho.

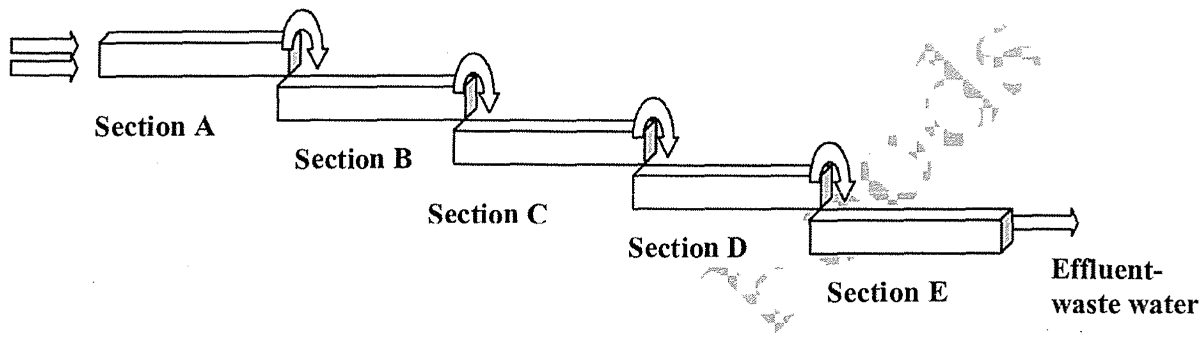
The scientific understanding of rainbow trout physiologic needs, how those needs vary with life stage, and development of efficient feeds allows determination of fish farm carrying capacity and permissible stocking density. The goal of intensive, high-density aquaculture is to maximize carrying capacity (Clark 2003). Carrying capacity is the maximum permissible loading rate (fish weight per unit of water flow) that results in effluent dissolved oxygen at the predetermined minimum allowable oxygen tension (Soderberg 1995; Procarione 1999). In Idaho fish farms must comply with State water quality standards. Dissolved oxygen must be discharged to public waters at 6 mg/L or greater. Fish density is primarily dependent on volume of water flow because flow determines the amount of oxygen available for fish respiration and the degree of metabolite dilution. The maximum permissible density (fish weight per volume unit of rearing space) is determined by the hydraulic characteristics of the rearing unit (e.g. raceway) and the physical, physiological and behavioral spatial requirements of the fish. Environmental factors that deviate from spring water quality (including water volume) and reliability diminish a fish farms carrying capacity and maximum permissible loading rate and fish density, and diminish the utility of water associated with Clear Springs Foods water rights. **Because pumping waste water, or pumping spring or ground water cause all of these things, it is unacceptable.**

B. Flowing water fish culture

Many texts describe the intricacies of aquaculture production systems including flowing water fish culture techniques (e.g. Piper et al. 1982; Soderberg 1995; Black and Pickering 1998; and Wedemeyer 2001). In this part of the report I will only describe the general process of flowing water aquaculture because it has relevance for understanding how water quality changes with use. Flowing water systems afford the greatest control compared to other types because they allow for quantitative analysis of production capacity based on water quality measurements and amount of waste generated (Westers

2001). Because rainbow trout are sensitive to water quality conditions compared to many other types of fish, such control is critical. **Use of waste water or water of suspect water quality is consequently not acceptable.**

Over-view of Flow-through System at Snake River Farm



Flow-through aquaculture using raceways, as occurs at the Snake River Farm, is characterized by linear flow from one raceway to the next. The typical hydraulic pattern approximates plug flow in which all elements of the water move with the same horizontal velocity. The consequence is that there is a water-quality gradient from the inlet (entering Section A) to the outlet (Section E effluent). In the diagram above, Section A water quality or fish production utility is greater than Section B and so on (A>B>C>D>E). Aquaculturists often refer to this as serial reuse but in fact the water in each section is only used once in that section. There is no recirculation of water and water is not re-used in that sense but it is used multiple times. There is an attempt at each raceway to remove solid wastes and replace some dissolved oxygen used during fish production. At the end of each raceway is a quiescent zone that captures solid wastes. These wastes are removed at least weekly. With each drop in elevation there is some degree of dissolved oxygen replenishment. In this process there is some rejuvenation in the utility of the water for fish production. If a single very long raceway were used there would not be much opportunity to control waste accumulation or opportunity to replenish some of the dissolved oxygen used during fish production.

The use of this type of aquatic animal production system requires various production accommodations. The best water quality is used to husband the youngest, most immunologically naïve fish. In the diagram above this occurs in Section A. As the fish grow and become more immunologically competent they are moved to water of poorer quality (less production value), Section B. This process continues until the fish reach Section E where they are held until harvest. To accommodate the progressive decline in water quality there is a progressive decrease in fish loading (lbs/cfs) and a decrease in feeding rate.

The general fish production flow is from egg to fry to grow-out and harvest. The process is continuous year-round. As fish increase sufficiently in size and physiological tolerance, they are graded and moved along to downstream raceways with fish of similar size. Flow-through farms are designed so that water flows from one raceway to another in series. As fish become more tolerant of diminished water quality they are moved down-stream. Harvest of rainbow trout for human consumption occurs when they reach market size which varies generally from 1 to 2 lbs each. Such grow-out may require as much as 2 years depending on water quality and feed conversion.

Pristine spring water is referred to as first use water and it is used for egg incubation and hatching, for first stocking of fry and/or fingerlings, and for brood stock. First use water at the Snake River Farm generally has optimal water quality conditions. It is saturated (or nearly so) with dissolved oxygen, has no or very limited waste products from other fish, and no or very few numbers or quantity of fish pathogens. At Snake River Farm we also use some excellent quality water that has been used for our brood stock and selective breeding programs. This water, while some of its production utility has diminished from the pristine spring water, nevertheless retains most of the quality deemed essential for early life stage rearing in the Snake River Farm Section A. The reason the water retains significant utility is that brood stock production is not nearly as intensive as at Snake River Farm where fish are raised for food production. Additionally the brood stock have been reared in essentially pathogen free water so it is unlikely fish pathogens occur that could impact naïve, downstream fish.

Juvenile fish for grow-out are in first use water for approximately 3 months during which they grow, consume dissolved oxygen, excrete wastes, become more physiologically tolerant of less optimal water quality conditions and become more immuno-competent (better able to withstand bacterial or viral pathogens). At the end of three months these fish are now large enough and old enough to be graded and moved to less optimal water quality conditions immediately down stream. It is at this time they are better able to withstand greater challenge from pathogens that are more likely to occur (and at higher density) in downstream locations. This process continues through each serial use of water. The worst water quality on the farm is the last use (Section E). Generally only about 50-55% of the dissolved oxygen in first use raceways is present. Un-ionized ammonia concentration has increased as has carbon dioxide, total suspended solids (TSS), biological and chemical oxygen demand (BOD and COD) and the concentration of bacteria and other pathogens in the water column. pH has decreased relative to first use ponds. During late spring and summer the water temperature may have increased about 1° C from first use water. In the winter the water temperature may decrease 1-2° C. **Each of these changes affect the quality and quantity of rainbow trout produced.**

The production capacity of raceways denied water of suitable quality will suffer reduced production which causes financial injury. Use of waste water in any raceway will cause injury because its carrying capacity will be reduced. Using water subject to intermittent supply will diminish certainty which will increase operating costs and diminish carrying capacity.

C. Water supply

Just as with dairies and other animal production systems, one of the most important factors impacting production is water quality (Westers 2001). Water used at dairies is not re-circulated to the dairy because it is incompatible with milk production and dairy cow survival- so too with rainbow trout in flow-through production systems. Water quality is a term used to describe the chemical, physical, and biological characteristics of water, usually in respect to its suitability for a particular purpose which in this case is to maximize fish production. The impact of water quality (in all of its dimensions) on fish production capacity is profound and it has been scientifically dissected over time to characterize many of the individual factors that make up "water quality." It is the speciation of water quality into its component parts that will constitute much of the remainder of this report. There are however other factors besides water quality that are important to fish farming and that impact production and market success. **Recirculation of waste water directly impacts some of these factors as does use of water from uncontrolled and unreliable sources.**

Food Safety and Marketability

Federal food safety requirements ultimately require that water and animal feed used for growing fish for human consumption must not contaminate the fish. Contaminated fish are then classified as adulterated. The Federal Food, Drug, and Cosmetic (FD&C) Act (1938) (US Code, vol. 21, section 342) provides that food is "adulterated" if it meets any one of the following criteria: (1) it bears or contains any "poisonous or deleterious substance" which may render it injurious to health; (2) it bears or contains any added poisonous or added deleterious substance (other than a pesticide residue, food additive, color additive, or new animal drug, which are covered by separate provisions) that is unsafe; (3) it bears or contains a pesticide chemical residue that is unsafe. The Environmental Protection Agency (EPA) establishes tolerances for pesticide residue in foods which are enforced by the FDA. For rainbow trout, there are no established pesticide tolerances. **This means that even trace amounts of pesticide, herbicides or other "deleterious" substances would adulterate the food. Water entering a fish farm consequently needs to be free of substances that could be absorbed by the fish and contaminate the flesh, even with minute quantities.**

In this case, if the spring water proposed for mitigation purposes is exposed to pesticides associated with golf course care, potential exists for fish contamination by uptake from the water. Typical chemicals used on golf courses include 2,4-D, pendimethalin, 2,4-DP, dicamba and chlorpyrifos. **Clear Springs Foods does not know what pesticides or**

herbicides are used by the Clear Lake Country Club but any use that contaminates spring water would be unacceptable.

Further, spring or ground water diversions and water flow paths must be secured from intentional or inadvertent contamination. Because of heavy automobile traffic in the area of Snake River Farm and potential access by individuals of ill-intent, Clear Springs Foods has attempted to protect its water sources. Much of the property surrounding the area where currently used springs emanate is owned by Clear Springs Foods or Idaho Trout Company. Clear Springs Foods owns much of the talus slope up to the canyon rim in the area where the Snake River Farm Complex of springs are believed to emanate. The Snake River Farm Complex water collection system has been physically covered. **Biosecurity of water supply is an essential component of the Snake River Farm complex and it would be unacceptable to deliver water to this complex that is less biosecure than currently delivered.**

Contaminants also include microbiological and parasitological elements. Certain parasites of fish can cause marketability issues in addition to "adulterating" the food. Various nematodes, cestodes, digenetic trematodes and copepods can infect trout appearing in eyes, gills, skin or muscle. The life cycle of these parasites is often complex involving intermediate hosts (such as birds) or snails. Water sources should be protected from the intermediate host or other vectors. Water supplying a fish farm needs to be bio-secure so that such parasites do not have opportunity to infest trout. At Clear Springs Foods, all farms are enclosed in bird netting in part to prevent piscivorous birds from defecating in water and releasing infective parasites and moving diseased fish from one raceway to another. **At Snake River Farm, Clear Springs Foods has covered much of the water diversion and collection area significantly reducing public access and access to potential pathogen vectors. Spring water not generally protected poses a biosecurity threat to Clear Springs Foods Snake River Farm complex research, selective breeding and fish production and would be unacceptable.**

Contaminants can also arise from plants and blue-green bacteria. These contaminants taint the taste (off-flavor) or organoleptic qualities of the flesh making them less desirable to consumers. Certain plants are more prone to releasing off-flavor compounds than others. Two chemical compounds, Geosmin and 2-Methylisoborneol (MIB), have been identified as the cause of a majority of off-flavor incidents in rainbow trout although others occur as well (Selli et al. 2006). Geosmin and MIB are secondary metabolic products of some species of bluegreen algae and actinomycete bacteria. MIB causes a flavor to be imparted to the flesh described as "musty" or "lagoon" and geosmin results in "earthy" or "woody" flavors. These two compounds are extremely potent. Geosmin and MIB can be tasted in the water by humans at concentrations of 0.01 and 0.03 parts per billion (ppb), respectively. A number of other less frequent off-flavors have been recognized such as moldy, astringent, rotten and sewage for which no chemical compounds have been identified. Off-flavor compounds can be absorbed in a matter of minutes once they are present in water. Fish absorb chemical compounds through their gill membranes as well as through their digestive tract. The compounds are fat soluble and are stored in fatty tissues. The amount of off-flavor absorbed by fish seems to be related to water temperatures, environmental concentrations and exposure time. Off-flavor in recirculating aquaculture systems (a production system in which water is reused over again after re-conditioning) is a common and a persistent problem (Masser et al. 1999). Algae may also affect fish health through the production of toxins and through mechanical damage to fish gills (Munro and Roberts 1989). What is clear is that off-flavor causing compounds can be produced in water which if used to culture rainbow trout could impart unacceptable flavor properties making the fish un-marketable.

Water in which extensive plant and algae growth occurs cannot be used by Clear Springs Foods because of the likely occurrence of off-flavor compounds. The Ground Water Districts have proposed combining Spring 1 and Spring 2 for diversion to the headwaters of the Snake River Farm Complex. Examination of Spring 1 during summer months indicate extensive plant growth (including algae) occurs. It is likely off-flavor compounds would be produced which would render the water unuseable for rainbow trout aquaculture.

Drugs and chemicals used at trout farms are potential contaminants of other fish. The FDA limits the use of drugs to those drugs that are federally approved as a New Animal Drug Application (NADA), an Investigative New Animal Drug Application (INAD), or through an extra-label application under veterinary prescription. Drugs approved under an NADA (http://www.fda.gov/cvm/aqualibtoc.htm) include oxytetracycline (NADA 038-439), florfenicol (NADA 141-246), and ormetoprim-sulfadimethoxine (NADA 125-933). These are all antimicrobial therapeutic agents that have mandatory withdrawal times (the time after treatment when fish are not exposed to the antibiotic before they can be harvested for human or animal consumption). For oxytetracycline the withdrawal time is 21 days, for florfenicol the withdrawal time is 12 days and for ormetoprim-sulfadimethoxine the withdrawal time is 42 days. Populations of fish (those in a raceway) exposed to an antimicrobial are segregated so they do not enter the food chain before they have met their withdrawal requirements. A veterinarian may prescribe the extra-label use of a drug and typically ensure sufficient withdrawal time has occurred by ensuring harvest does not occur for at least 180 days. Other drugs potentially used include formalin (NADA 137-687, 140-831, and 140-989) and hydrogen peroxide (NADA 141-255). Potassium permanganate and copper sulfate are water treatments for disease management that may also be used at various locations within a raceway series. The use of any drug or water treatment chemical is highly variable depending on the incidence of specific diseases. **Use of re-circulated waste water is unacceptable to Clear Springs Foods because such action would likely and unpredictably contaminate fish with drugs and chemicals used to treat diseases in fish held in later water uses.**

Water supplies for fish farms must also be bio-secure. International and domestic events in the past 10 years have elevated concern that foods, including that produced at fish farms, could be subject to terrorist attack. The concern is that contaminants could be placed in water that makes the farmed fish unsafe for human or animal consumption. These could include chemicals or microbiologic agents. Control of access to the water supply is thus important. Biosecurity has become a critical issue to third parties (consumers) and has become subject to scrutiny during Clear Springs Foods third party audits. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 prescribe actions that are intended to prevent malicious, criminal or terrorist actions that could impact food safety. As part of that effort FDA developed "Food Producers, Processors, and Transporters: Food Security Preventive Measures Guidance" (Oct. 2007) that identifies various actions all food producers and manufacturers are expected to implement. These actions include steps to secure water supplies from access to the public. Control of the water supply is an important element based on this guidance. **Spring water supplies open to the public are less desirable than secure sources and would be inconsistent with food safety expectations. Clear Springs Foods has gone to considerable effort where practicable to prevent public access to water used on its fish farms. The Snake River Farm Complex water supply is relatively well protected from public access. The springs proposed for use by the Ground Water Districts are not bio-secure.**

<u>Risk</u>

Certainty of water supply is also an important element of fish farming risk considerations and investment decisions. Water supplies that are of predictable quality and quantity allow certainty for investment in fish, feed and the other elements of normal business planning. Gravity delivered spring water flow, while some of the quantity is variable over a year and varies from year-to-year, is not subject to immediate curtailment interruptions. The Snake River Farm 1955 water right had been available with constant quantity (no seasonal variation) throughout the year until 2001. In 2001 and since then even this supply has become more variable. **The relative constancy of gravity fed spring water permits quantitative estimates of important environmental needs** (e.g. dissolved oxygen) and allows estimates of potential metabolite concentrations so that stocking of fish in each section (A-E) for production purposes can be optimized. The Ground Water Districts propose to pump waste water, or to pump spring water or to pump ground water to the Snake River Farm Complex head waters. Because pumped water is subject to unpredictable failure due to loss of power (Masser at al. 1999; Soderberg 1995), there is increased uncertainty and hence risk to the water supply for the complex. Pumped water is occasionally used in public, conservation hatcheries or in small scale commercial farms but is too unreliable for large commercial ventures with an established customer base. Use of waste water increases risk of catastrophic disease and risk of product adulteration. Use of unsecured Spring 1 and Spring 2 water (contaminated by golf course herbicide and pesticides) or with access to the public increases the risk of product adulteration. Use of pumped ground water is subject to increased risk from interrupted delivery.

<u>Importance of water quality constancy</u>

As previously discussed, various environmental factors can shift the bioenergetics of rainbow trout away from somatic growth. The more constant is the water quality and the closer it is to optimum (i.e. to gravity fed pristine spring water) the less energy resources need be diverted toward homeostasis, and greater resource (energy and protein from feed) can be directed toward somatic growth.

To the fish farmer, impaired food conversion efficiency can be a sensitive indicator of stress (Smart 1981). Poor water quality, or even fluctuations in water quality, may cause significant reductions in appetite, growth and food conversion efficiency. Fluctuation in dissolved oxygen content adversely affects feed conversion (Smart 1981). Smart (1981) also reports that elevated CO_2 concentration may accompany the use of groundwater or may occur when water is re-cycled. Affected fish may show not only a depression of appetite, growth or food conversion, but decreased ability to withstand stress associated with normal hatchery procedures.

Variation in water quality also affects the predictive ability of fish farmers. Operational adjustments are already made to accommodate diminished water quality associated with serial use. These adjustments include reduction in fish loads, reduced feeding, and extra water aeration. These adjustments are predictable and accounted for in the method of production. The adjustments are based on modeling and practical experience. Re-circulation of waste water, the quality of which by its very nature would be in-constant, would unpredictably contaminate first use water making management problematic. **No matter what life stage or raceway use, re-circulated waste water would reduce**

production or carrying capacity and quality of fish. It would reduce the over-all carrying capacity of Snake River Farm and would be incompatible with our selective breeding and research program.

D. Re-circulating waste water would reduce dissolved oxygen and pumped water, whether from springs or from the ground could deliver gas supersaturated water. Ground water may also have diminished dissolved oxygen. These will decrease the carrying capacity of the Snake River Farm Complex.

There are a variety of factors that can limit rainbow trout survival, fish performance (e.g. food consumption, efficient feed conversion, and fillet yield) and fish-farm production capacity but dissolved oxygen is most often considered the first limiting factor. This is because the availability of oxygen in water is in finite supply and can be rapidly depleted biologically and chemically.

Oxygen is essential for all types of aerobic life including rainbow trout through all life stages. Oxygen is a terminal electron acceptor in cellular metabolism. Without oxygen the ability to efficiently convert energy into a usable form is curtailed. Thus the aerobic metabolic capacity of a fish is primarily limited by the ability of the gills to extract oxygen from the water. Unfortunately, unlike air that is composed of about 21% oxygen, fresh water contains only a fraction of this in a useable gaseous (O_2) form. Only when oxygen is in its gaseous form can it be used by rainbow trout. The oxygen composing nearly 89 % (by atomic mass unit) of the water molecule (H_2O) is tightly bonded to hydrogen and consequently not available for respiration. Only the dissolved oxygen occurring in molecular "pockets" that exist in the loose hydrogen-bonded networks of water molecules can be used.

The physical chemistry delineating how gases such as oxygen behave in water was established over 200 years ago (Dalton's Law and Henry's Law). Essentially the amount of oxygen or any other gas that can be dissolved in freshwater is a function of the partial pressure of gas (e.g. oxygen) in the atmosphere and its solubility. Solubility is dependent on the partial pressure of oxygen in air above the water and on water temperature. In this case air is composed of 20.95% oxygen and its (oxygen) partial pressure is 159 mm Hg at sea level. At higher altitude the partial pressure of oxygen decreases in proportion to the decrease in barometric pressure (approximately 11.2 per cent per kilometer) (Prosser, 1973). At 3200 ft elevation (approximate elevation of Snake River Farm) the solubility of oxygen in water is decreased about 10% from sea level. The colder the water temperature, the more soluble is oxygen. The maximum solubility of oxygen in pure freshwater occurs at about 0-4° C so that the maximum oxygen content of water at equilibrium with air at sea level is 13.2 mg/L while at 20° C there is only 9.4 mg/L (Prosser 1973) possible. At 15° C and 3200 ft elevation, the maximum dissolved oxygen content of water is 9.0 - 9.2 mg/L. As previously mentioned, the minimum acceptable concentration of dissolved oxygen for rainbow trout is 5-6 mg/L. This means that there is only 3-4 mg/L dissolved oxygen available for fish production. Oxygen gas must diffuse into the water and this occurs optimally at the air-water interface. The solubility of oxygen in water is only about 1/30 of that in air (Nikinmaa and Salama 1998) and 1/8 that of carbon dioxide (CO_2). The rate of oxygen diffusion into water is also quite different from air. The rate of oxygen diffusion is only 1/100,000 of that in air (Nikinmaa and Salama 1998). The amount of dissolved oxygen in water is most often expressed in concentration units (mg/L or ppm). When water is saturated (100%) with dissolved oxygen it means the maximal amount of dissolved oxygen the water can contain is occurring. This is the ideal situation for trout farming and occurs with first use spring water.

Since dissolved oxygen exerts no measurable pressure itself, for fish respiration considerations, the amount of dissolved oxygen is more usefully expressed as tension with the tension of dissolved oxygen in water defined as the pressure of oxygen with which the gas is in equilibrium (Jobling 1994). In practice, the terms partial pressure and tension are used interchangeably. **The end result is that the amount of oxygen in water is far less than in air and from a practical standpoint constitutes the first limiting factor for fish.** The fish gill however capitalizes on the diffusion process (movement of oxygen from an area of higher tension to lower) to capture sufficient oxygen for life. This property has significant implications for re-oxygenation of rearing water in flow-through aquaculture systems and how fish are able to efficiently remove oxygen for respiration.

Whilst the oxygen concentration determines the volume of water that must be pumped over the gills in order for the fish to obtain a given amount of oxygen, the rate at which oxygen will diffuse from the water to the blood will be dependent upon the oxygen tension. Thus, oxygen tension is also an important factor determining the performance of fish (Jobling 1994). The oxygen requirement of fish depends mainly on species, activity and fish size. Sensitivity to oxygen is very species dependent. Salmonid species such as the rainbow trout have been shown to be among the most sensitive to oxygen concentration (Dean and Richardson 1999). Food intake and growth of rainbow trout may become depressed if oxygen concentrations fall below 6-7 mg/L (Jobling 1994). Westers and Pratt (1977) report the minimum (in contrast to the optimum) dissolved oxygen concentration for salmonids is 5-6 mg/L. Smart (1981) considers the minimum dissolved oxygen concentration to be 5 mg/L. Water about to be discharged from the Snake River Farm (Section E) has a dissolved oxygen concentration of 4-5 mg/L.

While there is disagreement among experts as to the practical minimum, there is not disagreement that fish performance is best when the water is saturated with dissolved oxygen. Not only can fish performance as measured by feed consumption be impacted by dissolved oxygen saturation levels, fillet qualities can be impacted. Lefevre et al. 2007) report that fillet yield is greater at 100 % dissolved oxygen saturation or slightly above saturation compared to lower (74%) saturation levels.

What is less clear is whether **variation** in dissolved oxygen content can also have an adverse impact on feed conversion or fish growth. Thus Smart (1981) reports that at least in some production situations such variation causes the conversion ratio to be adversely impacted. Further, while dissolved oxygen above some minimum may not impact feed conversion efficiency, it does impact carrying capacity. The greater the quantity of dissolved oxygen present, the greater is the carrying capacity of the system (Clark 2003). Carrying capacity is the maximum permissible loading rate and loading rate is the weight of fish per water flow unit (Soderberg 1995). Glencross (2008) demonstrated that reduced dissolved oxygen concentration down to a minimum of 5.7 mg/L did not impact feed conversion efficiency but it did decrease feed intake in rainbow trout. **The net result is decreased fish production.**

Carrying Capacity

Knowledge of the oxygen consumption rate of fish allows direct calculation of their water requirements (Soderberg 1995). Willoughby (1968), Liao (1971), and Muller-Fuega et al (1978) all developed empirical formulae for oxygen consumption rates for trout. Westers (2001) summarizes the process stating that there are several ways to express and determine carrying capacity of intensive, flow-through aquaculture systems. Capacity can be expressed as the maximum allowable weight of fish per unit of flow (loading), per unit of space (density), or as maximum production per year. Regardless of how carrying capacity is expressed, it is dependent on the fish's tolerance of rearing water quality and its changes caused by metabolic activity (Westers 2001). Dissolved oxygen, ammonia, carbon dioxide, nitrite and suspended solids are particularly important (Westers 2001). Theoretical estimates of carrying capacity are frequently made (e.g. Klontz 1991; Soderberg 1995; Westers 2001; and Fish Factory 2004) but it is not until fish are placed into production over several years, where all of the vagaries of biology, fish strain, water quality, operations management, feed quality, and pathogens are in place that the actual carrying capacity can be determined. **Pristine, first use spring water contains the maximum possible useful dissolved oxygen so is the preferred water source.**

E. Waste water will have elevated concentrations of carbon dioxide. Ground water could have elevated and or supersaturated levels of CO_2.

Carbon dioxide (CO_2) is a waste product of respiration and is potentially toxic to rainbow trout. Physiological disequilibria may cause upsets in acid-base balance which could have impacts on fish health. As fish respire, CO_2 gas is excreted into the water as it passes over the gills. The physiology of CO_2 excretion is intimately tied to oxygen and the gills. A brief review of CO_2 excretion physiology is appropriate because damage to gills (e.g. bacterial gill disease) or upsets in its excretion impact overall fish health and fish production capacity.

Tufts and Perry (1998), Brauner and Randall (1998) and Henry and Heming (1998) well describe the physiology of carbon dioxide excretion. Basically metabolism produces CO_2 at variable rates that are dictated by aerobic metabolic requirements. In aqueous solution, CO_2 acts as a weak acid, and consequently the processes of CO_2 transport/excretion and acid-base balance are closely linked. To avoid acid-base imbalances, CO_2 production is matched by CO_2 excretion under steady-state conditions. The processes of O_2 uptake and CO_2 excretion share common pathways, are governed by several mutual principles, and are intricately related. As blood arrives at the gill, it contains carbon dioxide predominantly in the form of HCO_3 dissolved in the plasma. Within the transit time through the gill vasculature (approximately 0.5 to 2.5 seconds; Cameron and Polhemus 1974), sufficient HCO_3 is converted to molecular CO_2 and in healthy gills, is excreted at a rate that matches production at the tissues. In a single passage through the gill, approximately 12-35% of blood CO_2 is excreted (Perry 1986). The rapid change from bicarbonate to carbon dioxide appears to be catalyzed by carbonic anhydrase in erythrocytes. The CO_2 then enters the plasma and traverses the gill epithelium by diffusion. CO_2 entering the water is removed physically by ventilatory convection and chemically by hydration to HCO_3 and H^+ within a boundary layer adjacent to the gill epithelium. This conversion appears to be mediated by carbonic anhydrase. The physical and chemical removal of CO_2 from the ventilatory water serves to maintain the diffusion gradients as blood flows through the gill. Factors that can dramatically impair this process include bacterial gill disease and epitheliotropic IHNv infection. In these cases the gill disease causes increased production of mucus that enlarges the boundary layers and increases both the diffusion resistance to oxygen and CO_2 transfer and the resistance to water flow through the gills.

CO_2 in freshwater is nearly 200 times more soluble in freshwater than is oxygen (Wetzel 1975) and obeys normal solubility laws within the conditions of temperature and pressure encountered in lakes and streams. The amount of CO_2 dissolved in water from atmospheric concentrations is about 0.6 mg/L at 15° C. Critical to fish culture is the buffering capacity of the CO_2-HCO_3-CO_3 equilibrium system. Thus waters such as the spring water delivered to the Snake River Farm have an alkalinity that generally resists changes in pH as CO_2 is excreted during intensive fish farming. Indeed, the differential between influent pH and effluent pH at Snake River Farm is generally only about 0.5 units. Nevertheless, CO_2 concentrations can rise above 10 – 20 mg/L which is believed to be toxic to rainbow trout (Oelßner et al. 2002).

Smart (1981) reports that elevated concentrations of free CO_2 are of significance to fish culturists when ground water is used. He also found that in intensive rearing systems employing re-oxygenation, metabolically produced CO_2 will accumulate in the water and would be detrimental to fish.
The harmful effects of CO_2 on fish are well characterized. They include reductions in oxygen affinity and oxygen capacity of the blood (Alabaster et al. 1957; Basu 1959; Saunders 1962). Klontz (1973) has suggested that 12 mg/L CO_2 may be detrimental to growth and 20 mg/L may be lethal.

F. Waste water will have a lower pH than first use spring water. Ground water and spring water proposed for mitigation has an unknown pH.

The pH of water is an important factor in fish production because of direct physiological impacts but also because of the significance pH has on other water quality factors such as ammonia (pH and temperature shifts ammonia between the toxic un-ionized and non-toxic ionized forms). pH also impacts the toxicity of copper (Lauren and McDonald 1985).

Generally a pH of 6.5-9.0 is recommended (Piper et al. 1982) for good physiological functioning of most fish including rainbow trout. Given the total alkalinity (buffering capacity) of Snake River Farm spring water (ca. 150 mg/L as $CaCO_3$), it is unlikely pH alone would be a limiting factor for rainbow trout aquaculture. However, environmental pH affects the toxicity of ammonia, hydrogen sulfide and metals.

G. Waste water will have elevated or reduced water temperature from optimum. The water temperature of the proposed spring water or ground water is unknown.

Temperature is well known to significantly affect the metabolism of poikilothermic animals such as rainbow trout (Prosser 1973). Temperature can impact rates of development (Blaxter 1988), membrane permeability (Alderdice 1988), and oxygen consumption and solubility in water. Temperature has significant impact on chemical equilibria especially that associated with ammonia. In summer months the water temperature of Snake River Farm effluent can exceed 16° C. This temperature could adversely impact embryonic development or at the least would change the rate of embryonic development. Elevated temperature would also increase the amount of un-ionized ammonia which is toxic to all life stages of rainbow trout.

Thermal windows of animals likely evolved to be as narrow as possible to minimize physiological maintenance costs (Portner and Farrell 2008). Thus different species populations may have different optimal and critical temperatures. For example, two populations of sockeye salmon in the Fraser River in British Columbia, Canada have optimal and critical temperatures that differ by 2° to 3° C (Farrell et al. 2008). **Changes in water temperature resulting from re-circulation of waste water or pumped water (from other springs or ground water) could have a significant impact.**

H. Waste water will have elevated total ammonia nitrogen and toxic un-ionized ammonia concentrations which will decrease fish growth and could affect survival. The concentration of ammonia nitrogen and un-ionized ammonia concentrations in the spring and ground water proposed for mitigation is unknown.

Ammonia is the primary nitrogenous waste product of rainbow trout and depending upon its form, may be toxic. Ammonia excretion increases in fish following a meal (Handy and Poxton 1993) reflecting increased production associated with the breakdown of ingested protein. Ammonia is considered by many to be the second limiting factor (after dissolved oxygen) for rainbow trout aquaculture (Fornshell 2002). When ammonia is dissolved in water, a pH and temperature dependent equilibrium is established between un-ionized ammonia (NH_3) and ammonium ions (NH_4^+): $NH_3 + H_2O = NH_4^+ + OH$. Un-ionized ammonia in the water is keenly toxic to rainbow trout while it is the NH_4^+ that is internally toxic. Un-ionized ammonia is freely diffusible across gill membranes into the blood and it is the ammonia form that is excreted from fish across the branchial epithelium (Henry and Heming 1998). Consequently even slightly elevated concentrations of un-ionized ammonia in water will decrease the diffusion gradient, impairing ammonia excretion. Ammonia is 1000 times more soluble in water than is CO_2 (Ip et al. 2001). Elevated ammonia concentration in the water leads to reduced swimming and depressed growth.

The toxicity of ammonium in the fish is due to a multitude of actions (Ip et al. 2001). Ammonium causes muscle depolarization and interference with energy metabolism through impairment of the tricarboxylic acid (TCA) cycle, inhibition of key enzymes including isocitrate dehydrogenase, α-Ketoglutarate dehydrogenase and pyruvate dehydrogenase. Ammonium affects ionic balance reducing Na^+ influx and K^+ loss through substitution of NH_4^+ for K^+ in Na^+, K^+ ATPase. Ammonium acts on the central nervous system causing hyperventilation, hyperexcitability, coma, convulsions and ultimately death. Unfortunately most studies dealing with ammonia toxicity of fish are conducted on starved, resting and stress free animals under static conditions. In aquaculture, fish are likely more sensitive to ammonia toxicity during feeding when oxygen would be lowest, and because of the general elevation of stress due to crowding and other water quality factors (Ip et al. 2001).

High energy-high protein diets currently fed to fish (including at Clear Springs Foods Snake River Farm) in intensive culture systems result in high levels of ammonia as the principal nitrogen-containing excretory product so that where water is re-circulated without any treatment, toxic ammonia levels will build up (Munro and Roberts 1989). A safe level of un-ionized ammonia, where no other physiological stresses exist, is 0.02 mg/L (Munro and Roberts 1989; Westers 2001).

Smart (1981) suggests that because water flow is usually sufficiently high ammonia in serial use systems is rarely a problem unless the farm is receiving ammonia-polluted water or there is water re-cycling. The Ground Water Districts propose to re-cycle waste water from the Snake River Farm which is unacceptable.

I. Waste water could have elevated nitrite concentrations which would be hazardous to trout survival. Spring or ground water could have elevated nitrate or nitrite concentrations.

Nitrite causes a functional anemia in which the oxygen-carrying properties of hemoglobin are blocked. Nitrite and other oxidizing agents convert functional hemoglobin to methemoglobin, which does not bind oxygen (e.g. Jensen et al. 1987; Jensen 1990). This decreases the oxygen capacity of blood markedly which reduces oxygen transport to the tissues. Under these circumstances less than 100 % oxygen saturation in the water may be very detrimental as would even low levels of CO_2 (Hughes 1981).

At the Snake River Farm progressively increasing concentrations of nitrate-nitrite nitrogen have occurred in spring water since 1990. In one particular spring, the concentration has increased to over 13 mg/L. The maximum contaminate level for nitrate nitrogen in Idaho is 10 mg/L (IDAPA 58.01.11.200.01). While the source of nitrate-nitrite nitrogen in the spring water feeding the Snake River Farm complex is unknown, its occurrence has increased fish production concerns for Clear Springs Foods. Rainbow trout actively take up nitrite and chloride ions as part of their osmoregulatory efforts. This allows nitrite to be concentrated in the plasma with respect to the environment and magnifies the toxic effects of nitrite in water (Westers 2001). In addition to diminished oxygen carrying capacity of the blood, nitrite toxicity is associated with necrosis of trout retina (Hofer and Gatumu 1994). Nitrate in water can also be detrimental to salmonid egg survival. Kincheloe et al. (1979) demonstrated that concentrations of nitrate at 5-10 mg/L was toxic to developing eggs and early fry stages of rainbow trout. **Use of waste water, spring water or ground water with increased nitrite or nitrate concentration would be unacceptable to Clear Springs Foods.**

J. Waste water will have elevated concentrations of total suspended solids (TSS) which will stress all life stages of trout. This is unacceptable to Clear Springs Foods.

Total suspended solids (TSS) is composed of uneaten fish feed and fecal material at Snake River Farm. Influent spring water has no detectable TSS. When solids settle to raceway bottoms and in quiescent zones it contributes to biological oxygen demand and potential areas of anoxia and hydrogen sulfide production (Colt and Tomasso 2001). Because TSS at the Snake River Farm is by nature organic, it also fosters increased bacterial loads in the water column. Such loads arguably increase the prevalence of bacterial gill disease. TSS is most frequently associated with interference with gas exchange but can also abrade gill tissue causing inflammation. Coughing is a response to such irritation but may also precipitate upon gill surfaces and interfere with the diffusion of oxygen into the blood (Herbert et al. 1961).

K. Waste water will have higher loads of fish pathogens. Pathogens such as IHNv are not present in spring water. Unsecured, unprotected spring water could have foreign pathogens and could have common pathogens which would expose naïve fish to significant threat. Ground water containing foreign or opportunistic pathogens would expose naïve fish to threat. This is unacceptable to Clear Springs Foods.

<u>Pathogens typically impacting commercial culture of rainbow trout.</u>

Groff and LaPatra (2000) identify the various pathogens that are most likely to impact intensive rainbow trout aquaculture. They also discuss the epizootiology, pathogenesis, diagnosis, and treatment and prevention of the diseases associated with the pathogens. Of the common bacterial pathogens they identify *A. hydrophila, A. sobria, A. caviae and*

A. salmonicida, F. columnare, F. psychrophilum, and F. branchiophilum, Yersinia ruckeri, and *Renibacterium salmoninarum.* The cause of strawberry disease remains uncertain but has most recently been associated with a rickettsial agent (Lloyd et al. 2008). Of the bacterial diseases most problematic in commercial trout aquaculture in the 1000 Springs area, coldwater disease (caused by *F. psychrophilum*) and bacterial gill disease associated with *F. branchiophilum* are most problematic.

F. psychrophilum appears to be wide spread in freshwater environments and is endemic in freshwater salmonid facilities (Groff and LaPatra 2000). The bacterium has been isolated from the external surfaces of salmonids (Holt et al. 1993). Cutaneous lesions may predispose fish to infection and subsequent disease. Juvenile fish have increased susceptibility to disease. Transmission is horizontal and can occur either directly from fish to fish or **indirectly through the water** (Holt et al. 1993). Disease is most severe at 15° C (Groff and LaPatra 2000). Coldwater disease often occurs in association with IHN disease (LaPatra 2003). Infection with *F. psychrophilum* is not only associated with mortality and morbidity but may cause significant deformity (LaPatra 2003). Deformity of rainbow trout results in quality downgrading in the market or is a complete loss.

Bacterial gill disease is most frequently associated with *F. branchiophilum* although other environmentally common bacteria may be isolated as well (Ferguson 1989). *F. branchiophilum* causes chronic morbidity with low mortality in rainbow trout although mortality may approach 25% (Speare et al. 1991). The primary economic impact of the disease is due to the chronic morbidity that results in reduced feed conversion and, consequently, reduced growth rate (Groff and LaPatra 2000). The bacteria are transmitted horizontally through the water. Disease is generally associated with poor environmental quality such as increased turbidity, ammonia concentrations, and density or decreased dissolved oxygen concentrations (Turnbull 1993). BGD is associated with chronic hyperplasia of secondary lamellar epithelium (Ferguson et al. 1991; Turnbull 1993). Fusion of adjacent primary filaments may occur with severe disease. Morbidity and mortality are most probably due to compromised branchial respiration and osmoregulation (Groff and LaPatra 2000). As with most diseases of trout there are few remedies. The general strategy is to prevent BGD by ensuring optimal environmental quality is maintained and to reduce stress (Groff and LaPatra 2000). The use of copper sulfate and potassium permanganate is common but this result's in only temporary relief. The use of copper sulfate is a compromise decision between its therapeutic efficacy and its potential toxicity. Copper is known to adversely impact osmoregulation and ammonia excretion (Lauren and McDonald 1985). **There are no FDA approved treatments specific for BGD.**

Of the viral pathogens likely to impact rainbow trout aquaculture, IHNv has the greatest impact and will be reviewed here. IHNv is a rhabdovirus that is endemic in the 1000 Springs area (LaPatra 2003). Mortality associated with IHNv can be very high (up to 80%; LaPatra 2003). The primary reservoir of infection is carrier salmonids (Wolf 1988). Viral transmission can occur directly and indirectly. IHNv is known to be present in fish mucus (LaPatra et al. 1989) of naturally infected rainbow trout. Fish with clinical disease have high titers of virus in the feces, urine, and mucous that will facilitate viral transmission during epizootics. The virus is stable in freshwater and can remain infectious for several months. The virus enters trout via the gills and ingestion (Drolet et al. 1994). Severe disease generally occurs in juvenile and subadult fish less than two years old with the highest mortality in fish less than six months of age (Wolf 1988). Temperature appears to be a significant modifying factor with warmer temperatures (above 15.5 C) reducing problems (LaPatra 1998). Life stage also appears to be a significant factor. Thus the hematopoietic form of infection occurs typically in the youngest, most naïve life stage (LaPatra et al. 2008). The neurotropic form occurs most often in more mature fish and the epitheliotropic form occurring on the gills most often occurs in much larger fish. **Treatment in all cases is by avoidance of the virus. Waste water will contain IHNv and its use in any raceway is unacceptable to Clear Springs Foods. Use of spring water containing sources of IHNv is unacceptable to Clear Springs Foods.**

Infectious pancreatic necrosis virus (IPNv) is a birnavirus that can cause disease in a variety of salmonids. Rainbow trout are among the most susceptible of salmonids (Groff and LaPatra 2000). Like IHN and some of the bacterial pathogens, the reservoir of infection is other salmonids, i.e. rainbow trout. Transmission is directly from contact with infected fish or indirectly through the water. Carrier fish can shed virus indefinitely in the feces and urine. All ages are susceptible to infection although clinical disease generally occurs in fish less than six months of age (Wolf 1988; Traxler et al. 1998). Mortality can be rapid and severe. **Treatment is by avoidance. Re-circulation of waste water would spread IPNv and is unacceptable to Clear Springs Foods. Use of spring water containing sources of IPNv is unacceptable to Clear Springs Foods.**

A variety of parasites can infest rainbow trout but the most problematic is due to the protozoan *I. multifiliis*. Infestation by *I. multifiliis* (Ich) or white spot can cause significant morbidity and mortality. This particular parasite invades into various epithelial surfaces such as the skin and gills causing osmoregulatory challenges for the fish (ref). As long as water flows are sufficient, the parasite while present is not likely to reach critical concentrations. **Treatment is by avoidance** and occasionally copper sulfate may be used. **Re-circulation of waste water would spread *I. multifiliis* and is unacceptable to Clear Springs Foods. Use of spring water containing sources of *I. multifiliis* is unacceptable to Clear Springs Foods.**

Ichthyophonus hoferi, a mesomycetozoaen parasite (Mendoza et al 2002), also occurs in rainbow trout at the Snake River Farm. The epizootiology of this parasite is poorly understood but pathologically can occur in a variety of tissues especially the heart. The consequence is that rainbow trout infected by *I. hoferi* have reduced swimming stamina (Kocan et al. 2006). It does occur most often in larger fish. **Use of re-circulation waste water or other spring water containing this parasite is unacceptable to Clear Springs Foods.**

Bacterial gill disease (BGD) is a particularly serious problem of intensive salmonid culture (Ferguson 1989). All of the conditions predisposing to the disease are not known although overcrowding is thought to be a significant factor. The disease is characterized by filamentous bacteria on the gill surface of fish suffering clinically from respiratory distress (Ferguson 1989). Adverse environmental and disease conditions (elevated TSS, diminished oxygen, elevated CO_2 or ammonia, epitheliotropic IHN virus infection) and other factors appear to pre-dispose to BGD. **Introduction of re-circulated waste water to early life stage rearing areas is likely to increase the prevalence and severity of BGD and is unacceptable to Clear Springs Foods.**

Flavobacterium spp. Are commonly recovered from affected gills. With time there is lamellar fusion with entrapment of debris, obliteration of interlamellar spaces and frequently mucous metaplasia. *Flavobacter columnari* can also occur on the gills causing severe wide-spread necrosis of all gill elements.

L. Waste water will contain fish disease treatment drugs and chemicals which will expose all fish to potential toxicity and potential contamination. This is unacceptable to Clear Springs Foods.

Copper sulfate is a parasiticide used to treat Ich, other external parasite infestations and to help manage bacterial gill disease. While it can be effective it is also detrimental. Exposure of rainbow trout to copper at 12.5 to 200 ppb for 12 to 24 hours causes osmo-regulatory problems (Lauren and McDonald 1986). Apparently copper exposure affects tight junctions between cells allowing Na^+ and Cl^- to more readily diffuse across the gill epithelium. Copper treatment has also been found to adversely impact ammonia excretion in trout (Lauren and McDonald 1986). Wootten and Williams (1981) report that a $CuSO_4$ treatment of 0.5 mg/L for 1 hr caused changes in various hematological parameters and serum enzyme levels. These effects lasted at least for 24 hrs. Serum enzymes elevated included lactate dehydrogenase (LDH), hydroxybutyric dehydrogenase (HBDH), glutamic oxaloacetic transaminase (GOT) and gultamic pyruvate transaminase (GPT)- all enzyme elevations associated with liver damage. **Use of Snake River Farm waste water in a pump-back process will expose healthy fish to copper sulfate thereby causing toxicity and diminishing growth potential.**

Because pump-back of effluent would increase the prevalence and severity of gill disease, it is likely increased use of copper sulfate would be required. Even if the copper was effective, it would increase osmo-regulatory and ammonia excretion problems thereby diminishing fish production capacity. If pumped spring water or ground water were to fail to be delivered, environmental conditions would occur for even short times that would likely increase the prevalence and severity of gill diseases thereby necessitating increased use of copper. Increased copper use causes increased osmoregulatory and ammonia regulation problems which diminishes fish growth and farm production.

Potassium permanganate ($KMnO_4$) is an oxidizing agent used to manage bacterial gill disease. It oxidizes organic matter including bacteria on gills. Use of pump-back water during times of $KMnO_4$ use would expose healthy fish to this chemical potentially adversely impacting their sensitive respiratory surfaces, their feeding response and growth potential. It would expose eggs and very sensitive young fish to this chemical.

Various antibiotics are used to treat certain fish diseases. These antibiotics are mixed in feed and fed to affected populations for 7-10 days. During that time antibiotic contaminated TSS and solids are generated. Use of waste water in a pump-back procedure would contaminate healthy fish including those near harvest. There are no rapid tests for antibiotics in farmed fish so fish could potentially be marketed that are contaminated with antibiotics. **This would not be acceptable to Clear Springs Foods.**

M. Waste water will contain hormones released by female trout that could impact the developmental physiology of eggs and fry, and could alter growth characteristics of early life stages. Use of waste water is unacceptable to Clear Springs Foods. Spring and ground water could also contain hormones or endocrine disrupting compounds. Spring or ground water containing hormones or endocrine disrupting compounds would not be acceptable to Clear Springs Foods.

The impact of environmental, hormonally active substances on the endocrine and immune system of fishes is only beginning to be studied yet appears significant (Segner et al. 2006). Rainbow trout are known to release chemical cues to con-specifics through the gills and in the urine. These chemical cues are typically water soluble and include alarm pheremones, steroids and prostaglandins. The rainbow trout releases urine borne pheremones in bursts and these can have physiological impacts on other rainbow trout including on the immune system, growth, and cross-talk between different endocrine systems. These substances may modulate growth and disease. Stress itself may cause the release of stress hormones that may in turn elicit components of the stress response in non-stressed fish (Toa et al. 2005). The stress response has been clearly shown to be disadvantageous for somatic growth and thus considerable management effort is directed at minimizing stress. **Waste water that contains stress hormones and used in place of spring water would be unacceptable to Clear Springs Foods.**

The elevated nitrate-nitrite nitrogen detected in some of the incoming spring water entering the Snake River Farm complex has raised concern that other compounds may also be present. Among these compounds are hormones and endocrine disrupting chemicals. Hormones conceivably could include those derived from cows. **Spring or ground water containing such hormones would be unacceptable to Clear Springs Foods.**

V. Research requirements for first use spring water

The Research and Development Division, which includes the Snake River Brood Station, at Clear Springs Foods conducts primarily applied research focused on optimizing fish production and ensuring consistent, high quality trout are produced economically and in an environmentally responsible manner. Work areas include environmental science, fish health management, fish culture, reproduction and breeding, and fish nutrition. The Division also serves as a major service organization for the company. These work areas include water quality analyses that are conducted according to "Good Laboratory Procedures" including a quality assurance program to satisfy water discharge requirements specified by the US Environmental Protection Agency. Feed ingredients and finished feed and food fish products are monitored for their nutritional qualities. Clinical diagnostics are conducted on juvenile fish from Operations and spawning adult fish are routinely monitored for pathogens that could be detrimental to egg production. New adult rainbow trout with enhanced genetics produced at the Division through the selective breeding program are certified specific-pathogen-free before they are allowed entry into the egg production program. Additionally, vaccines are produced and tested used on juvenile and adult fish that are owned by Clear Springs.

The Research and Development Division is a state-of-the-art facility not equaled anywhere else in the world for a single private aquaculture company. Research faculty consists of two PhD and two MS scientists along with 13 technical and administrative staff. The dry laboratories consist of sophisticated scientific instrumentation used in medical research, microscopy, clinical chemistry, immunology and analytical chemistry. The wet laboratory consists of a specific pathogen free room for future broodstock and selective breeding of about150 families annually and a specific pathogen infected room for fish health research. A fish rearing facility (1/3 operations size) completes the Research and Development Division but the Division also serves as an important egg production facility for Farm Operations primarily during the spring and summer months. Clear Springs Foods has a significant economic investment in and need for this Division.

The Research and Development Division currently uses about 37 cfs of first-use spring water originating from the Eastern Snake Plain Aquifer. Over time the quantity of water devoted to this operation has diminished because of declines in spring water flow and the need to provide sufficient water flow to the Snake River Farm for fish production purposes. The Research Division could use more water if it were available. Use of pump-back water is infeasible for the same reasons it is not feasible in the Snake River Farm itself- waste water has pathogens and decreased water quality which would cause production failure, significantly reduce the utility of the water and eliminate its use for research purposes. Spring water however has constant temperature (15° C), has a high dissolved oxygen content (9.2 ppm) and is virtually pathogen free which are all critical water quality parameters that must be maintained to ensure success of all of the Division's programs. Mitigation of flows to this facility by pumping back water from our Snake River Farm after passing through five successive production raceways where fish are continually fed and grown and occasionally get sick would significantly jeopardize the research and service activities described above. Currently there are over 12,000 adult rainbow trout at this facility that provide eggs for the company in the spring and summer months. Within these adult populations are the 4,000 selected adults which possess the most superior genetic stock of the Clear Springs strain of rainbow trout. Each fish is tagged with a small computer chip for individual identification and this code corresponds to the entire pedigree of that particular animal. In addition to these fish there are 16,700 yearling rainbow trout with enhanced genetics produced through the selective breeding program which must be certified specific-pathogen-free before they are allowed entry into the egg production program on an annual basis. Additionally, 13 million eggs are produced annually by the Division that must be specific-pathogen-free before they are shipped to our production facilities.

Besides not being pathogen free, if fish were reared or incubated in water contaminated with the Snake River Farm's effluent or Clear Lake, many of these young fish and eggs would not survive or would be significantly compromised. Additionally, the research activities that go on in the Research and Development Division require pristine spring water or the results are not valid and will not be reproducible because of the extreme variability of effluent water after it has passed through the Snake River Farm. Selected breeding evaluations, nutritional studies for sustainable aqua-feeds, and the development of vaccines for enhanced animal welfare would no longer be possible. In summary mitigation of spring water flows to the Clear Springs Foods Research and Development Division with water from our Snake River Farm after passing through five successive production raceways where fish are continually fed and grown would have a significant economic impact on the production of valuable fish and eggs. **Additionally it would significantly compromise the capability of the company to conduct economically viable research. The Division's activities and personnel would**

have to be significantly reduced if this type of water flow mitigation were put into place.

The use of pumped spring or pumped ground water at Research and Development is also infeasible. Experiments, brood stock selection, and production of eggs are all highly dependent on continuous water flow. Pumped water, subject to unpredictable curtailment is unacceptable to Clear Springs Foods.

Water from the Snake River Brood program is subsequently used at the Snake River Farm. It is used for some early life stage grow-out occurring in the A section of the farm. While the quality or growth potential of this water is not as good as pristine spring water, we nevertheless accept the water quality compromise because otherwise there is insufficient water flows for production expectations and because the utility of the water is still good. We do not want to waste the quality of water that still exists. Water for brood stock and eggs produced at the Snake River Brood Station is not used as intensively as would occur at the Snake River Farm itself. It maintains a substantial growth potential, does not usually contain dangerous fish pathogens such as IHNv and would not contain treatment drugs.

VI. NPDES Permit

The Federal Water Pollution Control Act, also known as the Clean Water Act (CWA, 33 U.S.C. § 1251 et seq.) is a comprehensive water quality statute designed to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters" (33 U.S.C. § 1251(a)). In order to meet this objective, the CWA imposes certain obligations on the federal Environmental Protection Agency (EPA) and state environmental protection agencies (Idaho Department of Environmental Quality; IDEQ). EPA must establish technology-based standards for discharges from point sources, including fish farms, to waters of the United States. These technology-based limits are imposed through National Pollutant Discharge Elimination System (NPDES) permits (33 U.S.C. §§ 1311, 1314, 1342). In Idaho the NPDES permits are issued by EPA. While EPA is required to set technology-based limits, Idaho, as with all other states, are required to adopt Water Quality Standards (IDAPA 58.01.02). The Water Quality Standards consist of designated uses of state waters, water quality criteria to protect those uses, and an antidegradation statement (33 U.S.C. § 1313c: 40 C.F.R. § 131.6). NPDES permit limits are consistent with State WQS. The Snake River Farm discharges to Clear Lakes which in turn discharges to the Snake River. These water bodies are both designated for cold water biota and primary recreation (IDAPA 58.01.02.140). In addition, the Snake River is designated for salmonid spawning. The water quality criteria developed for these designations are as follows:

Cold water biota: dissolved oxygen needs to exceed 6 mg/L at all times; the maximum daily average temperature must be 19° C or less; the ammonia concentration is dependent on temperature and pH.

Salmonid spawning: dissolved oxygen needs to be greater than 6 mg/L or 90% of saturation; the water temperature must be 13° C or less with a maximum daily average of 9° C; the ammonia concentration is dependent on temperature and pH.

To determine if a water body meets beneficial uses, IDEQ relies on a Water Body Assessment Program and has developed a River Fish Index. The Index measures the biological integrity of a water body based on fish assemblages. If a water body is meeting the cold water biota or salmonid spawning beneficial use, it will have a species composition, diversity and functional organization comparable to that of the natural (undisturbed by man) habitats of the region. For cold water biota the fish assemblage consists of salmonids, sculpin, sucker and dace. The water quality criteria would be consistent with habitat in which this particular assemblage occurs.

A commercial trout farm is not intended to mimic the natural habitat or circumstances for which cold water biota or salmonid spawning conditions would occur. Commercial trout farms raise a single species of fish (i.e. rainbow trout) at far higher densities than would occur in their natural habitat. Farmed fish are fed a nutrient dense-high energy diet very different than what is available in the natural habitat. Farmed trout have been domesticated for over 100 years. At Clear Springs Foods, a selective breeding program has selected fish for peak performance in first use spring water. Farmed fish are raised under far more stressful conditions than occurs in natural habitats. Consequently water quality requirements are considerably different. Indeed, the water quality requirements for farmed fish are more stringent than for wild fish. Mere compliance with the water quality requirements stipulated in the NPDES permit would not suffice for intensive commercial production of rainbow trout as occurs at the Snake River Farm complex. The carrying capacity of the farm would be significantly reduced if only NPDES permit requirements were fulfilled.

VII. The process of drilling a well as proposed so near to the Snake River Farm production facility may adversely impact fish behavior and compromise fish growth and health.

The impact of noise or music on fish performance has only recently been explored. In recently published research, Papoutsoglou et al. (2008) demonstrates that certain types of music (Mozart, K525) had a positive stimulative impact on gilthead seabream raised in a re-circulation system. Whether Mozart merely masked adverse sounds associated with pumps and filters used for re-circulation aquaculture is unknown. However, regardless of the reason, sound does appear to have an impact on fish. **Clear Springs Foods is opposed to drilling near our Snake River Farm where potential sounds could have an adverse impact on ongoing fish production. We also are opposed to noise from pumps used to deliver water from any source because of its probable negative impact on fish production.**

VIII. Pumped water of any kind is subject to interrupted delivery and to gas super-saturation. Fish, whether for research, brood stock, or food production require un-interrupted water delivery 24 hrs/day, 7 days/week, 365 days/year. Water used for early life stages and production must not be supersaturated with gases that cause gas bubble disease or trauma.

In air the partial pressures of nitrogen and oxygen are 0.78 and 0.21 respectively (Munro and Roberts 1989). With pumped water supplies, air and water may be drawn into the pump together so that the air is compressed by the pump, resulting in greater solution of oxygen and nitrogen. Additionally, ground water would likely contain gases at higher pressure than surface water. Ground water is typically not saturated with oxygen, but is supersaturated with nitrogen and may have high levels of carbon dioxide (Munro and Roberts 1989; Batzios et al. 1998). Once drawn up to higher elevation some gas will come out of solution leading to gas bubble disease in all life stages of rainbow trout. Gas bubble disease occurs under supper-saturation conditions when gas, typically nitrogen, accumulates in the blood stream of fish (similar to the bends in SCUBA divers). The bubble blocks blood flow causing focal and disseminated necrosis. Advanced sac-fry and newly "buttoned-up" fry will develop visible bubbles in the body cavity, pressing the yolk sac out of the way or opening the seam on newly "buttoned-up" fry (Wood 1968). Gas bubbles that occur in fingerling, yearlings and adults often lead to blindness and death. Distress and ultimately death follows. The economic consequences of gas bubble disease can be significant (Batzios et al. 1998).

IX. Recirculation aquaculture is subject to catastrophic failure, is very expensive, causes bio-accumulation of drugs and has water quality problems.

Recirculation aquaculture systems are subject to catastrophic fish losses (Masser et al. 1999; Sumerfelt et al. 2001, Ismond 1996; Lee 1992; and Summerfelt et al. 2004). Additionally they are complex because they require extensive water treatment, space consumptive to house the treatment system, and are expensive.

X. Ground water may be chemically different than current spring water, could contain elevated arsenic and other chemicals, and be saturated with gases that could cause gas bubble disease.

The ground water proposed for mitigation purposes is of unknown character. We do know from Clear Springs Foods own processing plant well that ground water quality may have significantly different physico-chemical differences from the spring water currently received. The water temperature of the ground water may be significantly warmer and arsenic concentrations significantly higher. We do know that rainbow trout are sensitive to toxicity from chronic arsenic exposure (Kotsanis and Illiopoulou-Georgudaki 1999). **It is unacceptable to Clear Springs Foods to use mitigation ground water whose physico-chemical character is significantly different from currently used surface water.**

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EXHIBIT "1"

Curriculum Vitae

John R. MacMillan
Clear Springs Foods, Inc.
P.O. Box 712
Buhl, Idaho 83316
208-543-3456
randy@clearsprings.com

Education

B.Sc. University of Maryland, College Park, Maryland, USA. 1974. Conservation and Resource Development.
M.Sc. Michigan State University, East Lansing, Michigan, USA. 1976. Fishery Biology.
Ph.D. University of Washington, Seattle, Washington, USA. 1980. Fish Pathology.

Professional Experience

1998-present. Vice President of Research and Environmental Affairs. Clear Springs Foods, Inc., Buhl, Idaho, USA. Responsible for all research, environmental management, regulatory affairs, and quality assurance (seafood safety) for large, vertically integrated seafood (farm raised rainbow trout) company.

1990-1998. Director of Research and Development. Clear Springs Foods, Inc., Buhl, Idaho, USA.

1985-1990. Associate Professor of Veterinary and Aquatic Animal Medicine. College of Veterinary Medicine, Mississippi State University, Starkville, Mississippi, USA.

1982-1985. Area Extension Fisheries Specialist. Mississippi State University, Stoneville, Mississippi, USA.

1980-1982. Senior Research Fellow. Department of Pathology, School of Medicine, University of Washington, Seattle, Washington, USA.

1976-1980. Research Microbiologist. United States Fish and Wildlife Service. Seattle, Washington.

Ancillary Experience

2007-present. Comprehensive Aquifer Management Plan (CAMP) Advisory Committee. Idaho Governor Appointment as Spring representative.

1999-present. President, National Aquaculture Association.

2000-present. Idaho Board of Environmental Quality. Vice-chairman (2002-2004) and Chairman (2004-2006). Appointment by Idaho Governor and confirmation by Idaho Legislature.

2007. Testified before US House of Representatives Committee on Natural Resources; Subcommittee on Fisheries, Wildlife and Oceans. National Offshore Aquaculture Act of 2007.

2006. Expert. Joint FAO/WHO/OIE Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance. Seoul, Republic of Korea, June 13-16, 2006.

2006. Testified before US Senate Committee on Science, Commerce, and Transportation; National Ocean Policy Subcommittee. Senate Bill 1195.

2000-2005. Minor Use Minor Species (MUMS) Coalition. Chairman (2000-2005). Successfully passed through US Congress the Minor Use and Minor Species Animal Health Act of 2004.

2000-2004. United States Department of Agriculture (USDA) Aquaculture Effluents Task Force. Member.

2000-2002. American Academy of Microbiology. Colloquium steering committee "The role of antibiotics in agriculture (2002).

2001-present. Alliance for Prudent Use of Antibiotics (APUA). Facts About Antibiotics in Animals and Their Impact on Resistance (FAAIR II). Scientific expert.

2000-2001. Chairman, Joint National Association of State Aquaculture Coordinators-National Aquaculture Association Committee on National Aquatic Animal Health Management Plan Development.

1995-1997. President United States Trout Farmers Association.

1994-1995. President Idaho Aquaculture Association.

1992-1993. President, American Fisheries Society, Fish Health Section.

1990-2008. Joint Subcommittee (FDA-USDA) on Aquaculture, Quality Assurance in Aquaculture Working Group.

Honors, Awards, and Certifications

FDA Commissioners Award, 2005.
AFS/FHS Board Certified Fish Pathologist. 1985-present.
U.S. Jaycees Outstanding Young Man of the Year. 1982.
Raymond J. Huff Memorial Scholarship. 1978, University of Washington.
E.P.A. Scholarship. 1974-1976. Michigan State University.
Eagle Scout. 1968.

Publications

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EXHIBIT "2"

History of Aquaculture in Idaho

The artificial propagation (production of young) of fish began in ponds in China about 3000 years ago but it was not until market demand for seafood significantly increased (sometime after 1950), and the application of scientific methods and technology development occurred that commercial aquaculture (fish grow out at high density) became feasible in Idaho and globally. In Idaho, this conjunction occurred in late 1950-1965. A time line of rainbow trout aquaculture follows.

1700-1790: over-fishing, pollution and dams deplete various wild species of fish in US and in Europe. This creates demand for wild fish stock replacement.

1790-1850: Fish culture becomes well established in Western Europe, the Balkans, and in Scandinavia. Fry for culture are captured from the wild and used for re-stocking in public waters.

1853: First artificial propagation of brook trout occurs in the US (Theodatus Garlick and H.A. Ackley) in Ohio. Trout feed consists of boiled lean meat, egg yolk, liver, heart, and clabbered milk. Maggot factories established (meat and entrails suspended over fish ponds) to feed fish.

1866-1870: Brook trout, Atlantic salmon, American shad, whitefish, lake trout, and yellow perch successfully propagated and cultured. All fish raised for stocking in public waters.

1870: Fish culture practiced in 19 of 37 states plus territories of Colorado and Kansas. State Fish Commissions culture designed for restoration of fishery resources. Foundation of fish culture well established for fishery conservation.

1870-1950: Fish diets continue to be composed of ground meat (horse, cattle, and carp) particularly liver, heart and spleen.

1909: First commercial fish farm in Idaho at Devil's Corral Spring near Shoshone Falls. Farm closed one year later in 1910 presumably because there was no fish market.

1915-1930: Warren Meader pioneers rainbow trout brood stock and egg production at farms near Pocatello (Papoose Springs) and Soda Springs (Caribou Trout Farm later sold to Clear Springs Trout Co. and renamed Soda Springs).

1919: Frame Trout Farm in Twin Falls opens. Farmed continuously until 1973.

1920: Snake River bottomland opened to homesteading allowing land below the Snake River Canyon rim to be developed, thus allowing for fish farm development at headwaters of springs. In the late 1920's Burt Perrine, son of L.B. Perrine, began raising trout in the Snake River Canyon near Twin Falls at a site close to the current Blue Lakes Trout Farm. This farm became Royal Catfish Industries and operated until 1975. Also in the 1920's, the Southern Idaho Fish & Game Association (a sportsman's club) began construction of a hatchery in Rock Creek Canyon. The club leased the facility to the Idaho Fish & Game in 1931, and then to the College of Southern Idaho in 1976. CSI now uses the farm for its aquaculture training program.

1928: Jack Tingey starts Snake River Trout Company (located at the current location of Clear Springs Foods Snake River Farm). Farm consists of earthen ponds. Ted Eastman and Percy Greene employed by Jack Tingey. Tingey's vision was to develop a trout farm dedicated to producing food fish. He was successful in developing fresh trout markets as far away as Chicago where he shipped product with ice departing from Shoshone on the old REA rail system.

1930-1933: Hagerman National Fish Hatchery (USFWS) built for conservation fishery.

1932: In response to Idaho Power's filing on all springs in the Hagerman Valley, the "1932 Decree or New International Decree" resolved water rights for those people who settled the area and claimed water rights since the late 1800's from Billingsley Creek, Riley Creek and various springs. This decree also established that the common source of water for this area was the underground aquifer generally to the east of Hagerman. Many of these properties would later expand the beneficial use of their water to include fish propagation on small farm ponds when technology advanced to the point that aquaculture became profitable through the "Clear Springs Farm Pond Program".

1935: Percy Greene establishes Greene's Trout Farm on south side near Twin Falls.

1930-1940: Joint research conducted at a New York State laboratory and Hagerman's Tunison lab (now the University of Idaho Hagerman Experiment Station) developed dry feed formulations that replaced those originally made from decaying animal carcasses. In the early 1940's, dry diets were first tested at Tupper's Trout Farm in Hagerman.

1938: George Isaac purchases Caribou Trout Farm for trout egg production from Warren Meader.

1940: IDF&G acquires Tucker Ranch property for Hagerman State Fish Hatchery and Wildlife Management Area. Thirteen ponds for bass, bluegill and catfish were constructed by 1942. First IDF&G trout hatchery building built by 1942 with full construction completed by 1949.

1946: Art Wylie establishes Canyon Trout Farm on Rock Creek. Ted Eastman returns from WWII again finding employment with Jack Tingey and then with Bob Erkins at Snake River Trout Company.

1948: Earl Hardy and Al Iverson establish Rainbow Trout Farm at head of Cedar Draw (now part of Idaho Trout Company).

1949: Rangen Inc., founded in 1925, starts its Aquaculture Division, providing high quality dry diets based on formulations developed by the Tunison lab in Hagerman. Food conversion ratios drop from 5:1 using carcasses to present efficiencies of 1.25:1 using dry feeds.

1950- present: Selective breeding of rainbow trout for growth in flowing water culture conditions begins in Washington.

1951-1952: Rimview Trout Company started near Niagara Springs by Milford Schmekpepper. Ralph Nelson starts Crystal Springs Trout Farm near Niagara Springs.

1952: Rainbow trout aquaculture starts in Great Britain. Bob Erkins purchases Snake River Trout Company from Jack Tingey. Eventually changes name to 1000 Springs Trout Company.

1953: US Trout Farmers Association formed to enhance communication and technology transfer throughout the United States.

1956: Snake River Trout Company builds first local processing plant- previous processing capacity in area very limited. Automated processing equipment installed thereafter. Blue Lakes Trout Farm built by Percy Greene and Stan Miller. A processing plant was added to Rainbow Trout Farm (now Idaho Trout Company).

1958: David Haskell (New York Fish Conservation Department) establishes scientific principles of flowing water fish culture. Definition of chemical and biological parameters affecting fish in confinement takes fish culture from art to science.

1960: Al Dunn purchases Caribou Trout Farm from George Isaac.

1962: Rangen Inc.'s Research Hatchery established. Notable research accomplishments include: development in mid-1980's of a stable form of Vitamin C now included in all aquatic animal feeds world wide (Rangen sold the formula to Hoffman-LaRoche); collecting efficacy data in mid-1990's to support FDA approval of BASF's pigment canthaxanthin (dietary pigment that turns trout and salmon flesh red); collecting manufacturing data in late 1970's to support FDA approval of the first of only 3 medicated feeds ever to be approved for fish; fish vaccine development; and feed product and ingredient testing.

1964: Idaho Trout Company builds new processing plant in Filer.

1965: Rainbow trout market demand spurs growth of trout industry in California, Colorado, Montana, Missouri, Wisconsin, West Virginia and North Carolina.

1966: Clear Springs Trout Company formed (Ted Eastman President). Clear Springs Trout Company builds Clear Lake Farm. Earl Hardy acquires trout farm at the Clear Lake site.

1966-1979: Clear Springs Trout Company successively builds and expands seafood processing plant at current location.

1968: Norman Standal starts building ponds for Whitewater Farm.

1969: Clear Springs Trout Company purchases Crystal Springs Trout Farm (near Niagara Springs). Eliminates existing facility which consisted of earthen ponds, develops efficient water capture structure and builds existing modern farm. Idaho Power sells properties with spring water, allowing for larger hatchery development. George Lemmon and Norman Standal establish Magic Springs Trout Farm near the Hagerman National Fish Hatchery on one of those properties.

1970: Jones Trout Farm (Billingsly Creek) built on family ground owned since 1896.

1972: 1000 Springs Trout Farm is sold to Inmont Corporation of New Jersey. Clear Springs Trout Company starts farm pond grow-out system. Production of rainbow trout and other farmed aquatic species expands greatly through the 1980's.

1973: Clear Springs Trout Company builds Box Canyon Trout Farm and expands its processing plant. Babington demand feeders designed and built.

1975-1980: First fish pump, automatic live fish grader, and boning tool built and patented by George Lemmon. Idaho Trout Company acquires Rim View Trout Farm and builds a second processing plant at Clear Lakes Trout Farm (?).

1978: Clear Springs Trout Company builds fish feed mill in Buhl.

1981: Clear Springs Trout Company purchases 1000 Springs Trout Company from Inmont Corporation. Rebuilds Snake River farm and builds research building. Rebuilding completed in 1988.

1983: Clear Springs Trout Company installs hydroelectric operation at Box Canyon.

1985: Clear Springs Trout Company purchases Caribou Trout Farm from Al Dunn and builds Soda Springs Brood Farm.

1987. Magic Valley Steelhead Hatchery built. Part of the Lower Snake River Fish and Wildlife Compensation Plan to mitigate for dams.

1991: Clear Springs Trout Company purchases Coast Oyster Company in Washington. Clear Springs Trout Company changes name to Clear Springs Foods, Inc. to reflect broader product offerings. Clear Springs Foods further automates processing plant with introduction of robotic cutting machines and pin-bone removal equipment.

1996: Clear Springs Foods acquires existing Pillsbury Oven Baked Bean plant in Buhl and reconstructs to form a specialty products plant.

2000: An Employee Ownership Plan and Trust (ESOP) is established and the 400 Clear Springs Foods employees purchase 100 % ownership of the company through the beneficial trust.

2001: Clear Springs Foods completes long-term trout supply contract with Chilean trout producer.

2003: Clear Springs Foods completes two long-term trout production facility leases at Briggs Creek.

2005: Clear Springs Foods completes additional long-term supply agreement with additional South American trout producers.

2006: Idaho produces 70-75% of all farm raised trout in the US. Approximately 561 trout farms are located throughout the US (42 states). United Nations projects aquaculture supplies 40-45% of all seafood consumed globally.

2006-2007: Clear Springs Foods completes major automation update at processing and specialty products facilities.

Global Seafood Market and Aquaculture

In the US there has been a seafood trade deficit for well over 20 years. In 2006 this trade deficit was over $8 billion. Imports of shrimp, salmon, tilapia, and other seafood create an extremely competitive market in which product price, quality, product availability and choice determine consumer purchasing decisions. These conditions prevail in the current seafood market compelling all US fish farmers and seafood processors to seek production cost reductions, greater production and processing efficiencies and product choice if they are to remain competitive. Natural resource barriers (i.e. availability of suitable water) and the technologic aquaculture challenge associated with some species (e.g. rainbow trout) preclude the excessive production of these species in many countries.

Capture fisheries have historically provided all seafood in the US and most of the world. As wild stocks have dwindled from over fishing and effects of pollution, and sustainable catch has been maximized, aquaculture has become an increasingly important supplier of seafood for human consumption (in 2007 about 45% according to the United Nations Food and Agriculture Organization). Seafood consumption itself has grown steadily in the US since the early 1980 (from about 12 lb/capita to 16.5 lb/capita). Starting sometime in the 1950s interest in commercial fish farming began to grow throughout the US and globally. This interest occurred because of market demand for consistent supply and quality of seafood. According to the United Nations, the phenomenal growth in world aquaculture over the last fifty years has been most notable in Asia and the Pacific regions. World aquaculture has grown at an average annual rate of 8.8 percent from 1950 to 2004. Production in the last fifty years has grown from less than a million tones in the early 1950s to 60 million tones in 2004 (United Nations). Nearly 70% of aquacultured products are produced in China. The potential to enhance food supply in low income, food deficit countries and the economic opportunity for all fish farmers fostered increased emphasis on aquaculture science and technology development ultimately leading to today's modern aquaculture industry. Over 442 aquatic animal species are farmed for human consumption, sport fishing and stock enhancement. The year round availability of some farmed species such as Idaho rainbow trout and consistency of high quality allow Idaho rainbow trout to compete for consumer purchase in the North American market.

Rainbow trout competes in the US market with other seafood and with poultry, pork and beef. Consumer price remains a significant factor in purchase decisions. Much of the imported farm raised seafood arrives at significantly lower price than domestic seafood because international labor costs (particularly China, SE Asia and South America) are very much lower. Additionally, environmental constraints on international production are much less than in the US further creating significant operational cost disadvantages to US producers.

Idaho produces 70-75% of all rainbow trout produced in the US for human consumption. Total production of rainbow trout in the US has been essentially constant over the past 20 years averaging around 55 million lbs per year. Fluctuations in total production arise because of variation in water flows, drought, floods, disease and predators, and market forces. Barriers to trout production in the US are lack of suitable water resources and production costs. Rainbow trout production volume in Idaho varies but is about 40 million pounds per year. The production capacity of Idaho, and any other trout producer, is determined by water availability, water quality, and the application of technology.

Aquatic animal production method significantly impacts production costs. Some aquatic animal species can be intensively raised in stagnant warm water ponds (e.g. channel catfish, basa, and tra). Others are primarily raised in open water (ocean, lake, large river) net pens (e.g. salmon, tuna and sea bass). Most rainbow trout grown in the US are intensively produced in flowing water culture systems because of the stringent water quality requirements of this species. Commercial success of rainbow trout farming demands intensive culture practices provided by flow-through water systems.