Enhancing the Growth and Health of Sockeye Salmon (*Oncorhynchus nerka*) in Land-Based Freshwater Aquaculture

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Abstract

The goal of this research was to provide data to assist in optimizing freshwater aquaculture practices for sockeye salmon (*Oncorhynchus nerka)*, and specifically, for LSL a land-based, freshwater sockeye salmon farm. Thus, this study successfully conducted inaugural trials using 17β-estradiol waterborne treatments (200 µg/L, 400 µg/L and 800 µg/L) to feminize genetic males to develop an enhanced male population to achieve larger sized sockeye at slaughter. In addition, this study tested the effects of weekly netting stress over 100 days and revealed a significant reduction in body weight and length of juveniles, and a change in the abundance of three liver proteins involved in the immune-responsive gene regulation, protein processing and cytoskeletal structure organization. However, bacterial kidney disease prevalence, leukocyte count, hematocrit, and whole-body cortisol level were not affected. This research shows that mild physical stress does compromise growth in juvenile sockeye salmon and would restrict commercial production substantially.

Keywords: Sockeye salmon; Proteomics; Husbandry stress; Hematology; Whole-body cortisol; Bacterial kidney disease

献给我的爸爸妈妈，奶奶，姥姥，谢谢你们一直爱着我。

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List of Acronyms

ACN	Acetonitrile
ACTH	Adrenocorticotropic hormone
ANOVA	One-way analysis of variance
BC	British Columbia
BKD	Bacterial kidney disease
CapZα1	F-actin-capping protein subunit alpha-1-like
Cq	Cycle quantification
CRF	Corticotropin-releasing factor
CV	Coefficient of variation
DTT	Dithiothreitol
ELISA	Enzyme-linked immunosorbent assay
gDNA	Genomic DNA
Hb	Hemoglobin
HPI	Hypothalamic-pituitary-interrenal
IAA	Iodoacetamide
IFN	Interferon
IHNV	Infectious haemopoietic necrosis virus
ILF2	Interleukin enhancer-binding factor 2 homolog
IPNV	Infectious pancreatic necrosis virus
K	Condition factor
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
Leg1	Liver-enriched gene 1
lfc	Log_2-fold change
LFQ	Label-free quantification
LSL	Living Seafoods Ltd.
LysC	Lysyl endopeptidase
Man1A1	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA-like isoform X2
MS	Mass spectrometry
MS-222	Tricaine methanesulfonate
NCBI	National Center for Biotechnology Information
nPCR	Nested polymerase chain reaction
NOAA	National Oceanic and Atmospheric Administration

NTC	No template controls
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
qPCR	Quantitative real-time polymerase chain reaction
R. salmoninarum	Renibacterium salmoninarum
sdY	Sexually dimorphic on the Y-chromosome
SE	Standard error
SFU	Simon Fraser University
TFA	Trifluoroacetic acid
UBC	University of British Columbia
UN	United Nations

Chapter 1. Introduction

1.1. Salmonids

Salmonid is the collective name of ray-finned fish that includes salmon, trout, chars, graylings, and freshwater whitefishes (Crête-Lafrenière et al., 2012). Salmonids are not only important ecologically but are also of economic and cultural significance globally. Indeed, as one the major seafood producers, Canada had an export value of $90 million for wild salmon in 2008 (Fisheries and Oceans Canada). In terms of ecological services, salmonids serve as a rich food resource for multiple species, particularly during spawning in their natal streams at the end of their life cycle. Interestingly, despite the loss of a large percentage of stored lipid and protein during migration to spawning grounds, there is still a considerable amount of protein (16% in wet mass) left in the carcasses (Gende et al., 2002). For predators that directly prey on the high-energy returning salmonids, the access to salmonids can impact their life cycle and even mortality rate (Willson et al., 1998). For example, some species such as minks and bald eagles will adjust their time of breeding according to salmonid availability (Willson et al., 1998). Furthermore, the minerals brought by salmonids is another contributor to local environment. Many low productivity coastal terrestrial ecosystems depend on salmonid runs for nutrients such as phosphorous (Hilderbrand et al., 2004). Though only 0.5% of salmonid body weight is phosphorous, it is still an enormous input when taking the total number of returning salmonids into account (Gende et al., 2002). The annual phosphorous contribution, for instance, made by southwest Alaska sockeye salmon (*Oncorhynchus nerka*) run alone to Lake Illiamna was up to 170 tons (Willson et al., 1998). Considering salmonid economic and ecological roles, their protection and conservation is critical to humans and wildlife.

Many of the salmonid's populations are declining and some of them are even at the stage of commercial extinction (Limburg & Waldman, 2009; Cohen, 2012). Since early the 1990s, Fraser River basin, the world's greatest producing system for Pacific salmon (*Oncorhynchus* spp.), has experienced a sharp decline in salmon abundance (Northcote & Atagi, 1997; Grant et al., 2018). Of all the salmon species spawning in Fraser River, the sockeye salmon population takes up a significant portion of the total salmon abundance (Grant et al., 2018). However, nearly half of the identified sockeye

conservation units in this region are classified as red or red/amber biological status which requires immediate action to prevent the loss of production and ecological benefits (Grant et al., 2018). In 2009, Fraser River sockeye salmon return was at the lowest point in history (Mckinnell et al., 2012), with only 1,590,000 adults, substantially below the forecasted 10,488,000 fish (Pacific Salmon Commission, 2009). Given multiple factors can lead to population collapse, the Canadian federal government established the Cohen Commission of Inquiry at the end of 2009 that concluded based on scientific evidence that the possible causes included habitat destruction, disease or parasites, pollution, climate change, and intense commercial fisheries (Pacific Salmon Commission, 2010; Cohen, 2012).

Considering Pacific salmon in general are species known to thrive under high quality habitat and specific temperature conditions, the increased salmon-bearing surface water temperature due to climate warming is of particular concern (Martins et al., 2010). In particular, optimum spawning and hatching temperatures for sockeye salmon range from 10.6 – 12.2°C (Bjornn & Reiser 1979), and optimum growth temperature is between 7.2 – 15.6°C (Bjornn & Reiser, 1979; Chen et al., 2013). However, in 2009 the summer water temperature was 17.4°C during sockeye salmon migration in Fraser River at Qualark Creek (Stiff, 2018), which is 1.8°C warmer than optimum. Studies have demonstrated when water temperate reaches 18°C, sockeye salmon may experience low migration performance and early physiological stress responses (Hyatt et al., 2015; Pellett et al., 2015). Moreover, prolonged exposure to these extreme temperatures can result in metabolic disorders and pre-spawning mortality (Sauter et al., 2001). Experiments conducted in the Fraser River have shown that sockeye salmon exposed to temperatures of 18°C have more than 67% mortality compared to untreated sockeye with 33% mortality (Crossin et al., 2008). It is possible that as the water temperature increases, the distribution and abundance of fish pathogens also change and results in a more widespread and severe infection (Kent, 2011). Previous studies have demonstrated how warmer temperatures would contribute to infection progression by facilitating the replication process of the pathogens (Poulin, 2006; Marcogliese, 2001; Prucell et al., 2015). Purcell et al. (2014) conducted a temperature study on chinook salmon and showed that fish held at relatively higher water temperature (14˚C) experienced early onset of *Renibacterium salmoninarum* infection.

In addition to temperature compromising the sockeye salmon populations, the loss of habitat also contributes to the collapse of salmon populations. The reduced water quality of salmon habitat is identified as one of the most serious challenges to be addressed during the conservation of salmon. The toxic urban runoff from various anthropogenic activities causes premature death in adult salmon in northwestern America as they return to freshwater spawning habitat (McIntyre et al., 2018). The common contaminants that pose such threat to salmon survival rate are heavy metals, i.e., copper, lead and cadmium, from mining activities and persistent organic pollutants often used for agricultural purposes (Gerig et al., 2020). Even at low environmental concentrations, these chemical contaminants have the potential to suppress immune system, alter metabolic activity and cause malnutrition and even sometimes lead to death in salmon (Dietrich et al., 2014, McIntyre et al., 2018, Gerig et al., 2020). Two widely used commercial pesticide formulations in British Columbia, atrazine and chlorothalonil were proved to affect the emergence, growth, and survival of early-stage sockeye salmon (Gas et al., 2017). Aside from the loss of suitable habitat caused by chemical substances, dam construction has substantial impacts on these migratory sockeye salmon as not only do those dams deny the access to original spawning sites, but also wash away the spawning gravels (Ferguson et al., 2010; Thompson et al., 2018). Consequently, there is a complete loss of one sockeye run in the upper Adams River (Ferguson et al., 2010).

1.2. Global Salmonid Farming

At present, one method employed globally to reduce overfishing of wild salmonids and allow humans access to fish as a protein source is the commercial aquaculture of various salmonids in numerous countries (i.e., Scotland, Norway, Chile, and Canada (Food and Agriculture Organization of the United Nations, 2018). Estimated by the United Nations (UN), trade in salmon has had a steady 10% rise in value term per year since 1976 (Food and Agriculture Organization of the United Nations, 2018), and consequently, by the year of 2011, the aquaculture production of salmon in Canada was 102,064 tonnes whereas the commercial landing of salmon in Canada's Pacific coast was only 20,670 tonnes (Fisheries and Oceans Canada, 2012). From the salmon catch logbook reports retrieved from Fisheries and Oceans Canada website (2019), the majority of farmed salmonids are from ocean net pens like Atlantic salmon (*Salmo salar*),

coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*), while a small portion are totally land-based, like rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*).

Although ocean based aquaculture of fish in general produces large quantities of salmon, several concerns have been identified with such methods, such as: 1) the diseases and/or parasites carried by the farmed fish can be passed to wild stocks (Harvell et al., 2004; Krkošek et al., 2006; Miller et al., 2014); 2) contaminants released to local ocean environment could harm the ocean ecosystems (US Commission on Ocean, 2004; Pacific Salmon Commission, 2010); and 3) environmental impacts caused by escapees (Marine Aquaculture Task Force, 2007). Firstly, the freely exchanged aquaculture system provides a path for pathogens to be transmitted from farms to surrounding environment. The parasite infection pressure induced by one commercial salmon farm built along the wild salmon migratory route was around 70 times higher than original natural background level (Krkošek et al., 2005). Furthermore, microparasites can change their virulence depending on the environment they are in, and their sublethal effects have more influence on wild fish populations than farmed ones (Miller et al., 2014). Secondly, the tendency of open marine aquaculture farm clustering geographically heightens the impacts of wastes discharged into coastal and ocean waters. Such marine aquaculture pollution has been demonstrated to be harmful to marine organisms in the surrounding waters (US Commission on Ocean, 2004; Marine Aquaculture Task Force, 2007; Hutchings et al., 2012). The release of nitrogen and phosphorus could cause the issue of eutrophication and ultimately, dead zones depleted of oxygen (Ngatia et al., 2019). The chemical treatments used for diseases and parasites in open net pen aquaculture are another concern. For example, sea lice treatment with pesticides, such as the commonly seen emamectin benzoate and azamethiphos in fish farming, was reported to disrupt the endocrine and neuromuscular system and have sub-lethal to lethal effects in in lobsters (Waddy et al., 2002, Dounia et al., 2016). Additionally, the escape of fish from open net aquaculture poses challenges to native species on an ecological level, including, more competition for food, resources, and habitat as well as at the genetic level due to compromised traits via interbreeding (Marine Aquaculture Task Force, 2007). The latter argument refers to reared fish acquiring certain traits that are favored in a domesticate setting, but not in the natural environment resulting in less fit wild populations. Hence once those fish manage to

escape and mate with closely related or same species, their offspring could have reduced adaptable ability and survival rate (Marine Aquaculture Task Force, 2007; Thorstad et al., 2008; Forseth et al., 2017). Studies have confirmed that the hybrid Atlantic salmon offspring (wild X farm) had less smolt production and low sea survival rate (Skaala et al., 2012; Forseth et al., 2017). Collectively, the challenges to overcome the risks of open-net pen aquaculture are many, and few innovations have been able to address these problems. Currently on the west coast of Canada in BC, Fisheries and Oceans Canada has proposed a transition of open-net pen system to land-based out of both economic and environmental considerations.

1.3. Production of Monosex Population to Enhance Land-based Sockeye Salmon (*Oncorhynchus nerka*) Farming

1.3.1. Land-based Freshwater Sockeye Salmon Farming

Compared to the ocean-based farms, land-locked aquaculture facilities have the opportunity to decrease pollution and disease discharge into the local environment in closed systems or perform water treatment prior to discharge to waterways. Indeed, few land-based aquaculture facilities exist, like the Danish Salmon in Danmark and Kuterra in British Columbia, for anadromous salmonid species (Evans, 2019), and only one exists globally to our knowledge for successful sockeye salmon production in Langley BC (Living Seafoods Ltd., LSL). Unlike well-established salmonid species currently in aquaculture, sockeye exhibit more stringent rearing requirements not easily replicated under aquaculture conditions. LSL in Langley has overcome many difficulties and managed to domesticate several generations of sockeye salmon as a food fish in a land-based, freshwater condition. The Langley farm cultures the sockeye salmon that are originated from Pitt River, a tributary of Fraser River, in British Columbia, Canada. The dry fertilization of brood fish is carried out during the second week of September when eggs and milt are mixed gently in water. After 20 minutes the fertilized eggs are transferred to the hatching apparatus (Heath stacks). The hatching time is around 13 to 14 weeks based on the water temperature and flow rate on the farm. Fry are subsequently transferred to round, flow through tanks from Heath stacks at an approximate weight of 0.4 g each. By using the current pond system, the production cycle lasts for 28 – 30 months. During the whole production process, no common therapeutants from the Health Canada-authorized veterinary drugs list, including

parasiticides (e.g., formalin) or antimicrobials (e.g., oxytetracycline hydrochloride), are used. The historical mortality rate is less than 5% from the swim-up fry stage to harvest season, which is about 6 weeks prior to spawning. Upon slaughter and harvest, the fish ranges from 3 to 4 pounds each. Currently, the LSL has a small niche market of buyers, but for economic viability, a farmed sockeye that is 5 pounds or larger is preferred. Therefore, further studies on improving certain traits of these farmed sockeye salmon to increase body size and fish health are necessary.

1.3.2. Male Cultures of Sockeye Salmon for Superior Growth Performance

The production of monosex population is essential to have large-scale industrial production and broodstock management in aquaculture due to the difference in growth rates between sexes (Martínez et al., 2014; Budd et al., 2015). According to aquaculture records at the Living Seafoods fish farm in Langley, BC, the average slaughter weight of freshwater reared sockeye males in the facility is 16% higher than that of females approximately 2 months prior to sexual maturation (Albright, personal communication). This sexual dimorphism in body size can be harnessed to increase the mean weight of these farmed salmon by increasing the total number of males in a fish cohort under freshwater aquaculture conditions. One of the methods of culturing more males is to employ estrogen treatments during early life stage development of salmon to induce ovary development in genetic males and conduct subsequent controlled breeding experiments to produce males with two copies of the Y chromosome.

In teleost, the maturation of gonad is triggered by genetic sex determining factors and steroid hormones and can be modified by environmental factors and endocrine disrupting chemicals (Wang & Shen, 2018). The typical gonad development pathway is comprised of 2 parts: sex determination and gonad differentiation (Martínez et al., 2014). The salmonid sex determination system is controlled by the action of sex-determining genes with a male heterogametic sex-determination system followed by gonad differentiation influenced by sex steroid hormones (Devlin & Nagahama, 2002; Martínez et al., 2014). Most salmonids exhibit an XY chromosome system, whereas sockeye salmon diverge slightly by having an XO system where the Y chromosome has attached to an autosome (designated X_2) and males possess one less chromosome than females (Thorgaard, 1978; Gomelsky, 2011; Faber-Hammond et al., 2012). This special

$X_1X_2X_1X_2/X_1X_2Y$ sex chromosome system results in sockeye males with a diploid chromosome number of 2N = 57 (one copy each of the X_1, X_2, and Y chromosomes), while females possess two copies each of the X_1 and X_2 chromosomes and have a diploid number (2N) of 58 (Larson et al., 2015; Faber-Hammond et al., 2012).

It is well established that sensitivity to sex steroids during embryogenesis and early fry stages in salmonids causes sex reversal such that the phenotypic sex does not correspond to genotypic sex (Budd et al., 2015). Specifically, it has been confirmed that 11-ketotestosterone induces masculinization of the gonad while 17β-estradiol feminizes during gonad differentiation (Fitzpatrick et al., 1994; Chang et al., 1999; Sandra & Norma, 2010; Budd et al., 2015). The liable period and required dosage for steroid treatments varies between species during sexual differentiation in salmonids (Budd et al., 2015). However, under most circumstances, the most appropriate period to initiate hormone treatment is during the larval hatching stage while the most effective dosage and exposure duration for immersion is around 50 – 1000 µg/L and 2 h, respectively (Hunter & Donaldson, 1983; Piferrer & Donaldson, 1991; Devlin & Nagahama, 2002). Generally, if the exposure is performed during the sensitive larval hatching stage, the transient hormone administration is sufficient to sustain the altered sex differentiation course in most tested fish species and the genetic programming cannot override the hormone treatment (Devlin & Nagahama, 2002).

The viability and fertility of YY sockeye salmon is undetermined, yet some studies suggest that the Y chromosome may have all the necessary genetic components as some other salmonids, e.g., YY chinook and YY Atlantic salmon, are indeed viable and fertile (Devlin et al., 2001; Fjelldal et al., 2020). For example, after a successful breeding trial to produce the YY chinook salmon, Devlin et al. (2001) speculated that Y chromosomes are not highly differentiated enough to cause essential genes to lose function in chinook salmon and thus the YY chinook survived. Considering chinook salmon and sockeye salmon belong to the same genus, it is possible that the Y chromosome in sockeye salmon also contains most of the vital genes to let the YY sockeye salmon survive and reproduce. Thus, the administration of exogenous 17β-estradiol to feminize genetic sockeye males that can be used to mate with normal males to produce YY offspring are viable possibilities that may lead to larger, more economically sustainable products for land-based, freshwater fish farms such as LSL.

1.4. Husbandry Stress Impacts on Juvenile Sockeye Salmon Growth

Domesticated fish are subjected to a variety of husbandry manipulations in aquaculture facilities, including but not limited to feeding, capture via netting, grading, non-lethal tissue sampling, transport, and other pre-slaughter management processes (Huntingford & Kadri, 2014). These practices may introduce a number of environmental, physical or social stressors which threaten or disturb various physiological processes in fish (Mateus et al., 2017). For example, such stressors can trigger hormonal imbalances, metabolic changes, and osmo-ionic disequilibrium (Davis, 2006) that result in suppressed growth, compromised immune system and elevated mortality in farmed fish (Rehman et al., 2017; Aerts, 2020). Hence, understanding the physiology of stress, determining the consequences on fish welfare, and identifying stress markers are crucial to optimize aquaculture production (Huntingford & Kadri, 2014; Hoem & Tveten, 2019).

1.4.1. Stress Responses in Fish

In fish, there are three stages of the stress response primary, secondary, and tertiary mainly mediated by the sympatho-chromaffin axis and the hypothalamic-pituitary-interrenal axis (HPI) (Barton, 2002). The first phase of stress response happens quickly and involves the release of catecholamines from chromaffin tissue and corticosteroid hormones due to the activation of the HPI axis into circulation upon recognizing the stressor by central nervous system (Barton, 2002; Mateus et al., 2017; Robinson et al., 2019). The plasma catecholamines (mainly adrenaline) stimulate the cardiovascular and respiratory systems resulting in increased heart rates, stroke volume, blood perfusion in gills and muscle, providing glucose supply to critical tissues (Reid et al., 1998). Additionally, the activated HPI axis contributes to the re-organization of resources by increasing gluconeogenesis, glycogenolysis and lipid degradation rates to meet the increased energy demands associated with stressful situations (Madaro et al., 2015). The secondary response generally takes place within 48 hours of the perception of stimuli, and it is where most changes are associated with physiological systems that entails the alterations in plasma and ion metabolite levels, hematological characteristics, and heat-shock proteins to re-establish homeostasis (Mateus et al., 2017). The subsequent tertiary response encompasses performance changes at the whole-organism level as common consequences are reduced growth or disease resistance

(Barton, 2002). An acute stressor is known to mostly induce adaptive processes (mostly primary and secondary phase) to reallocate energy for coping, modulate immune responses, and stimulate learning and neural plasticity due to its high severity but short duration nature (Mateus et al., 2017; Hoem & Tveten, 2019). As a result, some acute stress can be beneficial for fish in extending their normal adaptive ability for future perturbations (Madaro et al., 2015). For example, Atlantic salmon have been observed to exhibit superior growth after being treated with cold-shock and air exposure during embryogenesis and post-hatching (Mogahdam et al., 2017), and this difference in growth performance was maintained for an additional year after the termination of stress exposure confirming the positive and long-lasting effects of certain acute stress (Madaro et al., 2015; Mogahdam et al., 2017). Though the exact impacts of stress responses depend on the timing, duration, magnitude and nature of threatening factors, the stress responses would be maladaptive once it shifts to the tertiary level (Hoem & Tveten, 2019). In general, sustained high cortisol levels have adverse effects in animals (McEwen & Wingfield, 2003). A recent study in fish corroborates this as chronic cortisol treatment resulted in a significant reduction in mass gain in rainbow trout (Madison et al., 2015). Furthermore, many studies have shown that persistent stressors impair farmed fish health condition by compromising metabolic, immune, and endocrine systems (Mateus et al., 2017; Webster et al., 2018; Robinson et al., 2019). The chronic stressor will cause a less intense and slow onset stress response to ultimately create a prolonged stress situation (Tort, 2011). Being confined to certain culturing conditions, farmed fish cannot escape from the continuous stress exposure and eventually the chronic stress results in the physiological and behavioral balances becoming harder to restore (Madaro et al., 2015) and result in declined growth rate. This has been shown to be through elevated gluconeogenesis activity and increased disease susceptibility in fish (Davis, 2006). Therefore, chronic stress in fish should receive more attention because it is clear that it strongly correlates to the compromised fish welfare (Sadoul & Geffroy, 2019).

1.4.2. Cortisol as An Indicator of Stress in Juvenile Sockeye Salmon

Cortisol is recognized as the “stress hormone” because it is not only the principal hormone involved in primary stress response but also capable of promoting several mechanisms of secondary and tertiary stress responses (Hoem & Tveten, 2019). Upon the perception of stressful situations, the CNS signals the hypothalamus to release

corticotropin-releasing factor (CRF) into the circulatory system (Zuberi et al., 2014). Once CRF reaches the anterior pituitary gland, it then stimulates the secretion of adrenocorticotropic hormone (ACTH) into circulation that in turn activates the release of cortisol by the interrenal tissue (Mommsen et al., 1999; Sopinka et al., 2016; Mateus et al., 2017). After being distributed into blood circulation, cortisol exerts its actions via binding to glucocorticoid receptors and mineralocorticoid receptors and modulating gene expression, activating glycogenolysis or gluconeogenesis, and triggering stress-induced immunosuppression (Mommsen et al., 1999; Balasch & Tort, 2019; Sadoul et al., 2019). The regulation of both basal and stress-induced cortisol levels is responsible for stress resilience as cortisol also participates in the negative feedback control of the HPI axis (Robinson et al., 2019).

Cortisol is the key hormone in stress-related surveys to characterize disturbance levels (Madaro et al., 2015; Balasch & Tort, 2019), therefore the changes in cortisol levels in different species including salmonids in aquaculture conditions has been studied. For example, Atlantic salmon (Fast et al., 2008; Madaro et al., 2018; Delfosse et al., 2020) and chinook salmon (Quigley & Hinch, 2006; Herron et al., 2018; Cogliati et al., 2019) both experienced a spike in plasma cortisol concentration after being exposed to handling, confinement, or high temperature. Similarly, rainbow trout had a significantly elevated plasma cortisol level when subjected to low pH, crowding and handling (Ghaedi et al., 2014; Mota et al., 2016). Sockeye salmon has not been intensively studied as the aforementioned species, yet several studies provide some insights into how cortisol concentration fluctuates after stress. Kennedy and Picard (2012) showed low pH could increase cortisol level in juvenile sockeye salmon plasma while Lin et al. (2020) reported higher plasma cortisol level after exposed to the water-solution fraction of diluted bitumen. However, despite the extensive use of cortisol as physiological indicator for stress response analysis, there is much debate about its reliability in chronic stress conditions due to inconsistencies regarding cortisol induction across studies (Magalhães et al., 2020). For instance, Kousha et al. (2013) examined the plasma cortisol in juvenile Atlantic salmon after 65 days of chronic low water level as a stressor and found that no significant differences exist between control and treatment groups. It was hypothesized that the failure to observed increased cortisol as an indicator of chronic stress may be due to : 1) cortisol levels returning to resting levels within hours following a stimulus; 2) fish experiencing a “learning-adaption” response from the repeated disturbances

resulting in a desensitized HPI axis and attenuated cortisol response; and 3) factors like age, sex, reproductive stage, population, sampling method and stressor influencing the secretion of cortisol (Baker & Vynne, 2014; Magalhães et al., 2020). Additional studies are needed to explore these phenomena and better understand the utility of cortisol as an indicator of stress in farmed fish species, and ultimately, ensure optimal husbandry of poorly studied aquaculture species such as land-based freshwater sockeye cultures.

1.4.3. The Use of Hematological Parameter to Analyze the Effects of Chronic Stress

The secondary response is characterized by the change in physiological aspects including alterations in plasma and ion metabolite levels, hematological characteristics, and heat-shock proteins to re-establish homeostasis (Mateus et al., 2017). Key diagnostic markers to evaluate metabolism, the immune system, and other long-term impacts due to adverse husbandry conditions are hemoglobin (Hb) content, erythrocytes (red blood cells) percentage, and leukocyte (white blood cell) profiles (Lambert et al., 2018; Seibel et al., 2021). To satisfy the higher demand of tissue oxygen under stress, stored erythrocytes will be released from fish spleen when being promoted by cortisol, and thus the volume percentage of erythrocytes, also known as the hematocrit value, elevates (Pearson & Stevens,1991). Aside from the influence on hematocrit, stress can cause the leukocyte counts to differ. Since leukocytes are involved in processes such as immunoglobulin production, immune defense, and inflammation, these immune cells proliferate in response to damaged tissues or infections caused by stress (Davis et al., 2008). For example, Maheswaran et al. (2008) and Witeska et al. (2010) reported a similar pattern of change in total count of leukocytes whereby fish subjected to metal exposure had significantly higher total white blood cell count. In contrast to other testing techniques, white blood cell count and hematocrit measurement offer several advantages when assessing stress. First, these two types of blood analysis procedures are simple and require inexpensive equipment. Hematocrit values can be determined once blood is centrifuged in heparinized microhematocrit capillary tubes and cell counts are performed using a microscope after rapid, simple dilution blood and staining procedures. Second, the change in blood parameters of each individual organism over the entire experiment period can be tracked if animals are of sufficient size using non-lethal sampling with only small amount of blood is needed for each sample. Third, the baseline is easy to obtain because it takes hours to see any noticeable response in

leukocyte profiles (Davis et al., 2008). In light of these strengths, blood analysis, in association with other investigative approaches, has been frequently used to assess the severity of stress and obtain a picture of the fish health profile.

1.4.4. Disease Prevalence as An Indicator of Chronic Stress in Juvenile Sockeye Salmon

The possibility of compromised physical barriers against the pathogens, such as mucus shedding, skin abrasions and surface lesion due to chronic aquaculture husbandry stress, the invasion of parasites, fungi, bacteria, and viruses could alter immune functions and disease resistance (St-Hilaire et al., 1998). Therefore, the change in disease rate could be a useful measure for assessing chronic stress response in farmed fish. For example, the occurrence saprogleniosis disease, a common fungal disease in salmonids, is strongly related to handling stress and can compromise immune and endocrine system (Beckmann et al., 2020). After vaccination handling procedure, the experimental Atlantic salmon pre-smolts were observed to have pale cottony spots on the skin or gill (Beckmann et al., 2020). Not only did none of control exhibit such visible signs of saprolegniasis disease, their plasma cortisol and glucose level were also significantly lower than those handled fish (Beckmann et al., 2020). Similarly, the number of attached *Lepeophtheirus salmonis,* an ectoparasite of concern in salmon farming*,* found on Atlantic salmon had a significant increase after being handled (net and air exposure) compared to those not handled (Delfosse et al., 2020). The viruses affecting Pacific salmon species in cold water conditions (Gill, 2000; e.g., infectious haemopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) tend to be more problematic when fish are stressed. In one study, reduced water flow and hyperoxygenation increased the susceptibility to IPNV challenge in Atlantic salmon as mortality was 14% higher in chronically stressed salmon (Fridell et al., 2007). As described in Larson et al. (2020), the prevalence of Bacterial kidney disease (BKD) was indeed increased in stressed juvenile chinook salmon. The interactions of low nutritional regime and handling significantly increased the probability of disease in experimental fish (Larson et al., 2020).

The majority of health issues found in aquaculture are caused by viruses and bacteria, while fungal infections are categorized as low-impact diseases that can be easily resolved (Johansen et al., 2011; Fisheries and Oceans Canada, 2020). According

to Gill (2000), common bacterial pathogens to cause main disease problems, for example anemia, haemorrhagic septicaemia, and lesions, in cultured salmonids are from genera *Aeromonas, Pseudomonas, Vibrio*, and *Renibacterium*. Among all the above pathogens, *Renibacterium salmoninarum* (*R. salmoninarum*) is considered as one of the most prevalent and influences both wild and domesticated salmonids (Bruno, 2004). The geographic extent of this bacterium is considered to be global since it affects the entire salmonid family, even those in remote regions (Mimeault et al., 2020). This Gram-positive diplococcobacillus is a fastidious, slow growing yet very prevalent and virulent pathogen in salmonids that causes BKD (Kaattari & Piganelli, 1997), and the outbreak is usually seen when the water temperature is low as this naturally occurring bacterium is most active under 15°C (Delghandi et al., 2020). *R. salmoninarum* can be passed to other individuals both horizontally through direct or water contact and vertically as well through eggs or sperm (Annette et al., 2016). The symptoms of BKD seldom appear until salmon are 6 – 12 months old, making it almost impossible to tell whether the juvenile fish is infected (European Commission, 1999). And while some BKD-infected salmon display external clinical signs like irregular swimming behavior, shallow ulcers caused by broken skin blisters and swollen belly due to the accumulation of ascetic fluid, occasionally there are infected salmon that are either asymptomatic or only showing internal signs (European Commission, 1999; NOAA, 2018; Delghandi et al., 2020). For instance, Delghandi et al. (2020) demonstrated that swelling of the heart, spleen and liver can be found in fish, along with granulomatous lesions on the surface of the viscera.

Some plausible ways of controlling BKD so far are vaccination, antibiotherapy, nutritional supplements and switching into less stressed aquaculture management (Delghandi et al., 2020). As mentioned in one study (Arnason et al., 2013), neither the vaccines nor the antibody could sufficiently eradicate the disease. Renogen® is the only commercial vaccine licensed for administration in Canada at this point, but its efficacy and relative percent survival vary with each salmonid species (Elliott et al., 2014; Mimeault et al., 2020). When the vaccine was delivered via intraperitoneal injection into Atlantic salmon parr, the efficacy and relative percent survival was estimated to be 72-91% and 80%, respectively (Salonius et al., 2005). In contrast to the significant boost in survival rate for vaccinated Atlantic salmon parr, there was no significant difference between vaccinated and control juvenile chinook salmon which implies Renogen® conferred little protection against BKD in juvenile chinook salmon (Alcorn et al., 2005;

Elliott et al., 2014). With the various efficacies of vaccine between salmonid species, there is no guarantee that sockeye salmon will receive similar post-vaccination protection. Besides, although every member in salmonid population is susceptible to BKD, sockeye and pink (*Oncorhynchus gorbuscha*), have been reported to be more vulnerable than Atlantic salmon or rainbow trout (ICES, 2015; Delghandi et al., 2020). As a result, the most effective way to treat BKD at fish farms is hypothesized to be avoidance by culling infected fish to prevent the spreading of pathogens.

In order to build a culling program, a couple of conventional detection techniques are implemented in aquaculture including histopathology, immunohistochemical and molecular detection of pathogens such as *R. salmoninarum* (Delghandi et al., 2020). In the past, ELISA (enzyme-linked immunosorbent assay) was used as the major BKD diagnostic method. Monoclonal ELISA can detect major soluble antigen (MSA) which is the predominant cell surface antigen of *R. salmoninarum.* MSA is required for virulence as it renders several immunosuppressive patterns (Alcorn et al., 2005; Halaihel et al., 2009). Compared to monoclonal ELISA, polyclonal ELISA is more sensitive as it detects the whole bacterium instead of single surface antigen (Arnason et al., 2013; Delghandi et al., 2020). Though immunodiagnostic method is relatively quick, sensitive, and reproducible, and still serves a great biotechnological tool in many scientific labs (Hosseini et al., 2018), it may not be the ideal option for BKD detection. Since limited numbers of companies are producing the antibody for BKD ELISA test, poor antibody quality control would potentially cause discrepancies between different antibody batches (Powell et al., 2005). Recently, the molecular techniques such as nested polymerase chain reaction (nPCR) and real-time quantitative PCR (qPCR) have been developed to slowly replace ELISA and serve as primary detection tool (Delghandi et al., 2020). During nPCR analysis, two primer sets are designed to reduce non-specific binding and improve sensitivity and two rounds of PCR reaction are needed before getting any result (Carr et al., 2010). Though the World Organization for Animal Health (OIE) recommends the use of nPCR to confirm the presence of *R. salmoninarum* in 2006 as the two-step amplification could ensure the sensitivity and specificity of the test, it is a laborious technique that may not be suitable for large-scale BKD screening in aquaculture applications. On the other hand, qPCR has been proved to be a quicker and more reliable surrogate for the detection of *R. salmoninarum* in chinook and coho salmon (Sandell & Jacobson, 2011). In contrast to conventional PCR, qPCR is a fluorescence-

based quantitative technology which is capable of detecting with higher precision and increased sensitivity under approximately 2 hours (Bustin et al., 2009). At the same time, it enables the elimination of potential post-PCR manipulation to further avoid sample contamination and allows the quantification of target nucleic acid (Chase et al., 2006). Because the colonization of *R. salmoninarum* is the causative agent of BKD and the MSA on the surface of bacteria is the actual virulence factor (Coady et al., 2006; Suzuki & Sakai, 2007; Halaihel et al., 2009), several studies using qPCR method for detecting *msa* gene of *R. salmoninarum* in salmonids were reported. The relative high detection rates of *msa* gene in BKD-infected chum salmon (Suzuki & Sakai, 2007), chinook salmon (Chase et al., 2006) and rainbow trout (Jansson et al., 2008) indicates qPCR is a reliable screening method for *R. salmoninarum*. Therefore, *msa* gene will also serve as the target nucleic acid in the qPCR assay for the diagnostic of BKD in juvenile sockeye salmon to determine the consequence of netting stress in terms of disease susceptibility.

1.4.5. Proteomics to Discover Candidate Biomarkers of Chronic Stress

With the rapid development of omics technologies such as genomics, transcriptomics, proteomics, or metabolomics, stress-monitoring in fish aquaculture will no longer solely depend on the traditional endpoints (e.g., growth parameter and mortality rate). Proteomics is the large-scale study of proteins and their related properties, e.g., expression level and post-translational modification. (Blackstock & Weir, 1999; Graves & Haystead, 2002). Mass spectrometry (MS) and protein fractionation techniques can provide insight into many aspects of proteins such as spatial distribution, temporal dynamics, response under environmental stimuli, etc. (Hixson et al., 2017). Being the actual functional units in metabolism, transportation and biosynthesis within organism, proteins are more structurally diverse and have more direct influences on physiological functions and are more directly related to phenotypes than RNA or DNA at a given time point (Nallagangula et al., 2018). Indeed, proteomics analyses have been increasingly used in fields like pharmacology, ecotoxicology (Sanchez et al., 2010; Rabilloud & Lescuyer, 2014) and microbial pathogenesis in aquaculture (Peng, 2013). Several proteomics studies using various organs to measure growth under natural environments have been conducted to assess wild salmon welfare (e.g., ovarian fluid of chinook salmon (Johnson et al., 2014), liver of Atlantic salmon

(Nuez-Ortín et al., 2018), skeletal muscle in coho salmon (Causey et al., 2019) and serum in sockeye salmon, (Alderman et al., 2017)).

Aside from the use of proteomic analyses under normal circumstances, knowledge of alterations in protein abundance associated with stress in fish and the development of protein biomarkers of stress are of great interest in aquaculture (Magalhães et al., 2021). In teleosts, the liver plays an essential role in metabolic and biochemical functions, i.e., protein synthesis, and detoxification, and hence is an ideal target to assess and characterize the stress outcomes (Nallagangula et al., 2018; Nuez-Ortín et al., 2018). Scientists have studied the influence of stress on liver metabolic responses through investigating the relationship between the stressed-induced cortisol level change and carbohydrate metabolism and lipid mobilization (Wiseman et al., 2007; López-Patiño et al., 2014; Hernández-Pérez et al., 2019). Indeed, the contributions of hepatic proteins in coping with various stress events, including elevated water temperature (Nuez-Ortín et al., 2018), compromised immune defense (Hartley et al., 1996), acute and chronic handling practice (López-Patiño et al., 2014; Alves et al., 2010), and starvation (Mente et al., 2017), have all been demonstrated. Researchers have reached a consensus and it is widely accepted that hepatic metabolic performance during stress exposure is ultimately connected with fish overall health condition (Johnson et al., 2013). More importantly, with the time lag associated with protein profile change, proteomics is an appropriate candidate in searching for biomarkers to assess chronic stress (Magalhães et al., 2021). Even though the use of proteomics in salmonids aquaculture has been limited and mainly focused on rainbow trout and Atlantic salmon, these previously published results have underscored its importance in identifying physiologically relevant molecules and robust indicators for aquaculture stress management (Forne et al., 2010). Thus, liver protein analysis could potentially serve as a means to complement other stress assessment measures to investigate the responses in cellular processes (e.g., metabolic systems) relevant to growth induced by chronic stressors that are commonly seen in aquaculture in order to improve the culture conditions for higher production yield (Silvestre et al., 2012; Magalhães et al., 2021).

1.5. Objectives

1.5.1. Feminize Genetic XY Male for Enhanced Land-based Sockeye Salmon Farming

Upon consideration of the sexual dimorphism in growth, male sockeye salmon are more valuable than female sockeye salmon, therefore, techniques to produce all-male fish cohorts can be applied to increase farmed sockeye salmon production. This project will focus on the first stage of having an enhanced male sockeye salmon population, specifically, feminization using 17β-estradiol and viability assessment which includes survival rate and growth rate. To achieve sex reversal, 17β-estradiol (≥98%, Sigma Aldrich, USA) will be administered to sockeye salmon at early life stages, specifically, during pre-feeding and feeding, to produce phenotypic females despite the genetic complement. The XY females will be subsequently selected out by screening for the Y chromosome using qPCR experiments. This 17β-estradiol exposure study will be conducted at Simon Fraser University (SFU) in the Alcan Aquatic Research Centre (protocol No. 1291B-18) and LSL farm, and the immersion concentration and dietary regimen will adhere to those previously described by Devlin et al. (2001). Although beyond the scope of this M.Sc. thesis, the phenotypic females that test positive for the Y chromosome will then be utilized for breeding experiments with the goal of producing genetic males with two Y chromosomes.

1.5.2. Investigate Husbandry Stress in Juvenile Sockeye Salmon to Improve Sockeye Salmon Farming

It is likely that husbandry practices induce chronic stress responses in sockeye salmon at the freshwater, land-based Living Seafoods Ltd. fish farm and may have detrimental impacts on fish health, yet there are no certified farming procedures to breed wild sockeye salmon. Consequently, quantification and interpretation of chronic stress response is essential to evaluate husbandry conditions, adjust potential disruptive factors, avoid economic loss, and maintain a sustainable production (Sopinka et al., 2016). In order to study the consequences of chronic stress, two 100-day stress exposures were conducted with the netting as the stressor. Most of the husbandry practices including grading, vaccination, transportation, etc., require capturing fish beforehand via netting (a combination of chasing and air exposure) and this would be a

major physical stressor repeated over the entire production cycle. Accordingly, the juvenile sockeye salmon will be subjected to netting stress and the endpoints to evaluate whether this stressor influences the immune system will be hematocrit, total white blood cell count, and whole-body cortisol level. The values of body weight and length will be used to assess the stress effects on growth performance while hepatic protein profile will be obtained using proteomics analyses to identify protein changes indicative of netting stress in sockeye salmon. Netting stress was hypothesized to affect the immune and endocrine system in reared sockeye salmon. Specifically, fish that are netted weekly are predicted to have higher *R. salmoninarum* infections, white blood cell counts, cortisol levels and impaired growth compared to control fish.

Chapter 2. Methods and Materials

2.1. Experiment 1: Feminize Genetic XY Male for Enhanced Land-based Sockeye Salmon Farming

2.1.1. Animals

Sockeye sperm and eggs from Pitt River stock were obtained from Inch Creek Hatchery (Dewdney, BC) on September 11th, 2019, and transported to LSL farm at 4 – 8°C and were dry fertilized according to Patterson et al. (2004) on the same day. Dry fertilization was performed by combining egg sample from one female with milt sample from one male in a sterilized plastic container. Artesian groundwater was slowly poured into the container to activate the sperm. After 30 seconds of gently swirling, the embryo sample was left in container for 45 minutes to allow water to harden. Embryos were randomly sorted into three different treatment groups after fertilization and placed into six 10 L Heath trays made of non-toxic plastic for rearing under a constant flow of artesian groundwater until the swim-up fry stage. In addition, a subset was transferred to Alcan Aquatic Research Center (Simon Fraser University) once the eyed embryo stage was reached and was reared until the swim-up fry stage under the same rearing conditions and apparatus, except the source of water was municipal dechlorinated tap water. The fish were reared in water under the following conditions: temperature, 6.8 ± 1.1°C; dissolved oxygen, 100.18 ± 1.1%; and pH of 7.54 ± 0.15. Water quality in each tank was recorded every Monday, Tuesday, Thursday, and Friday at Alcan Aquatic Research Center using a HACH portable HQ40d multimeter to measure dissolved oxygen, conductivity, and temperature. The pH was monitored at the same time using a pocket OAKTON pH Tester 30.

2.1.2. Feminization of Sockeye Salmon Using 17β-estradiol

Phase 1: The first phase of feminizing genetic male sockeye salmon was to administer waterborne 17β-estradiol to newly hatched alevins. Based on the concentration previously described in Devlin et al. (2001) and the effective dose range suggested by Devlin and Nagahama (2002), this steroid treatment was administered via the water and carried out in duplicate (200 alevins per replicate) with 17β-estradiol concentrations of 200 µg/L, 400 µg/L and 800 µg/L. The stock 17β-estradiol was

prepared beforehand by dissolving in anhydrous ethyl alcohol (EtOH, Commercial Alcohols, Canada) and stored in the dark at room temperature. The details of the 17β-estradiol stock preparation methods for each concentration are summarized in Table 2.1. Before each immersion of alevins in 17β-estradiol treated water, the water flow to the Heath tray holding the alevins was shut down to ensure the final 17β-estradiol was 0.001%. Once over 90% sockeye salmon had hatched (December 6th, 2019) at the LSL farm and the Alcan Aquatic Research Center, the first stage of *in vivo* 17β-estradiol waterborne exposure was conducted by immersing sockeye salmon alevins every week in rearing water containing different concentrations of 17β-estradiol for 2h at 10°C over the course of four weeks at both facilities. Twenty days after administration of waterborne 17β-estradiol, the alevins at Alcan Aquatic Research Center suffered from an unexpected chlorine exposure due to an equipment failure on December 26th, 2019, and over 50% of the alevins died. Therefore, the 17β-estradiol exposure at Alcan Aquatic Research Center was terminated.

Phase 2: In the second phase of the feminization experiment, 17β-estradiol-infused feed was given to the alevins. Due to the loss of fish at Alcan Aquatic Research Center from chlorine exposure, this hormone treatment phase was carried out using the backup sockeye salmon alevins from LSL once over 90% exhibited vertical swimming behavior and no/minimal visible yolk sac was observed. On January 13th, 2020, 600 fry (about 40 days post-hatch) that were reared in Heath stacks and had received administration of waterborne 17β-estradiol at LSL were transferred to Alcan Aquatic Research Center, and kept in six indoor 150 L, flow-through fiberglass tanks (length x width x height = 110 cm x 50 cm x 40 cm) with municipal dechlorinated water to acclimate for 4 days. The 70-day oral administration of 17β-estradiol at 10 mg/kg feed (Complete Fish Feed for Salmonids, EWOS Pacific, Surrey BC, Canada) was performed (Devlin et al., 2001) after four-days acclimatation period in new environment. The steroid treated feed was prepared by crushing the aforementioned commercial feed into fine particles in a coffee grinder, dissolving 1 mg of 17β-estradiol (≥98%, Sigma Aldrich, USA) in 10 mL EtOH and adding this steroid solution to 100 g of the ground feed while stirring. After mixing, the feed was left standing for 30 minutes at room temperature to allow the feed to dry out. The feed was stored below 0°C until further use. From January 17th, 2020, fry were fed daily until apparent satiation.

This trial was also prematurely terminated due to another malfunction at Alcan Aquatic Research Centre whereby the thiosulfate tank for dechlorinating water broke down again on February 14th, 2020, killing all fish in this experiment. At this point, due to time and lack of availability of animals, no further experiments on feminization of sockeye salmon were performed.

2.1.3. Use of Male Specific *sdY* qPCR Screening Experiments to Identify Genetic Sex

With the goal of the 17β-estradiol treatments resulting in ovaries developing in all fish despite the genetic complement, quantitative real-time PCR (qPCR) experiments to identify genetic males was to be employed when the fish reached ~2 years of age along with histological or ultrasound determination of phenotypic sex (i.e., testis or ovary). Female sockeye salmon naturally only have X chromosomes while the males possess both X and Y chromosomes. Furthermore, the *sdY* (*sexually dimorphic on the Y-chromosome*) gene has been demonstrated to be the conserved master sex-determining gene responsible for the gonadal sex determination and differentiation cascade in most salmonids (Yano et al., 2013), including in sockeye salmon (Larson et al., 2016; Royle et al., 2018). Therefore, during phase 1 and 2 of 17β-estradiol exposures to feminize sockeye salmon (see 2.1.2), the optimization of a screening method to identify genetic sex using the male specific *sdY* gene was performed.

Genomic DNA (gDNA) samples from adult sockeye salmon muscle with known genotypic sex generously donated by Thomas Royle and Dr. Dongya Yang from SFU were used for the *sdY* primer (Royle et al., 2018) validation and optimization experiments. Sockeye salmon muscle gDNA samples was first isolated using E.Z.N.A® Tissue DNA Kit (Omega Bio-tek Inc., Georgia, USA) according to manufacturer's instructions. Briefly, the 200 µL of TL buffer and 25 µL of Proteinase K solution were added to muscle samples that were less than 30 mg in mass followed by homogenization using a Retsch® MM 300 TissueLyser Lab Vibration Mill Mixer (Retsch, Haan, Germany) at 30 Hz for eight minutes. The supernatant was transferred to a new sterile 1.5 mL tube along with 220 µL BL buffer, and this solution was then incubated at 70°C for 10 minutes using Heratherm™ General Protocol Microbiology incubator (Thermo Fisher Scientific Inc., Massachusetts, USA). A volume of 220 µL of 100% ethanol (Commercial Alcohols, Ontario, Canada) was added before transferring the

entire sample to a spin column inserted into a collection tube followed by a one-minute centrifugation at 12,000 x g. Upon discarding the filtrate in the collection tube, 500 µL of binding solution (HBC Buffer) was added to the spin column and the sample was centrifuged at 12,000 x g for 30 seconds. The filtrate was discarded after the completion of DNA binding, and the spin column was rinsed by the addition of 700 µL ethanol-diluted DNA Wash Buffer and centrifuged in order to remove any remaining cell debris that might interfere with downstream analysis. Finally, the purified gDNA was obtained after two rounds of elution in 200 µL Elution Buffer heated to 70°C. The 400 µL of eluted DNA was stored at -20°C. DNA concentration was measured quantified using Epoch™ microplate spectrophotometer and a Take 3 Micro-Volume Plate (BioTek Instrument Inc., Vermont, USA).

A standard curve using four-fold serial dilution of a 60 ng/µL gDNA stock extracted from male sockeye muscle was used to determine detection limits of the qPCR assay, verify the primer set efficiency, and to select the optimal concentration of gDNA to be added. All qPCR experiments were conducted on a Bio-Rad CFX384™ Real-Time PCR Detection System with Hard-Shell 384 well PCR plates (Bio-Rad Laboratories, Inc., California, USA). Every plate included three technical replicates per gDNA sample as well as no template controls (NTC) by which gDNA template was replaced by same amount of DNase/RNase-free water. In each reaction well, the following components were added to have a total reaction volume of 12.5 µL: 2.5 µL of the gDNA standard sample at 15 ng/µL, 3.75 ng/µL, 0.94 ng/µL, and 0.23 ng/µL; 0.375 µL of 10 µM forward and reverse primers; 6.25 µL of SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories, Inc., California, USA); and 3 µL of DNase/RNase-free water. Detailed information about the *sdY* primer set is provided in Table 2.2. Amplification reaction for *sdY* qPCR experiment consisted of the following thermal profile: 1 cycle of enzyme activation at 95°C for 30 s, followed by 45 cycles of denaturation (95°C for 5 s) and primer annealing (50°C for 5 s). A melt curve analysis was performed after 45 cycles to demonstrate reaction specificity. The distinct peak indicated primer-dimers were absent and amplicons were a single discrete species. The melt curve analysis was conducted by slowly increasing the temperature from 65.0°C to 95.0°C at an increment of 0.5°C for 5 s. The final results were analyzed using CFX Manager™ Software (Bio-Rad Laboratories, Inc., California, USA). The primer satisfied the following criteria is to be

considered as acceptable for future *sdY* qPCR: an efficiency ranging from 90% – 110%; a standard curve R^2 value greater than 0.900 (Bustin et al., 2010).

2.2. Experiment 2: Investigate Husbandry Stress in Juvenile Sockeye Salmon

2.2.1. Animals

Given the netting procedures routinely used during aquaculture practices, the physical stressor tested in this study was netting. One thousand four-month-old, sexually immature sockeye salmon (average initial weight of 0.5 g) originating from the Inch Creek Hatchery (Pitt River, BC stock) were transported to outdoor fiberglass flow through fish tanks (length x width x height = 110 cm x 50 cm x 40 cm) using a special insulated fish tote at 8 – 10°C and randomly divided into one reserve tank (400 fish in total), three 150 L, replicate control tanks (300 fish in total) and three 150 L, replicate stress exposed tanks (300 fish in total). Upon receipt of fish, a 30-day acclimation period ensued to allow the sockeye salmon to recover from the transport stress and resume normal feeding.

2.2.2. Netting Stress Experimental Design

Trial 1: After the acclimation period, the first trial of netting experiment whereby fish were netted weekly for a duration of 100 days was conducted. The netting activity entailed the use of a net with a frame size of 6″ x 8″ and bag depth of 7.25″ which was placed in water to chase the sockeye until 20% were captured. Captured fish were kept in the net and out of the water for 20 seconds. Each of the 3 replicates tanks were netted weekly during the 100-day experiment (May 24th, 2019 – August 31st, 2019). Fish were fed once a day from Monday to Friday at a rate of 2% of body weight using commercial salmonid feed (Complete Fish Feed for Salmonids, EWOS Pacific, Surrey, BC, Canada), and all rearing containers including the reserve tank were cleaned via siphon on a daily basis before feeding. Measurements of water quality (temperature, dissolved oxygen, pH, and conductivity) were conducted three times a week on all tanks using the equipment mentioned in section 2.1.1.

Trial 2: Another 100-day netting experiment was conducted (initiated October 7th, 2019 and planned to be terminated on January 14th, 2020) by using the juvenile fish reared in the outdoor fiberglass reserve tanks to investigate whether or not the effects on an older life stage of sockeye salmon fish would vary. The fish were transported into indoor 150 L fiberglass tanks and reared with municipal dechlorinated tap water at 8 – 12°C. This juvenile netting trial was performed in quadruplicate with thirty juvenile sockeye salmon per tank. The water quality along with mortality rate was recorded the same way as before. The measurements of temperature, dissolved oxygen, pH, and conductivity were conducted three times a week on all tanks between 1 – 3 pm as previously describe in section 2.1.1. Unfortunately, this experiment was not completed due to a failure at Alcan Aquatic Research Centre whereby the dechlorination process failed (thiosulfate pump failure on the night December 26th, 2019) after 81 days of the experiment. All the fish in all tanks died within 2 days, therefore the results of this experiment were survival up until 1 day before this failure only (i.e., survival from initiation to December 26th, 2019).

2.2.3. Stress Experiment Termination and Analyses

Termination of the first trial of netting experiment using fingerling sockeye salmon was performed in a single day. Fish were fasted for 24 hours prior to termination and the last netting event was 2 days prior to termination. To prevent the changes in cortisol levels due to capture upon termination, fish (5 fish/tank from tank 2 – 6, 3 fish from tank 1) were captured first and euthanized in 0.4 g/L of tricaine methanesulfonate (MS-222) buffered to pH = 7 with sodium bicarbonate at 10-12°C, body weight, fork length and external deformities were recorded for individual fish, and whole fish were immediately frozen on dry ice. Fish were then transferred to -80°C for long term storage and subsequent whole-body cortisol analysis.

The second batch of fish (6 fish/tank from tank 2 – 6, 3 fish from tank 1) collected on termination day were used in subsequent hematocrit, kidney DNA extraction for *R. salmoninarum* screening, and liver proteomics. For hematocrit sample collection, once the opercular flap stopped moving or the fish did not respond to prodding with forceps after immersion in neutral buffered MS-222, measurements on growth parameters (body weight, body length and deformity) were performed. The caudal peduncle was then severed, and blood was collected using heparinized microhematocrit capillary tubes

(Fisher Scientific International, Inc., USA). The collected blood was placed on ice for 20 minutes which was followed by a 2-minute centrifugation in IEC Micro-MB centrifuge (International Equipment Company, USA). After centrifugation, the upper clear layer was collected into a labeled 0.2 mL flat cap tube (Thermo Fisher Scientific Inc., Massachusetts, USA) along with white blood cell diluting fluid at a 1:10 ratio. The diluted white blood cell was then stored at 4°C overnight. The white blood cell diluting fluid was prepared as follows: 3 µL of glacial acetic acid (Sigma Aldrich, Ontario, Canada), 3 drops of methylene blue (Ricca Chemical Company, USA) and 97 µL of distilled water, and stored at 4°C afterwards. White blood cell count was performed using an improved Neubauer Hemocytometer according to Shah and Altindag (2005) within 24 hours. Briefly, the diluted white blood cell solution was added to both chambers of the hemocytometer slide under coverslip. The slide was allowed to sit at room temperature for 2 minutes until cells settled. The cells inside four large corner squares (1 mm^3) were counted under 100X total magnification for each Neubauer chamber. After counting the cells in both chambers, the total number of white blood cells per mm^3 were calculated using this formula: counted cells / (counted area x dilution factor).

During kidney and liver tissue extraction, all tools were cleaned between each dissection with 10% hydrogen peroxide and rinsed with DNase/RNase-free water to prevent DNA cross contamination and degradation. Tissues were immediately placed in labeled 1.5 mL RNase/DNase-free tubes (Thermo Fisher Scientific Inc., Massachusetts, USA) and frozen on dry ice before storing them in -80°C.

2.2.4. Prevalence of *R. salmoninarum* Using qPCR Analysis to Assess Netting Stress

For the detection of *R. salmoninarum* infection to assess the effect of netting on disease susceptibility, *msa,* the virulence gene was measured in control and netting-stressed fish. A primer set was previously developed by Suzuki and Sakai (2007) and was used for qPCR assays on fish from the each of the 3 replicate tanks in the stress experiment trial 1 (6 fish/tank from tank 2, 3, 5 and 6, 2 fish/tank from tank 1 and 4). The gDNA isolation method and laboratory equipment used for *sdY* qPCR assays (see section 2.1.3) were used during kidney gDNA isolation procedure for the *msa* qPCR assays.

All *msa* qPCR assays used to measure *R. salmoninarum in the kidney* adhered to the conditions outlined by Suzuki and Sakai (2007), and the primer/probe details are provided in Table 2.3. To validate these primers in our lab, gDNA isolated from *R. salmoninarum* positive sockeye kidney tissue that was generously donated by Dr. Christy Thompson (Fisheries and Oceans Canada) and utilized as template for qPCR standard curve experiments. Specifically, the primer and probe set efficiency was tested using a 5-point standard curve generated by a 4-fold dilution of a 25 ng DNA/µL of water. Each reaction well contained the following master mix in total reaction volume of 11.25 µL: 1.125 µL of forward and reverse primers (initial concentration = 10 µM), 0.5 µL of 10 µM customized TaqMan™ probe (Thermo Fisher Scientific Inc., Massachusetts, USA), 6.25 µL of TaqMan™ universal master mix II (Thermo Fisher Scientific Inc., Massachusetts, USA) and DNase/RNase-free water for the remaining volume. In addition to each well containing 11.25 µL of the qPCR master mix, a volume of a 1.25 µL of standard/test sample gDNA template was added (i.e., standard curve test gDNA concentrations of: 25 ng/µL, 6.25 ng/µL, 1.56 ng/µL, 0.39 ng/µL, and 0.01 ng/µL). The standard curve was performed in triplicate wells on Hard-Shell 384 well PCR plates and analyzed via CFX Manager™ Software (Bio-Rad Laboratories, Inc., California, USA) according to the manufacturer's instructions to confirm amplification efficiency. Each plate contained three technical replicates per gDNA sample, and no template controls (NTC) comprised of 1.25 µL of DNase/RNase-free water instead of gDNA sample. The *msa* gene amplification parameters using on a Bio-Rad CFX384™ Real-Time PCR Detection System with Hard-Shell 384 well PCR plates (Bio-Rad Laboratories, Inc., California, USA) were: enzyme activation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 15 s and primer annealing at 60°C for 1 min. After 45 cycles, a melt curve analysis was conducted using the following instrument settings: initial temperature of 65.0°C that was increased by an increment of 0.5°C for 5 s to a maximum of 95.0°C. The presence of a single peak was used to confirm only one type of amplicon was amplified. The standard curve acceptability criteria by Bustin (2010) were assessed as follows: a single peak melt curve, efficiencies between 90% – 110%, amplification in at least 4 concentrations of the standard curve, and an R^2 of the standard curve > 0.900.

2.2.5. Whole-body Cortisol Assay to Assess Netting Stress

Whole-body cortisol levels in fish from all six tanks (8 fish in total per treatment) were measured using ELISA kit purchased from Cayman Chemical Company (Michigan, USA). The hormone extraction protocol was described by Arukwe et al. (2008) and Gas et al. (2017). For the purpose of this assay, the protocol was further adapted for fingerling sockeye salmon. Whole-body sockeye sample stored in -80°C were removed from freezer and immediately homogenized on ice in 1:4 volume of 0.1 M Na-phosphate buffer (pH = 7.4) using Wheaton® overhead stirrer (DWK Life Sciences LLC, Tennessee, USA). The buffer was prepared beforehand and contained 3.1 g of $NaH_2PO_4 \cdot H_2O$ (VWR International Co., Pennsylvania, USA), 10.9 g of anhydrous Na_2HPO_4 (ACP Chemicals Inc., Quebec, Canada) and 1 L of distilled water. The sample was then transferred to a 50 mL falcon tube and centrifuged at 5,000 x g for 25 minutes at 4°C. The supernatant was poured into a 20 mL glass test tube and then was purified by extraction using an organic solvent to remove lipids and proteins insert reference. Specifically, 4 mL diethyl ether (Fisher Scientific International, Inc., USA) was added to each tube and then mixed using a vortex for 20 seconds. The test tubes were left at room temperature for 10 minutes to complete phase separation and then placed in acetone/dry ice bath to freeze. The test tube was hand rolled to pellet out the floating debris to the bottom frozen layer. The top lipophilic phase remained liquid and was decanted into a clean 10 mL glass test tube. After another two rounds of diethyl ether addition and phase separation were performed, and then the decanted samples were left in the fume hood uncovered at room temperature overnight to evaporate ether phase. The resulting dry extract was reconstituted in 300 µL ELISA buffer (Cayman Chemical Company, Michigan, USA) by vortexing 30 seconds followed by a 10-minute incubation period.

A 96-well plate format ELISA was then performed as described by the manufacturer (item number: 500360). Briefly, the 96-well plate was set up with the following contents: duplicate blank wells; duplicate non-specific binding wells; triplicate maximum binding wells; one total activity well; duplicate 8-point standard curve concentrations (6.6 pg/mL to 4000 pg/mL); and duplicate whole-body extracted samples. The type and volume of reagents in each reaction well are summarized in Table 2.4. The final step included an incubation period of 95 minutes for optimal development. The absorbance of the assay was read using an EPOCH2 microplate reader (BioTek

Instruments Inc., Vermont, USA) and Gen 5.02 Software (BioTek Instruments Inc., Vermont, USA) at 410 nm wavelength. The assay precision was determined using intra-assay coefficient of variation (CV). The Intra-assay CV represented the degree of variability between sample measurements in different wells from one sample plate. The final hormone concentration data were determined against a cortisol standard curve which was linearized using a logit transformation of B/B_0 (bound sample/maximum bound) according to the manufacturer's instructions.

2.2.6. Hepatic Tissue Protein Extraction

Total proteins were extracted from sockeye liver samples using TRIzol™ Reagent (Invitrogen, Ontario, Canada) according to manufacturer instructions, and submitted for proteomics analysis to Proteomics Core Facility at University of British Columbia (UBC), Vancouver, BC. Briefly, less than 100 mg sockeye livers were homogenized in 1 mL of TRIzol™ until a homogenous liquid was obtained using Retsch Mixer Mill MM 400 (Fisher Scientific, Ontario, Canada) at 30 Hz for 10 minutes. After adding 200 µL of chloroform (Anachemia, Quebec, Canada) to each tube, a 15-minute centrifugation at 12,000 x g at 4°C (Sorvall ST 16R Centrifuge, Thermo Fisher Scientific Inc., Massachusetts, USA) was needed to allow phase separation. Only the middle and lower layers resulted from the centrifugation were retained to proceed to extract protein. Once DNA was precipitated out of the solution with the addition of 300 µL ethanol, the phenol-ethanol supernatant was transferred to a new 2 mL tubes for protein isolation and purification. Isopropanol (Caledon Lab Chemicals, Ontario, Canada) was added until it filled the 2 mL tube, and a 10-minute incubation was performed on ice to allow protein precipitation followed by centrifuging the sample at 12,000 x g for 10 minutes at 4°C. The protein washing step started with resuspending the pellet with 2 mL of wash solution consisting of 0.3 M guanidine hydrochloride (Sigma-Aldrich, Missouri, USA) in 95% ethanol. After the resuspension, the sample was incubated for 20 minutes on ice and centrifuged at 7,500 x g at 4°C for 5 minutes to collect the protein pellet. The whole protein washing procedure was repeated 3 times before storing final pellet in 2 mL of 100% ethanol and delivered to UBC Proteomics Core Facility (Vancouver, British Columbia) on ice for subsequent proteomics analyses.

2.2.7. Hepatic Proteomics Analyses

The following proteome-wide label-free quantification using mass spectrometer (MS) was conducted on the hepatic protein pellet samples at the UBC Proteomics Core Facility (Vancouver, British Columbia) and adhered to Foster et al. (2003) and Tyanova et al., (2016). Briefly, all protein pellets were resuspended in 6M urea/2M thiourea to obtain a final concentration of 1-2 µg/uL. Reduction of disulfide bonds of proteins was achieved by adding 1 µg dithiothreitol (DTT) to every 50 µg protein and incubating for 30 min at room temperature. Blocking free sulfhydryl groups was performed by iodoacetamide (IAA) solution (5 µg IAA to every 50 µg protein) and incubated for 20 min at room temperature in the dark. Samples were then digested with lysyl endopeptidase (LysC) enzyme (1 µg LysC to every 50 µg protein) and incubated 3 hours at room temperature. Next, samples were diluted by four volumes of 50 mM ammonium bicarbonate buffer (pH ~ 8) for pH correction, followed by the addition of 1 µg Trypsin per 50 µg protein and a 19-hour incubation at room temperature. Trypsin activity was quenched by acidifying samples by adding 1% Trifluoroacetic acid (TFA) for a final pH < 2.5 of the samples.

For peptide clean-up, approximately one quarter of each of the acidified samples (~25-50 µg) was first forced through a conditioned (with MeOH) and equilibrated (twice with 2% acetonitrile (ACN)) BioPureSPN™ Midi RPC Desalting Columns of PROTO™ 300 C18 (The Nest Group Inc., Massachusetts, USA), and then washed with 2% ACN twice to wash out any traces of salts from protein samples. Finally, samples are eluted into clean tubes by buffer containing 40% ACN, 0.1% TFA, then dried down.

For the LC-MS/MS (liquid chromatography with tandem mass spectrometry) analyses, first the samples were reconstituted in 2% ACN, 0.5% formic acid, and then the peptides were analyzed using a quadrupole – time of flight mass spectrometer (Impact II; Bruker Daltonics Inc., Massachusetts, USA), coupled to an EasyLC 1000 HPLC (Thermo Fisher Scientific Inc., Massachusetts, USA) using a Captive spray nanospray ionization source (Bruker Daltonics Inc., Massachusetts, USA) and a C18 analytical column with Gen2 nanoZero® and CSI fitting (IonOpticks, Aurora Series Emitter Column, AUR2-25075C18A-CSI 25 cm × 75 µm ID, 1.6 µm FSC C18) and a Thermo Scientific™ Acclaim™ PepMap™ µ-Precolumn (5 mm x 300 µm ID, 5 µm FSC C18, pore size: 100 Å, Thermo Fisher Scientific Inc., Massachusetts, USA). The

analytical column was heated to 50°C using a tape heater (SRMU020124, Omega Engineering, Inc., Connecticut, USA) and an in-house build microprocessor temperature controller. Mobile phase A consisted of 0.1% aqueous formic acid and 2% acetonitrile in water, and mobile phase B consisted of 0.1% formic acid in 90% acetonitrile. Samples were loaded at 2 µg per injection, injected in triplicates. Standard 90 min run the gradient was from 5% B to 18% B over 45 min, then to 35% B from 45 to 90 min, then to 90% B over 2 min, held at 90% B for 13 min. Before each run, the analytical column was conditioned with 4µL of buffer A, and precolumn was conditioned with 20 µL of buffer A. The LC thermostat temperature was set at 7°C. The analysis was performed at 0.35 µL/min flow rate. Impact II was run with Bruker OTOF Control v. 4.1 (Bruker Daltonics Inc., Massachusetts, USA). LC and MS were controlled with Bruker HyStar version 4.1.21.2 (Bruker Daltonics Inc., Massachusetts, USA). The Impact II was set to acquire in a data-dependent auto-MS/MS mode with inactive focus fragmenting the 20 most abundant ions one at the time at 18 Hz rate after each full-range scan from m/z 200 Th to m/z 2000 Th at 5 Hz rate. The isolation window for MS/MS was 2 to 3 Th depending on parent ion mass to charge ratio and the collision energy ranged from 23 to 65 eV depending on ion mass and charge. Parent ions were then excluded from MS/MS for the next 0.3 min and reconsidered if their intensity increased more than 5 times. Singly charged ions were excluded since, in ESI mode, tryptic peptides usually carry multiple charges. The strict active exclusion was applied. For mass accuracy, the error of mass measurement is typically within 5 ppm and is not allowed to exceed 10 ppm. The CaptiveSpray source was operated at 1900 V capillary voltage, 0.25 bar pressure with methanol in the nanoBooster, 3 L/min drying gas, and 150°C drying temperature.

2.2.8. Statistical Analysis

To evaluate the difference in growth and immune response, statistical comparisons were conducted on the average body mass, fork length, condition factor, hematocrit, and white blood cell count between control and stressed group. All statistical analyses were performed with IBM® SPSS® Statistics Standard version 25 (New York, USA) with data presented as the mean ± standard error (SE). The homogeneity of variances was examined by using Levene's test while the normality of the data distribution was assess using Shapiro-Wilk normality tests. Significant differences between two treatments were determined by an Independent Samples t-test ($p < 0.05$).

A one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test was performed to compare the average survival of three treatment groups of juvenile sockeye salmon after being immersed in different 17β-estradiol concentrations. The condition factor (K) was calculated using Fulton formula as follows (Datta et al., 2013): $K = (W \times 100) / L^3$ where W is the weight of fish in grams and L is length of fish in centimeters.

After obtaining the raw LC-MS/MS data from UBC Proteomics Core Facility, the data was processed in Perseus computational Proteomics Platform (V1.6.7.0) and the shiny app within R-4.0.5, and then searched against National Center for Biotechnology Information (NCBI) protein database to extract high confidence peptide and protein identifications that are translated from recently published genomic sequence and the relative ratios between treatments. The label-free quantification (LFQ) intensities extracted and normalized using the MaxQuant LFQ algorithm (PMID: 24942700), with 20ppm and 30ppm mass accuracies for precursor and product ion masses, respectively, and a 1% false discovery rate cut-off. The data were filtered for common contaminants and checked for reproducibility of technical replicates (Pearson correlation > 0.95) (Tyanova & Cox, 2018). Differential enrichment analysis of proteomics data was conducted in R-studio version1.4.1106 interfacing with R version 4.0.5 (DEP package, Massachusetts, USA). Specifically, rows were cleansed based on categorical column to filtered out proteins that were 1) only identified by peptides that carry one or more modified amino acids ("only identified by site"); 2) false positive hits from being matched to a decoy database ("reverse"); or 3) the contaminants generated from extraction process ("potential contaminant"). Then, the data were filtered based on valid values in each row and the mean of three technical replicates were taken if two out three values were found valid for each protein. The Perseus files were then loaded into R-studio for subsequent statistical tests to identify the significantly expressed proteins between treatments. To complete the differential protein expression analysis, an interactive DEP built-in tool called label-free quantification shiny app was launched. By running this application, normalization was automatically applied through variance-stabilizing transformation (Zhang et al., 2018). Proteomics data sometimes suffers from a high rate of missing values caused by the limit of detection ability, and therefore is usually left-censored (Schwämmle et al., 2019). This type of missingness is categorized as missing not at random (MNAR) and the use of imputation method from MSnbase

should be specific to such type of missingness (Zhang et al., 2018; Mcgurk et al., 2019). Other quality control methods (filtering and normalization) during bioinformatics stage were applied to have robust, reproducible, and unbiased results by eliminating process-based sources of variation and extreme biological outliers (Bittremieux et al., 2017; Zhang et al., 2018; Stratton et al., 2019). Next, differential enrichment analysis was performed by applying empirical Bayes statistics on protein-wise linear models using limma and the multiple visualizations were generated after adjusting the p value using empirical Bayes adjustment and $\log_2$-fold change (lfc) value (Zhang et al., 2018). The change in expression level can be considered as biological significant if the fold change is greater than 2 (Ting et al., 2009; Aguilan et al., 2020).

Table 2.1 Summary of preparation of 17β-estradiol stock solutions for phase 1 of feminizing genetic male sockeye salmon.

Stock solution			**Volume of stock solution added to 10 L Heath tray (mL)**	**Nominal 17β-estradiol exposure concentration per 10 L Heath tray (µg/L)**
17β-estradiol stock solution concentration (mg/L)	**Volume of EtOH (mL)**	**Mass of 17β-estradiol (mg)**		
20,000	0.2	4	0.1	200
40,000	0.2	8	0.1	400
80,000	0.2	16	0.1	800

Table 2.2 **qPCR primer set used to amplify *sexually dimorphic on the Y-chromosome (sdY)* in sockeye salmon in order to identify feminized males and males. National Center for Biotechnology and Information Accession numbers, primer sequences (5' to 3'), product size, melting temperature (Tm) and publication are listed (https://www.ncbi.nlm.nih.gov/).**

Gene of interest	Accession numbers	Sequence (5'- 3')	Product size (bp)	Tm (°C)	Reference
sdY (sexually dimorphic on the Y-chromosome)	KU556851	Forward: CCCAACACCCTTCCTATCTCC	95	57.0	Royle et al., 2018
		Reverse: CCTTCCTCCCTAGAGCTTAAAAC		54.8	

Table 2.3 **Primer and probe set used to amplify the virulent gene, *msa1*, in sockeye salmon for detection *Renibacterium salmoninarum* using qPCR assay to assess the effects of netting on disease suscepibility in sockeye salmon. National Center for Biotechnology and Information (https://www.ncbi.nlm.nih.gov/) accession number, primer and probe sequences (5' to 3'), product size, metling temperature (Tm) and publication are listed.**

Gene of interest	Accession number	Sequence (5'- 3')	Product size (bp)	Tm (°C)	Reference
msa1	AF123890	Forward: CCCAGATATCCATGCACCAGAT	137	56.6	Suzuki and Sakai (2007)
		Reverse: CAACTGAAACGGAACCAGCATT		56.2	
		Probe: FAM-TGGCGACAACACGTA-MGB			

Table 2.4 Detailed information of reagents added into each reaction well during whole-body cortisol ELISA for one 96-well plate.

Well type	Reagents				
	ELISA buffer (µL)	AChE tracer (µL)	Monoclonal antibody (µL)	ELISA standard (µL)	Whole-body sample (µL)
Blank well	0	0	0	0	0
Non-specific binding well	100	50	0	0	0
Maximum binding well	50	50	50	0	0
Total activity well	0	0	0	0	0
ELISA standard well	0	50	50	50	0
Sample well	0	50	50	0	50

Chapter 3. Results

3.1. Feminize Genetic XY Male for Enhanced Land-based Sockeye Salmon Farming

3.1.1. The Evaluation of Male Specific *sdY* Primer Prior to qPCR Screening Experiments to Identify Genetic Sex

In preparation for confirming the success in producing XY females with 17β-estradiol, the efficiency and specificity of *sdY* published in Royle et al. (2018) were tested. The *sdY* standard curve test using sockeye salmon male gDNA resulted in a standard curve with an efficiency of 100.5%, a slope of standard curve at -3.311, and an R^2 value of 0.978. A single peak was observed during the melt curve analysis demonstrating a single type of amplicon was generated. The cycle quantification (Cq) values from the primer validation test indicated the amount of DNA template added in each reaction well should be within 0.3 -15.6 ng/µL. Based on the qPCR result (Table 3.1), male sockeye salmon were being successfully amplified while the female sockeye salmon were not, indicating the *sdY* qPCR screening assay worked.

Table 3.1 qPCR assay results for *sdY* screening in fingerling sockeye for primer efficiency and specificity determination. A total of 4 samples with known sex (2 fish/sex) were tested. A no template control (NTC) negative control (did not contain any gDNA) was included in each assay. All samples were tested in triplicate wells.

Sample	Detectable (Yes/No)
Male sockeye 1	Yes
Male sockeye 2	Yes
Female sockeye 1	No
Female sockeye 2	No
NTC	No

3.1.2. Survival Data for Phase 2 of Feminization of Genetic XY Male

Because of the equipment failure at Alcan Aquatic Research Center, percent survival of sockeye fry during phase 2 of hormone treatment was only available for 28 days (Fig. 1). The sockeye fry exposed to lower 17β-estradiol level exhibited a slightly

higher mortality rate, however this difference was not significant as shown in Fig. 1 ($p > 0.05$). Overall mean percent survival was above 75% for all treatments.

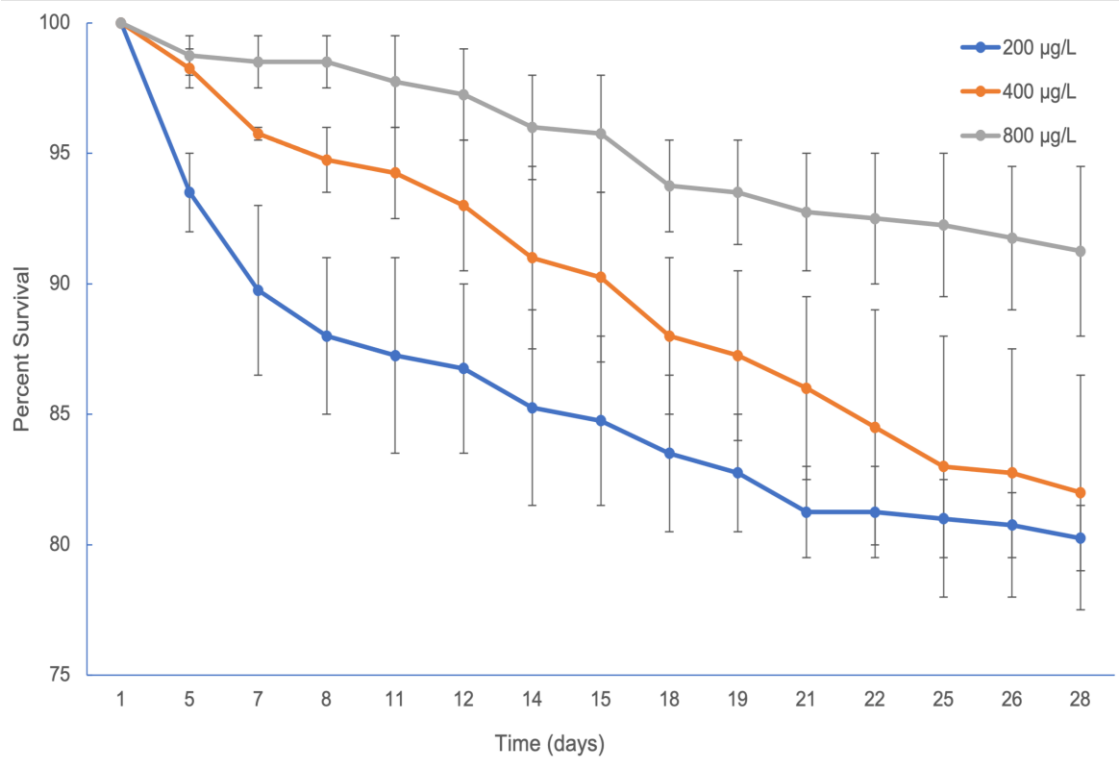


Figure 1 **Survival in sockeye fry over time during phase 2 of 17β-estradiol treatment to feminize genetic male sockeye salmon using various concentrations (200 µg/L, 400 µg/L and 800 µg/L; 100 fry from each replicate tank). Hormone treatments were waterborne and administered weekly from 40 days post-hatch to 68 days post-hatch at a water temperature of 6.8°C. Data are presented as mean ± SE. There were no significant differences in mortality over time or between hormone treatments (one-way ANOVA followed by a Tukey's post hoc test; $p > 0.05$).**

3.2. Impacts of Chronic Stress in Juvenile Sockeye Salmon

3.2.1. Effects of Repeated Netting on Growth Performance and Hematological Features

Both mean fork length ($p < 0.0001$) and body mass ($p < 0.0001$) were significantly reduced in netting-stressed fish compared to the control fish during trial 1 of weekly netting experiment (Table 3.2). The stressed fish had an average weight of 7.18

± 0.13 g while the unstressed sockeye salmon were significantly heavier (7.99 ± 0.12 g; Table 3.2). Fork length was also diminished in stressed sockeye salmon with a mean length of 8.44 ± 0.44 cm while the control fish mean length was 8.69 ± 0.05 cm. Despite these body weight and length decreases, condition factor was not significantly different between treatments (Table 3.2). In addition, no significant differences between treatments were observed in the total white blood cells counts ($p = 0.17$) or in hematocrit ($p = 0.88$) (Table 3.2).

Table 3.2 **Effects of weekly netting in trial 1 of the 100-day husbandry stress experiment on sockeye salmon fingerling growth, total white blood cell count, hematocrit and whole-body cortisol concentration. Each value represents the mean ± standard error of 3 replicate tanks. An asterisk indicates significant difference between netting-stressed and the control treatment for each measure using Independent Samples t-test ($p < 0.05$).**

Treatment group	Body weight (g)	Fork length (cm)	Condition factor	Total white blood cell count (cell/mm^3)	Hematocrit (%)	Whole-body cortisol concentration (pg/g body weight)
Netting-stressed	7.18 ± 0.13*	8.44 ± 0.05*	1.18 ± 0.01	4752 ± 628	39.03 ± 1.77	17.36 ± 5.67
control	7.99 ± 0.12	8.69 ± 0.05	1.20 ± 0.01	3693 ± 401	39.34 ± 0.83	17.55 ± 5.52

3.2.2. Effect of Weekly Netting Activity on Whole-body Cortisol Concentration

Whole-body cortisol level was measured in juvenile fish to determine the influence of handling treatment on stress hormone level in early life stages of sockeye salmon (trial 1). The mean intra-assay CV was 11.43%, and there was no significant difference in the whole-body cortisol level ($p > 0.05$) in juvenile sockeye salmon between treatments. The average cortisol concentration was 17.55 ± 5.52 pg/g body weight in the control group and 17.36 ± 5.67 pg/g body weight in the netting-stressed treated fish from trial 1.

3.2.3. Effect of Weekly Netting Activity on Disease Susceptibility

For the purpose of disease susceptibility evaluation, *R. salmoninarum* qPCR primer and probe set was first validated using fingerling sockeye salmon kidney collected from LSL and the aforementioned *R. salmoninarum* positive sockeye kidney tissue. The final curve efficiency was 99.4% and R^2 was 0.988 with the Cq value were ranging from 23 to 32 using a nominal template concentration ranging from 0.1 to 24.8 ng/µL of sockeye salmon kidney gDNA.

Based on the qPCR result (Table 3.3), none of the kidney samples from trial 1 of the weekly netting experiment tested positive for BKD across 6 tanks. The trial 1 of weekly netting practice, therefore, did not affect the prevalence of *R. salmoninarum* in fingerling sockeye.

Table 3.3 qPCR assay results for *R. salmoninarum* screening in fingerling sockeye after trial 1 of netting experiment. A total of 30 samples (15 fish/treatment group) were tested. An *R. salmoninarum* gDNA positive control was included in each qPCR assay, and a no template control (NTC) negative control (did not contain any gDNA) was included in each assay. All samples were tested in triplicate wells. Cq values are presented as mean ± standard error of 3 technical wells (- refers to Cq below detection limits of qPCR assay).

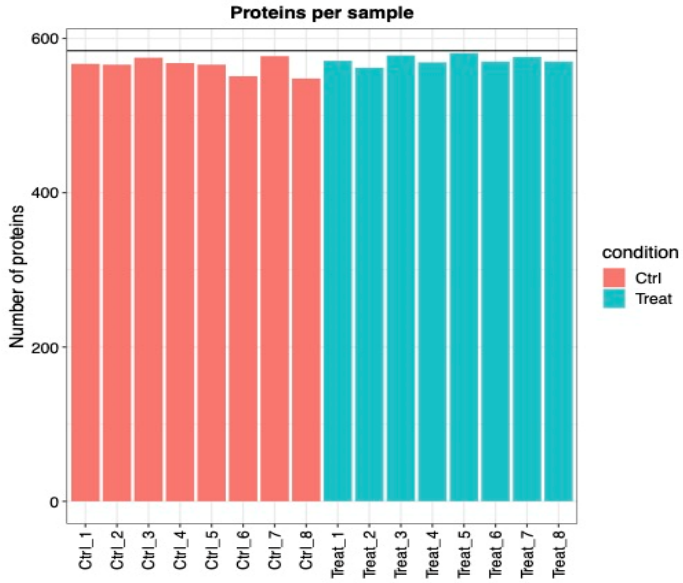
Sample	Detectable (Yes/No)	Cq
NTC	No	-
Positive control	Yes	23 ± 0.05
Control group from trial 1	No	-
Netting-stressed group from trial1	No	-

3.2.4. Change in Liver Proteome Profile and Potential Biomarkers

A total of 1810 proteins were detected by LC-MS/MS in 16 liver protein samples (n = 8 for both control and netting-stressed group). Among these proteins, only 584 proteins (Supplementary Table A1) were retained following the filtration of proteins in Perseus based on the three criteria described in section 2.2.8 and the Shiny built-in stringent filtering strategy. The number of identified and quantified proteins per sample after the data filtering step which includes the removal of potential contaminant sequence, reverse sequence and proteins only identified by site are presented in Fig. 2A. During the data processing using shiny app, the imputation method was selected based on the nature of missingness. The heatmap of protein abundance pattern in all fish liver samples was plotted to verify by visual inspection that the missing protein values were not biased to specific liver samples (Fig. 2B), and also mainly for proteins with low average intensity (Fig. 2C). After filtering out proteins with too many missing values, the protein coverage plot revealed the number of proteins that were consistently identified in all 16 of the juvenile sockeye salmon liver samples tested (Fig. 3). Approximately 400 proteins were identified in all 16 liver samples and over 100 proteins were identified in 15 of the 16 liver samples tested (Fig. 3).

As presented in Fig. 4, three proteins were differentially expressed between the control and netting-stressed fish when the adjusted p value was 0.05. Compared to the control juvenile sockeye salmon (Fig. 4), fish exposed to netting had one downregulated protein (interleukin enhancer-binding factor 2 homolog, ILF2) with an average $\log_2$-fold change (lfc) of -1.17 $\pm$ 0.31 (mean $\pm$ standard error) and two upregulated proteins: F-actin-capping protein subunit alpha-1-like (CapZα1, lfc: 1.18 $\pm$ 0.34) and mannosyl-oligosaccharide 1,2-alpha-mannosidase IA-like isoform X2 (Man1A1, lfc: 1.59 $\pm$ 0.38). Since all three proteins have a fold change greater than 2 (2.3-foldchange for ILF2, 2.3-fold change for CapZα1 and 3-fold change for Man1A1), the changes are biological significant (Ting et al., 2009; Aguilan et al., 2020).

(A)


Proteins per sample
Number of proteins
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400
200
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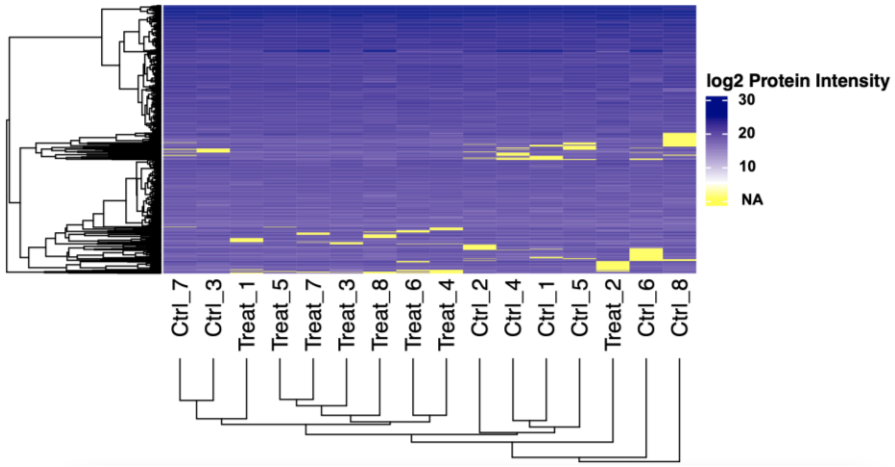
Ctrl_2
Ctrl_3
Ctrl_4
Ctrl_5
Ctrl_6
Ctrl_7
Ctrl_8
Treat_1
Treat_2
Treat_3
Treat_4
Treat_5
Treat_6
Treat_7
Treat_8
condition
Ctrl
Treat

(B)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20
10
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Treat_5
Treat_7
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(C)

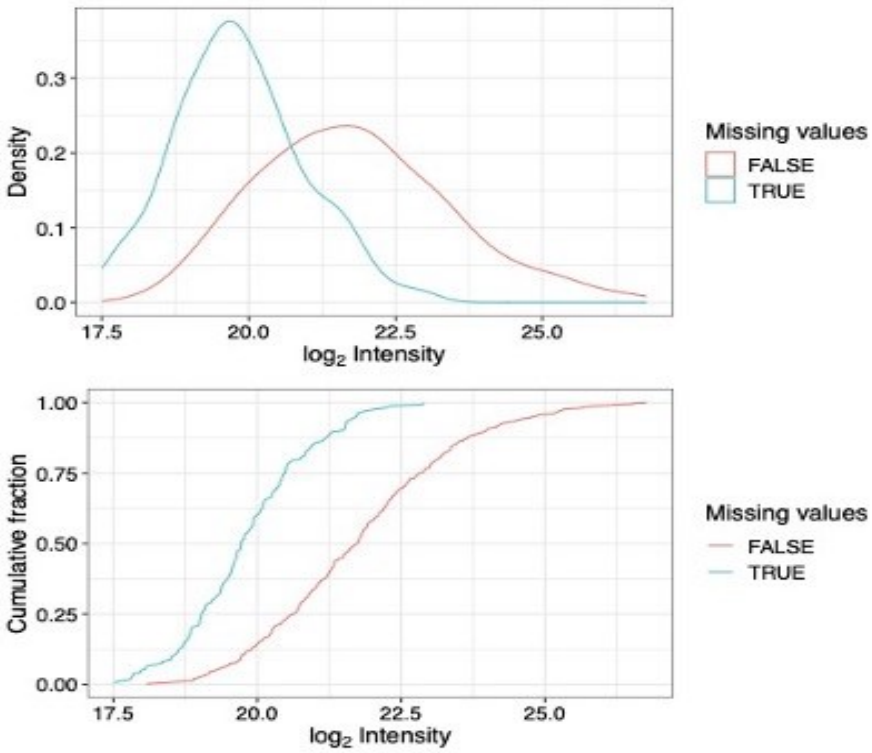

Figure 2 **Juvenile sockeye salmon protein profiles in control treatment (Ctrl) and after weekly netting stress for 100 days (Treat): A) number of identified proteins per sample (black horizontal line represents the proteins identified in all 16 samples); B) heatmap of liver proteins with log_2 protein intensity for every sample; C) pattern of missing values with respect to protein intensity**

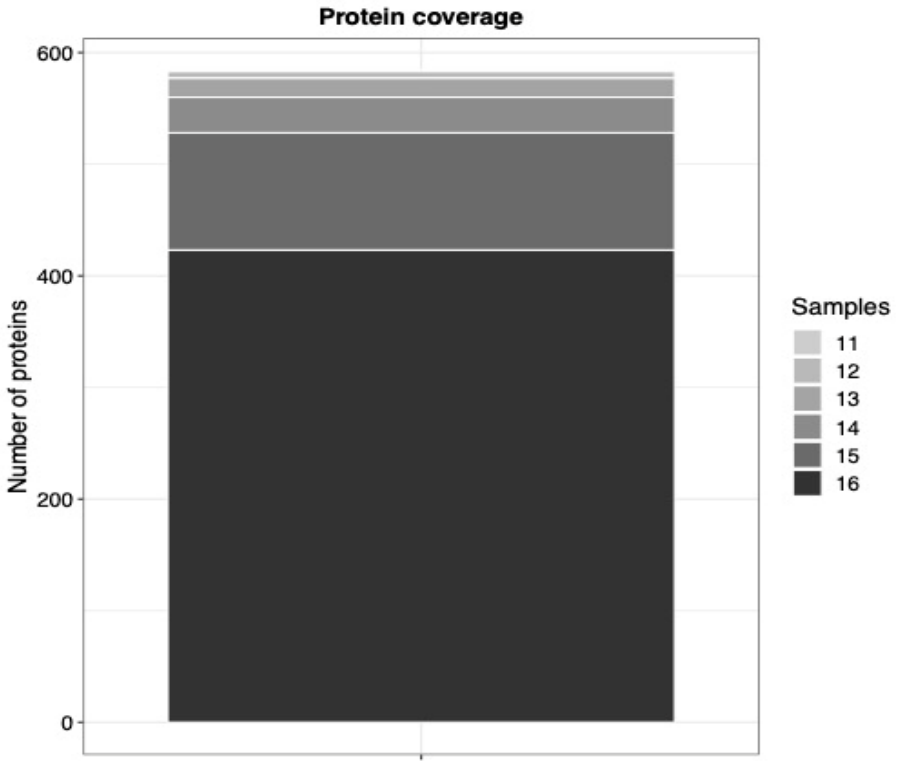

Figure 3 **Protein coverage plot of all 16 liver samples examined after 100 day netting experiment in juvenile sockeye salmon. The number of proteins classified and the number of juvenile sockeye salmon liver samples they were identified in is presented. Approximately 400 proteins were identified in all 16 liver samples, while ~100 proteins were identified in 15 of the 16 liver samples tested.**

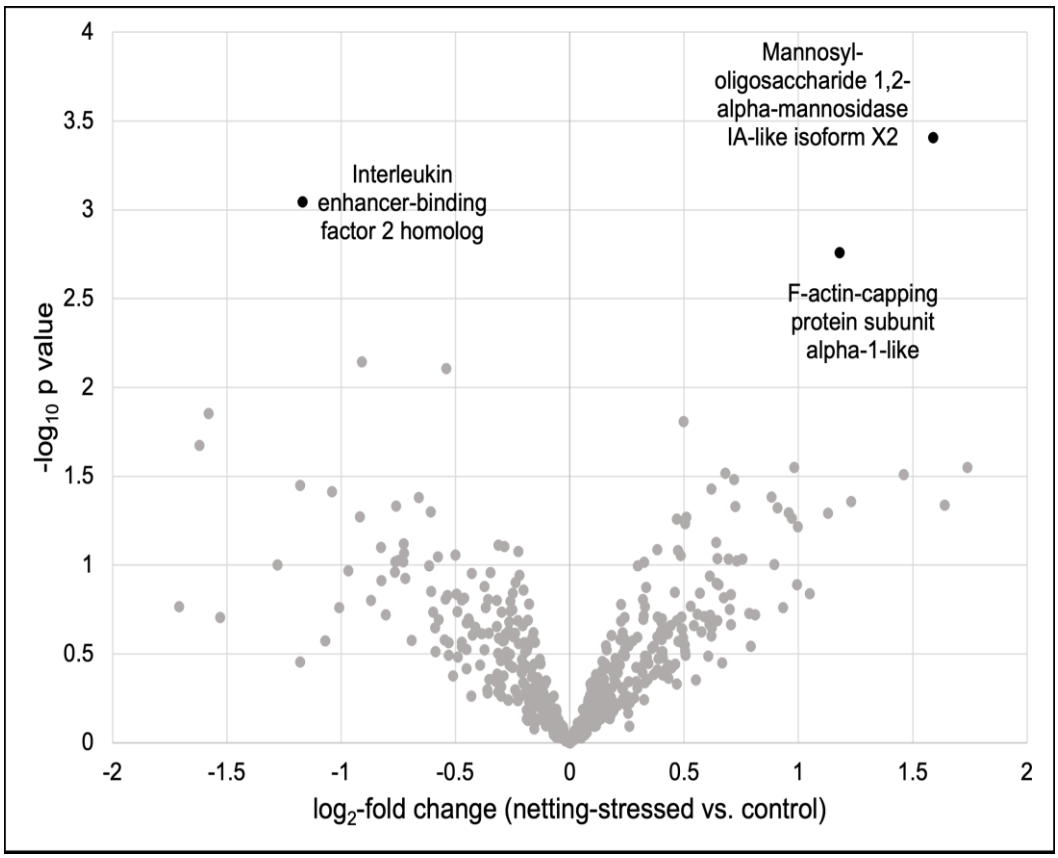

Figure 4 **Volcano plot of the juvenile sockeye salmon liver proteins detected by LS-MS/MS. Each point represents the log_2-fold change in abundance between netting-stressed fish and control fish plotted against the level of statistical significance. Black dots represent proteins significantly up- and down-regulated (empirical Bayes moderated t-test, adjusted $p < 0.05$).**

3.2.5. Survival of Juvenile Sockeye Salmon During Stress Treatments

During the first netting trial initiated on juvenile sockeye salmon 5 months post-hatch in age there was no significant change in percent survival between treatment groups; the average percent survival in control group was 76% while the netting-stressed group was 82% ($p > 0.05$, Figure 5A). However, in trial 2 of the netting trial initiated on older juvenile sockeye (9 months post-hatch) there was significantly higher survival in the control fish (100%) compared to the netting-stressed fish (98.1 ± 0.625%; $p = 0.02$; Figure 5B).

In addition, percent survival for sockeye salmon under control and stressed environment were both higher as the age of fish increases ($p < 0.05$). In consideration of the variation in trial location and water temperature, the extent to which the survival rate

can be affected by that 4-month difference in age remains unresolved and is worth further investigation.

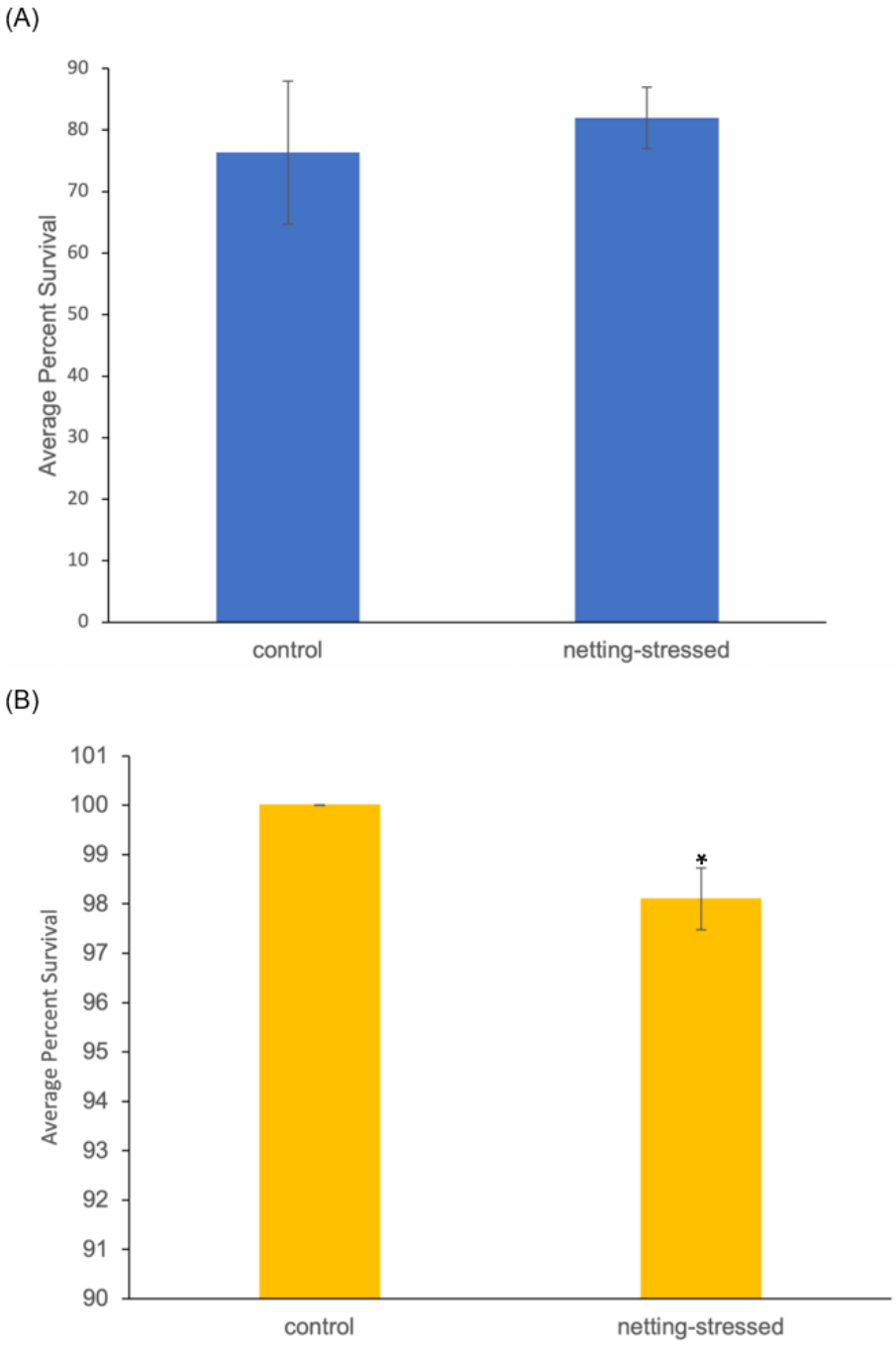

Figure 5 **Effects of weekly netting on average survival in juvenile sockeye salmon A) initiated in fish 5 months post-hatch over 100 days (trial 1) and B) and initiated at 9 months post-hatch over 81 days (trial 2). Values are shown as mean ± standard error and asterisk indicates significant difference between control or netting-stressed groups (Independent Samples t-test, $p < 0.05$).**

Chapter 4. Discussion

Atlantic salmon, rainbow trout, Arctic char, and coho and chinook salmon are currently the most popular salmonids to aquaculture in either fresh and/or sea water systems. The aquaculture of sockeye salmon through their life cycle remains a problem. Several individuals and groups have attempted to culture sockeye salmon in either fresh or sea water in Canada, Japan, and New Zealand (Albright, personal communication). Accordingly, the main objective of this research was to provide data to assist in optimizing land-based aquaculture practices for sockeye salmon, and specifically, for LSL a land-based, freshwater sockeye salmon farm. The key findings demonstrated that weekly netting less than one minute in duration imposed upon juvenile sockeye salmon (5 months post-hatch) resulted in ~10% reduction in body weight and length and some changes in the liver proteome. However, this reduction in body size was not associated with alterations in immune system indicators (i.e., BKD prevalence, leukocyte count, hematocrit) or induction of the stress response via cortisol levels. Additionally, even though difference in percent survival after netting stress experiment when sockeye salmon are older (9 months post-hatch) was statistically significant, the 1.9% decrease in survival may not be biologically significant. Collectively, this study revealed that weekly netting for 100 days does compromise growth in juvenile sockeye salmon, and this could restrict commercial production substantially if the aquacultural operator is unaware of this phenomenon.

4.1. Netting Causes Reduced Growth Performance

The negative impact of stressful stimuli on juvenile salmonids (5 months post-hatch) growth rate observed in this study was in accordance with the findings published by McCormick et al. (1998), Madaro et al (2015), and Vindas et al (2016). For example, Atlantic salmon (6 months post hatch) experienced impaired growth rate after being exposed to either daily 15 min chasing, 15 min crowding or water draining activity for 30 days (McCormick, 1998). Though the duration and frequency of induced stimuli changed, Vindas et al. (2016) and Madaro et al. (2015) reported similar results as McCormick (1998) after challenging the 10 months old Atlantic salmon parr with chronic stressors (5 min low water level, 3 min netting and air exposure and 5 min chase, etc.) three times a day for 23 days. In line with the results from present study, the body mass,

condition factor and growth rate of the untreated fish were all significantly higher than the stressed fish by the end of experiment (Vindas et al., 2016; Madaro et al., 2015). Similar stress responses to sub-optimal water conditions were found by Kennedy and Picard (2012) and Olsvik et al (2013). Kennedy and Picard (2012) demonstrated that juvenile sockeye salmon would have a significant low body weight following the 126-day chronic low pH exposure while Olsvik et al (2013) confirmed that high temperature and low oxygen stress contributed to the reduction in growth after 45 days.

One characteristic behavioral response to stress in fish that negatively affects growth performance appears to be the reduction in feed intake (Conde-Sieira et al., 2018). For example, juvenile rainbow trout subjected to weekly netting and weighing over an eight-week period in one study consumed significantly less food and the final weight gain was strongly related to the amount of consumed food (Hoskonen & Pirhonen, 2006). Similarly, the feed intake following one hour of confinement in Atlantic salmon pre-smolts decreased (Pankhurst et al., 2008) and Atlantic salmon parr suffered from a decreased appetite and a reduced growth rate after being handled daily for a month (McCormick et al., 1998). The compromised food consumption is believed to be correlated with stress (Bernier & Peter, 2001). More specifically, the various components along the HPI axis, such as CRF and cortisol level, are known to regulate appetite when fish are under stress (Conde-Sieira et al., 2018). In support of the suppressing influence on food intake due to cortisol, Madison et al. (2015) conducted a chronic cortisol exposure experiment on rainbow trout and found that cortisol indeed impacted growth by decreasing food intake. The inhibitory effect on feed conversion efficiency was observed by Bernier et al. (2004) in goldfish (*Carassius auratus*) after administering cortisol via the diet. Consequently, despite no changes in cortisol levels at the end of present study in juvenile sockeye salmon, a decrease in body weight was observed and supports several studies in fish that demonstrate decreased body weight is indicative of stress in fish.

Although in the present study, the approximate 1 g difference in average weight between treatment groups may seem trivial, the netting-stressed fish were actually gaining almost 10% less in biomass than control fish after ~3 months. Given that sockeye salmon will experience a more rapid growth before the beginning of the spawning season, the weight difference may go up to 15% – 20% when harvesting if the stress continues throughout the entire rearing period (Albright, personal communication).

Hence, if the netting activity persists and no adjustments are made, there will be a remarkable loss of profit.

4.2. Effects on Disease Susceptibility

The present study supports previous observations that LSL farm sockeye salmon exhibit a low rate of BKD infections caused by *R. salmoninarum,* and that netting does not induce BKD outbreaks in juvenile fish. Indeed, compared to high *R. salmoninarum* prevalence of 10% observed in sockeye salmon obtained from the LSL farm using qPCR in the present study, the fish in the netting experiment were all negative for this bacterium. This is contrary to other studies showing chinook salmon that were handled, crowded or subjected to lower nutritional regiments were more susceptible to R. salmoninarum (Elliott & Pascho 2001; Larson et al., 2020). However, this lack of increased rate of BKD after netting in the present study could potentially be due in part to the different water quality between the LSL farm and the rearing location of sockeye during the netting experiment at Simon Fraser University (SFU). Specifically, the relatively more frequent cleaning activity required for the stress experiment conducted at SFU reduced feces accumulation at the bottom of the tanks, and these cleaning procedures are not routinely performed in the larger scale aquaculture tanks at the LSL farm. Given that *R. salmoninarum* can survive outside the host animals for a short period of time and the ingestion or even contact with feces from BKD-infected fish results in the horizontal transfer of BKD (BC center for Aquatic Health Sciences, 2010), it is probable that *R. salmoninarum* is more prevalent on the farm. Therefore, more work should be done to further investigate the relationship between infection rate and the *R. salmoninarum*-specific mortality rate using husbandry practices and rearing conditions (i.e., feces removal, loading densities and netting frequencies) that more closely reflect aquaculture conditions are warranted. For example, if smaller scale laboratory-based studies are used then study designs that entail exposure to *R. salmoninarum* after netting to better mimic horizontal transfers prevalent in aquaculture facilities could be included.

Despite cultured sockeye salmon on the farm having a higher prevalence of *R. salmoninarum* (~10%), the mortality rate remains low to date (~1%) at the LSL farm, with visible signs of *R. salmoninarum* infection such as irregular swimming behavior and shallow ulcers not observed until sexual maturation is complete shortly before spawning.

Mortality near spawning season increases because the innate anti-bacterial effector proteins required to initiate a proper immune response would normally decrease dramatically as the spawning season approaches (Dolan et al., 2016). Previous studies suggested that certain preventative measures such as dietary supplementation and temperature control can be taken to alleviate the impact from netting on *R. salmoninarum* prevalence or to diminish the post-infection prevalence (Larson et al., 2020; Purcell et al. 2015). The present stress study examined sockeye salmon that were less than one year of age and not sexually mature. Thus, future studies are needed to determine if older life stages would exhibit different *R. salmoninarum* infection rates after various husbandry and rearing practices, including netting.

4.3. Effects on Blood Parameters and Whole-body Cortisol

The blood samples that were assayed for leukocyte count and hematocrit percentage determination revealed no significant changes between stressed and non-stressed fish, which concurs with previous studies reporting modest effects on the innate immune system of fish after chemical stress-type exposures. However, in the present study, only one time point was examined after a chronic physical stress (i.e., netting) and it is possible that in the present study the leukocyte number may have increased significantly at a more acute time point after netting and then recovered. This pattern has already been documented in some fish, for example total white blood cell count reached the highest point in common carp two days after lead exposure and then gradually returned to the baseline (Witeska et al., 2010). After 3-hour lead exposure at 10 mg/L, 6-month-old common carp were transferred back to metal-free culturing condition and subjected to blood sampling every other day (Witeska et al., 2010). It turned out that the white blood cell count fluctuated throughout the entire 16-day experimental process and once it was at the highest concentration around day 2, the white blood cell number slowly decreased to a level before the metal exposure (Witeska et al., 2010). Another study conducted on Javanese carp (Shariff et al., 2001) also revealed a similar pattern. The total white blood cell number in the treatment groups increased significantly during the initial stage of chronic copper exposures and then steadily recovered and remained at a level that resembles the control groups after 2 weeks (Shariff et al., 2001). In view of this rapid fluctuation of white blood cell numbers after chemical exposure, it is worthwhile to monitor the changes more frequently after physical stress such as netting in the

present study to better reflect the status of non-specific immune system when faced with similar prolonged stress event.

Chronic weekly netting activity did not have significant effects on whole-body cortisol levels in the present study. This finding is consistent with the plasma cortisol level change in juvenile Atlantic salmon after a long-term daily handling experiment which entailed keeping the treated fish out of water for 15 seconds (Fast et al., 2008). After this 4-week stress regime, no significant differences were observed between treatment groups, including total and free plasma cortisol level (Fast et al., 2008). One proposed explanation for the absence of a sustained chronic elevation of resting cortisol is that chronic stress events may desensitize and exhaust the HPI axis via mediation of various hormones (i.e., cortisol, corticosterone), receptors (glucocorticoid receptors, mineralcorticoid receptors) and enzymes (i.e., 11β-hydroxylase, 11β-hsd2) involved in the HPI axis at the transcriptional level (Madaro et al., 2016; Barton et al., 2002). For example, when rainbow trout were infected with *Cryptobia*, suppressed expression of head kidney genes involved in cortisol production and secretion, including *star*, *p450scc* and *11β-hydroxylase* was observed (Madison et al., 2013). As a result, the attenuated HPI axis may either mount a smaller and insignificant rise in cortisol level or delay the release of cortisol (Barton et al., 2002). In agreement with this theory, Madaro et al. (2015) introduced another handling stress event to both the control and stress-treated group at the end of 23-day stress experiment and found that plasma cortisol level changed before and after the novel stressor and was more pronounced in control Atlantic salmon parr compared to post-smolts. Furthermore, the plasma cortisol level in Atlantic salmon parr during the experimental period already exhibited a more attenuated increase after being handled for 9 days (Madaro et al., 2015). Together, these studies suggest that the amplitude of plasma cortisol change was greater when the stressor was first introduced and then gradually decreased, presumably due to a desensitized HPI axis. In addition, it is well established that the cortisol profile is strongly associated with developmental stage in salmon (Baker and Vynne, 2014), thus, cortisol response to the same stressor may differ between age groups. Baker and Vynne (2014) have characterized the basal cortisol level for sockeye salmon at three reproductive stages and showed that sockeye salmon at early maturation stages have significantly lower cortisol levels on a per mass basis. Likewise, Atlantic salmon parr were confirmed to be less responsive to acute crowding stress than Atlantic salmon smolt (Carey and

McCormick, 1998). Thus, it is possible that the lack of induction of cortisol synthesis during the early life stage tested in the present study may be attributed to the developmental stage or not capturing the temporal response of cortisol changes after a chronic, physical stressor in sockeye salmon.

4.4. Effects on Liver Protein Profile

The label-free proteomics methods identified 1810 proteins, similar to some other proteomics studies in the liver of pre-harvest Atlantic salmon exposed to warmer temperature (total of 1386, Nuez-Ortín et al., 2018), skeletal muscle of sexually immature coho salmon (total of 1365, Causey et al., 2019) and liver of chinook salmon (total of 2433, Esmaeili et al., 2021) but more than the number of protein number quantified in one-year old sockeye salmon serum after diluted bitumen exposure (total of 513, Alderman et al., 2017). And, after quality-control steps and stringent filtering of the data, only 584 were included in further analyses. This is the first study to examine liver proteome changes in sockeye salmon after chronic, repeated netting stress and showed that after 100 days only 3 proteins were differentially expressed despite a significant decreased body weight. Many other studies on the influence of pathogens on the proteins' abundance revealed larger scale changes in spleen, plasma and kidney proteome as summarized by Moreira et al. (2021). Collectively, these data suggest that global proteome changes in the liver may not be useful indicators of mild and permanent physical stress in juvenile sockeye salmon. These findings could be attributed to liver function being unaffected or to compensation/adaption after repetitive netting practice, whereby proteome changes were occurring but returned to baseline at the termination time point in this experiment. The latter phenomenon whereby changes may have occurred prior to the liver sampling time point (day 101) is also proposed as a plausible explanation for the absence in changes associated with circulating cortisol and white blood cells in this study, despite the decreased body weight indicative of a stress response in the netting-stressed fish. Nonetheless, a key finding from the present study is that the low-intensity, repetitive netting stress decreased the abundance of a protein (i.e., ILF2) responsible for the regulation of immune-responsive genes and increased the abundance of two proteins involved in protein processing (i.e., CapZα1) and cytoskeletal structure organization (i.e., Man1A1) in juvenile sockeye salmon.

The decrease in hepatic ILF2 protein levels suggests some impact on immune system function manifested in the netting-stressed fish, despite not finding other indicators of immune system malfunction (i.e., prevalence of *R. salmoninarum*, white blood cells count) in fingerling sockeye in this study. Although less well studied in lower vertebrates, substantial (i.e., >90%) conservation of amino acid sequence of across vertebrates studied to date suggests that ILF2 in bony fish likely functions in a similar fashion as observed in mammals (Wang et al., 2006; Cui et al., 2011). In mammals ILF2 is known to regulate the expression of interleukin-2 as well as other genes that are crucial in T-cell responses and amplification of naive lymphocytes (Wang et al., 2006; Jin et al., 2018) by binding to the interleukin-2 promoter region and influencing immune-associated gene transcription (Lin et al., 2006; Wang et al., 2006; Jin et al., 2018). Several *in vivo* and *in vitro* studies demonstrated that mice with deficient interleukin-2 activity have less efficient viral clearance (Boyman & Sprent, 2012). Similarly, an *in vitro* study in rainbow trout supports interleukin 2 function by demonstrating that the addition of trout recombinant interleukin-2 into head kidney cells induced the expression of various interferons (IFN), namely *IFNγ1, IFNγ2* and *TNFα2*, required for mounting a proper defense against infections (Wang et al.,2018). In 2021, Wang et al. investigated the bioactivity interleukin-2 in leucocytes and the expression level of *interleukin-2* after challenging grass carp (*Ctenopharyngodon Idella*) reovirus and *Flavobacterium columnare*. They found that interleukin-2 can stimulate the proliferation of primary cell cultures from head kidney leucocytes and its expression was upregulated following the challenges (Wang et al., 2021). Chi et al. (2014) have explored the effects of ILF2 on bacterial and viral infection and observed that both the viral copies and bacterial load were significantly lower in tongue sole (*Cynoglossus semilaevis*) with overexpressed ILF2. Thus, the reduction in ILF2 observed in present study should be further investigated with other pathogens and stressors in fish to more comprehensively study the implications of changes in this protein on immune system function in fish and its utility as an indicator of long-term, repetitive stress.

The significance of the increased in abundance of CapZα1 in the liver of juvenile sockeye salmon after netting stress exposure in the present study merits further research since this protein in fish is not well studied. However, in higher vertebrates it is involved in actin cytoskeleton organization and is known to bind to the growing ends of actin and arrest the exchange of subunits at these ends (Cooper et al., 1991; Mejillano

et al., 2004; Elbediwy et al., 2012). Actin filaments are involved in many crucial cytoskeletal structures and functions of eukaryotic cells, including cytokinesis during cell division, membrane protrusions during cell migration, spine development and maturation, formation of functional synapses, and is an essential part of the cytoskeleton to build many higher order structures in cells (Pollard & Cooper et al., 2009; Hu & Papoian, 2010; Fan et al., 2011; Edwards et al., 2014; Terry et al, 2018). Studies in humans have demonstrated that overexpression of CapZα1 was associated with smaller gastric tumor size, absence of lymph node metastasis, lower recurrence rate and longer survival times, which suggests that excessive capping proteins can stall cell growth and intracellular motility (Lee et al., 2013). In addition, by encapsulating CapZα1 and actin into giant unilamellar vesicles, researchers discovered that low capping protein activity favored the formation membrane protrusions in this reconstitution system (Dürre et al., 2018). Ultimately, an increase in this protein that influences cytoskeletal structure and function, and cellular motility may have implications on numerous cellular processes, such as cell proliferation or motility of immune system cells. Future *in vivo* studies examining this protein in fish liver as an indicator of stress are needed to determine if it is a possible biomarker of stress. And *in vitro* and *in vivo* studies aimed at establishing CapZα1 function and role in cell structure, motility, and proliferation in vertebrate species to better understand the implications of abnormal levels of this protein at the cellular and organismal level are warranted.

Man1A1 also increased in abundance (~ three-fold) in netting-stressed juvenile sockeye salmon relative to the control fish and is also a potential biomarker of handling stress in salmonids that merits further investigation. Man1A1 is a subtype of α-1,2 mannosidase which participates in trimming N-glycan during post-translational protein modification in eukaryotes (Bednarska et al., 2017; Tu et al., 2017). In general, protein glycosylation increases the diversity of proteins due to the variety of oligosaccharide side chains, extends the range of protein functionality and has a defining impact on protein folding pattern (Bednarska et al., 2017; Tu et al., 2017; Lin et al., 2020). Some studies have also demonstrated that the glycosylated proteins are crucial parts in modulating immune functions as they can affect cell-cell adhesion and cell motility (Hong et al., 2014; Tu et al., 2017; Lin et al., 2020). For example, once glycosylated, fish skin gelatin exhibited antimicrobial activity and higher antioxidant activity (Hong et al., 2014). Similarly, when Atlantic salmon (post-smolt) were reared in high loading density, the

glycosylation of skin mucus was induced (Benktander et al., 2021). Hu et al. (2016) also examined the effects of glycosylation of a secretory protein, namely liver-enriched gene 1 (leg1) in zebrafish liver. Specifically, Hu et al. (2016) injected the glycosylated leg1 into zebrafish embryos at the one-cell stage and demonstrated that the N-glycosylation was required to protect and promote liver development under stress conditions, such as warmer temperature, high density, and ultraviolet irradiation. Interestingly, in transgenic zebrafish overexpressing Man1A1 in liver was associated with progression of hepatocarcinogenesis along with an enhanced protein expression in binding immunoglobulin protein, and although further studies are necessary it was proposed as a novel oncogene (Tu et al., 2017). Collectively, the studies to date suggest that Man1A1 is inducible in fish liver under various stress conditions, but additional studies investigating if overexpression of this protein in liver translates into hepatocarcinogenesis or is an adaptive response are needed.

Chapter 5. Conclusion

In light of the dramatic declines in wild salmon stocks, optimizing fish welfare and growth in captivity is increasingly important for sustainable, humane and productive aquaculture of salmonids. Collectively, this study has revealed that weekly netting stress for 100 days does compromise growth in juvenile sockeye salmon and would restrict commercial production. This study also shows that global proteome changes in the liver, BKD prevalence, leukocyte count, hematocrit and cortisol levels were not useful indicators of mild and permanent physical stress in juvenile sockeye salmon in a ~3-month experiment. Future studies aimed at identifying earlier onset biomarkers of stress are needed to better predict and understand the underlying pathways that cause a reduction in growth in juvenile sockeye salmon. In particular, additional time-course experiments investigating proteome, hematological and physiological measures on a daily basis throughout chronic studies are needed. For juvenile sockeye salmon in this study, blood volume was limited but several studies have demonstrated blood proteome profiles change in response to pathogens (Magalhães et al., 2020; Moreira et al., 2021), so testing multiple tissue as well as blood plasma will increase the likelihood of identifying more consistent acute and chronic husbandry stress biomarkers. Longer term studies are also necessary to gauge the extent of age specific sensitivity to stressors in sockeye salmon aquaculture and the ability of this species to adapt over time, and the implications these factors have on growth and welfare.

In conclusion, the data from the present study helps to build hematology reference values and paves the way for further investigations to elucidate the molecular and cellular pathways that eventually lead to suppressed growth in response to a common and frequent husbandry stressor in sockeye salmon, a species that has potential in aquaculture but is not currently prevalent in this industry. We also emphasize the need for ongoing research to optimize management of husbandry practices in aquaculture, as fish in general are often not afforded the same level of concern for welfare as other vertebrates.

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Appendix.

Supplementary Data

Table A.1. Accession numbers from National Center for Biotechnology and Information protein database (https://www.ncbi.nlm.nih.gov/protein), average log2-fold changes, p values and adjusted p values of all 584 liver proteins retained in control and netting-stressed juvenile sockeye salmon.

		Netting-stressed vs. control					
Accession number	Data imputed	95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029476040.1	TRUE	-0.61366	1.080647	0.233492	0.984914	0.568164	FALSE
XP_029476453.1	FALSE	-0.34003	0.506042	0.083007	0.987466	0.683765	FALSE
XP_029476563.1	FALSE	-1.62905	0.566683	-0.53118	0.973079	0.32129	FALSE
XP_029476592.1	TRUE	-1.28274	0.49854	-0.3921	0.97642	0.365587	FALSE
XP_029476614.1	TRUE	-0.40474	1.204254	0.399759	0.971955	0.308778	FALSE
XP_029476799.1	TRUE	-0.67583	0.780501	0.052335	0.990261	0.881135	FALSE
XP_029477266.1	FALSE	-0.47435	0.609816	0.067734	0.989214	0.795033	FALSE
XP_029477284.1	FALSE	-0.2027	1.48741	0.642356	0.928817	0.127036	FALSE
XP_029477305.1	TRUE	0.509285	1.847634	1.178459	0.046559	0.001744	TRUE
XP_029477310.1	FALSE	-1.51896	0.538771	-0.4901	0.973696	0.328616	FALSE
XP_029477473.1	TRUE	-2.05434	0.441505	-0.80642	0.953571	0.1903	FALSE
XP_029477675.1	TRUE	-0.45516	1.457295	0.501069	0.969422	0.283975	FALSE
XP_029477873.1	FALSE	-0.44715	0.354639	-0.04625	0.989417	0.8104	FALSE
XP_029478459.1	FALSE	-0.38981	1.612236	0.611211	0.959081	0.214649	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029478639.1	TRUE	-1.49516	1.374746	-0.06021	0.990774	0.930425	FALSE
XP_029478640.1	FALSE	-0.65735	1.075988	0.20932	0.986101	0.616564	FALSE
XP_029478700.1	FALSE	-0.47801	0.393352	-0.04233	0.989786	0.839858	FALSE
XP_029478750.1	FALSE	-0.2725	0.343608	0.035552	0.989416	0.810348	FALSE
XP_029478981.1	TRUE	-1.14899	0.260325	-0.44433	0.956097	0.200706	FALSE
XP_029479233.1	FALSE	-0.42718	0.453335	0.013077	0.990969	0.950709	FALSE
XP_029479234.1	FALSE	-0.18815	0.660166	0.236008	0.96599	0.256283	FALSE
XP_029479267.1	TRUE	0.15165	2.775647	1.463649	0.739502	0.030914	FALSE
XP_029479401.1	FALSE	-0.28558	0.504258	0.10934	0.984867	0.566432	FALSE
XP_029479627.1	TRUE	0.011284	1.804321	0.907802	0.809759	0.04749	FALSE
XP_029479742.1	FALSE	-0.43735	0.11743	-0.15996	0.963593	0.240051	FALSE
XP_029479772.1	TRUE	-1.56629	0.114413	-0.72594	0.892224	0.085935	FALSE
XP_029479824.1	TRUE	-2.51209	0.489664	-1.01121	0.948681	0.173039	FALSE
XP_029479959.1	TRUE	-0.50824	1.090172	0.290966	0.981024	0.452558	FALSE
XP_029480118.1	FALSE	-0.29395	0.520487	0.113266	0.98482	0.56466	FALSE
XP_029480541.1	TRUE	-0.1891	1.980071	0.895484	0.907709	0.099479	FALSE
XP_029480584.1	FALSE	-0.19007	0.414205	0.112066	0.980665	0.444256	FALSE
XP_029480618.1	FALSE	-0.56198	0.721294	0.079656	0.989231	0.796325	FALSE
XP_029480626.1	FALSE	-0.43054	0.243502	-0.09352	0.984844	0.565578	FALSE
XP_029480708.1	FALSE	-0.42345	0.242568	-0.09044	0.985062	0.573789	FALSE
XP_029480711.1	FALSE	-0.45983	0.130889	-0.16447	0.96594	0.255927	FALSE
XP_029480767.1	FALSE	-0.77361	0.68157	-0.04602	0.990415	0.895305	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029480847.1	FALSE	-0.65942	1.021414	0.180998	0.986916	0.654981	FALSE
XP_029481009.1	FALSE	-0.5454	0.282552	-0.13142	0.983235	0.511597	FALSE
XP_029481061.1	FALSE	-0.75466	1.115698	0.18052	0.987554	0.688603	FALSE
XP_029481133.1	FALSE	-0.60115	0.266459	-0.16734	0.979851	0.426567	FALSE
XP_029481272.1	FALSE	-0.53842	0.327704	-0.10536	0.986043	0.614011	FALSE
XP_029481527.1	TRUE	-0.84075	0.926669	0.042961	0.990664	0.919423	FALSE
XP_029481617.1	FALSE	-0.23975	0.28562	0.022936	0.989976	0.855878	FALSE
XP_029482050.1	FALSE	-0.33924	0.359099	0.009929	0.990989	0.95281	FALSE
XP_029482128.1	FALSE	-0.36003	0.763887	0.201927	0.981268	0.458369	FALSE
XP_029482345.1	FALSE	-0.53606	0.169558	-0.18325	0.969873	0.288081	FALSE
XP_029482364.1	FALSE	-0.45342	1.267913	0.407246	0.973952	0.331754	FALSE
XP_029482378.1	FALSE	-0.49793	0.473341	-0.0123	0.991037	0.95798	FALSE
XP_029482405.1	TRUE	-1.11501	0.190886	-0.46206	0.941781	0.153565	FALSE
XP_029482453.1	TRUE	-1.53826	0.084818	-0.72672	0.877267	0.075979	FALSE
XP_029482463.1	FALSE	-0.34775	0.648519	0.150387	0.983891	0.532256	FALSE
XP_029482630.1	FALSE	-0.52586	0.773255	0.123699	0.987625	0.692558	FALSE
XP_029482753.1	FALSE	-1.17814	0.325195	-0.42647	0.964719	0.247402	FALSE
XP_029482999.1	TRUE	-0.75796	0.694087	-0.03194	0.990741	0.927061	FALSE
XP_029483201.1	FALSE	-0.20152	0.456691	0.127587	0.979742	0.424316	FALSE
XP_029483387.1	TRUE	-1.15128	0.271131	-0.44008	0.957889	0.208845	FALSE
XP_029483436.1	FALSE	-0.57462	0.253996	-0.16031	0.979784	0.425184	FALSE
XP_029483471.1	FALSE	-0.17385	0.370424	0.098287	0.981174	0.456108	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029483474.1	FALSE	-0.13473	0.771776	0.318521	0.942847	0.15627	FALSE
XP_029483576.1	FALSE	-0.51469	0.229914	-0.14239	0.980036	0.430473	FALSE
XP_029483967.1	FALSE	-0.01114	0.948054	0.468456	0.828053	0.054953	FALSE
XP_029484070.1	TRUE	-1.02401	0.576414	-0.2238	0.984762	0.562544	FALSE
XP_029484114.1	FALSE	-0.75392	0.15643	-0.29875	0.951842	0.1838	FALSE
XP_029484323.1	FALSE	-0.7874	0.755348	-0.01602	0.991107	0.965515	FALSE
XP_029484339.1	TRUE	-0.38983	1.682214	0.64619	0.957148	0.205398	FALSE
XP_029484345.1	FALSE	-0.12818	1.517677	0.694748	0.900501	0.092671	FALSE
XP_029484462.1	TRUE	-0.33078	1.135021	0.40212	0.966854	0.26271	FALSE
XP_029484469.1	FALSE	-0.16998	0.632649	0.231335	0.96362	0.240225	FALSE
XP_029484496.1	TRUE	-1.5456	0.451415	-0.54709	0.966937	0.263346	FALSE
XP_029484790.1	FALSE	-0.96789	0.109845	-0.42902	0.917981	0.11117	FALSE
XP_029485145.1	FALSE	-0.83901	0.3319	-0.25355	0.976914	0.373242	FALSE
XP_029485153.1	FALSE	-0.87299	0.124793	-0.3741	0.9316	0.131902	FALSE
XP_029485422.1	FALSE	-0.25777	0.648918	0.195575	0.977028	0.375059	FALSE
XP_029485728.1	FALSE	-0.2103	0.466633	0.128168	0.980245	0.434951	FALSE
XP_029485786.1	FALSE	-0.27649	0.331405	0.027456	0.989919	0.850961	FALSE
XP_029485832.1	TRUE	-0.28791	1.170412	0.441249	0.95985	0.218576	FALSE
XP_029486296.1	FALSE	-0.30009	0.331044	0.015477	0.990657	0.918714	FALSE
XP_029486446.1	FALSE	-0.9724	0.263304	-0.35455	0.96394	0.242268	FALSE
XP_029486767.1	FALSE	-0.63807	0.249108	-0.19448	0.976547	0.367523	FALSE
XP_029486768.1	FALSE	-1.72304	0.190598	-0.76622	0.916451	0.109253	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029486815.1	FALSE	-0.22763	0.408967	0.090667	0.984566	0.555413	FALSE
XP_029486818.1	FALSE	-0.60771	0.036315	-0.2857	0.881376	0.078475	FALSE
XP_029486819.1	FALSE	-0.92857	0.504457	-0.21206	0.984131	0.540264	FALSE
XP_029486874.1	FALSE	-0.42676	0.684862	0.129052	0.986401	0.630153	FALSE
XP_029486902.1	FALSE	-0.2809	0.662	0.190549	0.978773	0.405264	FALSE
XP_029487233.1	TRUE	-0.85149	0.622132	-0.11468	0.988514	0.74639	FALSE
XP_029487268.1	FALSE	-1.26386	0.72682	-0.26852	0.985128	0.576314	FALSE
XP_029487608.1	FALSE	-0.40497	0.426793	0.010913	0.991023	0.956449	FALSE
XP_029487743.1	TRUE	-1.10746	0.279363	-0.41405	0.960952	0.224471	FALSE
XP_029488010.1	TRUE	-0.33184	1.506957	0.587561	0.954744	0.194991	FALSE
XP_029488152.1	FALSE	-0.2845	0.571953	0.143727	0.98242	0.488071	FALSE
XP_029488168.1	FALSE	-0.24052	0.600571	0.180025	0.977252	0.378671	FALSE
XP_029488186.1	FALSE	-0.86139	1.797234	0.467921	0.981635	0.46743	FALSE
XP_029488323.1	FALSE	-0.57334	0.599415	0.013039	0.991085	0.96309	FALSE
XP_029488524.1	TRUE	-0.87347	0.841472	-0.016	0.991139	0.969021	FALSE
XP_029488696.1	TRUE	-3.04255	0.89721	-1.07267	0.967303	0.266188	FALSE
XP_029488847.1	TRUE	-0.53898	1.551362	0.506193	0.973039	0.320821	FALSE
XP_029489045.1	FALSE	-0.56957	0.231156	-0.16921	0.977611	0.38462	FALSE
XP_029489086.1	TRUE	-0.85221	0.80515	-0.02353	0.99099	0.952882	FALSE
XP_029489166.1	FALSE	-0.22644	0.626616	0.20009	0.974274	0.335802	FALSE
XP_029489233.1	FALSE	-0.77282	0.559548	-0.10664	0.988407	0.739454	FALSE
XP_029489264.1	TRUE	-0.47879	1.718614	0.619912	0.96509	0.24993	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029489276.1	FALSE	-0.26708	0.477853	0.105389	0.984638	0.558021	FALSE
XP_029489412.1	FALSE	-0.77122	0.460092	-0.15556	0.985729	0.600507	FALSE
XP_029489436.1	TRUE	0.065083	1.37152	0.718301	0.752494	0.033068	FALSE
XP_029489562.1	TRUE	-0.82673	0.579261	-0.12373	0.988007	0.714695	FALSE
XP_029489577.1	FALSE	-1.02319	0.941829	-0.04068	0.990783	0.931341	FALSE
XP_029489742.1	TRUE	-0.92978	2.033549	0.551886	0.980581	0.442369	FALSE
XP_029489783.1	FALSE	-0.69665	0.954388	0.128868	0.988503	0.745663	FALSE
XP_029489846.1	FALSE	-1.48683	0.341559	-0.57264	0.956743	0.203564	FALSE
XP_029489976.1	FALSE	-0.27724	0.277719	0.000242	0.991399	0.998551	FALSE
XP_029490095.1	FALSE	-0.40712	0.263238	-0.07194	0.986938	0.656081	FALSE
XP_029490568.1	FALSE	-0.77296	0.211486	-0.28074	0.964363	0.24503	FALSE
XP_029490631.1	FALSE	-0.44606	0.673032	0.113486	0.987281	0.673785	FALSE
XP_029490707.1	FALSE	-0.7788	0.594533	-0.09214	0.989011	0.780308	FALSE
XP_029490843.1	TRUE	-0.38685	0.969707	0.29143	0.977146	0.376947	FALSE
XP_029490950.1	FALSE	-0.26814	0.431687	0.081775	0.986353	0.627937	FALSE
XP_029491083.1	FALSE	-0.36774	0.510633	0.071445	0.988343	0.735389	FALSE
XP_029491185.1	TRUE	-1.00397	1.133395	0.064715	0.990461	0.899733	FALSE
XP_029491241.1	FALSE	-1.8955	0.246755	-0.82437	0.926128	0.122678	FALSE
XP_029491424.1	TRUE	-1.52526	0.805911	-0.35967	0.983614	0.523337	FALSE
XP_029491466.1	FALSE	-0.35053	0.386335	0.017902	0.990665	0.919463	FALSE
XP_029491526.1	FALSE	-0.21006	0.385908	0.087925	0.984166	0.541473	FALSE
XP_029491641.1	FALSE	-1.64078	0.141792	-0.74949	0.901835	0.093858	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029491785.1	FALSE	-0.30819	0.322605	0.007208	0.991075	0.962066	FALSE
XP_029491808.1	FALSE	-0.74157	0.538677	-0.10144	0.988446	0.741947	FALSE
XP_029491853.1	FALSE	-0.83101	0.245298	-0.29285	0.967341	0.266485	FALSE
XP_029491854.1	FALSE	-1.29189	-0.02838	-0.66014	0.790995	0.041564	FALSE
XP_029491860.1	TRUE	-1.60196	0.144052	-0.72895	0.904161	0.096004	FALSE
XP_029491904.1	TRUE	-0.45217	0.781796	0.164815	0.985223	0.580026	FALSE
XP_029491970.1	FALSE	-0.64367	0.196398	-0.22364	0.968576	0.276579	FALSE
XP_029492128.1	FALSE	-0.14078	0.506105	0.182661	0.96503	0.249514	FALSE
XP_029492145.1	FALSE	-0.5768	0.763637	0.093417	0.988894	0.772037	FALSE
XP_029492161.1	TRUE	-0.44169	1.216206	0.387256	0.974427	0.337759	FALSE
XP_029492409.1	FALSE	-0.62089	0.872561	0.125833	0.988198	0.7263	FALSE
XP_029492654.1	TRUE	-0.00335	1.917673	0.957159	0.818111	0.050718	FALSE
XP_029492933.1	TRUE	-0.99009	0.777872	-0.10611	0.989319	0.80291	FALSE
XP_029492972.1	FALSE	-0.57962	0.610465	0.015425	0.991028	0.956978	FALSE
XP_029493022.1	FALSE	-0.4164	0.151947	-0.13223	0.974572	0.339635	FALSE
XP_029493161.1	TRUE	-0.58731	0.951717	0.182203	0.986254	0.623455	FALSE
XP_029493416.1	FALSE	-0.42411	0.202825	-0.11064	0.981587	0.466236	FALSE
XP_029493598.1	FALSE	-1.3627	0.133197	-0.61475	0.909084	0.100896	FALSE
XP_029493662.1	TRUE	-0.72927	2.31346	0.792094	0.969752	0.286968	FALSE
XP_029493930.1	FALSE	-0.20328	0.316012	0.056367	0.986865	0.652415	FALSE
XP_029493936.1	TRUE	-0.6715	1.191959	0.260227	0.984777	0.563067	FALSE
XP_029493967.1	FALSE	-0.45094	0.965872	0.257467	0.981056	0.453319	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029494197.1	FALSE	-0.50206	0.059302	-0.22138	0.920362	0.114297	FALSE
XP_029494729.1	FALSE	-0.32854	0.384883	0.028173	0.990132	0.869492	FALSE
XP_029494794.1	FALSE	-0.00926	1.026316	0.508529	0.825076	0.053774	FALSE
XP_029494810.1	TRUE	-1.0284	0.450283	-0.28906	0.979554	0.420473	FALSE
XP_029494841.1	TRUE	-0.33793	1.316313	0.489192	0.961709	0.228716	FALSE
XP_029494887.1	FALSE	-0.49597	0.352166	-0.0719	0.988172	0.724681	FALSE
XP_029495002.1	FALSE	-0.3481	0.443997	0.047951	0.989297	0.801245	FALSE
XP_029495018.1	FALSE	-1.27936	0.209719	-0.53482	0.939386	0.147827	FALSE
XP_029495180.1	FALSE	-0.58636	0.901576	0.157609	0.98702	0.660229	FALSE
XP_029495342.1	FALSE	-0.30838	0.728471	0.210045	0.978713	0.404145	FALSE
XP_029495406.1	FALSE	-0.51428	0.802097	0.143908	0.986818	0.650112	FALSE
XP_029496142.1	FALSE	-0.43373	0.130038	-0.15185	0.967926	0.271171	FALSE
XP_029496198.1	FALSE	-0.78446	0.762168	-0.01115	0.991202	0.97607	FALSE
XP_029496211.1	FALSE	-0.69672	0.366514	-0.1651	0.983531	0.520697	FALSE
XP_029496473.1	FALSE	-0.29306	0.356903	0.031923	0.989765	0.838113	FALSE
XP_029496588.1	TRUE	-0.35404	1.440109	0.543036	0.959822	0.218432	FALSE
XP_029496602.1	FALSE	-0.24595	0.446151	0.1001	0.984395	0.549365	FALSE
XP_029496659.1	FALSE	-0.32854	0.435395	0.053427	0.988883	0.771261	FALSE
XP_029496771.1	FALSE	-0.37156	0.335615	-0.01797	0.990627	0.915761	FALSE
XP_029496797.1	FALSE	-1.25678	1.356438	0.049829	0.990836	0.936746	FALSE
XP_029496815.1	TRUE	-1.51866	0.458492	-0.53008	0.968185	0.273299	FALSE
XP_029496820.1	FALSE	-0.48574	0.324432	-0.08066	0.987385	0.67939	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029496916.1	FALSE	-0.26191	0.317962	0.028024	0.989796	0.840671	FALSE
XP_029497068.1	FALSE	-0.55733	0.306491	-0.12542	0.984351	0.547834	FALSE
XP_029497254.1	TRUE	-0.8668	1.516462	0.324833	0.985025	0.572375	FALSE
XP_029497338.1	FALSE	-0.36483	0.664019	0.149596	0.984335	0.547264	FALSE
XP_029497513.1	FALSE	-0.4354	0.475989	0.020295	0.990732	0.92616	FALSE
XP_029497528.1	FALSE	-0.28535	0.577821	0.146236	0.98227	0.483991	FALSE
XP_029497647.1	FALSE	-1.11111	0.477861	-0.31663	0.979112	0.411721	FALSE
XP_029497711.1	FALSE	-0.81374	1.150271	0.168266	0.988126	0.7219	FALSE
XP_029498041.1	FALSE	-0.43751	0.081169	-0.17817	0.946104	0.165191	FALSE
XP_029498078.1	FALSE	-0.34409	0.226036	-0.05903	0.987159	0.667393	FALSE
XP_029498138.1	TRUE	-1.32053	0.691794	-0.31437	0.983451	0.518213	FALSE
XP_029498203.1	TRUE	-0.90956	1.058635	0.074539	0.990191	0.87479	FALSE
XP_029498400.1	FALSE	-0.49729	0.497624	0.000169	0.991407	0.999436	FALSE
XP_029498535.1	FALSE	-0.52742	0.839979	0.156282	0.986514	0.635435	FALSE
XP_029498801.1	FALSE	-0.41352	0.695495	0.140989	0.985675	0.598252	FALSE
XP_029498917.1	FALSE	-0.39783	0.22913	-0.08435	0.985153	0.577293	FALSE
XP_029499228.1	FALSE	-0.57052	0.172584	-0.19897	0.968258	0.273905	FALSE
XP_029499265.1	TRUE	-0.05127	2.044866	0.996797	0.845462	0.060946	FALSE
XP_029499883.1	FALSE	-0.69405	0.163784	-0.26513	0.957982	0.209287	FALSE
XP_029500021.1	FALSE	-0.21821	0.985406	0.3836	0.955012	0.196095	FALSE
XP_029500060.1	FALSE	-0.50828	0.664553	0.078138	0.989032	0.78179	FALSE
XP_029500567.1	FALSE	-0.60603	1.070632	0.2323	0.984859	0.566111	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029500636.1	FALSE	-0.24705	1.045742	0.399345	0.958033	0.209528	FALSE
XP_029500653.1	FALSE	-0.47674	0.10998	-0.18338	0.956938	0.204445	FALSE
XP_029501048.1	FALSE	-0.36211	0.455606	0.046749	0.989439	0.812066	FALSE
XP_029501121.1	FALSE	-0.33943	1.056474	0.358522	0.970422	0.29325	FALSE
XP_029501132.1	TRUE	-0.79909	1.026663	0.113787	0.98922	0.795521	FALSE
XP_029501401.1	FALSE	-0.18941	0.833091	0.321841	0.95626	0.201419	FALSE
XP_029501403.1	FALSE	-0.22779	0.387846	0.08003	0.985476	0.590097	FALSE
XP_029501410.1	FALSE	-0.58844	0.805218	0.108388	0.988517	0.746543	FALSE
XP_029501662.1	FALSE	-0.22824	0.709331	0.240545	0.970474	0.293753	FALSE
XP_029501710.1	TRUE	-1.51198	0.314004	-0.59899	0.951887	0.183965	FALSE
XP_029501818.1	FALSE	-0.52049	0.818123	0.148815	0.986706	0.644633	FALSE
XP_029501989.1	FALSE	-0.57654	1.496187	0.459822	0.976177	0.361945	FALSE
XP_029502069.1	FALSE	-0.58733	0.197699	-0.19482	0.972006	0.309324	FALSE
XP_029502234.1	TRUE	-1.25126	1.111583	-0.06984	0.990486	0.902109	FALSE
XP_029502285.1	FALSE	-0.4157	0.288454	-0.06362	0.987885	0.707455	FALSE
XP_029502449.1	FALSE	-0.36493	0.732475	0.183774	0.982453	0.488976	FALSE
XP_029502585.1	TRUE	-1.51748	0.613632	-0.45193	0.977514	0.382987	FALSE
XP_029502696.1	FALSE	-0.50813	0.195792	-0.15617	0.976176	0.361921	FALSE
XP_029502855.1	FALSE	-0.74433	0.757803	0.006734	0.991283	0.985113	FALSE
XP_029502935.1	TRUE	-2.97256	-0.27465	-1.6236	0.652007	0.021214	FALSE
XP_029502969.1	FALSE	-0.42039	0.512856	0.04623	0.989748	0.836745	FALSE
XP_029503104.1	TRUE	-1.29764	0.591468	-0.35309	0.980509	0.440762	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029503222.1	FALSE	-0.47214	0.381941	-0.0451	0.989618	0.826161	FALSE
XP_029503239.1	FALSE	-1.5806	0.400133	-0.59023	0.961106	0.22532	FALSE
XP_029503357.1	TRUE	-0.27362	1.088004	0.407189	0.96082	0.223749	FALSE
XP_029503474.1	TRUE	-0.45096	2.314095	0.931566	0.948668	0.172999	FALSE
XP_029503777.1	FALSE	-1.50855	-0.01277	-0.76066	0.807315	0.046623	FALSE
XP_029503801.1	FALSE	-0.41387	0.73699	0.161558	0.984721	0.561043	FALSE
XP_029503803.1	FALSE	-0.7488	0.398695	-0.17505	0.983759	0.527968	FALSE
XP_029503866.1	TRUE	-0.81594	2.147859	0.66596	0.975767	0.355957	FALSE
XP_029503907.1	TRUE	-1.68804	1.379029	-0.15451	0.989715	0.834024	FALSE
XP_029504062.1	FALSE	-0.24366	0.47794	0.117138	0.982919	0.502205	FALSE
XP_029504304.1	TRUE	-0.59433	1.485661	0.445666	0.977222	0.378179	FALSE
XP_029504508.1	FALSE	-0.06481	0.714386	0.324788	0.90468	0.096497	FALSE
XP_029504680.1	FALSE	-0.66285	0.038064	-0.31239	0.879324	0.077208	FALSE
XP_029504718.1	FALSE	-0.8599	0.334617	-0.26264	0.976455	0.366122	FALSE
XP_029504881.1	FALSE	-0.54694	0.293753	-0.12659	0.983921	0.533245	FALSE
XP_029504944.1	TRUE	0.209698	3.265924	1.737811	0.72051	0.028202	FALSE
XP_029505043.1	FALSE	-0.67818	0.668528	-0.00483	0.991309	0.988097	FALSE
XP_029505094.1	FALSE	-0.08174	1.05456	0.486409	0.895571	0.088535	FALSE
XP_029505241.1	FALSE	-0.41369	0.382162	-0.01576	0.990812	0.934305	FALSE
XP_029505400.1	TRUE	-0.71384	1.035924	0.16104	0.987796	0.702292	FALSE
XP_029505444.1	FALSE	-0.46735	0.489781	0.011214	0.991066	0.961105	FALSE
XP_029505689.1	FALSE	-0.51607	0.649043	0.066486	0.989443	0.812408	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029505924.1	FALSE	-0.52121	0.421525	-0.04984	0.989615	0.825949	FALSE
XP_029505927.1	FALSE	-0.5405	0.503349	-0.01858	0.990876	0.940956	FALSE
XP_029505934.1	FALSE	-0.65647	0.430931	-0.11277	0.987149	0.666876	FALSE
XP_029505971.1	FALSE	-0.41991	0.380613	-0.01965	0.990656	0.918633	FALSE
XP_029505983.1	FALSE	-0.31051	0.170057	-0.07023	0.984277	0.545275	FALSE
XP_029506119.1	FALSE	-1.21579	0.000531	-0.60763	0.816792	0.050179	FALSE
XP_029506177.1	FALSE	-0.46634	0.502844	0.018251	0.990843	0.937529	FALSE
XP_029506473.1	FALSE	-0.60564	0.371046	-0.1173	0.986144	0.618483	FALSE
XP_029506529.1	FALSE	-0.89604	0.796239	-0.0499	0.990489	0.902337	FALSE
XP_029506607.1	FALSE	-0.63487	0.113842	-0.26051	0.944292	0.160102	FALSE
XP_029506646.1	FALSE	-0.27813	0.716745	0.219305	0.976376	0.364928	FALSE
XP_029506692.1	FALSE	-0.07119	1.351842	0.640328	0.874984	0.074659	FALSE
XP_029506717.1	FALSE	-0.16953	0.453048	0.141757	0.975326	0.349751	FALSE
XP_029506730.1	FALSE	-0.66288	0.242948	-0.20997	0.974705	0.341368	FALSE
XP_029506737.1	FALSE	-0.99075	0.845525	-0.07261	0.99013	0.869314	FALSE
XP_029506782.1	FALSE	-0.45035	0.497703	0.023675	0.990642	0.917228	FALSE
XP_029507288.1	FALSE	-0.98658	0.980491	-0.00304	0.991367	0.994862	FALSE
XP_029507367.1	TRUE	-1.07159	0.745368	-0.16311	0.987915	0.709246	FALSE
XP_029507824.1	FALSE	-2.11454	0.373564	-0.87049	0.94349	0.157952	FALSE
XP_029507880.1	TRUE	-0.30126	1.067258	0.382999	0.965612	0.253573	FALSE
XP_029507909.1	TRUE	-0.59129	1.182029	0.295367	0.982536	0.491283	FALSE
XP_029507951.1	TRUE	-0.71585	1.097998	0.191076	0.987054	0.661968	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029507962.1	FALSE	-0.35392	0.403968	0.025024	0.990366	0.890719	FALSE
XP_029508039.1	TRUE	-0.6985	1.560061	0.430783	0.980091	0.431634	FALSE
XP_029508298.1	FALSE	-0.28237	1.215752	0.466692	0.957252	0.205873	FALSE
XP_029508535.1	FALSE	-1.96046	0.575312	-0.69258	0.967118	0.264747	FALSE
XP_029508539.1	FALSE	-0.74167	0.411079	-0.16529	0.984491	0.552756	FALSE
XP_029508613.1	FALSE	-0.95697	0.353111	-0.30193	0.974908	0.344067	FALSE
XP_029508647.1	TRUE	0.03308	3.249216	1.641148	0.805377	0.045958	FALSE
XP_029508741.1	FALSE	-0.53334	0.333507	-0.09991	0.986453	0.632586	FALSE
XP_029508948.1	FALSE	-0.63072	0.880618	0.124951	0.988277	0.731215	FALSE
XP_029509030.1	FALSE	-0.65853	0.560481	-0.04903	0.990105	0.867112	FALSE
XP_029509086.1	FALSE	-0.17376	0.403759	0.114997	0.979129	0.412051	FALSE
XP_029509197.1	TRUE	-0.90178	0.327924	-0.28693	0.974467	0.338265	FALSE
XP_029509236.1	TRUE	-0.14095	1.603601	0.731327	0.902825	0.094759	FALSE
XP_029509238.1	FALSE	-0.48332	0.598808	0.057741	0.989595	0.824365	FALSE
XP_029509346.1	TRUE	-0.41763	1.567357	0.574863	0.963277	0.238068	FALSE
XP_029509445.1	FALSE	-0.54574	0.134285	-0.20573	0.959862	0.218638	FALSE
XP_029509523.1	TRUE	-0.71736	0.446972	-0.13519	0.986399	0.630096	FALSE
XP_029509826.1	FALSE	-0.66995	0.583576	-0.04319	0.990315	0.886004	FALSE
XP_029510304.1	TRUE	-1.85306	0.015226	-0.91892	0.824333	0.05343	FALSE
XP_029510338.1	FALSE	-0.32477	0.394558	0.034892	0.989789	0.840092	FALSE
XP_029510357.1	TRUE	-1.67718	0.150778	-0.7632	0.904146	0.09599	FALSE
XP_029510487.1	FALSE	-0.38281	0.177158	-0.10283	0.980858	0.448676	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029510542.1	FALSE	-0.26806	0.560806	0.146371	0.981577	0.465962	FALSE
XP_029510577.1	FALSE	-0.42483	0.609071	0.092121	0.98795	0.711311	FALSE
XP_029510794.1	FALSE	-0.32736	0.208985	-0.05919	0.986756	0.647063	FALSE
XP_029511150.1	FALSE	-0.49888	0.520993	0.011058	0.991093	0.964003	FALSE
XP_029511192.1	FALSE	-0.35991	0.683724	0.161906	0.983543	0.521086	FALSE
XP_029511429.1	FALSE	-0.13578	0.58364	0.223929	0.957329	0.206227	FALSE
XP_029511522.1	FALSE	-0.39321	0.350988	-0.02111	0.990527	0.906028	FALSE
XP_029511835.1	TRUE	0.118653	1.843927	0.98129	0.720218	0.028164	FALSE
XP_029511964.1	FALSE	-0.23045	0.778272	0.273911	0.967459	0.267418	FALSE
XP_029511987.1	FALSE	-0.5227	0.251	-0.13585	0.981676	0.468472	FALSE
XP_029511994.1	FALSE	-0.44594	0.47603	0.015044	0.990923	0.945855	FALSE
XP_029512021.1	FALSE	-0.82854	0.33021	-0.24916	0.977119	0.376518	FALSE
XP_029512117.1	TRUE	-0.97481	0.630866	-0.17197	0.986951	0.656716	FALSE
XP_029512132.1	FALSE	-0.23429	0.748579	0.257143	0.969497	0.284649	FALSE
XP_029512149.1	FALSE	-0.78419	0.087721	-0.34823	0.917119	0.110081	FALSE
XP_029512357.1	FALSE	-0.35606	0.324602	-0.01573	0.990704	0.923377	FALSE
XP_029512438.1	TRUE	-0.28729	1.121285	0.416996	0.961624	0.228233	FALSE
XP_029512596.1	FALSE	-0.42091	1.66217	0.620632	0.961118	0.22539	FALSE
XP_029512711.1	TRUE	-1.06311	0.684775	-0.18917	0.986884	0.653366	FALSE
XP_029512965.1	FALSE	-0.21998	0.430466	0.105243	0.982966	0.503581	FALSE
XP_029513004.1	FALSE	-0.90565	0.757204	-0.07423	0.989939	0.85268	FALSE
XP_029513152.1	FALSE	-0.23018	0.257099	0.01346	0.990553	0.908479	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029513155.1	FALSE	-0.79424	0.829853	0.017808	0.991089	0.963598	FALSE
XP_029513369.1	TRUE	-1.43576	0.221746	-0.607	0.936038	0.140513	FALSE
XP_029513402.1	FALSE	-0.27874	0.557942	0.139601	0.982509	0.490543	FALSE
XP_029513504.1	FALSE	-0.34407	0.366195	0.011061	0.990947	0.948319	FALSE
XP_029513532.1	FALSE	-0.68484	0.277986	-0.20343	0.977616	0.384696	FALSE
XP_029513552.1	FALSE	-0.22633	0.562956	0.168313	0.977357	0.380392	FALSE
XP_029513635.1	TRUE	-0.42134	1.990429	0.784545	0.952831	0.187459	FALSE
XP_029513915.1	FALSE	-0.48592	0.033703	-0.22611	0.889352	0.083824	FALSE
XP_029513987.1	FALSE	-0.40327	0.428142	0.012436	0.990966	0.95036	FALSE
XP_029514550.1	FALSE	-0.99173	0.531171	-0.23028	0.983869	0.531558	FALSE
XP_029514818.1	FALSE	-0.42373	0.40105	-0.01134	0.991004	0.954375	FALSE
XP_029514915.1	TRUE	-2.8417	0.273998	-1.28385	0.908275	0.100057	FALSE
XP_029515138.1	FALSE	-1.07861	0.623608	-0.2275	0.985217	0.579786	FALSE
XP_029515209.1	FALSE	-0.2729	0.330855	0.028975	0.989809	0.841764	FALSE
XP_029515223.1	FALSE	-0.30114	0.239522	-0.03081	0.989446	0.812668	FALSE
XP_029515260.1	FALSE	-0.32607	0.309506	-0.00828	0.991026	0.956734	FALSE
XP_029515296.1	TRUE	-1.23974	0.255688	-0.49202	0.951542	0.182721	FALSE
XP_029515374.1	TRUE	-0.13883	1.651617	0.756394	0.900225	0.092428	FALSE
XP_029515421.1	TRUE	-1.18647	0.283237	-0.45162	0.958507	0.211812	FALSE
XP_029515455.1	FALSE	-0.31669	0.370176	0.026745	0.990152	0.871297	FALSE
XP_029515503.1	FALSE	-1.37984	0.436631	-0.4716	0.969888	0.288216	FALSE
XP_029515616.1	FALSE	-0.72496	0.306985	-0.20899	0.978721	0.404282	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029515659.1	FALSE	-0.72688	0.19105	-0.26792	0.962706	0.234571	FALSE
XP_029515829.1	FALSE	-0.34048	1.324582	0.492049	0.961763	0.229026	FALSE
XP_029515850.1	TRUE	-0.59762	1.436657	0.419521	0.978263	0.395918	FALSE
XP_029515875.1	TRUE	-0.95728	0.822514	-0.06738	0.990192	0.87483	FALSE
XP_029515952.1	TRUE	-1.16581	0.519155	-0.32333	0.979962	0.4289	FALSE
XP_029516144.1	FALSE	-0.68866	0.194223	-0.24722	0.965578	0.253332	FALSE
XP_029516245.1	FALSE	-0.48367	0.839731	0.178029	0.985154	0.577338	FALSE
XP_029516321.1	FALSE	-0.98262	0.502217	-0.2402	0.982969	0.50366	FALSE
XP_029516377.1	FALSE	-0.32769	0.445219	0.058767	0.9886	0.752004	FALSE
XP_029516785.1	FALSE	-0.75887	0.636213	-0.06133	0.989965	0.854887	FALSE
XP_029517008.1	TRUE	-0.27288	1.680578	0.703846	0.938857	0.146618	FALSE
XP_029517109.1	FALSE	-0.67963	0.647913	-0.01586	0.991059	0.960344	FALSE
XP_029517171.1	FALSE	-0.36407	0.265413	-0.04933	0.988488	0.744697	FALSE
XP_029517177.1	FALSE	-0.13627	0.613562	0.238648	0.955152	0.19668	FALSE
XP_029517221.1	FALSE	-0.77542	0.137453	-0.31899	0.943672	0.158435	FALSE
XP_029517435.1	FALSE	-1.23849	0.932418	-0.15304	0.988858	0.769491	FALSE
XP_029517536.1	FALSE	-0.1803	0.828473	0.324088	0.954179	0.1927	FALSE
XP_029517588.1	TRUE	-1.09279	1.052023	-0.02038	0.991134	0.968449	FALSE
XP_029517603.1	TRUE	-0.35059	1.750211	0.69981	0.950075	0.177619	FALSE
XP_029517714.1	FALSE	-1.33833	0.43647	-0.45093	0.970939	0.298305	FALSE
XP_029517819.1	FALSE	-0.50095	1.508677	0.503863	0.971551	0.304515	FALSE
XP_029517882.1	FALSE	-0.6553	0.177366	-0.23897	0.963923	0.242158	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029517954.1	FALSE	-0.18679	0.373486	0.09335	0.982531	0.491145	FALSE
XP_029518112.1	FALSE	-0.19264	0.397066	0.102214	0.981897	0.474107	FALSE
XP_029518171.1	FALSE	-0.26323	0.502925	0.119845	0.983434	0.51768	FALSE
XP_029518218.1	FALSE	-0.16479	0.343307	0.089261	0.981667	0.468235	FALSE
XP_029518459.1	TRUE	-0.16711	1.391578	0.612232	0.921348	0.115646	FALSE
XP_029518688.1	FALSE	-0.76042	1.252014	0.245798	0.98601	0.612576	FALSE
XP_029518789.1	TRUE	-0.40065	2.500168	1.049757	0.93812	0.144969	FALSE
XP_029518888.1	FALSE	-0.63911	0.320357	-0.15938	0.982578	0.492445	FALSE
XP_029519032.1	FALSE	-0.06878	1.014792	0.473005	0.888089	0.082929	FALSE
XP_029519207.1	TRUE	-1.77775	-0.55409	-1.16592	0.020569	0.000904	TRUE
XP_029519295.1	FALSE	-0.30555	0.553407	0.123927	0.984423	0.550338	FALSE
XP_029519418.1	TRUE	-0.46558	1.101917	0.318167	0.978665	0.403244	FALSE
XP_029519438.1	TRUE	-1.76192	0.588426	-0.58675	0.971744	0.306541	FALSE
XP_029519445.1	FALSE	0.107492	0.889458	0.498475	0.561575	0.01555	FALSE
XP_029519453.1	FALSE	-0.56224	0.186569	-0.18784	0.971528	0.304286	FALSE
XP_029519457.1	FALSE	-0.51265	0.46139	-0.02563	0.990597	0.912807	FALSE
XP_029519465.1	FALSE	-0.44304	0.747339	0.152148	0.985628	0.596299	FALSE
XP_029519556.1	FALSE	-0.65329	0.133893	-0.2597	0.951228	0.181603	FALSE
XP_029519714.1	FALSE	-0.77139	0.363436	-0.20398	0.981259	0.458168	FALSE
XP_029519719.1	FALSE	-0.77445	0.398497	-0.18798	0.983102	0.507598	FALSE
XP_029519874.1	FALSE	-0.2633	0.558965	0.147834	0.981255	0.458058	FALSE
XP_029519933.1	FALSE	-0.27847	1.254578	0.488056	0.955124	0.196561	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029520094.1	FALSE	-0.82168	0.827951	0.003138	0.991357	0.993683	FALSE
XP_029520099.1	TRUE	-1.0404	1.549574	0.254586	0.987456	0.683195	FALSE
XP_029520107.1	FALSE	-0.40583	0.585836	0.090002	0.987863	0.70621	FALSE
XP_029520181.1	TRUE	-1.14698	0.201304	-0.47284	0.943134	0.157018	FALSE
XP_029520410.1	FALSE	-0.54581	0.464838	-0.04049	0.990111	0.867623	FALSE
XP_029520641.1	FALSE	-0.45423	0.662992	0.104383	0.987721	0.698014	FALSE
XP_029520928.1	FALSE	-0.40786	0.279387	-0.06424	0.987719	0.697888	FALSE
XP_029521057.1	FALSE	-0.25839	0.740072	0.240843	0.973196	0.322654	FALSE
XP_029521097.1	FALSE	-0.3262	0.267345	-0.02943	0.989747	0.836617	FALSE
XP_029521118.1	FALSE	-0.24999	0.651995	0.201004	0.976034	0.359833	FALSE
XP_029521126.1	FALSE	-0.15703	0.814959	0.328964	0.948084	0.171155	FALSE
XP_029521156.1	TRUE	-0.99241	1.120011	0.063799	0.990464	0.899983	FALSE
XP_029521170.1	FALSE	-0.32299	0.253457	-0.03477	0.989306	0.801967	FALSE
XP_029521226.1	FALSE	-0.60261	1.097771	0.247582	0.984319	0.546714	FALSE
XP_029521403.1	FALSE	-0.25246	0.337375	0.042456	0.98879	0.764794	FALSE
XP_029521419.1	TRUE	-1.35437	0.978113	-0.18813	0.988377	0.737514	FALSE
XP_029521498.1	TRUE	-1.11271	0.364266	-0.37422	0.971069	0.299603	FALSE
XP_029521565.1	FALSE	-0.21465	1.353075	0.569212	0.937552	0.143725	FALSE
XP_029521651.1	TRUE	-0.62589	1.440832	0.407473	0.97936	0.416579	FALSE
XP_029521703.1	FALSE	-0.91414	0.178237	-0.36795	0.948695	0.173085	FALSE
XP_029521787.1	TRUE	-0.76461	1.288683	0.262038	0.985641	0.596857	FALSE
XP_029521790.1	FALSE	-0.3221	0.376241	0.027072	0.990159	0.871858	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029521810.1	FALSE	-1.0826	0.082902	-0.49985	0.894882	0.087987	FALSE
XP_029522027.1	FALSE	-0.40073	0.508993	0.05413	0.989341	0.80457	FALSE
XP_029522115.1	FALSE	-0.37782	0.273449	-0.05219	0.988402	0.739158	FALSE
XP_029522195.1	TRUE	-0.25177	1.308088	0.52816	0.948026	0.170973	FALSE
XP_029522225.1	FALSE	-0.28081	0.440483	0.079834	0.986736	0.646081	FALSE
XP_029522313.1	FALSE	-0.33001	0.384249	0.027118	0.990188	0.87448	FALSE
XP_029522510.1	FALSE	-1.31915	0.22891	-0.54512	0.942519	0.155428	FALSE
XP_029522797.1	FALSE	-0.86439	0.150791	-0.3568	0.942807	0.156167	FALSE
XP_029523213.1	FALSE	-0.57586	0.646539	0.035341	0.990509	0.904234	FALSE
XP_029523339.1	FALSE	-0.38795	0.368192	-0.00988	0.991025	0.956638	FALSE
XP_029523625.1	TRUE	-0.45439	1.862402	0.704006	0.959485	0.216694	FALSE
XP_029523644.1	FALSE	-0.47384	0.31006	-0.08189	0.987105	0.664596	FALSE
XP_029523680.1	FALSE	-0.06425	0.657791	0.296769	0.909044	0.100854	FALSE
XP_029523800.1	FALSE	-0.23523	1.04027	0.40252	0.956004	0.200304	FALSE
XP_029523830.1	TRUE	-1.24282	1.207133	-0.01784	0.9912	0.975818	FALSE
XP_029523882.1	FALSE	-0.33016	0.376671	0.023257	0.99037	0.891094	FALSE
XP_029524040.1	TRUE	-0.66956	0.757238	0.043839	0.990446	0.898259	FALSE
XP_029524057.1	TRUE	-0.27523	1.622662	0.673714	0.941273	0.15231	FALSE
XP_029524325.1	TRUE	-0.31961	1.176637	0.428512	0.964069	0.243103	FALSE
XP_029524435.1	FALSE	-0.40655	0.698359	0.145905	0.985331	0.584287	FALSE
XP_029524483.1	FALSE	-0.15887	0.483825	0.162478	0.971172	0.300641	FALSE
XP_029524546.1	FALSE	-0.26297	0.425665	0.081347	0.986271	0.624213	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029524646.1	TRUE	-0.87113	0.25003	-0.31055	0.966265	0.258292	FALSE
XP_029524838.1	FALSE	-0.14276	0.455931	0.156584	0.969513	0.284792	FALSE
XP_029524929.1	FALSE	-0.4241	0.477985	0.026942	0.990476	0.90109	FALSE
XP_029524933.1	FALSE	-0.19013	0.49774	0.153804	0.975927	0.358268	FALSE
XP_029524992.1	TRUE	0.038072	2.41204	1.225056	0.798779	0.043831	FALSE
XP_029525052.1	TRUE	-0.77059	0.815879	0.022646	0.990987	0.952622	FALSE
XP_029525240.1	FALSE	-0.53041	1.293611	0.381599	0.977885	0.389277	FALSE
XP_029525521.1	FALSE	-0.46134	0.924889	0.231775	0.982477	0.489652	FALSE
XP_029525836.1	FALSE	-0.56429	0.449576	-0.05736	0.989464	0.813991	FALSE
XP_029525839.1	TRUE	-1.76359	0.109281	-0.82716	0.883275	0.079685	FALSE
XP_029525844.1	FALSE	-0.63002	0.127117	-0.25145	0.950441	0.178863	FALSE
XP_029526351.1	FALSE	-0.23366	0.826271	0.296306	0.965683	0.254082	FALSE
XP_029526402.1	FALSE	-0.65226	0.75633	0.052033	0.990225	0.877843	FALSE
XP_029526411.1	TRUE	-0.71799	1.282429	0.282221	0.984668	0.559108	FALSE
XP_029526496.1	FALSE	-0.74989	0.726436	-0.01173	0.99118	0.973621	FALSE
XP_029526565.1	FALSE	-3.92591	0.872917	-1.5265	0.955207	0.196909	FALSE
XP_029526910.1	FALSE	-0.58716	1.243284	0.328061	0.981312	0.459447	FALSE
XP_029526967.1	TRUE	-3.77061	1.416814	-1.1769	0.975447	0.351435	FALSE
XP_029527064.1	FALSE	-0.31298	0.440771	0.063894	0.988172	0.724711	FALSE
XP_029527257.1	FALSE	-1.06008	0.28868	-0.3857	0.964172	0.243772	FALSE
XP_029527330.1	FALSE	-0.86417	0.508285	-0.17794	0.9855	0.59107	FALSE
XP_029527585.1	FALSE	-0.46448	0.611904	0.07371	0.988949	0.775885	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029527676.1	FALSE	0.011333	1.434346	0.72284	0.807945	0.046844	FALSE
XP_029527778.1	FALSE	-0.19939	0.480483	0.140548	0.9782	0.394795	FALSE
XP_029527864.1	FALSE	-0.2131	0.374795	0.080847	0.984935	0.568969	FALSE
XP_029528562.1	FALSE	-0.56845	0.637106	0.034326	0.990524	0.905678	FALSE
XP_029528627.1	FALSE	-0.36036	0.330853	-0.01475	0.990762	0.92922	FALSE
XP_029528719.1	FALSE	-0.54845	0.605558	0.028556	0.99065	0.917976	FALSE
XP_029528724.1	FALSE	-0.4016	1.349212	0.473806	0.967656	0.268985	FALSE
XP_029528770.1	FALSE	-0.8333	0.279423	-0.27694	0.971879	0.307967	FALSE
XP_029528878.1	FALSE	-1.17922	0.190104	-0.49456	0.938455	0.145714	FALSE
XP_029529099.1	FALSE	-0.68776	0.38863	-0.14956	0.984829	0.564997	FALSE
XP_029529148.1	FALSE	-0.31527	0.41521	0.049968	0.988952	0.776126	FALSE
XP_029529322.1	FALSE	-0.42459	0.445233	0.010319	0.991062	0.960617	FALSE
XP_029529412.1	FALSE	-1.53883	-0.28205	-0.91044	0.308667	0.007181	FALSE
XP_029529460.1	FALSE	-0.51857	0.881587	0.18151	0.985501	0.591122	FALSE
XP_029529479.1	TRUE	-1.97102	2.490123	0.259551	0.989397	0.808817	FALSE
XP_029529514.1	TRUE	-2.27555	-0.08929	-1.18242	0.766128	0.035659	FALSE
XP_029529671.1	TRUE	-0.55346	1.284464	0.365502	0.979159	0.412637	FALSE
XP_029530077.1	TRUE	-0.20945	1.511038	0.650793	0.929811	0.128731	FALSE
XP_029530337.1	FALSE	-0.24849	0.546814	0.149164	0.980439	0.439203	FALSE
XP_029530567.1	TRUE	-2.80349	-0.36471	-1.5841	0.527606	0.013983	FALSE
XP_029530605.1	FALSE	-0.34166	0.290253	-0.0257	0.990088	0.865619	FALSE
XP_029530701.1	FALSE	-0.85018	0.211871	-0.31915	0.960422	0.221593	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029530943.1	FALSE	0.072728	1.288665	0.680696	0.73608	0.03039	FALSE
XP_029530972.1	FALSE	-0.44197	0.310189	-0.06589	0.988028	0.715942	FALSE
XP_029531137.1	TRUE	-1.24966	0.89121	-0.17923	0.988225	0.727979	FALSE
XP_029531323.1	FALSE	-1.16367	1.080902	-0.04138	0.990856	0.938836	FALSE
XP_029531752.1	FALSE	-0.76582	0.370201	-0.19781	0.981819	0.472118	FALSE
XP_029532124.1	TRUE	-0.02111	1.96296	0.970924	0.826654	0.054521	FALSE
XP_029532177.1	FALSE	-0.30863	0.362243	0.026807	0.990115	0.86796	FALSE
XP_029532190.1	TRUE	-0.54646	1.405055	0.429299	0.97644	0.365885	FALSE
XP_029532211.1	FALSE	-0.49148	0.336569	-0.07746	0.987715	0.697684	FALSE
XP_029532390.1	FALSE	-0.20678	0.525167	0.159193	0.976785	0.371207	FALSE
XP_029532796.1	TRUE	-1.38144	1.01406	-0.18369	0.988569	0.749967	FALSE
XP_029532914.1	FALSE	-0.24046	0.365777	0.062658	0.987169	0.667929	FALSE
XP_029532941.1	FALSE	-0.30552	0.317801	0.00614	0.991123	0.967295	FALSE
XP_029533416.1	FALSE	-0.52238	0.77839	0.128004	0.987449	0.682863	FALSE
XP_029533479.1	FALSE	-0.28573	1.081428	0.39785	0.962925	0.235901	FALSE
XP_029533521.1	FALSE	-0.45628	0.817166	0.180443	0.984621	0.557411	FALSE
XP_029533570.1	FALSE	-0.53688	0.386604	-0.07514	0.988342	0.735312	FALSE
XP_029533592.1	FALSE	-0.47103	0.930096	0.229533	0.982786	0.498343	FALSE
XP_029533630.1	FALSE	-0.2184	0.341654	0.061628	0.986775	0.647983	FALSE
XP_029533720.1	TRUE	-0.88499	1.234372	0.174691	0.988289	0.731993	FALSE
XP_029533962.1	FALSE	-0.72973	0.306405	-0.21166	0.978505	0.400291	FALSE
XP_029534103.1	FALSE	-0.79046	0.719412	-0.03552	0.99069	0.922001	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029534161.1	TRUE	-0.69425	0.967729	0.136742	0.988297	0.732459	FALSE
XP_029534162.1	TRUE	-0.11748	1.408033	0.645279	0.899794	0.092053	FALSE
XP_029534217.1	FALSE	-0.4012	0.356036	-0.02258	0.990477	0.901235	FALSE
XP_029534265.1	FALSE	-1.05214	0.69463	-0.17875	0.987228	0.670998	FALSE
XP_029534473.1	FALSE	-0.59227	0.09405	-0.24911	0.937607	0.143845	FALSE
XP_029534727.1	FALSE	-0.89442	0.683942	-0.10524	0.98903	0.781621	FALSE
XP_029534942.1	FALSE	-0.30055	0.283455	-0.00855	0.990976	0.951431	FALSE
XP_029535429.1	FALSE	-0.32234	0.25116	-0.03559	0.989232	0.796374	FALSE
XP_029535723.1	FALSE	-0.10306	0.551117	0.224029	0.94652	0.166409	FALSE
XP_029535791.1	TRUE	0.828333	2.347462	1.587897	0.007591	0.000392	TRUE
XP_029535794.1	FALSE	-0.70144	0.391751	-0.15484	0.984625	0.557556	FALSE
XP_029535825.1	FALSE	-0.46024	0.562883	0.05132	0.989724	0.834722	FALSE
XP_029535855.1	FALSE	-0.50783	0.43611	-0.03586	0.990187	0.874406	FALSE
XP_029535927.1	FALSE	-0.48771	0.256456	-0.11563	0.983522	0.520444	FALSE
XP_029535953.1	TRUE	-0.57026	1.25291	0.341327	0.980476	0.440019	FALSE
XP_029536212.1	FALSE	-0.33814	0.643732	0.152796	0.983502	0.519814	FALSE
XP_029536264.1	TRUE	-0.00621	2.258992	1.126389	0.819104	0.051132	FALSE
XP_029536283.1	TRUE	-0.90308	0.997165	0.047044	0.990649	0.917938	FALSE
XP_029536374.1	TRUE	0.039009	1.72422	0.881615	0.790135	0.041327	FALSE
XP_029536699.1	FALSE	-0.3439	0.223144	-0.06038	0.986987	0.658581	FALSE
XP_029536846.1	FALSE	-0.26558	0.558471	0.146446	0.981464	0.463177	FALSE
XP_029537014.1	TRUE	-0.37294	1.049406	0.338236	0.973756	0.329348	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029537122.1	TRUE	-0.35732	1.015928	0.329304	0.973431	0.325418	FALSE
XP_029537185.1	TRUE	-0.81151	1.027758	0.108125	0.989371	0.806866	FALSE
XP_029537250.1	FALSE	-0.54674	0.671522	0.062391	0.989682	0.831309	FALSE
XP_029537701.1	FALSE	-0.67077	0.183999	-0.24338	0.964468	0.245723	FALSE
XP_029537753.1	FALSE	-0.21238	0.251254	0.019437	0.990041	0.861537	FALSE
XP_029537997.1	FALSE	-0.46359	0.354055	-0.05477	0.989016	0.780653	FALSE
XP_029538031.1	FALSE	-0.38456	0.617973	0.116708	0.98638	0.629215	FALSE
XP_029538083.1	FALSE	-0.40153	1.052291	0.325379	0.975896	0.357816	FALSE
XP_029538097.1	FALSE	-0.4927	0.348529	-0.07208	0.988126	0.721854	FALSE
XP_029538196.1	FALSE	-0.31818	0.705979	0.193902	0.980246	0.434972	FALSE
XP_029538249.1	FALSE	-0.30313	1.418331	0.557599	0.953301	0.189253	FALSE
XP_029538250.1	FALSE	-0.0537	0.817975	0.382137	0.886359	0.081733	FALSE
XP_029538252.1	FALSE	-0.51472	0.742167	0.113723	0.987878	0.707066	FALSE
XP_029538266.1	TRUE	-1.64601	0.205327	-0.72034	0.923605	0.11886	FALSE
XP_029538332.1	FALSE	-0.62082	0.673531	0.026353	0.990794	0.932469	FALSE
XP_029538547.1	FALSE	-0.95366	0.325827	-0.31392	0.972497	0.314682	FALSE
XP_029538558.1	FALSE	-0.31431	0.38535	0.035519	0.9897	0.832759	FALSE
XP_029538619.1	FALSE	-0.60473	0.491375	-0.05668	0.989662	0.829703	FALSE
XP_029538821.1	FALSE	-0.18088	0.62234	0.22073	0.96675	0.261919	FALSE
XP_029538959.1	FALSE	-0.40755	0.277285	-0.06513	0.987631	0.692895	FALSE
XP_029539226.1	FALSE	-0.83356	0.452973	-0.19029	0.984136	0.540447	FALSE
XP_029539237.1	TRUE	-0.82871	0.822293	-0.00321	0.991356	0.993547	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029539313.1	FALSE	-0.59202	0.137573	-0.22722	0.957276	0.205984	FALSE
XP_029539373.1	FALSE	-0.39419	1.386021	0.495913	0.965908	0.255691	FALSE
XP_029539518.1	TRUE	-0.33718	1.069798	0.36631	0.969747	0.286916	FALSE
XP_029539730.1	FALSE	-0.81601	0.240506	-0.28775	0.967283	0.266033	FALSE
XP_029539893.1	FALSE	-0.80105	0.276639	-0.2622	0.972846	0.318607	FALSE
XP_029540078.1	FALSE	-1.47647	0.753648	-0.36141	0.982943	0.502908	FALSE
XP_029540176.1	FALSE	-0.44909	1.453164	0.502037	0.969041	0.280594	FALSE
XP_029540272.1	FALSE	-0.80435	0.645728	-0.07931	0.989541	0.820048	FALSE
XP_029540279.1	TRUE	-0.70128	1.249755	0.274237	0.984708	0.560546	FALSE
XP_029540471.1	FALSE	-0.34693	0.357043	0.005054	0.991203	0.976159	FALSE
XP_029540564.1	TRUE	-0.58224	1.41713	0.417444	0.977938	0.390207	FALSE
XP_029540643.1	FALSE	-0.70823	0.599378	-0.05442	0.990053	0.862522	FALSE
XP_029540809.1	TRUE	-1.32994	0.729178	-0.30038	0.984298	0.545968	FALSE
XP_029540856.1	TRUE	-0.78773	0.786113	-0.00081	0.991397	0.998294	FALSE
XP_029540888.1	FALSE	-1.20505	0.563319	-0.32087	0.981085	0.453991	FALSE
XP_029541006.1	FALSE	-0.40821	1.218483	0.405137	0.971849	0.307644	FALSE
XP_029541016.1	TRUE	-1.18782	0.586112	-0.30085	0.982253	0.483535	FALSE
XP_029541023.1	FALSE	-4.24825	0.822389	-1.71293	0.948325	0.171909	FALSE
XP_029541038.1	FALSE	-0.77336	0.997065	0.111855	0.989183	0.79278	FALSE
XP_029541053.1	TRUE	-0.32025	2.306068	0.992908	0.929919	0.128919	FALSE
XP_029541310.1	FALSE	-0.91934	-0.16264	-0.54099	0.332782	0.007819	FALSE
XP_029541379.1	FALSE	-0.59464	0.297029	-0.14881	0.982506	0.490453	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029541384.1	TRUE	-2.00996	-0.06123	-1.0356	0.779368	0.03858	FALSE
XP_029541389.1	FALSE	-0.61602	1.029908	0.206944	0.98577	0.60225	FALSE
XP_029541519.1	FALSE	-0.67958	0.267435	-0.20607	0.97677	0.370967	FALSE
XP_029541556.1	FALSE	-0.33599	0.405145	0.03458	0.989861	0.846099	FALSE
XP_029541636.1	TRUE	-0.33992	1.568258	0.614171	0.953983	0.191921	FALSE
XP_029541653.1	FALSE	-0.67445	0.605135	-0.03466	0.990571	0.910255	FALSE
XP_029541697.1	TRUE	-0.39728	0.654828	0.128772	0.985993	0.611843	FALSE
XP_029542039.1	FALSE	-0.21825	0.28812	0.034933	0.988926	0.774272	FALSE
XP_029542078.1	TRUE	-0.44427	2.065541	0.810635	0.95361	0.190453	FALSE
XP_029542145.1	FALSE	-0.41406	0.158625	-0.12772	0.97601	0.359474	FALSE
XP_029542171.1	FALSE	-0.54873	0.073187	-0.23777	0.927555	0.124952	FALSE
XP_029542310.1	FALSE	-0.66218	0.534512	-0.06383	0.989596	0.824423	FALSE
XP_029542329.1	FALSE	-0.27926	0.68027	0.200505	0.977916	0.38982	FALSE
XP_029542518.1	TRUE	-1.35071	0.405807	-0.47245	0.968004	0.271809	FALSE
XP_029542560.1	FALSE	-0.83478	0.900523	0.03287	0.99084	0.937163	FALSE
XP_029542688.1	FALSE	-0.24534	0.2353	-0.00502	0.991105	0.965309	FALSE
XP_029542741.1	FALSE	-0.58332	0.476467	-0.05343	0.989714	0.833903	FALSE
XP_029542794.1	FALSE	-0.27963	0.381103	0.050735	0.988564	0.749642	FALSE
XP_029542921.1	FALSE	-0.64424	0.479027	-0.08261	0.988717	0.75985	FALSE
XP_029542977.1	FALSE	-0.31578	0.246154	-0.03481	0.989236	0.796709	FALSE
XP_029542999.1	TRUE	-0.8126	0.721913	-0.04534	0.990487	0.902134	FALSE
XP_029543214.1	TRUE	-2.17281	0.235672	-0.96857	0.915279	0.107829	FALSE

Accession number	Data imputed	Netting-stressed vs. control					
		95% Confidence interval lower limit	95% Confidence interval upper limit	Average log2-fold change	Adjusted p value	p value	Significant
XP_029543221.1	FALSE	-0.24175	0.708096	0.233172	0.972474	0.314418	FALSE
XP_029543224.1	FALSE	-0.17144	1.089502	0.459033	0.937093	0.142735	FALSE
XP_029543309.1	TRUE	-1.26853	0.726964	-0.27078	0.985069	0.574052	FALSE
XP_029543323.1	TRUE	-0.42365	1.11075	0.343548	0.975883	0.357631	FALSE
XP_029543600.1	FALSE	0.04107	1.19888	0.619975	0.773806	0.037298	FALSE
XP_029543691.1	FALSE	-0.71773	0.731995	0.00713	0.99127	0.983668	FALSE
XP_029543739.1	FALSE	-0.33224	0.614965	0.141362	0.98403	0.536858	FALSE
XP_029543793.1	FALSE	-0.11285	0.777856	0.332505	0.932454	0.133472	FALSE
XP_029544010.1	FALSE	-0.49474	0.347937	-0.0734	0.988053	0.717476	FALSE
XP_029544014.1	FALSE	-0.47926	0.07266	-0.2033	0.934983	0.138361	FALSE
XP_029544126.1	FALSE	-1.82184	0.799375	-0.51123	0.979605	0.421517	FALSE
XP_029544310.1	FALSE	-0.57473	0.489388	-0.04267	0.990109	0.867496	FALSE
XP_029544459.1	TRUE	-0.01981	1.029481	0.504837	0.83812	0.058272	FALSE
XP_029544470.1	TRUE	-0.65471	1.865161	0.605223	0.973368	0.324674	FALSE
XP_029544747.1	FALSE	-0.82208	1.049667	0.113792	0.989286	0.80042	FALSE
XP_029544752.1	FALSE	-0.26554	0.300116	0.01729	0.990451	0.898782	FALSE
XP_029544862.1	FALSE	-0.86592	0.651938	-0.10699	0.988858	0.769507	FALSE
XP_029544927.1	FALSE	-0.3865	0.272583	-0.05696	0.988088	0.719585	FALSE
XP_029545032.1	TRUE	-1.90252	1.041459	-0.43053	0.984269	0.544979	FALSE
YP_908767.1	FALSE	-1.25519	0.100509	-0.57734	0.897427	0.090048	FALSE

Note: Values based on liver proteomics data from 16 juvenile sockeye salmon (8 fish/treatment group) after first trial of chronic netting stress experiment. The significant column indicates whether the protein in netting-stressed fish is differentially expressed compared to control fish (adjusted p value < 0.05).