THE INFLUENCE OF ACCLIMATION ON THE ORGANISMAL AND MOLECULAR THERMOTOLERANCE PARAMETERS IN TWO ARCTIC TELEOSTS

By

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Abstract

The nearshore Beaufort Sea is a highly dynamic thermal environment that is faced with climate change-driven increases in temperature. Analyzing the thermotolerance of important Arctic subsistence and prey fishes, such as broad whitefish *Coregonus nasus* and saffron cod *Eleginus gracilis,* will provide an understanding of the relative species-specific responses to current and future temperature changes. The objectives of this study were to determine if acclimating broad whitefish and saffron cod to two different temperatures (5 and 15°C) affected their critical thermal maximum (CT_{max}) and their HSP70 protein and mRNA transcript concentrations in brain, muscle, and liver tissues. Following acclimation, fish were exposed to a thermal ramping rate of $3.4°C \cdot h^{-1}$. The CT_{max} temperature was recorded when the fish expressed a loss of equilibrium. Tissue samples were then collected and analyzed via western blotting and transcriptome sequencing. Broad whitefish and saffron cod acclimated to 15°C had a significantly higher mean CT_{max} (27.3°C and 25.9°C, respectively) than 5°C fish (23.7°C and 23.2°C, respectively). Broad whitefish had a significantly higher CT_{max} than saffron cod at 15°C in addition to significantly higher HSP70 protein concentrations in liver and muscle tissues at both acclimation temperatures. Brain and muscle tissues had the highest and lowest HSP70 protein concentrations, respectively, for both species and acclimation temperatures. The only significant difference in protein concentration between acclimation temperatures was in saffron cod liver tissues where 5°C samples had a significantly higher concentration than 15°C. Brain and liver tissues for broad whitefish acclimated to 15°C had significantly higher HSP70 mRNA transcript concentrations than the control group that remained in lab-acclimation conditions of 8°C. Transcript B expressed a higher quantity of transcripts than transcript A, but both transcripts followed similar expression profiles and there were no differences in transcript

concentration between tissues. The molecular data from this study demonstrates the cellular mechanisms that are, in part, responsible for the observed shifts in broad whitefish and saffron cod organismal thermotolerance, and this plasticity could be used to respond to changing thermal conditions in the nearshore Beaufort Sea in the future.

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General Introduction

Anthropogenic-driven ecological and environmental changes in the Arctic are occurring at a rate faster than in any other region, which has directly impacted the extent, quality, and duration of Arctic sea ice (Schuur *et al.,* 2015; Drost *et al.,* 2016; Reusser *et al.,* 2016). These changes in ice quality and quantity have affected abiotic and biotic parameters including temperature, salinity, and aquatic organism abundance and distribution (Reist *et al.*, 2006; Schuur *et al.*, 2015; Khalsa *et al.*, 2021). The nearshore Beaufort Sea is no exception to these changes. Additionally, this area is highly dynamic as freshwater inputs from rivers and wind-driven currents can drastically influence salinity, temperature, pH, and sedimentation (Ross, 1988; Mccain *et al.*, 2014). Of the aforementioned abiotic parameters, temperature is arguably the most important in influencing aquatic populations, especially for teleosts, due to the regulations it imposes on physiological processes (Laurel *et al.*, 2016; Stefanovic *et al.*, 2016; Manzon *et al.*, 2022).

Fishes are poikilotherms and, given the high specific heat and thermal conductivity of water, the aquatic thermal environment is what determines internal body temperatures (Feder and Hofmann, 1999; Reist *et al.*, 2006). If the aquatic environment is unhabitable, fishes can move to suitable microhabitats (Feder and Hofmann, 1999). However, polar aquatic thermal parameters are becoming warmer, and for stenothermic species, this will present challenges to find habitats that fall within its restrictive thermal preferences, ultimately driving them farther north (Somero, 2010; Eissa and Zaki, 2011; Fossheim *et al.*, 2015). Eurythermal species will almost certainly be favored as they can access niches that their more stenothermal counterparts can no longer tolerate (Somero, 2010). For example, Reist *et al.* (2006) predicted that climate change will cause an overall increase in competition for suitable habitats. With temperature playing an ever-

increasing role in species distribution in the Arctic, it is becoming imperative to understand their response to thermal environmental parameters. However, there is limited thermal-physiology studies on most Arctic species, including broad whitefish *Coregonus nasus* and saffron cod *Elegimus gracilis* (Drost *et al.,* 2016).

Broad whitefish is a member of the Salmonidae family that reside in lakes and rivers throughout Alaska. Part of the impetus for studying broad whitefish is due to their subsistence and cultural importance to Indigenous coastal Alaskan communities (Fechhelm *et al.*, 1992; Tallman and Reist, 1997). Broad whitefish in Prudhoe Bay, Alaska is amphidromous, feeding in the brackish-nearshore area during the ice-free season before mature spawning adults migrate upriver in the fall to overwinter in deep pools within river deltas or channels (Fechhelm *et al.*, 1992; Griffiths *et al.*, 1992; Harper *et al.*, 2012). Most thermal preference experiments that have been conducted have revolved around comparing growth rates as a function of temperature. In general, there is a positive, linear relationship between growth rate and temperature, with peak growth occurring at 8°C in age 1–2 broad whitefish and higher abundances of amphidromous and anadromous fishes occurring at warmer temperatures and lower salinities (Fechhelm *et al.*, 1992; Griffiths *et al.*, 1992; Logerwell *et al.*, 2015; Khalsa *et al.*, 2021). However, there is a lack of information on the upper-temperature profile for this species. Further, broad whitefish are highly migratory, which is an energetically costly life-history strategy (Tallman *et al.*, 2002). With aquatic temperatures changing so rapidly, increased migratory patterns to locate suitable habitat may become more frequent for this species.

Saffron cod is a polar marine species that primarily resides offshore but moves to the nearshore Beaufort Sea to feed during the ice-free period (Dunn and Matarese, 1987; Logerwell *et al.*, 2015). Arctic cod *Boreogadus saida* and saffron cod are important for transferring energy

between lower and higher trophic levels and are integral to the diets of a variety of marine organisms (Frost and Lowry, 1981; Copeman *et al.*, 2016; Reusser *et al.*, 2016). Although saffron cod have limited subsistence importance, they compete with important commercial and subsistence gadids, such as walleye pollock *Gadus chalcogrammus* and Arctic cod, due in part to their thermal preferences (Frost and Lowry, 1981; Johnson *et al.*, 2009). Saffron cod is eurythermal and tends to occur in shallower and warmer waters than Arctic cod (Wolotira, 1985; Mueter *et al.*, 2016; Vestfals *et al.*, 2019). For example, saffron cod was the only gadid out of four species to survive a range of experimental temperatures from 0 to 20°C with a positive effect on growth past 16°C (Laurel *et al.*, 2016). While most thermal-physiology studies have focused on Arctic cod, this species does not show the same potential to acclimate to warmer temperatures as saffron cod (Drost *et al.,* 2016; Laurel *et al.,* 2016; Mueter *et al.,* 2016). With overlapping diets and foraging habitats that will only become more competitive with climate change, it is imperative to understand the thermotolerance of saffron cod and broad whitefish.

Species can survive in a range of temperatures, which is partially driven by a family of proteins known as heat shock proteins (Dietz and Somero, 1993; White *et al.*, 1994). Heat shock proteins (HSP) are highly conserved across all taxa, with large chromosome segments having been conserved for up to 480 million years since the divergence of zebrafish *Danio rerio* and humans *Homo sapiens* (Yamashita *et al.*, 2010). The HSP are roughly divided into groups based on their molecular weight and the unique functions they provide (Dietz and Somero, 1993). In general, these proteins are chaperones, which means they are important for localization and movement within the organelle, minimizing aggregation of non-native proteins or targeting aggregated proteins for degradation, protein assembly and integrity, translocation, oligomerization, and mediation of steroid and receptor binding (Dyer *et al.*, 1991; Dietz, 1994;

Iwama *et al.*, 1998, 1999; Feder and Hofmann, 1999; Molina *et al.*, 2000; Buckley *et al.*, 2004). The presence of HSP is ubiquitous and highly conserved, but one of the most highly conserved family is the 70-kDA HSP (White *et al.*, 1994; Iwama *et al.*, 1999; Basu *et al.*, 2002).

The family of 70-kDa HSP are considered some of the most highly conserved across fish and other taxa (Lindquist, 1986; White *et al.*, 1994; Iwama *et al.*, 1998). There are species-specific constitutive cognates (HSC70) that maintain daily cellular processes and homeostasis (Lindquist, 1986; Santoro, 1999; Basu *et al.*, 2002; Yamashita *et al.*, 2010). However, there is an inducible cognate (HSP70) that is produced during a stress response upon first recognition of a stressor via neuroendocrine pathways (Iwama *et al.*, 1999; Molina *et al.*, 2000; Buckley *et al.*, 2004). A multitude of stressors can elicit the response, including changes in temperature, salinity, pH, pathogens, and toxic metals or pollutants (Dietz, 1994). Before a stress response, HSP70 is bound to the heat shock factor, HSF1 (Santoro, 1999). During the stress response, the proteins become detached to assist in preventing denaturation and aggregation caused by an influx of non-native proteins (Feder and Hofmann, 1999; Santoro, 1999). This releases HSF1, which becomes active and binds upstream to specific sequences in the genome known as heat shock elements (HSE), resulting in the production of HSP70 mRNA transcripts (Santoro, 1999; Molina *et al.*, 2000; Yamashita *et al.*, 2010). Multiple transcripts of the inducible HSP70 can create HSP70 isoforms. These isoforms have been identified in rainbow trout *Oncorhynchus mykiss* and zebrafish *Danio rerio* and can have differential expression patterns based on the tissue and cell type (Yamashita *et al.*, 2010). The entire heat shock response, from recognition of the stressor to the production of proteins, occurs in a matter of minutes and remains detectable hours after the stressor is removed (Lindquist, 1986). Thermotolerance is based on evolutionary thermal history, but the production of 70-kDa HSP can shift based on external environmental

parameters, which allows homeostasis in a variety of environmental parameters (White *et al.*, 1994; Manzon *et al.*, 2022).

Through the mechanisms of phenotypic plasticity, acclimation to different temperatures facilitates a shift in organismal thermotolerance underscored by HSP production in accordance with new environmental parameters to achieve a new state of homeostasis (Dietz and Somero, 1992; Basu *et al.*, 2002; Somero, 2010). Living in a variable environment, such as the nearshore Beaufort Sea, might require organisms to have different levels of the constitutive cognate or change the induction temperature of the inducible protein to respond to changes in abiotic parameters (Hofmann, 1999; Tomanek, 2010; Manzon *et al.*, 2022). Seasonal changes can also result in differential HSP expression patterns (Dietz, 1994; Currie *et al.*, 2000). Summer-acclimated species have been shown to induce HSP at higher temperatures and increase the endogenous protein concentrations, which increases survival in warmer temperatures (Dietz and Somero, 1992; Feder and Hofmann, 1999; Hofmann, 1999; Buckley *et al.*, 2001; Zakhartsev *et al.*, 2005). Congeners and populations of the same species living in different latitudinal habitats have been shown to have unique thermal preferences depending on their specific environment (Somero, 2010; Kelley *et al.*, 2011). Inherent individual genetic variation alone can result in thermotolerance differences within populations (Iwama *et al.*, 1999). The HSP protein and mRNA transcript concentrations can be measured to determine a species' thermal preferences and how those preferences can shift in unique thermal environments. Additionally, there are physiological thermotolerance parameters that, influenced by HSP, can be measured in tandem with the molecular parameters.

Critical thermal maximum (CT_{max}), a superficially designed physiological parameter, is the maximum temperature that can be sustained right before the incipient lethal temperature

(ILT) that results in mortality (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997; Beitinger *et al.,* 2000). Both CT_{max} and ILT have been used to assess the thermotolerance of fishes, but there are important differences between each parameter. The ILT temperature results from subjecting fish to an abrupt change in temperature that results in mortality, whereas the CT_{max} value is determined from a gradual change in temperature that eventually leads to the fish losing the ability to "…escape from conditions that will lead to death" (Becker and Genoway, 1979). Most teleost studies measuring CT_{max} use the inability to remain upright and buoyant (e.g., a loss of equilibrium) as the CT_{max} endpoint (Becker and Genoway, 1979; Beitinger *et al.,* 2000). A variety of temperature increments have been used in CT_{max} experiments, which makes it difficult to compare values among studies. Nevertheless, the methodology tends to better replicate thermal parameters that fish might experience in natural habitats (Beitinger *et al.,* 2000). As a result, combining this physiological parameter with the HSP70 protein and mRNA production will provide detailed insight into overall thermotolerance and the response to environmental changes (Basu *et al.*, 2002).

Currently, there is limited to no information on the organismal and molecular thermotolerance parameters for broad whitefish and saffron cod. Although studies looking at molecular thermotolerance parameters have analyzed HSP70 protein concentrations or mRNA expression, there are few studies comparing both parameters. Teigen *et al.* (2015) noted that many times, the HSP protein levels do not change in tandem with the mRNA transcript quantities and recommended measuring both to garner a complete understanding of the role that HSP play in an individual's response to heat stress. Therefore, the goal of this project was to measure HSP70 protein and mRNA transcript concentrations that occurred at the CT_{max} endpoint in broad whitefish and saffron cod acclimated to 5 and 15°C. The objectives of this study were to quantify

(1) CT_{max} temperatures and determine if there are differences between the two acclimation temperatures and between the two species; (2) HSP70 protein concentration in brain, liver, and muscle tissues and determine if there are differences in concentration among tissue types, acclimation temperatures, and species; and (3) mRNA HSP70 concentration in broad whitefish liver and muscle tissues to determine significant differences between tissue-types and acclimation temperatures. With information garnered from this study, there will be an increased understanding as to why measured molecular parameters caused shifts in thermotolerance and if there are significant differences in thermotolerance between species. This information could aid future predictions in distributions and homeostasis of these two fishes in a warming climate regime (Reist *et al.*, 2006; Somero, 2010).

Chapter 1: The influence of acclimation on the organismal and molecular thermotolerance parameters in two Arctic teleosts[1]

Abstract

The nearshore Beaufort Sea is a highly dynamic thermal environment that is faced with climate change-driven increases in temperature. Analyzing the thermotolerance of important Arctic subsistence and prey fishes, such as broad whitefish *Coregonus nasus* and saffron cod *Eleginus gracilis,* will provide an understanding of the relative species-specific responses to current and future temperature changes. The objectives of this study were to determine if acclimating broad whitefish and saffron cod to two different temperatures (5 and 15°C) affected their critical thermal maximum (CT_{max}) and their HSP70 protein and mRNA transcript concentrations in brain, muscle, and liver tissues. Following acclimation, fish were exposed to a thermal ramping rate of 3.4°C · h^{-1}. The CT_{max} temperature was recorded when the fish expressed a loss of equilibrium. Tissue samples were then collected and analyzed via western blotting and transcriptome sequencing. Broad whitefish and saffron cod acclimated to 15°C had a significantly higher mean CT_{max} (27.3°C and 25.9°C, respectively) than 5°C fish (23.7°C and 23.2°C, respectively). Broad whitefish had a significantly higher CT_{max} than saffron cod at 15°C in addition to significantly higher HSP70 protein concentrations in liver and muscle tissues at both acclimation temperatures. Brain and muscle tissues had the highest and lowest HSP70 protein concentrations, respectively, for both species and acclimation temperatures. The only significant difference in protein concentration between acclimation temperatures was in saffron cod liver tissues where 5°C samples had a significantly higher concentration than 15°C. Brain and liver tissues for broad whitefish acclimated to 15°C had significantly higher HSP70 mRNA

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transcript concentrations than the control group that remained in lab-acclimation conditions of 8°C. Transcript B expressed a higher quantity of transcripts than transcript A, but both transcripts followed similar expression profiles and there were no differences in transcript concentration between tissues. The molecular data from this study demonstrates the cellular mechanisms that are, in part, responsible for the observed shifts in broad whitefish and saffron cod organismal thermotolerance, and this plasticity could be used to respond to changing thermal conditions in the nearshore Beaufort Sea in the future.

Introduction

Anthropogenic-driven climate change is increasing global aquatic temperatures, and these changes are occurring twice as fast in the nearshore Beaufort Sea (Overeem *et al.*, 2011; Graham *et al.*, 2017). Additionally, this area is inherently dynamic and can experience temperature changes as high as 2°C per hour due to wind-driven currents and river discharge (Figure 1.1; Ross, 1988; Mccain *et al.,* 2014; Hamman *et al.,* 2021). The aquatic thermal parameters in the nearshore Beaufort Sea are arguably the most important abiotic factors regulating fish abundance and distribution (Somero, 2010; Zhang and Kieffer, 2014; Moyano *et al.*, 2017; Khalsa *et al.*, 2021). Therefore, it is imperative to understand how species in this area may respond to these temperature changes through thermotolerance analysis. However, there have been limited thermotolerance examinations on Arctic teleosts, including broad whitefish *Coregonus nasus* and saffron cod *Eleginus gracilis.*

Although broad whitefish and saffron cod co-occur in the nearshore area, they have divergent and unique life-history strategies. Broad whitefish is amphidromous and utilize the nearshore environment to feed during the ice-free period (Fechhelm *et al.*, 1992; Griffiths *et al.*, 1992; Tallman and Reist, 1997; Harper *et al.*, 2012). This species is an important prey item for higher trophic-level organisms and is culturally important to Indigenous communities as a subsistence resource in coastal Alaska (Tallman and Reist, 1997; Tallman *et al.*, 2002). Saffron cod is a polar-marine species that occurs in warmer and shallower waters relative to other gadids and feed in the nearshore area (Wolotira, 1985; Mueter *et al.*, 2016; Vestfals *et al.*, 2019). Saffron cod provide an important energy-transfer link between trophic levels and are also used as a subsistence resource albeit at lower levels than broad whitefish (Frost and Lowry, 1981;

Copeman *et al.*, 2016; Reusser *et al.*, 2016). There is a distinct lack of information on the phenotypic plasticity of these two species in response to the changing thermal environments.

Organismal thermal tolerance is facilitated through the expression of molecular chaperones known as heat shock proteins (HSP; Dietz and Somero, 1993; White *et al.,* 1994; Iwama *et al.,* 1998; Buckley *et al.,* 2004; Somero, 2010). These proteins are categorized by molecular weight, which corresponds to their unique functions (Dietz and Somero, 1993). The most highly conserved family across teleost species is the 70-kDa HSP (Lindquist, 1986; White *et al.*, 1994; Iwama *et al.*, 1998; Basu *et al.*, 2002). There are constitutive levels of this protein (HSC70) that aid in daily cellular housekeeping and homeostasis while also demonstrating tissue and cell specific expression (Iwama *et al.*, 1998; Feder and Hofmann, 1999; Yamashita *et al.*, 2010). The inducible cognate (HSP70) is the most common HSP upregulated in response to a stressor, such as extreme temperatures, which helps maintain cellular homeostasis during conditions that would normally cause apoptosis (Feder and Hofmann, 1999; Santoro, 1999; Yamashita *et al.*, 2010). There are several HSP70 isoforms, partly due to alternative splicing of the transcripts, that can have unique species and tissue-specific expression patterns (Currie *et al.*, 2000; Basu *et al.*, 2002; Yamashita *et al.*, 2010). The presence of HSC70 and upregulation of HSP70 aids in the acquisition and manipulation of thermotolerance.

The production of HSP can change depending on the thermal environment (Hofmann, 1999). Phenotypic plasticity allowing for acclimation at different temperatures permits organisms to shift their HSP production and, ultimately, organismal thermotolerance (Dietz and Somero, 1992; Basu *et al.*, 2002; Somero, 2010). Seasonal thermal parameters have resulted in summer-acclimated fish having higher constitutive levels of HSP and higher induction temperatures for the inducible cognate versus winter-acclimated populations (Dietz and Somero,

1992; Dietz, 1994; Hofmann, 1999; Currie *et al.*, 2000). Living in a highly variable environment can result in organisms possessing elevated levels of the constitutive cognate to help maintain homeostasis during environmental fluctuations (Hofmann, 1999; Somero, 2010; Yamashita *et al.*, 2010). Additionally, there are physiological metrics related to thermotolerance that can be measured as a proxy for overall thermotolerance. The critical thermal maximum (CT_{max}) has been used in fish-thermotolerance studies, and it represents the maximum temperature that can be sustained before thermally induced mortality (Beitinger *et al.*, 2000; Zhang and Kieffer, 2014). This parameter, which is partially influenced by HSP70 production, will also shift in differing thermal environments (Becker and Geonway, 1979; Beitinger *et al.,* 2000; Zhang and Kieffer, 2014). Measuring CT_{max}, the underlying HSP70 mechanisms driving it, and how these parameters change in differing thermal conditions will provide a detailed understanding of thermotolerance and the extent that it can shift in response to environmental changes (Basu *et al.*, 2002; Teigen *et al.*, 2015).

The goal of this project was to measure HSP70 protein and mRNA transcript concentrations that occurred at the CT_{max} endpoint in broad whitefish and saffron cod acclimated to 5 and 15°C. The objectives of this study were to quantify: (1) CT_{max} values and determine if there are differences between the two acclimation temperatures and two species; (2) HSP70 protein concentration in brain, liver, and muscle tissues and determine if there are differences among tissue types, acclimation temperatures, and species; and (3) mRNA HSP70 concentration in broad whitefish liver and muscle tissues to determine significant differences between tissue types and acclimation temperatures. This information could aid future predictions in the responses and distribution of these two fishes in a warming climate regime (Reist *et al.*, 2006; Somero, 2010).

Materials and Methods

Study site description and sample collection

All fish sampling and transport were conducted in accordance with the Alaska Department of Fish and Game Aquatic Resource Permit (ADF&G; CF-20-021 and CF-21-009) and the University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC) protocol (protocol #1054743). Nearshore Prudhoe Bay is a shallow (~2 m) estuarine environment that is used by various anadromous, marine, and amphidromous fishes for feeding during the ice-free period (Ross, 1988; Mccain *et al.*, 2014). The Sagavanirktok River, located 5 km from Prudhoe Bay, is the second largest river on the North Slope of Alaska (Figure 1.2; Craig, 1984; Hodel, 1986; Carmack and Macdonald, 2002). The discharge from this river, coupled with the prevailing winds that drive currents, creates an estuarine environment in this region (Ross, 1988; Mccain *et al.*, 2014). The four locations sampled during this study, spanning approximately 27 km along the coastline, varied in topography and proximity to the Sagavanirktok River, which influenced their unique thermal microenvironments (Figure 1.2).

Paired fyke nets (1.8 m x 1.7 m, with 12.77-mm stretch mesh netting) were placed 60 m from shore, while a lead net (60 m x 1.8 m, 25-mm stretch mesh) and two block nets (15 m x 1.8 m, 25-mm stretch mesh) helped guide fish into the nets from the shore in either direction (Figure S1). Environmental variables were measured daily using a handheld YSI (YSI Inc., Yellow Springs, Ohio) during the field season, and these measurements were used to determine the experimental thermal parameters described later. Broad whitefish (n = 17) and saffron cod (n = 35) were sampled in 2020 and 2021, respectively. Fish were placed in polyethylene bags (15–20 individuals per bag) with Beaufort Sea water before being shipped by air to the University of Alaska Fairbanks (UAF). Upon arrival at UAF, fish were immediately placed into rearing tanks

maintained at 8°C for lab acclimation. Broad whitefish were kept at a salinity of 3.5 while saffron cod were held at a salinity of 9.0 throughout the duration of acclimation and experimentation periods and both species were fed 0.5 g per fish of frozen blood worms (*Glycera* spp.) daily.

Thermal ramping experiment and critical thermal maximum determination

All holding and experimental procedures were approved by the UAF IACUC (protocol #'s 1615559 and 197441). After a six-week laboratory acclimation period, fish were randomly divided into acclimation-temperature trials of 5 and 15°C. These temperatures were chosen because they represented the mean minimum and maximum temperatures experienced in the nearshore Beaufort Sea during the ice-free period (Gatt *et al.*, 2019). Broad whitefish ($n_{5°C}$ = 8; $n_{15°C}$ = 9; 93 – 148 mm; 8.59 – 32.0 g) and saffron cod ($n_{5°C}$ = 15; $n_{15°C}$ = 16; 121 – 170 mm; 8.0 – 26.1 g) were lab-acclimated to their respective temperature treatments for one week in experimental tanks (76.2 cm x 45.72 cm x 30.48 cm, 110 L) that were a part of a recirculating aquarium system. The 5°C-temperature treatment was maintained with a 1/13 HP Aquarium Chiller (AquaEuroUSA, Gardena, California), while the 15°C-temperature treatment was maintained using industrial chillers (Frigid Units, Toledo, Ohio). Each tank was also fitted with an aquarium air pump (Tetra, Blacksburg, Virginia) and two air stones to keep oxygen levels consistent. Temperatures were monitored and adjusted daily with a temperature monitor fitted with a probe (Finnex, Chicago, Illinois) to maintain target temperatures (± 1.0°C). Daily feeding was carried out as described previously, and water changes were conducted to maintain balanced water chemistry.

At the end of the acclimation period, individual plastic containers (14.0 cm x 8.9 cm x 5.1 cm for broad whitefish; 10.2 cm x 10.2 cm x 8.9 cm for saffron cod) were secured together and

placed in the acclimation aquaria so the water level in each container matched the aquarium water level. Each container was fitted with an air stone and aerator before fish were placed into individual containers. Thermal ramping was carried out using an 800-watt titanium heater fitted in each tank that held the individual plastic containers (Finnex, Chicago, Illinois). The ramping rate was set to 3.4°C · h^{-1}, which represented the largest change in water temperature observed in the Beaufort Sea nearshore area during the 2019 ice-free period (Gatt *et. al.*, 2019). Temperature was measured using the same temperature monitor and probe as described previously, and temperature was increased 0.85°C every 15 minutes to ensure there was a linear increase in water temperature (± 0.2°C h^{-1}).

Individuals were continually monitored until demonstrations of lost equilibrium (LOE), and the temperature at which this occurred was recorded as the CT_{max} endpoint (Becker and Geonway, 1979; Saravia *et al.*, 2021). The fish was then immediately euthanized, fork length (mm) and wet weight (g) were measured, and a 1-cm^2 section of pectoral-muscle and liver tissue and the entirety of brain tissue were removed and placed into individual cryovials, flash-frozen in liquid nitrogen, and stored at -80°C for future analysis. All dissecting instruments were sterilized with 70% molecular grade ethanol and wiped clean between each tissue collection.

Protein extraction, separation, and densitometry quantification

Ten percent (by volume) of an individual tissue sample was added to 500 µL of homogenization buffer (Table S1). Using a tissue homogenizer with a pestle attachment, each tissue sample was ground to make a homogenous solution and incubated at 100°C for 5 minutes before undergoing centrifugation at 15,000 RPM for 15 minutes. The supernatant, containing extracted protein, was then pipetted into new cryovials. The protein in each supernatant was quantified in cuvettes using the Pierce™ Coomassie (Bradford) Protein Assay Kit (ThermoFisher

Scientific, Waltham, Massachusetts) as well as following procedures outlined in Bradford (1976). Duplicates of each protein sample were made by mixing 1 µL of thawed protein sample with 499 µL of diH_2O. Protein concentration was determined by averaging the fluorescence values per sample and calculating the concentration based on an albumin standard curve.

A wet tank transfer Western Blot protocol was followed for protein separation and transfer (Supplementary Information 1). A concentration of 10 µg of sample was separated on a 10% SDS-Page gel before being transferred to a 0.45-nm nitrocellulose membrane overnight at 30 V. Membranes were blocked for 1.5 hours at 1:1,000 dilution with a monoclonal mouse anti-HSP70 antibody and 5% NFDM in 1xPBS (Table S1). This was followed by incubation for an hour in a donkey anti-Mouse IgG (H+L) secondary antibody at 1:10,000 dilution in 5% NFDM in 1xPBS. The membranes were exposed to a chemiluminescent substrate before being imaged using the Amerhsam Imager chemiluminescent setting (GE Healthcare, Chicago, Illinois). After imaging, it was ensured that the separated bands aligned with 70-kDa on the protein ladder (Figure S2). The HSP70 protein concentrations were quantified using ImageJ (v. 1.8.0_172) following the protocol outlined by ImageJ (imagej.nih.gov) and Gassmann *et al.* (2009) to calculate the optical density (OD) unit. This unit is the ratio of the density of the sample band to the density of the internal standard band and can be used to make proportionate comparisons between the relative levels of HSP70 concentration.

RNA-seq and HSP70 transcript quantification

Broad whitefish that had remained in lab-acclimation conditions at 8°C were used as control samples for mRNA quantification. Liver ($n_{5°C}$ = 6; $n_{15°C}$ = 6; $n_{control}$ = 4) and muscle ($n_{5°C}$ = 5; $n_{15°C}$ = 6; $n_{control}$ = 4) tissue samples from broad whitefish were sent in to GENEWIZ® (South Plainfield, New Jersey) for RNA-seq analysis to acquire a full

transcriptome dataset per sample. A rainbow trout *Oncorhynchus mykiss* transcriptome dataset was downloaded from Ensembl (v. 105, uswest.ensembl.org) to use as the target transcriptome since no categorized transcript dataset currently exists for broad whitefish and rainbow trout was the most closely related species with such a dataset (Shedko *et al.*, 2012).

Salmon software (v. 1.6.0, github.com/COMBINE-lab/salmon) was used to quantify broad whitefish transcripts. This software uses a series of alignment techniques that maps broad whitefish transcript sequences to corresponding, indexed rainbow trout sequences, which allowed the dataset to be filtered to show only HSP70 transcripts. The number of times an HSP70 transcript occurred was normalized using transcript per million (TPM), which proportions the read length and sequencing reads by the feature length and provides the relative abundance of a transcript in the dataset (Wagner *et al.*, 2012). The TPM values for each HSP70 transcript were summated to garner an overall value of HSP70 gene expression. Two transcripts of the HSP70 gene were quantified in this manner.

Data Analysis

All statistical analyses were performed in RStudio (v. 1.4.1106, https://www.rstudio.com/products/rstudio/). A linear-regression model was used to determine if fish weight, length, and the interaction of these two variables had any impact on the respective CT_{max} values. A second linear-regression model was used to determine the linear-relationship between the CT_{max} and acclimation temperatures. A Grubb's test was used to identify and remove outliers in the CT_{max} and HSP70 protein concentrations; there were too few data points in the mRNA expression dataset to remove outliers. Even after transformation and outlier removal, the CT_{max}, HSP70 protein concentration, and HSP70 mRNA expression datasets failed to meet the assumptions for parametric tests. As a result, non-parametric statistical analyses were used

instead with an $\alpha = 0.05$ and p-values were corrected using the Bonferroni method. A Wilcoxon rank-sum test was used to compare the CT_{max} values between each acclimation temperature and between species at the same acclimation temperature. The acclimation response ratio (ARR) for each species was calculated as follows: $\frac{\Delta CT_{max}}{\Delta Acclimation\ Temperature}$ (Claussen, 1977; Kelley, 2014). For the HSP70 protein expression, a Wilcoxon rank-sum test was used to compare means of the OD Unit values between the acclimation temperatures in the three tissue samples and between species at the same acclimation temperature. A Kruskal-Wallis test was used to determine if tissue-type made a significant difference in OD Unit values for each species, which was followed by a post-hoc Dunn's test to determine which pairwise comparisons were significant. Analyses were run on the TPM values for each transcript, but no analyses were run between the transcripts themselves. A Wilcoxon rank-sum test was used to compare the TPM values between muscle and liver tissue samples at the same temperature treatment. A Kruskal-Wallis test was used to determine if temperature treatment influenced mRNA expression, and a post-hoc Dunn's test was used to determine which pairwise temperature treatments were different.

Results

CT_{max} temperature comparisons

There was no effect of fish weight ($p = 0.672$), length ($p = 0.908$), or the interaction of weight x length ($p = 0.633$) on CT_{max} temperatures. For the linear model between acclimation temperature (T_a) and CT_{max}, there was a significant relationship for broad whitefish and saffron cod in addition to broad whitefish having a higher slope than saffron cod (Table 1). The ARR for broad whitefish was 0.3895 and 0.2890 for saffron cod. Mean CT_{max} values at 15°C were significantly higher than the 5°C group for both broad whitefish ($W = 72$; $p < 0.001$) and saffron cod ($W = 224$; $p < 0.0001$; Figure 1.3; Table 1.1). The mean CT_{max} temperature at 15°C was significantly higher for broad whitefish than saffron cod ($W = 110$; $p < 0.01$; Figure 1.3; Table 1.1), but there was no significant difference between the two species at 5°C ($W = 81$; $p > 0.05$).

HSP70 protein concentration

The antibody used in this study recognizes both the constitutive and inducible HSP70 protein, and thus the total 70-kDa protein concentration was measured (Dietz and Somero, 1993; Yoo and Janz, 2003). However, since the thermal ramping rate induces HSP70 production and the constitutive HSC70 should remain constant given the consistent acclimation conditions, the change in protein levels represents a relative change in HSP70 protein concentration. Mean OD unit and standard deviation in 5°C-acclimated broad whitefish was 0.949 ± 0.062 (brain), 0.803 ± 0.262 (liver), and 0.471 ± 0.135 (muscle). At 15°C, mean values were 0.866 ± 0.102, 0.611 ± 0.0728, and 0.317 ± 0.188 in brain, liver, and muscle tissues, respectively. There were no significant differences in the mean OD units between the two acclimation temperatures for the same tissue type ($W_{liver} = 12.5$; $W_{muscle} = 16.0$; $W_{brain} = 17.0$; $p > 0.05$; Figure 1.4). However, tissue type did influence the mean OD unit values ($\chi^2_{5°C} = 14.6$; $p_{5°C} < 0.0001$; $\chi^2_{15°C} = 21.0$, $p_{15°C}$

< 0.0001). At 15°C, brain tissue had a significantly higher mean OD unit than the liver and muscle ($T_{brain*liver} = -2.465$; $p_{brain*liver} < 0.05$; $T_{brain*muscle} = -4.573$, $p_{brain*muscle} < 0.0001$; Figure 1.5). However, at 5°C, brain tissue only had a significantly higher mean OD unit than the muscle samples ($T_{brain*muscle} = -3.783$; $p_{brain*muscle} < 0.001$; Figure 1.5).

The 5°C-acclimated saffron cod had mean OD unit values and standard deviations of 0.907 ± 0.0975, 0.214 ± 0.0946, and 0.0428 ± 0.0363 in brain, liver, and muscle tissues. At 15°C, the mean values were 0.933 ± 0.163 (brain), 0.0744 ± 0.0585 (liver), and 0.0339 ± 0.0157 (muscle). The 5°C liver tissue had a significantly higher mean concentration than the 15°C samples ($W_{liver} = 8.5$; $p < 0.05$) but there was no significant difference in mean OD unit in the other two tissues ($W_{muscle} = 118.5$; $W_{brain} = 148$; $p > 0.05$; Figure 1.4). Tissue type had a significant effect on mean OD unit values ($\chi^2_{5\,°C} = 38.5$; $p_{5\,°C} < 0.0001$; $\chi^2_{15\,°C} = 26.9$, $p_{15\,°C} < 0.0001$), with brain tissues having a higher mean OD unit than liver and muscle at 15°C ($T_{brain*liver} = -3.10$, $p_{brain*liver} < 0.01$; $T_{brain*muscle} = -5.05$, $p_{brain*muscle} < 0.0001$; Figure 1.5) and 5°C ($T_{brain*liver} = -3.19$, $p_{brain*liver} < 0.01$; $T_{brain*muscle} = -6.20$, $p_{brain*muscle} < 0.0001$). At 5°C, mean OD unit in liver tissue was significantly higher than in muscle ($T_{liver*muscle} = -2.79$, $p_{liver*muscle} < 0.05$; Figure 1.5). Broad whitefish had a significantly higher mean OD unit than saffron cod at both acclimation temperatures in the muscle ($W_{15°C} = 133$; $p_{15°C} < 0.001$; $W_{5°C} = 128$, $p_{5°C} < 0.001$) and liver ($W_{15°C} = 63$; $p_{15°C} < 0.01$; $W_{5°C} = 112$, $p_{5°C} < 0.0001$; Figure 1.4) tissues.

HSP70 mRNA Expression

Mean broad whitefish TPM values and standard deviations at 5°C were 135 ± 116 (Transcript A) and 238 ± 197 (Transcript B) in the liver and 106 ± 29.2 (Transcript A) and 210 ± 57.9 (Transcript B) in muscle tissues. At 15°C, mean values were 443 ± 389 (Transcript A) and 844 ± 734 (Transcript B) in liver and 760 ± 388 (Transcript A) and $1{,}657 \pm 953$ (Transcript B) in

muscle tissues. For the control group, mean TPM values were 3.97 ± 2.39 (Transcript A) and 8.34 ± 2.68 (Transcript B) in the liver and 42 ± 62.7 (Transcript A) and 92.2 ± 136 (Transcript B) in muscle tissues.

Temperature treatment had a significant effect on mean TPM values in liver ($\chi_A^2 = 9.94$; $p_a < 0.01$; $\chi_B^2 = 9.94$; $p_B < 0.01$) and muscle ($\chi_A^2 = 11.2$; $p_a < 0.01$; $\chi_B^2 = 10.7$; $p_B < 0.01$) tissues. Mean liver and muscle TPM values at 15°C were significantly higher than the control group in transcript A ($T_{liver} = -3.145$, $p_{liver} < 0.01$; $T_{muscle} = -3.204$; $p_{muscle} < 0.01$) and transcript B ($T_{liver} = -3.145$; $p_{liver} < 0.01$; $T_{muscle} = -3.031$; $p_{muscle} < 0.01$; Figure 1.6). The only difference between the two transcript was that transcript B had a significantly higher mean TPM at 15°C versus 5°C in muscle ($T = -2.400$; $p < 0.05$; Figure 1.6) tissues. There were no significant differences in mean TPM values between the two tissue types at the same acclimation temperature in transcript A ($W_{15°C} = 9$; $W_{5°C} = 13$; $W_{control} = 2$; $p > 0.05$) and transcript B ($W_{15°C} = 9$; $W_{5°C} = 13$; $W_{control} = 0.0$; $p > 0.05$).

Conclusions

CT_{max} comparisons

Broad whitefish and saffron cod successfully adjusted their thermotolerance in response to unique thermal parameters. Body mass has been shown to have a positive, linear relationship with CT_{max}, but there was no trend between these two variables in this study because fish from the same size classes were collected to avoid ontogenetic- or size-related bias (Zhang and Kieffer, 2014). However, acclimation temperature, which is considered the most important factor influencing thermotolerance, did have a significant effect on CT_{max} temperatures (Zhang and Kieffer, 2014). Both broad whitefish and saffron cod had significantly higher CT_{max} temperatures at 15 than 5°C, which has been observed in other fishes (Table 1.1). At 15°C, broad whitefish had a significantly higher CT_{max} than saffron cod. This is interesting because saffron cod can also be found in the North Pacific whereas broad whitefish are found in the Arctic and sub-Arctic (Fechhelm *et al.*, 1992; Reusser *et al.*, 2016). With their limited habitat and thermal range, it would be expected that broad whitefish would have a lower thermotolerance. The acclimation response ratios (ARR) between the two species followed similar trends as the CT_{max} temperatures. Broad whitefish had a higher ratio than saffron cod, indicating that it possessed a higher degree of thermal acclimation, which is consistent with the higher broad whitefish CT_{max} at 15°C (Kelley, 2014; Semsar-kazerouni and Verberk, 2018). The difference in CT_{max} and ARR between broad whitefish and saffron cod and acclimation temperatures can potentially be explained by underlying genetic differences, which is supported by the HSP70 molecular data. Molecular mechanisms will shift to respond to environmental parameters and maintain homeostasis (Dietz, 1994; Feder and Hofmann, 1999; Hofmann, 1999). The trends in ARR and

CT_{max} could also be due to suboptimal lab conditions for saffron cod, which is an idea that is explored later.

Although the CT_{max} temperatures for broad whitefish showed slightly lower values than other teleosts, this species had a larger overall thermal range (Table 1.1). For 5°C-acclimated fish, the CT_{max} of 23.7°C was lower than reported for like-acclimated coho salmon *Oncorhynchus kisutch* and lake whitefish *Coregonus clupeaformis* (Becker and Genoway, 1979; Manzon *et al.,* 2022). Further, this CT_{max} temperature was within 0.4°C of the value reported previously for 9°C-acclimated broad whitefish (Bilyk and Sformo, 2021). For 15°C-acclimated fish, the CT_{max} of 27.3°C was lower than values found for coho salmon, cutthroat trout *Oncorhynchus clarkii,* rainbow trout, and shortnose sturgeon *Acipenser brevirostrum* and higher than 18°C-acclimated lake whitefish *Coregonus clupeaformis* (Heath, 1963; Currie *et al.*, 1998; Beitinger *et al.*, 2000; Zhang and Kieffer, 2014; Manzon *et al.*, 2022). While the CT_{max} temperature tended to be lower, it appears that broad whitefish had a broader thermal range than these other species. The CT_{max} increased by 0.39°C for every 1°C increase in acclimation temperature for broad whitefish, which was higher than lake whitefish, rainbow trout, cutthroat trout, and saffron cod and lower than shortnose sturgeon and Arctic cod (Currie *et al.,* 1998; Beitinger *et al.,* 2000; Zhang and Kieffer, 2014; Drost *et al.,* 2016; Manzon *et al.,* 2022). A higher slope provided evidence of a wider thermal range, which broad whitefish may require to tolerate the thermally dynamic nearshore Beaufort Sea (Currie *et al.*, 1998). Broad whitefish had comparable, albeit slightly lower, CT_{max} temperatures compared to other salmonids, and their CT_{max} slope indicated a eurythermal species with broad temperature limits.

Saffron cod had a higher CT_{max} temperature relative to other gadids, but a lower value compared to other fish families (Table 1.1). The 5°C-acclimated saffron cod had a CT_{max} of

25.7°C, which was higher than reported for 6.5°C-acclimated Arctic cod (Drost *et al.,* 2016). Additionally, there was 100% mortality when Arctic cod were acclimated to 16°C versus 100% saffron cod survival during acclimation at both temperatures, which is consistent with saffron cod and Arctic cod being eurythermal and stenothermal, respectively (Drost *et al.,* 2016). The CT_{max} temperatures for saffron cod at both acclimation temperatures were lower than reported for like-acclimated lake whitefish, shortnose sturgeon, rainbow trout, coho salmon, and cutthroat trout (Becker and Genoway, 1979; Currie *et al.,* 1998; Beitinger *et al.,* 2000; Zhang and Kieffer, 2014; Manzon *et al.,* 2022). Interestingly, saffron cod had a CT_{max} gain of 0.255°C per 1°C acclimation temperature, which was lower than Arctic cod (Drost *et al.,* 2016). Typically, a higher slope value indicates a wider thermal range, and, given that saffron cod is eurythermal, it should have a higher slope (Currie *et al.*, 1998; Reusser *et al.*, 2016). However, this could potentially be explained by experimental conditions.

The salinity to which saffron cod were acclimated reflects conditions experienced in the brackish nearshore environment, but saffron cod is a species that is known to reside in marine conditions and utilize the nearshore, brackish environment (Reusser *et al.*, 2016; Helser *et al.*, 2017). For the laboratory experiment, saffron cod had to maintain homeostasis in continuous brackish conditions while also responding to stressful thermal changes. Multiple stressors can either evoke metabolic compensation, where the cells are able to delegate metabolic costs to continue to maintain homeostasis, or metabolic conservation where metabolism becomes anaerobic to maintain cellular processes (Petitjean *et al.*, 2019). The intensity of the stressors determines which metabolic strategy is invoked, but it is possible that the salinity conditions were sufficiently stressful so that saffron cod could not allocate as much metabolic energy to the thermal stress response, which lowered its overall thermotolerance. This reduction in

thermotolerance at suboptimal salinity conditions has also been observed for the European green crab *Carcinas maenas* (Munoz *et al.*, 2017). This response could also explain the lower thermotolerance that was observed for their CT_{max} temperature, ARR, and CT_{max} slope for saffron cod relative to broad whitefish. Compared to other species, the CT_{max} slope value for saffron cod was higher than lake whitefish and rainbow trout, the same as cutthroat trout, and lower than shortnose sturgeon and broad whitefish (Becker and Genoway, 1979; Currie *et al.*, 1998; Beitinger *et al.*, 2000; Zhang and Kieffer, 2014; Manzon *et al.*, 2022). Regardless, the CT_{max} dataset was consistent with saffron cod being a eurythermal species and having an intermediate thermal range.

HSP70 protein concentration

The only significant difference in HSP70 protein concentration between acclimation temperatures occurred in saffron cod when the 5°C liver tissues had a significantly higher mean protein concentration than 15°C samples. This is contrary to other studies that have reported enhanced *in vivo* and *in vitro* protein synthesis at higher acclimation temperatures. For example, longjaw mudsucker *Gillichthys mirabilis* had greater HSP70 protein expression in brain, gill, and liver tissues with higher temperature exposure (Dietz, 1994). The sun catfish *Horabagrus brachysoma* had higher HSP70 protein concentrations at 30 versus 20°C-acclimated fish (Dalvi *et al.*, 2012). Further, rainbow trout cell lines have shown an increase in a variety of HSP families in response to heat shock (Iwama *et al.*, 1999). Atlantic salmon *Salmo salar*, fathead minnow *Pimephales promelas,* and sun catfish showed an increase in protein concentration up to a peak temperature before concentrations began to decline at temperatures past this peak (Dyer *et al.*, 1991; Smith *et al.*, 1999; Dalvi *et al.*, 2012). The HSP70 protein concentrations from other teleosts have indicated temperature-dependent protein concentrations, but the only trend that

could be established here was contrary to these other studies (Dyer *et al.*, 1991; Smith *et al.*, 1999; Dalvi *et al.*, 2012).

Tissue-specific differences occurred in broad whitefish and saffron cod, with brain and muscle tissues having the highest and lowest HSP70 protein concentrations, respectively. Tissue-specific protein expression patterns have been reported in teleosts before, but the tissue type with the highest or lowest concentration tends to vary. Dyer *et al.* (1991) reported that fathead minnow had tissue-specific induction profiles in addition to each tissue expressing unique HSP families with gill and brain tissue expressing the most and fewest families, respectively. In Atlantic salmon, gill tissue had the most intense thermal response in terms of protein concentration while liver tissue had the smallest thermal response (Smith *et al.*, 1999). In sun catfish, liver tissue had the highest HSP70 protein concentration followed by brain and muscle tissues with gill tissue having the lowest concentration (Dalvi *et al.*, 2012). Underlying differences in the heat shock response between tissue types, including differences in the constitutive HSP levels, different induction temperatures for the inducible HSP, and variations in protein denaturation and proteolysis of damaged proteins, have been reported and could explain tissue-specific protein concentrations (Dietz and Somero, 1993; Smith *et al.*, 1999). Further experimentation would be needed to determine if and where the tissues differed in their heat shock response, but the tissue specific HSP70 concentrations in broad whitefish and saffron cod corroborate other teleost studies.

Broad whitefish had significantly higher protein concentrations than saffron cod in liver and muscle tissues at both acclimation temperatures. Differences in protein concentrations between species have been previously noted. For example, Atlantic salmon, a mesothermic salmonid, showed a unique HSP expression profile compared to the eurythermal fathead minnow

and mummichog *Fundulus heteroclitus* (Smith *et al.*, 1999). Further, significant differences in the induction profiles for HSP70 mRNA and protein concentrations were found between Atlantic salmon and Arctic charr *Salvelinus alpinus* (Lewis *et al.*, 2016). There are multiple isoforms, partly due to alternative splicing of the HSP70 mRNA transcripts, that vary between species and will impact HSP70 concentrations, which could explain the difference observed between broad whitefish and saffron cod (White *et al.*, 1994). The significantly higher protein concentration in broad whitefish could explain why it also had a higher CT_{max} at 15°C compared to saffron cod. Studies have reported higher levels of HSP70 with higher acclimation temperatures, which results in an overall increase in thermotolerance (Dalvi *et al.*, 2012). Since broad whitefish had a significantly higher CT_{max} than saffron cod at 15°C, they likely would have higher HSP70 protein concentrations to reflect this increase in thermotolerance. Underlying genetic differences between saffron cod and broad whitefish may explain the difference in HSP70 protein concentration between the two species, resulting in an overall thermotolerance difference.

HSP70 mRNA transcript concentration

The two HSP70 transcripts analyzed in the dataset associated with the current study followed previously established expression patterns. In rainbow trout, transcript A and B tended to follow similar expression profiles, with transcript B typically having higher concentrations (Yamashita *et al.*, 2010). This pattern was also observed for broad whitefish, with the only difference between the two transcripts being that mRNA concentration in 15°C-acclimated muscle tissue was significantly higher than the 5°C fish in transcript B. Although it is common for alternatively spliced transcripts to not follow exact expression profiles, the two broad whitefish transcripts behaved in a manner reflecting patterns that have been established for rainbow trout (Yamashita *et al.*, 2010).

Acclimation temperature significantly affected broad whitefish HSP70 mRNA transcript concentrations, with 15°C-acclimated fish having significantly higher concentrations than the control group in both transcripts. The upregulated expression at 15°C for broad whitefish could explain the significantly higher CT_{max} than saffron cod at the same temperature because higher HSP70 transcript levels have been correlated with warmer-acclimated organisms, including longjaw mudsucker and Mozambique tilapia *Oreochromis mossambicus* (Dietz, 1994; Hofmann, 1999; Molina *et al.*, 2000). Rainbow trout erythrocytes have demonstrated elevated levels of HSP70 mRNA after heat shock exposure, with transcript concentrations rising with increasing exposure temperature (Currie *et al.*, 2000; Yamashita *et al.*, 2010). Lewis *et al.* (2016) showed temperature dependent HSP70 transcriptional induction in Arctic charr and Atlantic salmon. Atlantic salmon parr also showed an increase in both HSP70 protein and mRNA concentration with increasing temperature treatments (Lund *et al.*, 2002). The higher mRNA transcript concentration in 15°C broad whitefish suggests that there is a temperature-dependent induction profile for HSP70 mRNA, which could explain the observed CT_{max} temperatures for this species.

There were no significant differences in transcript concentrations between tissue types, which was counter to the protein concentrations where liver tissue had a significantly higher protein concentration than muscle tissue. In rainbow trout, the HSP70 mRNA transcript concentrations varied between tissue type but, like broad whitefish, white muscle and liver had comparable HSP70 mRNA levels (Currie *et al.*, 2000). The lack of tissue-specific differences could be explained by prior-established patterns of expression between HSP70 mRNA transcript and protein levels. Smith *et al.* (1999) found an accumulation of HSP70 protein that preceded a sharp increase in mRNA. It is possible that the mRNA transcript levels had not yet increased to match the protein levels in liver tissues. The difference between broad whitefish mRNA and

protein levels suggests some post-transcriptional regulation during the heat shock response, which has been previously observed in Arctic charr and Atlantic salmon (Lewis *et al.*, 2016). Overall, differential expression patterns between mRNA transcript and protein concentrations likely explains why there were no tissue-specific differences for broad whitefish detected in the current study.

Implications with continued climate change

The results of this study suggest that broad whitefish and saffron cod in the nearshore Beaufort Sea can shift their thermotolerance due to phenotypic plasticity driven by underlying molecular mechanisms. Species living in variable environments have been shown to have a high diversity of HSP, which allows for continued homeostasis (White *et al.*, 1994). While HSP70 was the only HSP family measured in this study, both broad whitefish and saffron cod showed diversity in the HSP70 protein expression relative to each other and among tissue types, which allowed for a unique response to the given acclimation parameters. The heightened HSP70 mRNA transcript concentration in 15°C-acclimated broad whitefish could explain the higher CT_{max} at this temperature, and additional transcript production at higher temperatures will allow further phenotypic plasticity (Currie *et al.*, 2000). Additionally, the potential demonstrated here to acclimate to current thermal conditions in the nearshore environment could result in molecular changes that will increase tolerance to future temperature changes, which is known as "heat hardening" (Iwama *et al.*, 1999). The protein expression profiles and mRNA transcripts in the tissues of broad whitefish and saffron cod allowed the observed phenotypic plasticity that resulted in a shift in thermotolerance.

Although broad whitefish and saffron cod were able to successfully shift their thermotolerance, this response could come at a cost. Both species were able to acclimate to 15°C,

and there is the potential that they could acclimate to warmer temperatures as well. However, it has been shown that as acclimation temperature increases, the CT_{max} and incipient lethal temperatures converge, which minimizes continued acclimation and reduces overall homeostasis (Somero, 2010). It would be beneficial to determine the upper temperature limit for these species to gauge their acclimation potential at temperatures warmer than 15°C, which could already be at their acclimation limit. During the heat shock response, only inducible HSP mRNA are transcribed, which is energetically costly and not sustainable long term (Lewis *et al.*, 2016). The heat shock proteins themselves interfere with ongoing cellular processes and become detrimental to cellular homeostasis as more are produced (Feder and Hofmann, 1999). With the half-life of HSP70 being several days, the initial production of HSP70, while initially beneficial, could quickly become toxic and have negative consequences with additional production (Hofmann, 1999). The acclimation limit and energetic expenditure of continually producing HPS70 emphasizes the potential cost of inducible thermotolerance for these two species.

The evidence of phenotypic plasticity in broad whitefish and saffron cod suggests that they have the potential and underlying molecular mechanisms to respond to further abiotic changes in the nearshore Beaufort Sea. Further, the higher CT_{max}, ARR, and HSP70 protein concentration for broad whitefish indicates that they are more physiologically capable of responding to heat stress than saffron cod and could fair better as climate change continues. Additional CT_{max} data from other Arctic teleosts in this region shows varied thermotolerance responses (Drost *et al.,* 2016; Bilyk and Sformo, 2021). Given these unique responses, changing thermal parameters may influence the distribution and abundance of fishes in the nearshore Beaufort Sea (Reist *et al.*, 2006; Somero, 2010; Priest *et al.*, 2022). There have already been notable increases in broad whitefish and saffron cod abundances in this region in recent years,

with similar patterns also occurring for saffron cod in Prince William Sound, Alaska (Johnson *et al.*, 2009; Hamman *et al.*, 2021; Priest *et al.*, 2022). Overall, the current study provided detailed insights in to the physiological and molecular parameters driving the thermotolerance shifts demonstrated in broad whitefish and saffron cod, and this phenotypic plasticity may be used in response to future changes in thermal conditions in the nearshore Beaufort Sea.

References

Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK (2002) Heat shock protein genes and their functional significance in fish. *Gene* 295: 173–183. doi: 10.1016/s0378-1119(02)00687-x.

Becker CD, Genoway RG (1979) Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes* 4(3): 245–256. doi: 10.1007/BF00005481.

Beitinger TL, Bennett WA, Mccauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes* 58: 237–275. doi: 10.1023/A:1007676325825.

Bilyk KT, Sformo TL (2021) Varying heat tolerance among Arctic nearshore fishes. *Polar Biology* 44(3): 607–612. doi: 10.1007/s00300-021-02815-6.

Buckley BA, Place SP, Hofmann GE (2004) Regulation of heat shock genes in isolated hepatocytes from an Antarctic fish, *Trematomus bernacchii*. *Journal of Experimental Biology* 207(21): 3649–3656. doi: 10.1242/jeb.01219.

Carmack EC, Macdonald RW (2002) Oceanography of the Canadian shelf of the Beaufort Sea: a setting for marine life. *Arctic* 55: 29–45. doi: 10.14430/arctic733.

Copeman LA, Laurel BJ, Boswell KM, Sremba AL, Klinck K, Heintz RA, Vollenweider JJ, Helser TE, Spencer ML (2016) Ontogenetic and spatial variability in trophic biomarkers of juvenile saffron cod (*Eleginus gracilis*) from the Beaufort, Chukchi and Bering Seas. *Polar Biology* 39(6): 1109–1126. doi: 10.1007/s00300-015-1792-y.

Craig PC (1984) Fish use of coastal waters of the Alaskan Beaufort Sea: a review. *Transactions of the American Fisheries Society* 113(3): 265–282. doi: 10.1577/1548-8659(1984)113%3C265:FUOCWO%3E2.0.CO;2.

Currie RJ, Bennett WA, Beitinger TL (1998) Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes* 51: 187–200. doi: 10.1023/A:1007447417546.

Currie S, Moyes CD, Tufts BL (2000) The effects of heat shock and acclimation temperature on hsp70 and hsp30 mRNA expression in rainbow trout: in vivo and in vitro comparisons. *Journal of Fish Biology* 56(2): 398–408. doi: 10.1111/j.1095-8649.2000.tb02114.x.

Dalvi RS, Pal AK, Tiwari LR, Baruah K (2012) Influence of acclimation temperature on the induction of heat-shock protein 70 in the catfish *Horabagrus brachysoma* (Günther). *Fish Physiology and Biochemistry* 38(4): 919–927. doi: 10.1007/s10695-011-9578-9.

Dietz TJ (1994) Acclimation of the threshold induction temperatures for 70-kDa and 90-kDa heat shock proteins in the fish *Gillichthys mirabilis*. *Journal of Experimental Biology* 188: 333–338. doi: 10.1242/jeb.188.1.333.

Dietz TJ, Somero GN (1992) The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). *Proceedings of the National Academy of Science* 89: 3389–3393. doi: 10.1073/pnas.89.8.3389.

Dietz TJ, Somero GN (1993) Species-and tissue-specific synthesis patterns for heat-shock proteins HSP70 and HSP90 in several marine teleost fishes. *Physiological Zoology* 66(6): 863–880. doi: 10.1086/physzool.66.6.30163744.

Drost HE, Lo M, Carmack EC, Farrell AP (2016) Acclimation potential of Arctic cod (*Boreogadus saida*) from rapidly warming Arctic Ocean. *Journal of Experimental Biology* 219(19): 3114-3125. doi: 10.1242/jeb.140194.

Dyer SD, Dickson KL, Zimmerman EG, Sanders BM (1991) Tissue-specific patterns of synthesis of heat-shock proteins and thermal tolerance of the fathead minnow (*Pimephales promelus*). *Canadian Journal of Zoology* 69: 2021–2027. doi: 10.1139/z91-282.

Fechhelm RG, Dillinger RE, Gallaway BJ, Griffiths WB (1992) Modeling of in situ temperature and growth relationships for yearling broad whitefish in Prudhoe Bay, Alaska. *Transactions of the American Fisheries Society* 121(1): 1–12. doi: 10.1577/1548-8659(1992)121<0001:MOISTA>2.3.CO;2.

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243–282. doi: 10.1146/annurev.physiol.61.1.243.

Frost KJ, Lowry LF (1981) Trophic importance of some marine gadids in northern Alaska and their body-otolith size relationship. *Fishery Bulletin* 79(1): 187–192.

Gatt KP, Hamman CR, Priest JT, Green DG, Sutton TM (2019) Beaufort Sea long-term nearshore fish monitoring program: 2019 Annual Report. Report for Hilcorp Alaska, LLC by the University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, Department of Fisheries, Fairbanks, AK.

Graham RM, Cohen L, Petty AA, Boisvert LN, Rinke A, Hudson SR, Nicolaus M, Granskog MA (2017) Increasing frequency and duration of Arctic winter warming events. *Geophysical Research Letters* 44(13): 6974–6983. doi: 10.1002/2017GL073395.

Griffiths WB, Gallaway BJ, Gazey WJ, Dillinger RE (1992) Growth and condition of Arctic cisco and broad whitefish as indicators of causeway-induced effects in the Prudhoe Bay Region, Alaska. *Transactions of the American Fisheries Society* 121(5): 557–577. doi: 10.1577/1548-8659(1992)121<0557:GACOAC>2.3.CO;2.

Hamman CR, Bacon JA, Sutton TM (2021) Beaufort Sea long-term nearshore fish monitoring program: 2021 Annual Report. Report for Hilcorp Alaska, LLC by the University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, Department of Fisheries, Fairbanks, AK.

Harper KC, Harris F, Miller SJ, Thalhauser JM, Ayers SD (2012) Life history traits of adult broad whitefish and humpback whitefish. *Journal of Fish and Wildlife Management* 3(1): 56. doi: 10.3996/022011-JFWM-011.

Heath WG (1963) Thermoperiodism in sea-run cutthroat trout (*Salmo clarki clarki*). *American Association for Advancement in Science* 142(3591): 486–488. doi: 10.1126/science.142.3591.486.

Helser T, Colman J, Anderl D, Kastelle C (2017) Growth dynamics of saffron cod (*Eleginus gracilis*) and Arctic cod (*Boreogadus saida*) in the Northern Bering and Chukchi Seas. *Deep-Sea Research Part II: Topical Studies in Oceanography* 135: 66-77. doi: 10.1016/j.dsr2.2015.12.009.

Hodel KL (1986) The Sagavanirktok River, North Slope Alaska: characterization of an Arctic stream. USGS Open-File Report #86-267. 1-27. doi: 10.3133/ofr86267.

Hofmann GE (1999) Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. *American Zoology* 39: 889–900. doi: 10.1093/icb/39.6.889.

Iwama GK, Thomas PT, Forsyth RB, Vijayan MM (1998) Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35–56. doi: 10.1093/icb/39.6.889.

Iwama GK, Vijayan MM, Forsyth RB, Ackerman PA (1999) Heat shock proteins and physiological stress in fish. *American Zoologist* 39: 901–909. doi: 10.1093/icb/39.6.901.

Johnson SW, Thedinga JF, Neff AD (2009) Invasion by saffron cod *Eleginus gracilis* into nearshore habitats of Prince William Sound, Alaska USA. *Marine Ecology Progress Series* 389: 203–212. doi: 10.3354/meps08226.

Kelley AL (2014) The role thermal physiology plays in species invasion. *Conservation Physiology* 2(1): 1-14. doi: 10.1093/conphys/cou045.

Khalsa NS, Gatt KP, Sutton TM, Kelley AL (2021) Characterization of the abiotic drivers of abundance of nearshore Arctic fishes. *Ecology and Evolution* 11(16): 11491–11506. doi: 10.1002/ece3.7940.

Laurel BJ, Spencer M, Iseri P, Copeman LA (2016) Temperature-dependent growth and behavior of juvenile Arctic cod (*Boreogadus saida*) and co-occurring North Pacific gadids. *Polar Biology* 39(6): 1127–1135. doi: 10.1007/s00300-015-1761-5.

Lewis M, Götting M, Anttila K, Kanerva M, Prokkola JM, Seppänen E, Kolari I, Nikinmaa M (2016) Different relationship between hsp70 mRNA and hsp70 levels in the heat shock response of two salmonids with dissimilar temperature preference. *Frontiers in Physiology* 7: 1-12. doi: 10.3389/fphys.2016.00511.

Lindquist S (1986) The heat shock response. *Annual Review in Biochemistry* 55: 1151–1191. doi: 10.1146/annurev.bi.55.070186.005443.

Lund SG, Caissie D, Cunjak RA, Vijayan MM, Tufts BL (2002) The effects of environmental heat stress on heat-shock mRNA and protein expression in Miramichi Atlantic salmon (*Salmo salar*) parr. *Canadian Journal of Fisheries and Aquatic Sciences* 59(9): 1553–1562. doi: 10.1139/f02-117.

Manzon LA, Zak MA, Agee M, Boreham DR, Wilson JY, Somers CM, Manzon RG (2022) Thermal acclimation alters both basal heat shock protein gene expression and the heat shock response in juvenile lake whitefish (*Coregonus clupeaformis*). *Journal of Thermal Biology* 104: 1-11. doi: 10.1016/j.jtherbio.2021.103185.

Mccain KA, Raborn Scott W, Fechhelm RG, Raborn SW (2014) Year 31 of the long-term monitoring of nearshore Beaufort Sea fishes in the Prudhoe Bay region: 2013 Annual Report. Report for BP Exploration (Alaska) Inc. by LGL Alaska Research Associates, Inc. Anchorage, Alaska pp 72.

Molina A, Biemar F, Müller F, Iyengar A, Prunet P, Maclean N, Martial JA, Muller M (2000) Cloning and expression analysis of an inducible HSP70 gene from tilapia fish. *Federation of European Biochemical Societies Letters* 474: 5–10. doi: 10.1016/s0014-5793(00)01538-6.

Moyano M, Candebat C, Ruhbaum Y, Álvarez-Fernández S, Claireaux G, Zambonino-Infante JL, Peck MA (2017) Effects of warming rate, acclimation temperature and ontogeny on the critical thermal maximum of temperate marine fish larvae. *PLoS ONE* 12(7): 1-23. doi: 10.1371/journal.pone.0179928.

Mueter FJ, Nahrgang J, John Nelson R, Berge J (2016) The ecology of gadid fishes in the circumpolar Arctic with a special emphasis on the polar cod (*Boreogadus saida*). *Polar Biology* 39(6): 961-967. doi: 10.1007/s00300-016-1965-3.

Munoz L, Kelley AL, de Rivera C (2017) The effect of salinity acclimation on the upper thermal tolerance threshold of the European green crab. *Oceanography & Fisheries* 4(1): 1–5. doi: 10.19080/OFOAJ.2017.04.555627.

Overeem I, Anderson RS, Wobus CW, Clow GD, Urban FE, Matell N (2011) Sea ice loss enhances wave action at the Arctic coast. *Geophysical Research Letters* 38(17): 1-6. doi: 10.1029/2011GL048681.

Petitjean Q, Jean S, Gandar A, Côte J, Laffaille P, Jacquin L (2019) Stress responses in fish: from molecular to evolutionary processes. *Science of the Total Environment* 684: 371-380. doi: 10.1016/j.scitotenv.2019.05.357.

Priest JT, Green, DG, Fletcher BM, Sutton TM (2018) Beaufort Sea long-term nearshore fish monitoring program: 2018 annual report. Report for Hilcorp Alaska, LLC by the University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, Department of Fisheries, Fairbanks, AK.

Priest JT, Mueter FJ, Raborn SW, Sutton TM (2022) Effects of environmental variables on a nearshore arctic fish community, 2001–2018. *Polar Biology* 45(4): 585–599. doi: 10.1007/s00300-022-03013-8.

Reist JD, Wrona FJ, Prowse TD, Power M, Dempson JB, Beamish RJ, King JR, Carmichael TJ, Sawatzky CD (2006) General effects of climate change on Arctic fishes and fish populations. *Ambio* 35(7): 370–380. doi: 10.1579/0044-7477(2006)35[370:GEOCCO]2.0.CO;2.

Reusser DA, Frazier ML, Loiselle RA, Lee H, Thorsteinson LK (2016) Arctic climate change – a tale of two cods. In: Thorsteinson LK and Love, MS, eds. Alaska Arctic marine fish ecology catalog: U.S. Geological Survey Scientific Investigations Report 2016–5038 (OCS Study, BOEM 2016-048), pp 659-679. doi: 10.3133/sir20165038.

Ross BD (1988) Causeways in the Alaskan Beaufort Sea. U.S. Environmental Protection Agency, Region 10 1-33.

Santoro GM (1999) Heat shock factors and the control of the stress response. *Biochemical Pharmacology* 59: 55–63. doi: 10.1016/s0006-2952(99)00299-3.

Semsar-kazerouni M, Verberk WC (2018) It's about time: linkages between heat tolerance, thermal acclimation and metabolic rate at different temporal scales in the freshwater amphipod *Gammarus fossarum* Koch, 1836. *Journal of Thermal Biology* 75: 31–37. doi: 10.1016/j.jtherbio.2018.04.016.

Shedko S, Miroshnichenko I, Nemkova G (2013) Phylogeny of salmonids (salmoniformes: Salmonidae) and its molecular dating: analysis of mtDNA data. *Russian Journal of Genetics* 49(6): 623-637. doi: 10.1134/S1022795413060112.

Smith TR, Tremblay GC, Bradley TM (1999) Characterization of the heat shock protein response of Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 20: 279–292. doi: 10.1023/A:1007743329892.

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers.' *Journal of Experimental Biology* 213(6): 912–920. doi: 10.1242/jeb.037473.

Tallman RF, Abrahams M v, Chudobiak DH (2002) Migration and life history alternatives in a high latitude species, the broad whitefish, *Coregonus nasus* Pallas. *Ecology of Freshwater Fish* 11: 101–111. doi: 10.1034/j.1600-0633.2002.00001.x.

Tallman RF, Reist JD (1997) The proceedings of the broad whitefish workshop: the biology, traditional knowledge and scientific management of broad whitefish (*Coregonus nasus* (Pallas)) in the lower Mackenzie River. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2193: 1-227.

Teigen LE, Orczewska JI, McLaughlin J, O'Brien KM (2015) Cold acclimation increases levels of some heat shock protein and sirtuin isoforms in threespine stickleback. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 188: 139–147. doi: 10.1016/j.cbpa.2015.06.028.

Vestfals CD, Mueter FJ, Duffy-Anderson JT, Busby MS, de Robertis A (2019) Spatio-temporal distribution of polar cod (*Boreogadus saida*) and saffron cod (*Eleginus gracilis*) early life stages in the Pacific Arctic. *Polar Biology* 42(5): 969–990. doi: 10.1007/s00300-019-02494-4.

White CN, Hightower LE, Jack R (1994) Variation in heat-shock proteins among species of desert fishes (Poeciliidae, *Poeciliopsis*). *Molecular Biology and Evolution* 11(1): 106–119. doi: 10.1093/oxfordjournals.molbev.a040085.

Wolotira RJ (1985) Saffron cod (*Eleginus gracilis*) in western Alaska: the resource and its potential. NOAA Technical Memorandum NMFS F/NWC-79 1-127.

Yamashita M, Yabu T, Ojima N (2010) Stress protein HSP70 in fish. *Aquatic-BioScience Monographs* 3(4): 111–141. doi:10.5047/absm.2010.00304.0111.

Yoo JL, Janz DM (2003) Tissue-specific HSP70 levels and reproductive physiological responses in fishes inhabiting a metal-contaminated creek. *Archives of Environmental Contamination and Toxicology* 45(1): 110–120. doi: 10.1007/s00244-002-0109-7.

Zhang Y, Kieffer JD (2014) Critical thermal maximum (CTmax) and hematology of shortnose sturgeons (*Acipenser brevirostrum*) acclimated to three temperatures. *Canadian Journal of Zoology* 92(3): 215–221. doi: 10.1139/cjz-2013-0223.

Tables

Table 1.1 A comparison of different CT_{max} temperatures and the equations between CT_{max} and acclimation temperature (T_a) from other teleost studies.

Species Name	Acclimation Temperature (°C)	Thermal Ramping Rate (°C · h^{-1})	CT_{max} temperature ± SD (°C)	CT_{max} equation	Reference
Coregonus nasus (broad whitefish)	5	3.4	23.7 ± 1.36	*$CT_{max} = 0.390*T_a + 21.18393$, $R^2 = 0.6916$, $p < 0.0001$	This study
	15	3.4	27.3 ± 1.06		
Eleginus gracilis (saffron cod)	5	3.4	23.2 ± 0.786	*$CT_{max} = 0.289*T_a + 21.6273$, $R^2 = 0.7928$, $p < 0.0001$	This study
	15	3.4	25.9 ± 0.655		
Coregonus nasus (broad whitefish)	9	18.0	23.3 ± 0.84	NA	(Bilyk and Sformo, 2021)
Boreogadus saida (Arctic cod)	0.5	3.0	14.9[$]	$CT_{max} = 0.43*T_a + 14.24$, $R^2 = 0.74$, $p < 0.0001$	(Drost *et al.*, 2016)
	3.5	3.0	15.5[$]		
	6.5	3.0	17.1[$]		
Coregonus clupeaformis (lake whitefish)	6	12.0	23.9 ± 0.15	[1]$CT_{max} = 0.175*T_a + 22.867$	(Manzon *et al.*, 2022)
	12	12.0	25.0 ± 0.33		
	18	12.0	26.0 ± 0.58		
Acipenser brevirostrum (shortnose sturgeon)	10	6.0	27.6 ± 0.35	$CT_{max} = 0.52*T_a + 22.87$, $R^2 = 0.803$, $p < 0.001$	(Zhang and Kieffer, 2014)
	15	6.0	31.5 ± 0.91		
	20	6.0	32.8 ± 1.17		
Oncorhynchus mykiss (rainbow trout)	10	18.0	28.0 ± 0.36	$CT_{max} = 0.18*T_a + 26.23$, $R^2 = 0.975$, $p < 0.0001$	(Currie *et al.*, 1998)
	15	18.0	29.1 ± 0.27		
	20	18.0	29.8 ± 0.36		
Oncorhynchus kisutch (coho salmon)	5	6.0	24.84[$]	NA	(Becker and Genoway, 1979)
	5	18.0	25.32[$]		
	15	6.0	28.13[$]		
	15	18.0	28.70[$]		
Oncorhynchus clarkii (cutthroat trout)	10	24.0	27.63 ± 0.08	[#]$CT_{max} = 0.255*T_a + 25.5$	(Heath, 1963; Beitinger *et al.*, 2000)
	15	24.0	29.06 ± 0.05		
	20	24.0	29.88 ± 0.09		

[1] = calculated by this researcher based on the paper's data
[*] = only two acclimation temperatures were used to make the equation
[#] = no R^2 or p value was reported for the equation
[$] = no standard deviation values were reported

Figures

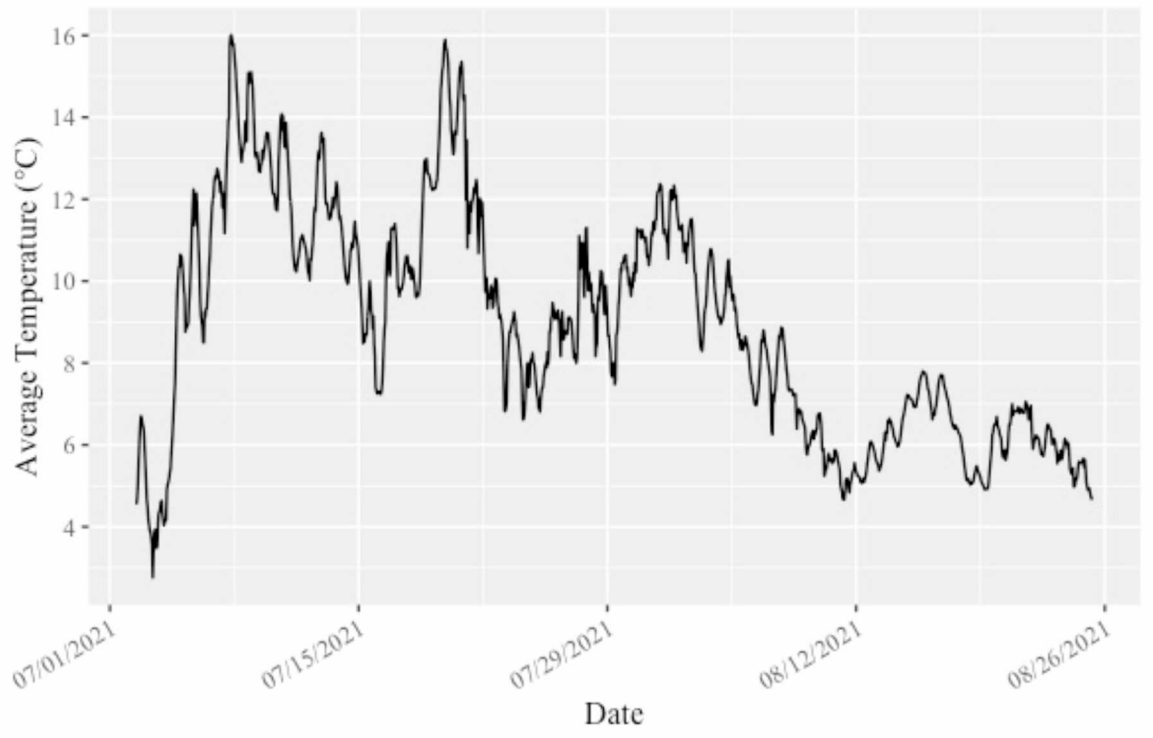


Figure 1.1 Hourly temperatures from nearshore Beaufort Sea during the 2021 field season. Temperature was collected with Star-Oddi CTD loggers placed at four sampling sites. Each hourly data-point is the average of the temperatures collected at the four sites (Hamman *et al.,* 2021).

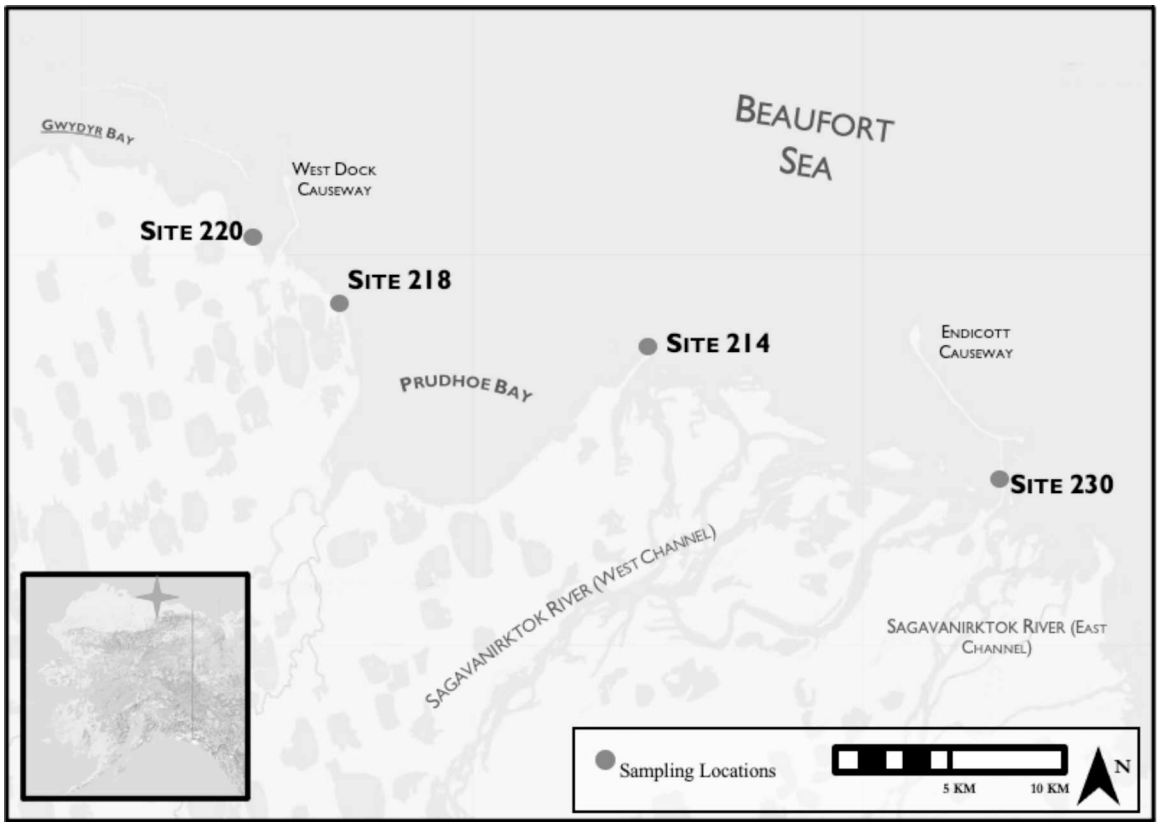


Figure 1.2 Each sample site within and around the Prudhoe Bay, Alaska. Broad whitefish and saffron cod were sampled from each of the four sites (Hamman *et al.* 2021).

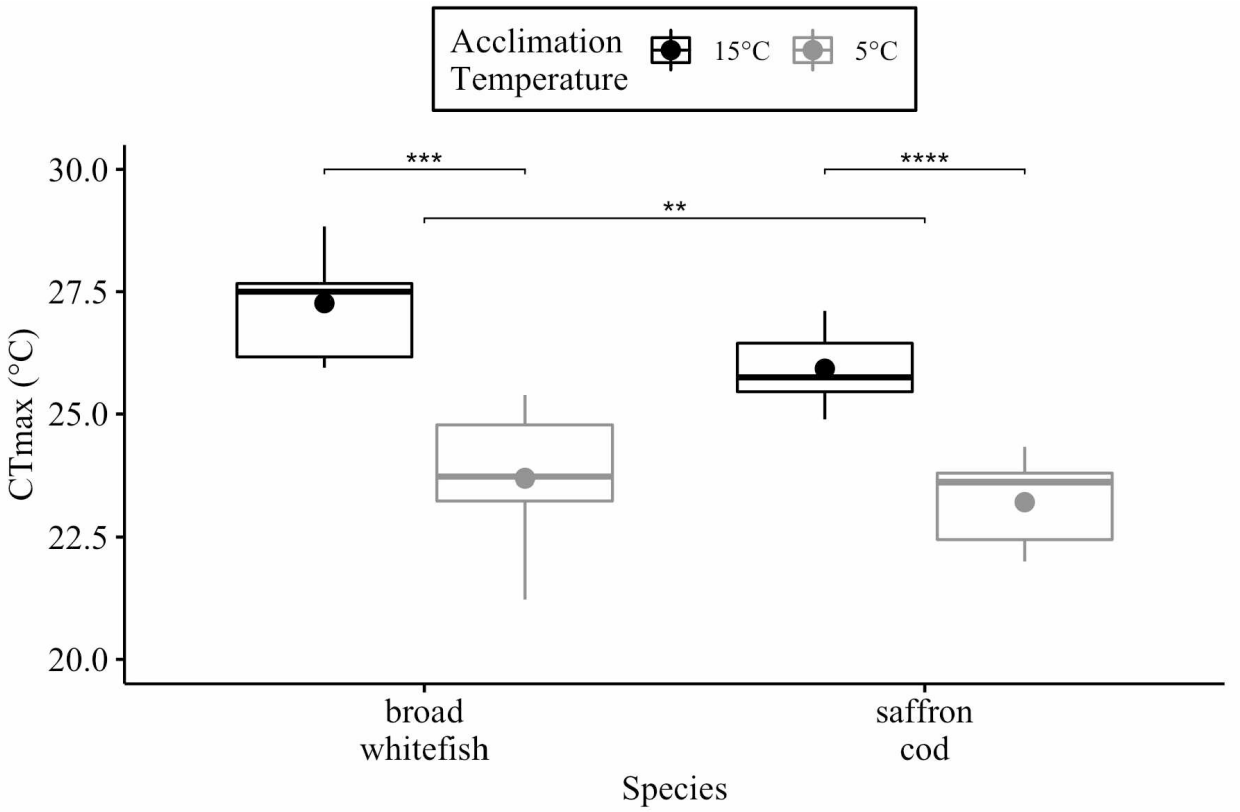


Figure 1.3 The CT_{max} values (°C) for broad whitefish and saffron cod at the 5 and 15°C acclimation temperatures. The median (line) and mean (dot) values are reported. Significant differences are denoted by *P < 0.05, **P<0.01, ***P<0.001, and ****P<1 x 10^{-4} from a Wilcoxon rank-sum test.

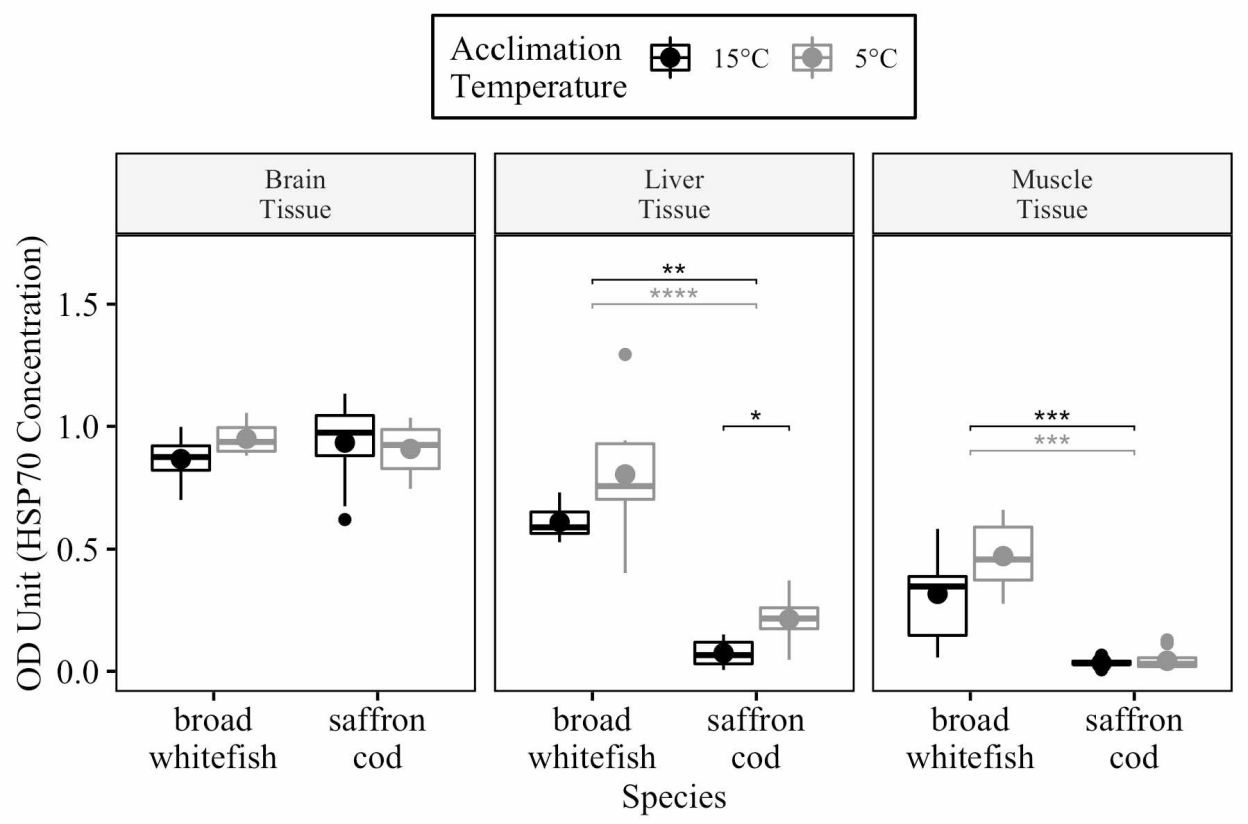


Figure 1.4 Comparisons of 70-kDa heat shock protein (HSP70) protein concentrations (optical density or OD unit) between acclimation temperatures 5 and 15°C and between broad whitefish and saffron cod. The median (line) and mean (dot) values are reported. Significant differences are denoted by *P < 0.05, **P<0.01, ***P<0.001, and ****P<1 x 10^{-4} from a Wilcoxon rank-sum test.

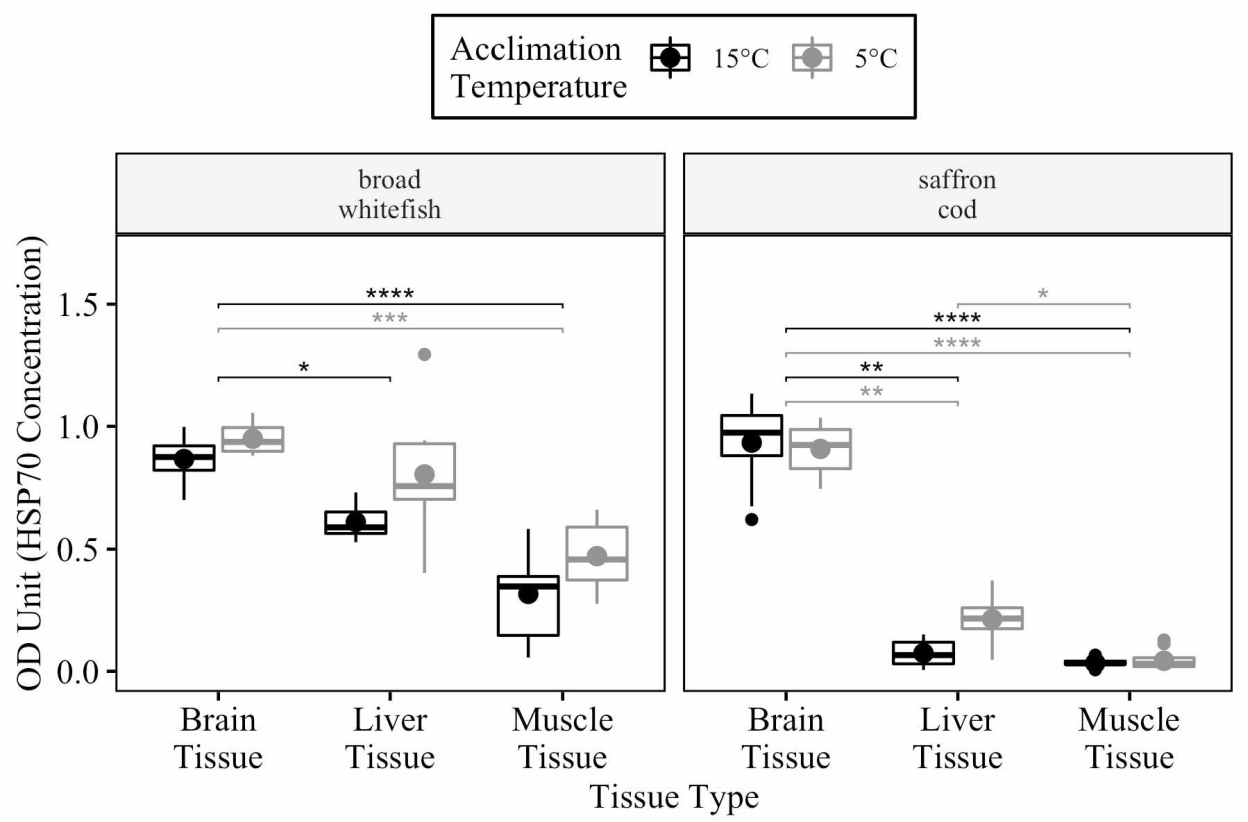


Figure 1.5 Comparisons of 70-kDa heat shock protein (HSP70) protein concentrations (optical density or OD unit) between brain, liver, and muscle samples in broad whitefish and saffron cod and between acclimation temperatures 5 and 15°C. The median (line) and mean (dot) values are reported. Significant differences are denoted by *P < 0.05, **P<0.01, ***P<0.001, and ****P<1 x 10^{-4} from a Kruskal-Wallis test followed by a Dunn's post-hoc test.

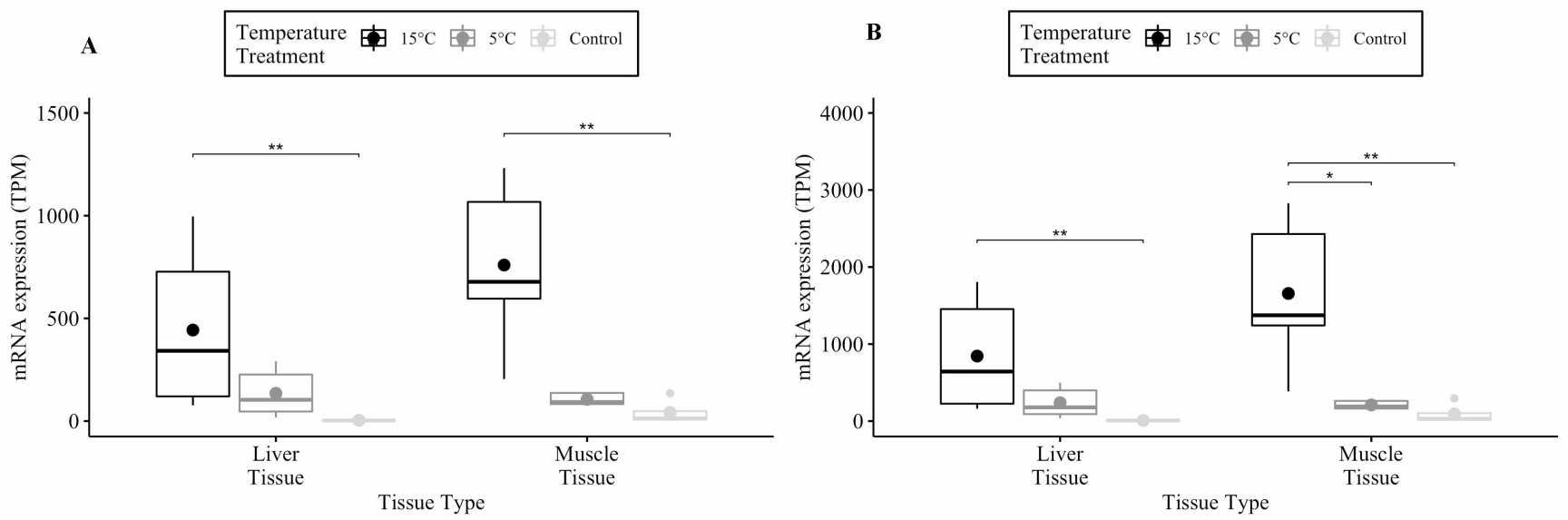


Figure 1.6 The mRNA 70-kDa heat shock protein (HSP70) transcript concentration (TPM) between broad whitefish liver and muscle tissue samples in addition to between the acclimation temperatures 5°C and 15°C and the control group. The control group were broad whitefish samples that were left in lab-acclimation conditions at 8°C. The image on the left are results from transcript A, and on the right is transcript B. The median (line) and mean (dot) values are reported. Significant differences are denoted by *P < 0.05, **P<0.01, ***P<0.001, and ****P<1 x 10^{-4} from a Kruskal-Wallis test followed by a Dunn's post-hoc test.

Appendix

Tables

Table 1.A.1 A concise list of all solutions and reagents used during tissue homogenization, HSP70 extraction, and Western Blot protein procedure.

10xPBS		**Running Buffer**	
$NaH_2PO_4H_2O$	2.03 g	Trizma Base	3.03 g
Na_2HPO_4	11.49 g	Glycine	14.4 g
NaCl	85 g	10% SDS	10 mL
	Add to 1 L dH_2O (pH 7.4)		Add to 1 L dH_2O
Separating Gel Buffer Stock		**Transfer Buffer**	
1.5 M Tris-HCl pH 8.8		Trizma Base	3.03 g
0.4% SDS		Glycine	14.4 g
		Methanol	20%
			Add to 1 L dH_2O
Stacking Gel Buffer Stock		**Blocking Buffer**	
0.5 M Trish-HCl pH 6.8		1xPBS	
0.4% SDS		5% NFDM	
Homogenization Buffer		**Other Necessary Reagents**	
32 mmol Tris-HCl		10% SDS	
4% SDS		29% Acrylamide : 1% Bis-acrylamide	
Add to 250 mL dH_2O (pH 6.8)		10% Ammonium persulfate	
		TEMED	
		ECL Reagent (Amersham)	
Recipe for 10% Separating Gel		**Recipe for Stacking Gel**	
Separating Buffer	5 mL	Separating Buffer	1.25 mL
dH_2O	8.2 mL	dH_2O	3.03 mL
29: 1% acrylamide	6.7 mL	29: 1% acrylamide	0.67 mL
10% APS	100 µL	10% APS	50 µL
TEMED	10 µL	TEMED	5 µL
0.1% Tween		**0.3% Tween**	
10xPBS	20 mL	10xPBS	10 mL
dH_2O	180 mL	dH_2O	90 mL
Tween	200 µL	Tween	150 µL

Table 1.A.2 The length, weight, and CT_{max} values for broad whitefish and saffron cod acclimated at 5 and 15°C.

Sample ID	Acclimation Temperature (°C)	CT_{max} Temperature (°C)	Weight (g)	Length (mm)
		broad whitefish		
BDWF_001	5	25.4	12.65	112
BDWF_003	5	25.9	8.60	93
BDWF_004	5	24.8	9.23	110
BDWF_005	5	21.2	11.67	111
BDWF_006	5	23.7	26.94	147
BDWF_007	5	24.8	10.52	110
BDWF_008	5	23.5	14.50	117
BDWF_009	5	22.4	10.70	112
BDWF_002	15	23.7	10.36	107
BDWF_010	15	26.2	13.80	117
BDWF_011	15	28.6	11.79	114
BDWF_012	15	27.5	31.97	148
BDWF_013	15	27.1	12.20	110
BDWF_014	15	27.7	8.59	102
BDWF_015	15	26.0	11.20	104
BDWF_016	15	28.8	12.02	111
BDWF_017	15	27.6	11.01	112
		saffron cod		
SFCD_001	15	24.9	15.05	152
SFCD_002	15	25.3	14.27	143
SFCD_003	15	25.3	11.87	140
SFCD_004	15	25.4	17.23	149
SFCD_005	15	26.1	12.81	131
SFCD_006	15	26.1	8.00	121
SFCD_007	15	26.6	14.40	130
SFCD_009	15	21.9	18.23	157
SFCD_010	15	25.5	12.16	133
SFCD_011	15	25.7	22.40	163
SFCD_012	15	25.6	17.39	152
SFCD_013	15	25.8	12.62	129
SFCD_014	15	26.8	19.20	157
SFCD_015	15	26.7	15.67	146
SFCD_016	15	27.1	9.84	130
SFCD_017	5	22.0	20.82	156
SFCD_018	5	22.1	19.47	153
SFCD_019	5	22.1	19.07	152
SFCD_020	5	23.8	23.02	159
SFCD_021	5	23.8	NA	159
SFCD_022	5	23.7	19.39	155
SFCD_023	5	23.8	15.70	149
SFCD_024	5	24.3	12.21	130
SFCD_025	5	22.0	25.26	164
SFCD_026	5	22.6	21.06	130
SFCD_027	5	23.3	26.09	170
SFCD_028	5	23.2	18.65	145
SFCD_029	5	23.8	15.82	149
SFCD_030	5	23.7	24.14	160
SFCD_031	5	23.6	16.49	146
SFCD_032	5	23.7	20.21	151

Table 1.A.3 The statistical parameters resulting from the Wilcoxon rank-sum test from comparing the CT_{max} values between each acclimation temperature in addition to between broad whitefish and saffron cod. All reported p-values are Bonferroni corrected. Any significant values ($\alpha = 0.05$) are bolded.

Comparative Groups	Test Group	N_1	N_2	Statistic	P-Value
15°C vs 5°C	broad whitefish	9	8	72	**6.26×10^{-4}**
	saffron cod	14	16	224	**3.48×10^{-6}**
broad whitefish vs. saffron cod	15°C	9	14	110	**6.78×10^{-3}**
	5°C	8	16	81	6.22×10^{-1}

Table 1.A.4 Statistical parameters resulting from the Wilcoxon rank-sum test from comparing the HSP70 concentration between acclimation temperature and species. All reported p-values are Bonferroni corrected. Any significant values ($\alpha = 0.05$) are bolded.

Comparative Group	Tissue	N_1	N_2	T-Statistic	P-Value
		broad whitefish			
15°C vs 5°C	Liver	9	8	12.5	0.1608
	Muscle	9	8	16.0	0.3552
	Brain	9	8	17.0	0.4470
		saffron cod			
15°C vs 5°C	Liver	7	14	8.5	**1.7×10^{-2}**
	Muscle	15	16	118.5	1.0000
	Brain	15	16	148.0	1.0000
		Acclimation Temperature: 15°C			
broad whitefish vs. saffron cod	Liver	9	7	63	**1.05×10^{-3}**
	Muscle	9	15	133	**6.36×10^{-4}**
	Brain	9	15	44	1.0000
		Acclimation Temperature: 5°C			
broad whitefish vs. saffron cod	Liver	8	14	112	**3.75×10^{-5}**
	Muscle	8	16	128	**6.00×10^{-4}**
	Brain	8	16	76	1.0000

Table 1.A.5 The Kruskal-Wallis statistical parameters for the HSP70 concentration between muscle, liver, and cranial tissue samples at the two acclimation temperatures for broad whitefish and saffron cod. A Dunn's post-hoc was performed if the p-value was significant, and these post-hoc values with a Bonferroni correction are italicized. Any significant statistical parameters are indicated by bolded values ($\alpha = 0.05$).

Comparative Group	χ^2	DF	p-value
broad whitefish (5°C)	14.615	2	**6.70×10^{-4}**
*CT*HT*			*4.719×10^{-1}*
*CT*MT*			***4.648×10^{-4}***
*MT*HT*			*5.353×10^{-2}*
broad whitefish (15°C)	20.956	2	**2.82×10^{-5}**
*CT*HT*			***4.113×10^{-2}***
*CT*MT*			***1.441×10^{-5}***
*MT*HT*			*1.050×10^{-1}*
saffron cod (5°C)	38.5	2	**4.47×10^{-9}**
*CT*HT*			***4.24×10^{-3}***
*CT*MT*			***1.73×10^{-9}***
*MT*HT*			***1.56×10^{-2}***
saffron cod (15°C)	26.9	2	**1.42×10^{-6}**
*CT*HT*			***5.81×10^{-3}***
*CT*MT*			***1.31×10^{-6}***
*MT*HT*			*1.000*

Table 1.A.6 Statistical parameters resulting from the Wilcoxon rank-sum test comparing the mRNA expression between liver and muscle samples in broad whitefish at each acclimation temperature. All reported p-values are Bonferroni corrected and any significant values ($\alpha = 0.05$) are bolded.

	Comparative group	Temperature Treatment	N_1	N_2	T-Statistic	P-Value
Transcript A	Liver vs. Muscle	15°C	6	6	9.00	0.54
		5°C	6	5	13.00	1.00
		Control (8°C)	4	4	2.00	0.342
Transcript B	Liver vs. Muscle	15°C	6	6	9.00	0.54
		5°C	6	5	13.00	1.00
		Control (8°C)	4	4	0.00	0.0858

Table 1.A.7 Kruskal-Wallis statistical parameters for the mRNA expression in the broad whitefish muscle and liver tissue samples. A Dunn's post-hoc test was performed if the p-value was significant, and these post-hoc values with a Bonferroni correction are italicized. Any significant statistical parameters are indicated by bolded values (α = 0.05).

Comparative Group	χ^2	DF	p-value
Liver Tissue – HSP70A	9.94	2	**6.94×10^{-3}**
*15°C *5°C*			*6.76×10^{-1}*
*15°C *Control (8°C)*			***4.97×10^{-3}***
*5°C *Control (8°C)*			*1.18×10^{-1}*
Muscle Tissue – HSP70A	11.2	2	**3.65×10^{-3}**
*15°C *5°C*			*7.29×10^{-2}*
*15°C *Control (8°C)*			***4.06×10^{-3}***
*5°C *Control (8°C)*			*8.81×10^{-1}*
Liver Tissue – HSP70B	9.94	2	**6.94×10^{-3}**
*15°C *5°C*			*6.76×10^{-1}*
*15°C *Control (8°C)*			***4.97×10^{-3}***
*5°C *Control (8°C)*			*1.18×10^{-1}*
Muscle Tissue – HSP70B	10.7	2	**4.78×10^{-3}**
*15°C *5°C*			***4.91×10^{-2}***
*15°C *Control (8°C)*			***7.31×10^{-3}***
*5°C *Control (8°C)*			*1.00*

Figures

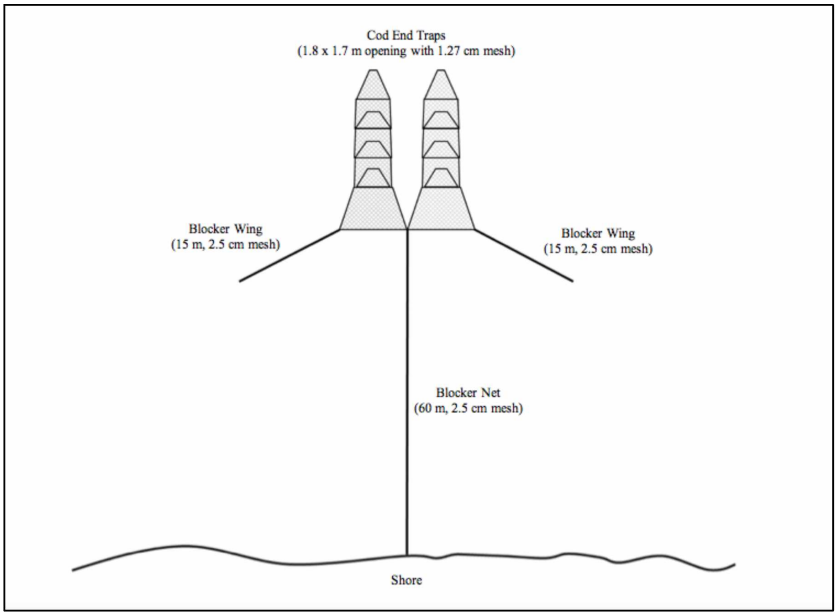


Figure 1.A.1 Overhead view of the nets used at each site during sampling for broad whitefish and saffron cod (Priest *et al.*, 2018).

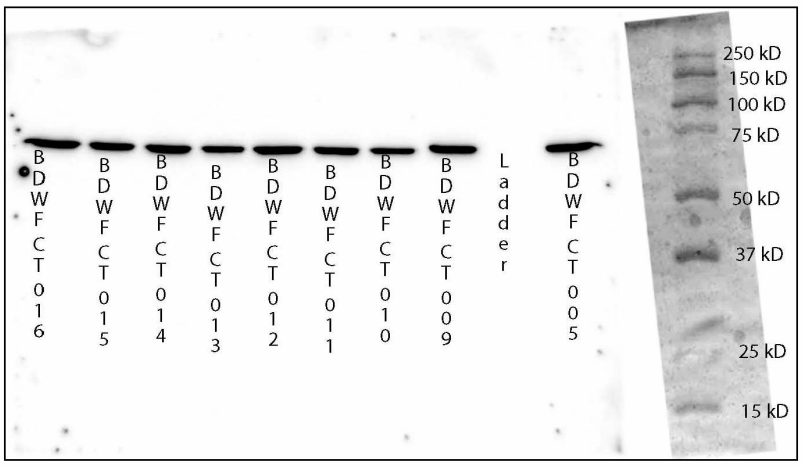


Figure 1.A.2 A sample of an imaged membrane that would be later used in densitometric analysis. This membrane contains samples from broad whitefish (BDWF) cranial tissue (CT) samples 009-016 with the internal standard and protein ladder. The ladder reference on the right was imaged using the colorimetric marker setting. Otherwise, the membranes were imaged without this setting, which is why there appears to be an empty well marked by ladder. The overlay of the ladder with the sample membrane was to confirm that the proteins separated and targeted were 70 kDa.

Supplementary Information

Wet tank transfer Western Blot Protocol

A 10% SDS gel was created by first pouring a 10% separating gel in a glass plate with 1 mm spacers, letting it set (~ 1 hour), and then pouring the stacking gel fitted with a 10-well comb and letting that polymerize (~45 minutes, Table S1). Once the gels had polymerized, the samples were prepared by diluting each sample with dH_2O so that the final concentration was 1.5 µg $µL^{-1}$. Next, 2 x Laemmli Sample Buffer (Bio-Rad, Hercules, California) was mixed 20:1 with ß-mercaptoethanol (Bio-Rad, Hercules, California) before adding 10 µL to each sample. Eight samples were prepared per gel in addition to the Precision Plus Protein™ Kaleidoscope™ Prestained Protein Standard (Bio-Rad, Hercules, California) and the internal standard, which was kept the same in every Western Blot. The internal standard used was the broad whitefish sample BDWF005_CT because it showed high, consistent specificity with the HSP70 antibodies. The Prestained Protein Standard was diluted 1:1 with diH_2O to make 10 µL before being mixed with 10 µL of the sample buffer. Every sample minus the protein standard was denatured in a heating block at 100°C for 3 minutes before being allowed to cool to room temperature. Finally, all samples were centrifuged at 15,000 RPM for 30 seconds.

The gels were assembled in the Mini-PROTEAN Tetra Cell (Bio-Rad, Hercules, California) followed by the addition of running buffer (Table S1). The samples were then loaded in to each well and run at 150 V until the dye front ran out of the bottom of the gel (~ 1 hour). The following components were soaked in transfer buffer (Table S1) before being assembled as described. A piece of nitrocellulose membrane (0.45 µm, GE Healthcare, Chicago, Illinois) was fitted over the gel before both were sandwiched between cardboard and sponge pads. The entire

ensemble was placed in the Tetra Blotting Module (Bio-Rad, Hercules, California), the tank filled with transfer buffer, and the transfer allowed to occur overnight at 30 V.

Once transfer was complete, the membrane was immediately blocked with blocking buffer (Table S1) for an hour on a rotating table. After an hour the buffer was discarded and the membranes were allowed to intubate with a monoclonal mouse anti-HSP70 antibody (MA3-006, ThermoFisher Scientific, Waltham, Massachusetts) at 1:1,000 dilution with blocking buffer for an hour and a half. The primary antibody was saved for future use, and the membranes were rinsed 3 x 10 minutes with 0.1% Tween (Table S1). The solution was then poured out and the membranes were mixed with Donkey anti-Mouse IgG (H+L) Secondary Antibody, HRP (ThermoFisher Scientific, Waltham, Massachusetts) at 1:10,000 dilution with blocking buffer for an hour. The secondary antibody was discarded, and the membrane rinsed 3 x 5 minutes with 0.3% Tween followed by 3 x 5 minutes with 0.1% Tween (Table S1). The membranes were prepared for imaging by adding SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific, Waltham, Massachusetts) and letting the membranes sit for five minutes. Finally, the membranes were imaged with the Amersham Imager 600 (GE Healthcare, Chicago, Illinois) using the chemiluminescent setting with exposure times of 30 seconds, 1 minute, 5 minutes, and 10 minutes (if needed).

General Conclusion

The nearshore Beaufort Sea experiences substantial diurnal and interannual temperature changes due to river discharge, wind-driven currents, and climate change (Ross, 1988; Overeem *et al.*, 2011; Mccain *et al.*, 2014; Graham *et al.*, 2017). Many Arctic teleosts in this region are important in subsistence fisheries for Alaska Native coastal communities and are integral in energy transfer as prey items within the nearshore food web (Frost and Lowry, 1981; Fechhelm *et al.*, 1992; Copeman *et al.*, 2016; Reusser *et al.*, 2016). As a result, understanding how these fishes are responding to changing thermal conditions is imperative to predicting long-term ecological impacts. Examination of the thermotolerance of Arctic teleosts, how their thermotolerance shifts given unique and changing thermal parameters, and the underlying molecular mechanisms causing these shifts will provide detailed insight as to how and to what extent these species may respond to future aquatic thermal changes.

Critical thermal maximum (CT_{max}) is the highest temperature that an organism can sustain immediately before mortality and can be measured as an overall proxy for thermotolerance (Becker and Genoway, 1979; Beitinger *et al.,* 2000). Organisms possess phenotypic plasticity that allow their thermotolerance to shift given different thermal parameters, and this is driven, in part, by chaperones known as heat shock proteins (Dietz and Somero, 1992; White *et al.*, 1994; Basu *et al.*, 2002; Somero, 2010). The 70-kDA heat shock protein family is one of the most common and highly conserved in teleost species (Lindquist, 1986; White *et al.*, 1994; Iwama *et al.*, 1998). The inducible cognate, known as HSP70, is upregulated during thermal stress events, such as at the CT_{max} temperature, and measuring the HSP70 protein and mRNA transcript concentrations will provide detailed analysis into the extent that thermotolerance can be adjusted by the organism (Iwama *et al.*, 1999; Teigen *et al.*, 2015). The

goal of this study was to acclimate broad whitefish *Coregonus nasus* and saffron cod *Eleginus gracilis,* both eurythermal species, to 5 and 15°C before subjecting them to a thermal ramping rate of 3.4°C · h^{-1} to determine the CT_{max} temperature and underlying HSP70 protein and mRNA concentrations in their brain, muscle, and liver tissues.

Broad whitefish and saffron cod had significantly higher CT_{max} temperatures when acclimated to 15 compared to 5°C, thus implying phenotypic plasticity. The CT_{max} temperature for broad whitefish was comparable, albeit slightly lower, to temperatures reported for salmonids (Table 1.1; Currie *et al.,* 1998; Beitinger *et al.,* 2000). Bilyk and Sformo (2021) recorded a CT_{max} temperature for 9°C-acclimated broad whitefish that was within 0.4°C of the 5°C-acclimated samples in this study. The degree of CT_{max} gained per 1°C of acclimation temperature tended to be higher than for other salmonids, possibly reflecting the need to maintain a large temperature range in such a dynamic environment (Currie *et al.*, 1998). In contrast, saffron cod had higher CT_{max} temperatures than reported Arctic cod values (Drost *et al.,* 2016). The gain in CT_{max} for saffron cod relative to acclimation temperature was lower than for Arctic cod, which is contrary to what is expected for the eurythermal saffron cod and could be due to suboptimal salinity conditions during acclimation (Currie *et al.,* 1998; Drost *et al.,* 2016; Petitjean *et al.,* 2019). Broad whitefish had a higher ARR than saffron cod, which means it has a higher thermal acclimation potential. Additionally, broad whitefish had a significantly higher CT_{max} temperature than saffron cod at 15°C, which could be a result of underlying genetic differences supported by the HSP70 molecular data.

There were tissue-specific differences in the HSP70 protein concentrations for broad whitefish and saffron cod that resulted in brain and white muscle tissues having the highest and lowest HSP70 protein concentrations, respectively, at both acclimation temperatures. Tissue-

specific differences in protein concentrations have been reported previously (Dyer *et al.*, 1991; Smith *et al.*, 1999). The only difference found between acclimation temperatures was that in saffron cod liver tissue, the 5°C samples had a significantly higher protein concentration than the 15°C samples. There were species-specific differences as well, with broad whitefish having significantly higher concentrations in liver and muscle tissues than saffron cod at both acclimation temperatures. There are multiple transcripts of the HSP70 gene that vary between species due to mechanisms such as alternative splicing, and this will create HSP70 isoforms that could impact HSP70 protein concentrations and potentially explain the difference observed between broad whitefish and saffron cod (White *et al.*, 1994). Additionally, the higher HSP70 concentration in broad whitefish could potentially explain the higher CT_{max} at 15°C compared to saffron cod (Dalvi *et al.*, 2012).

Concentrations of two HSP70 mRNA transcripts were analyzed in broad whitefish liver and muscle tissues. The 15°C-acclimated fish had the highest concentration, but there were no tissue-specific differences as observed with the protein concentrations. Differences between HSP70 protein and mRNA levels have been reported previously, with Smith *et al.* (1999) observing a spike in protein levels before an increase in mRNA transcripts, suggesting a post-transcriptional modification process (Smith *et al.*, 1999; Molina *et al.*, 2000; Lewis *et al.*, 2016). The high concentration of mRNA transcripts, in addition to the unique protein expression profile in broad whitefish tissues, may explain the reason for the higher CT_{max} temperature at 15°C.

The CT_{max} changes between the two acclimation temperatures, in conjunction with the underlying HSP70 protein and mRNA transcript levels, offers an unprecedented insight in to the thermotolerance of broad whitefish and saffron cod. The ability to respond to current thermal conditions could mean an increase in thermotolerance to future thermal parameter changes

(Iwama *et al.*, 1999; Santoro, 1999; Basu *et al.*, 2002). The difference in thermotolerance data between broad whitefish and saffron cod indicates that broad whitefish is more physiologically capable of responding to heat stress and could fair better as climate change continues. Additional thermotolerance data from other Arctic teleosts from the nearshore Beaufort Sea shows varied responses among species, which may result in a shift in distribution and abundance in this area, which has already been observed for broad whitefish and saffron cod (Reist *et al.*, 2006; Somero, 2010; Drost *et al.*, 2016; Bilyk and Sformo, 2021; Hamman *et al.*, 2021; Priest *et al.*, 2022).

Further research should be conducted to further increase our understanding of thermotolerance for broad whitefish, saffron cod, and other Arctic teleosts. The concentration of multiple inducible HSP families, including HSP70, should be measured to quantify the complete heat shock response in broad whitefish and saffron cod. This experiment could be repeated with additional acclimation temperatures to accurately predict the CT_{max} increase for every 1°C increase in acclimation temperature in addition to determining the upper temperature limit of these species. The HSP70 mRNA transcripts should be analyzed in saffron cod as a contrast to broad whitefish and to better understand the molecular mechanisms driving its phenotypic plasticity. This experiment could also be repeated using other Arctic teleosts in the same families of broad whitefish and saffron cod, such as Arctic cisco *Coregonus autumnalis* or Arctic cod, to better understand the thermotolerance potential of other species in the nearshore Beaufort Sea.

References

Basu N, Todgham AE, Ackerman PA, Bibeau MR, Nakano K, Schulte PM, Iwama GK (2002) Heat shock protein genes and their functional significance in fish. *Gene* 295: 173–183. doi: 10.1016/s0378-1119(02)00687-x.

Becker CD, Genoway RG (1979) Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes* 4(3): 245–256. doi: 10.1007/BF00005481.

Beitinger TL, Bennett WA, Mccauley RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes* 58: 237–275. doi: 10.1023/A:1007676325825.

Bilyk KT, Sformo TL (2021) Varying heat tolerance among Arctic nearshore fishes. *Polar Biology* 44(3): 607–612. doi: 10.1007/s00300-021-02815-6.

Buckley BA, Owen M-E, Hofmann GE (2001) Adjusting the thermostat: the threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *Journal of Experimental Biology* 204: 3571–3579. doi: 10.1242/jeb.204.20.3571.

Buckley BA, Place SP, Hofmann GE (2004) Regulation of heat shock genes in isolated hepatocytes from an Antarctic fish, *Trematomus bernacchii*. *Journal of Experimental Biology* 207(21): 3649–3656. doi: 10.1242/jeb.01219.

Copeman LA, Laurel BJ, Boswell KM, Sremba AL, Klinck K, Heintz RA, Vollenweider JJ, Helser TE, Spencer ML (2016) Ontogenetic and spatial variability in trophic biomarkers of juvenile saffron cod (*Eleginus gracilis*) from the Beaufort, Chukchi and Bering Seas. *Polar Biology* 39(6): 1109–1126. doi: 10.1007/s00300-015-1792-y.

Currie RJ, Bennett WA, Beitinger TL (1998) Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environmental Biology of Fishes* 51: 187–200. doi: 10.1023/A:1007447417546.

Currie S, Moyes CD, Tufts BL (2000) The effects of heat shock and acclimation temperature on hsp70 and hsp30 mRNA expression in rainbow trout: in vivo and in vitro comparisons. *Journal of Fish Biology* 56(2): 398–408. doi: 10.1111/j.1095-8649.2000.tb02114.x.

Dalvi RS, Pal AK, Tiwari LR, Baruah K (2012) Influence of acclimation temperature on the induction of heat-shock protein 70 in the catfish *Horabagrus brachysoma* (Günther). *Fish Physiology and Biochemistry* 38(4): 919–927. doi: 10.1007/s10695-011-9578-9.

Dietz TJ (1994) Acclimation of the threshold induction temperatures for 70-kDa and 90-kDa heat shock proteins in the fish *Gillichthys mirabilis*. *Journal of Experimental Biology* 188: 333–338. doi: 10.1242/jeb.188.1.333.

Dietz TJ, Somero GN (1992) The threshold induction temperature of the 90-kDa heat shock protein is subject to acclimatization in eurythermal goby fishes (genus *Gillichthys*). *Proceedings of the National Academy of Science* 89: 3389–3393. doi: 10.1073/pnas.89.8.3389.

Dietz TJ, Somero GN (1993) Species-and tissue-specific synthesis patterns for heat-shock proteins HSP70 and HSP90 in several marine teleost fishes. *Physiological Zoology* 66(6): 863–880. doi: 10.1086/physzool.66.6.30163744.

Drost HE, Lo M, Carmack EC, Farrell AP (2016) Acclimation potential of Arctic cod (*Boreogadus saida*) from rapidly warming Arctic Ocean. *Journal of Experimental Biology* 219(19): 3114-3125. doi: 10.1242/jeb.140194.

Dunn JR, Matarese AC (1987) A review of the early life history of northeast Pacific gadoid fishes. *Fisheries Research* 5: 163–184. doi: 10.1016/0165-7836(87)90038-5.

Dyer SD, Dickson KL, Zimmerman EG, Sanders BM (1991) Tissue-specific patterns of synthesis of heat-shock proteins and thermal tolerance of the fathead minnow (*Pimephales promelus*). *Canadian Journal of Zoology* 69: 2021–2027. doi: 10.1139/z91-282.

Eissa AE, Zaki MM (2011) The impact of global climatic changes on the aquatic environment. *Procedia Environmental Sciences* 4: 251–259. doi: 10.1016/j.proenv.2011.03.030.

Fechhelm RG, Dillinger RE, Gallaway BJ, Griffiths WB (1992) Modeling of in situ temperature and growth relationships for yearling broad whitefish in Prudhoe Bay, Alaska. *Transactions of the American Fisheries Society* 121(1): 1–12. doi: 10.1577/1548-8659(1992)121<0001:MOISTA>2.3.CO;2.

Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* 61: 243–282. doi: 10.1146/annurev.physiol.61.1.243.

Fossheim M, Primicerio R, Johannesen E, Ingvaldsen RB, Aschan MM, Dolgov AV. (2015) Recent warming leads to a rapid borealization of fish communities in the Arctic. *Nature Climate Change* 5(7): 673–677. doi: 10.1038/nclimate2647.

Frost KJ, Lowry LF (1981) Trophic importance of some marine gadids in northern Alaska and their body-otolith size relationship. *Fishery Bulletin* 79(1): 187–192.

Graham RM, Cohen L, Petty AA, Boisvert LN, Rinke A, Hudson SR, Nicolaus M, Granskog MA (2017) Increasing frequency and duration of Arctic winter warming events. *Geophysical Research Letters* 44(13): 6974–6983. doi: 10.1002/2017GL073395.

Griffiths WB, Gallaway BJ, Gazey WJ, Dillinger RE (1992) Growth and condition of Arctic cisco and broad whitefish as indicators of causeway-induced effects in the Prudhoe Bay Region, Alaska. *Transactions of the American Fisheries Society* 121(5): 557–577. doi: 10.1577/1548-8659(1992)121<0557:GACOAC>2.3.CO;2.

Hamman CR, Bacon JA, Sutton TM (2021) Beaufort Sea long-term nearshore fish monitoring program: 2021 Annual Report. Report for Hilcorp Alaska, LLC by the University of Alaska Fairbanks, College of Fisheries and Ocean Sciences, Department of Fisheries, Fairbanks, AK.

Harper KC, Harris F, Miller SJ, Thalhauser JM, Ayers SD (2012) Life history traits of adult broad whitefish and humpback whitefish. *Journal of Fish and Wildlife Management* 3(1): 56. doi: 10.3996/022011-JFWM-011.

Hofmann GE (1999) Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. *American Zoology* 39: 889–900. doi: 10.1093/icb/39.6.889.

Iwama GK, Thomas PT, Forsyth RB, Vijayan MM (1998) Heat shock protein expression in fish. *Reviews in Fish Biology and Fisheries* 8: 35–56. doi: 10.1093/icb/39.6.889.

Iwama GK, Vijayan MM, Forsyth RB, Ackerman PA (1999) Heat shock proteins and physiological stress in fish. *American Zoologist* 39: 901–909. doi: 10.1093/icb/39.6.901.

Johnson SW, Thedinga JF, Neff AD (2009) Invasion by saffron cod *Eleginus gracilis* into nearshore habitats of Prince William Sound, Alaska USA. *Marine Ecology Progress Series* 389: 203–212. doi: 10.3354/meps08226.

Kelley AL, de Rivera CE, Buckley BA (2011) Intraspecific variation in thermotolerance and morphology of the invasive European green crab, *Carcinus maenas*, on the west coast of North America. *Journal of Experimental Marine Biology and Ecology* 409(1-2): 70–78. doi: 10.1016/j.jembe.2011.08.005.

Khalsa NS, Gatt KP, Sutton TM, Kelley AL (2021) Characterization of the abiotic drivers of abundance of nearshore Arctic fishes. *Ecology and Evolution* 11(16): 11491–11506. doi: 10.1002/ece3.7940.

Laurel BJ, Spencer M, Iseri P, Copeman LA (2016) Temperature-dependent growth and behavior of juvenile Arctic cod (*Boreogadus saida*) and co-occurring North Pacific gadids. *Polar Biology* 39(6): 1127–1135. doi: 10.1007/s00300-015-1761-5.

Lewis M, Götting M, Anttila K, Kanerva M, Prokkola JM, Seppänen E, Kolari I, Nikinmaa M (2016) Different relationship between hsp70 mRNA and hsp70 levels in the heat shock response of two salmonids with dissimilar temperature preference. *Frontiers in Physiology* 7: 1-12. doi:10.3389/fphys.2016.00511.

Lindquist S (1986) The heat shock response. *Annual Review in Biochemistry* 55: 1151–1191. doi: 10.1146/annurev.bi.55.070186.005443.

Logerwell E, Busby M, Carothers C, Cotton S, Duffy-Anderson J, Farley E, Goddard P, Heintz R, Holladay B, Horne J, et al. (2015) Fish communities across a spectrum of habitats in the western Beaufort Sea and Chukchi Sea. *Progress in Oceanography* 136: 115–132. doi: 10.1016/j.pocean.2015.05.013.

Lutterschmidt WI, Hutchison VH (1997) The critical thermal maximum: history and critique. *Canadian Journal of Zoology* 75: 1561-1574. doi: 10.1139/z97-783.

Manzon LA, Zak MA, Agee M, Boreham DR, Wilson JY, Somers CM, Manzon RG (2022) Thermal acclimation alters both basal heat shock protein gene expression and the heat shock response in juvenile lake whitefish (*Coregonus clupeaformis*). *Journal of Thermal Biology* 104: 1-11. doi:10.1016/j.jtherbio.2021.103185.

Mccain KA, Raborn Scott W, Fechhelm RG, Raborn SW (2014) Year 31 of the long-term monitoring of nearshore Beaufort Sea fishes in the Prudhoe Bay region: 2013 Annual Report. Report for BP Exploration (Alaska) Inc. by LGL Alaska Research Associates, Inc. Anchorage, Alaska pp 72.

Molina A, Biemar F, Müller F, Iyengar A, Prunet P, Maclean N, Martial JA, Muller M (2000) Cloning and expression analysis of an inducible HSP70 gene from tilapia fish. *Federation of European Biochemical Societies Letters* 474: 5–10. doi: 10.1016/s0014-5793(00)01538-6.

Mueter FJ, Nahrgang J, John Nelson R, Berge J (2016) The ecology of gadid fishes in the circumpolar Arctic with a special emphasis on the polar cod (*Boreogadus saida*). *Polar Biology* 39(6): 961-967. doi: 10.1007/s00300-016-1965-3.

Overeem I, Anderson RS, Wobus CW, Clow GD, Urban FE, Matell N (2011) Sea ice loss enhances wave action at the Arctic coast. *Geophysical Research Letters* 38(17): 1-6. doi: 10.1029/2011GL048681.

Petitjean Q, Jean S, Gandar A, Côte J, Laffaille P, Jacquin L (2019) Stress responses in fish: from molecular to evolutionary processes. *Science of the Total Environment* 684: 371-380. doi: 10.1016/j.scitotenv.2019.05.357.

Priest JT, Mueter FJ, Raborn SW, Sutton TM (2022) Effects of environmental variables on a nearshore arctic fish community, 2001–2018. *Polar Biology* 45(4): 585–599. doi: 10.1007/s00300-022-03013-8.

Reist JD, Wrona FJ, Prowse TD, Power M, Dempson JB, Beamish RJ, King JR, Carmichael TJ, Sawatzky CD (2006) General effects of climate change on Arctic fishes and fish populations. *Ambio* 35(7): 370–380. doi: 10.1579/0044-7477(2006)35[370:GEOCCO]2.0.CO;2.

Reusser DA, Frazier ML, Loiselle RA, Lee H, Thorsteinson LK (2016) Arctic climate change – a tale of two cods. In: Thorsteinson LK and Love, MS, eds. Alaska Arctic marine fish ecology catalog: U.S. Geological Survey Scientific Investigations Report 2016–5038 (OCS Study, BOEM 2016-048), pp 659-679. doi: 10.3133/sir20165038.

Ross BD (1988) Causeways in the Alaskan Beaufort Sea. U.S. Environmental Protection Agency, Region 10 1-33.

Santoro GM (1999) Heat shock factors and the control of the stress response. *Biochemical Pharmacology* 59: 55–63. doi: 10.1016/s0006-2952(99)00299-3.

Schuur EG, McGuire AD, Schädel C, Grosse G, Harden JW, Hayes DJ, Hugelius G, Koven CD, Kuhry P, Lawrence DM, et al. (2015) Climate change and the permafrost carbon feedback. *Nature* 520(7546): 171-179. doi: 10.1038/nature14338.

Smith TR, Tremblay GC, Bradley TM (1999) Characterization of the heat shock protein response of Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry* 20: 279–292. doi: 10.1023/A:1007743329892.

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers.' *Journal of Experimental Biology* 213(6): 912–920. doi: 10.1242/jeb.037473.

Stefanovic DI, Manzon LA, McDougall CS, Boreham DR, Somers CM, Wilson JY, Manzon RG (2016) Thermal stress and the heat shock response in embryonic and young of the year juvenile lake whitefish. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* 193: 1–10. doi: 10.1016/j.cbpa.2015.12.001.

Tallman RF, Abrahams M v, Chudobiak DH (2002) Migration and life history alternatives in a high latitude species, the broad whitefish, *Coregonus nasus* Pallas. *Ecology of Freshwater Fish* 11: 101–111. doi: 10.1034/j.1600-0633.2002.00001.x.

Tallman RF, Reist JD (1997) The proceedings of the broad whitefish workshop: the biology, traditional knowledge and scientific management of broad whitefish (*Coregonus nasus* (Pallas)) in the lower Mackenzie River. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2193: 1-227.

Teigen LE, Orczewska JI, McLaughlin J, O'Brien KM (2015) Cold acclimation increases levels of some heat shock protein and sirtuin isoforms in threespine stickleback. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 188: 139–147. doi: 10.1016/j.cbpa.2015.06.028.

Tomanek L (2010) Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *Journal of Experimental Biology* 213(6): 971–979. doi: 10.1242/jeb.038034.

Vestfals CD, Mueter FJ, Duffy-Anderson JT, Busby MS, de Robertis A (2019) Spatio-temporal distribution of polar cod (*Boreogadus saida*) and saffron cod (*Eleginus gracilis*) early life stages in the Pacific Arctic. *Polar Biology* 42(5): 969–990. doi: 10.1007/s00300-019-02494-4.

White CN, Hightower LE, Jack R (1994) Variation in heat-shock proteins among species of desert fishes (Poeciliidae, *Poeciliopsis*). *Molecular Biology and Evolution* 11(1): 106–119. doi: 10.1093/oxfordjournals.molbev.a040085.

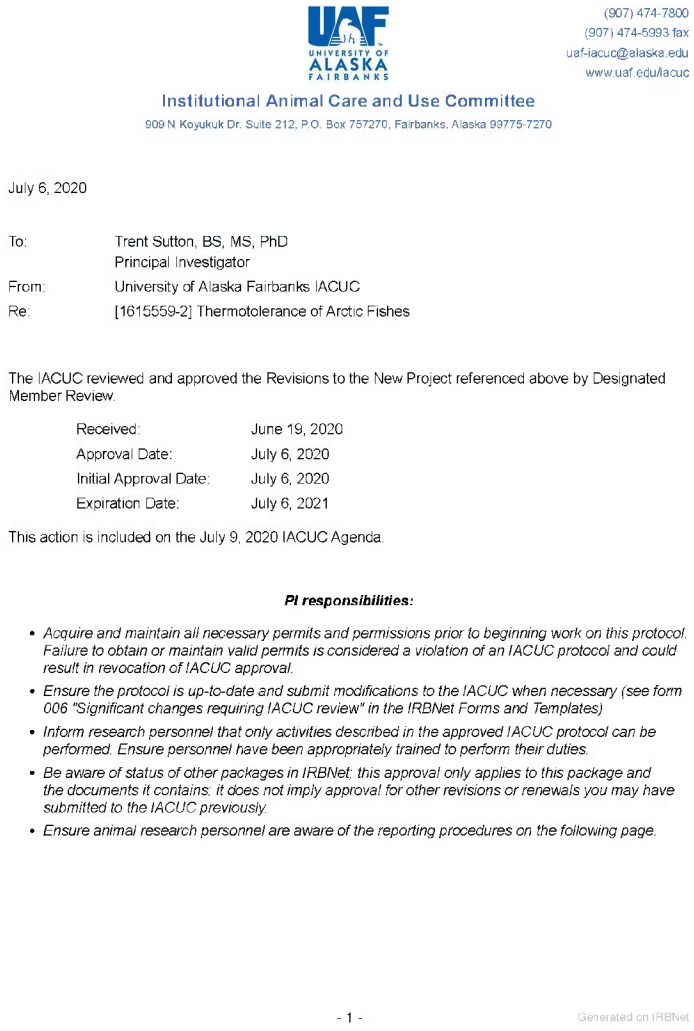
Wolotira RJ (1985) Saffron cod (*Eleginus gracilis*) in western Alaska: the resource and its potential. NOAA Technical Memorandum NMFS F/NWC-79 1-127.

Yamashita M, Yabu T, Ojima N (2010) Stress protein HSP70 in fish. *Aquatic-BioScience Monographs* 3(4): 111–141. doi:10.5047/absm.2010.00304.0111.

Zakhartsev M, de Wachter B, Johansen T, Pörtner HO, Blust R (2005) HSP70 is not a sensitive indicator of thermal limitation in *Gadus morhua*. *Journal of Fish Biology* 67: 767–778. doi: 10.1111/j.0022-1112.2005.00778.x.

Supplementary Approvals

Figure A1 Approval letter for the thermotolerance experimentation of broad whitefish and saffron cod under IACUC/IRB protocol #1615559-2.



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July 6, 2020

To: Trent Sutton, BS, MS, PhD
Principal Investigator
From: University of Alaska Fairbanks IACUC
Re: [1615559-2] Thermotolerance of Arctic Fishes

The IACUC reviewed and approved the Revisions to the New Project referenced above by Designated Member Review.

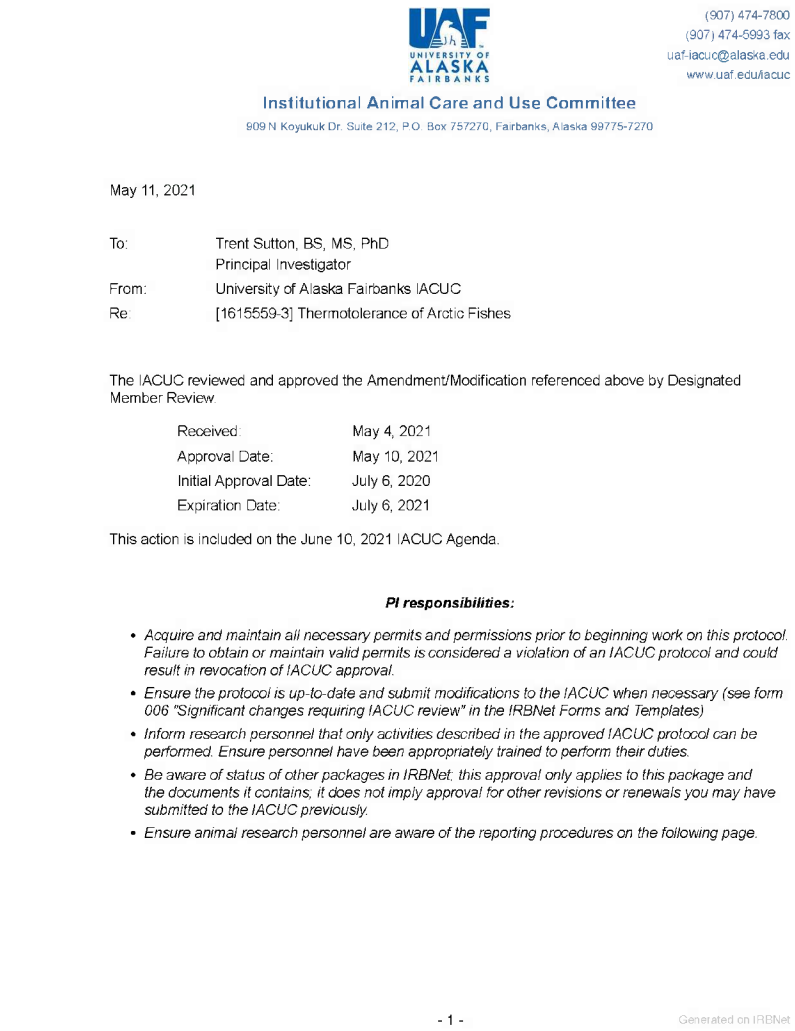
Received:	June 19, 2020
Approval Date:	July 6, 2020
Initial Approval Date:	July 6, 2020
Expiration Date:	July 6, 2021

This action is included on the July 9, 2020 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

Figure A2 Approval letter for the thermotolerance experimentation of broad whitefish and saffron cod under IACUC/IRB protocol #1615559-3.



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May 11, 2021

To: Trent Sutton, BS, MS, PhD
Principal Investigator
From: University of Alaska Fairbanks IACUC
Re: [1615559-3] Thermotolerance of Arctic Fishes

The IACUC reviewed and approved the Amendment/Modification referenced above by Designated Member Review.

Received:	May 4, 2021
Approval Date:	May 10, 2021
Initial Approval Date:	July 6, 2020
Expiration Date:	July 6, 2021

This action is included on the June 10, 2021 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
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Figure A3 Approval letter for the collection and transportation of broad whitefish and saffron cod from Prudhoe Bay under IACUC/IRB protocol #1054743-18.

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June 29, 2021

To: Trent Sutton, BS, MS, PhD
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [1054743-18] Beaufort Sea Fish Monitoring Project

The IACUC reviewed and approved the personnel list referenced above by Administrative Review.

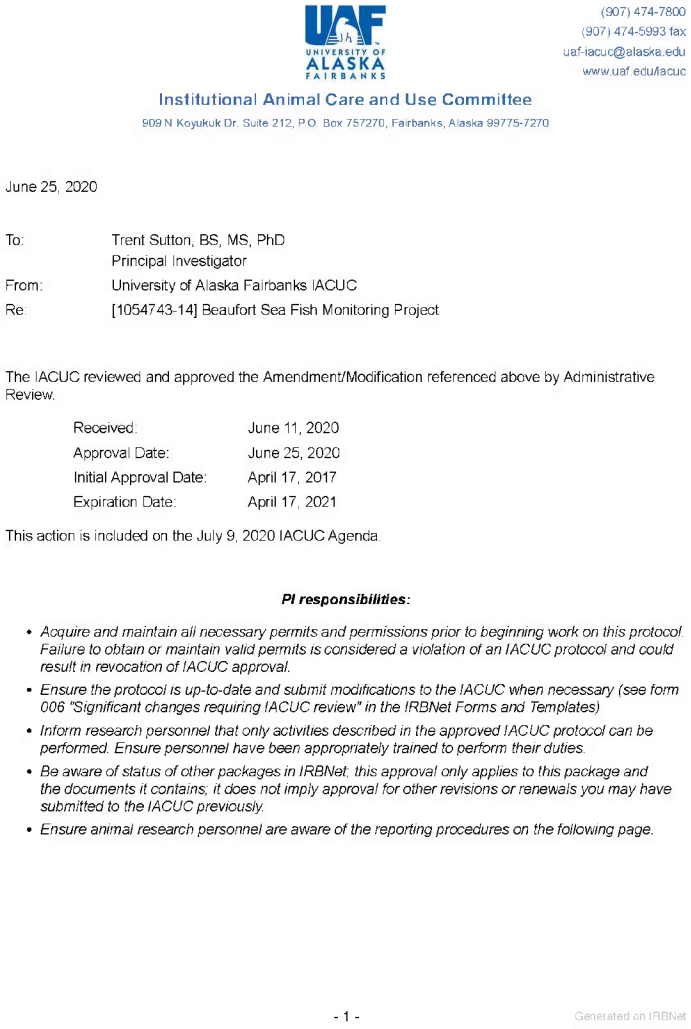
Received:	June 14, 2021
Approval Date:	June 29, 2021
Initial Approval Date:	April 17, 2017
Expiration Date:	April 17, 2022

This action is included on the July 8, 2021 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

Figure A4 Approval letter for the collection and transportation of broad whitefish and saffron cod from Prudhoe Bay under IACUC/IRB protocol #1054743-14.



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June 25, 2020

To: Trent Sutton, BS, MS, PhD
Principal Investigator
From: University of Alaska Fairbanks IACUC
Re: [1054743-14] Beaufort Sea Fish Monitoring Project

The IACUC reviewed and approved the Amendment/Modification referenced above by Administrative Review.

Received:	June 11, 2020
Approval Date:	June 25, 2020
Initial Approval Date:	April 17, 2017
Expiration Date:	April 17, 2021

This action is included on the July 9, 2020 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

Figure A5 Approval letter for the collection and transportation of broad whitefish and saffron cod from Prudhoe Bay under IACUC/IRB protocol #1054743-9.

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June 13, 2019

To: Trent Sutton, BS, MS, PhD
Principal Investigator
From: University of Alaska Fairbanks IACUC
Re: [1054743-9] Beaufort Sea Fish Monitoring Project

The IACUC reviewed and approved the Amendment/Modification to the Personnel List referenced above by Administrative Review.

Received:	June 11, 2019
Approval Date:	June 13, 2019
Initial Approval Date:	April 17, 2017
Expiration Date:	April 17, 2020

***Please be advise that training for Trent Sutton expires on July 11, 2019.*

This action is included on the July 11, 2019 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
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Figure A6 Approval letter for the husbandry of broad whitefish and saffron cod under IACUC/IRB protocol #197441-27.

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November 6, 2018

To: Trent Sutton, Ph.D.
Principal Investigator
From: University of Alaska Fairbanks IACUC
Re: [197441-27] Whitefish Husbandry

The IACUC reviewed and approved the Amendment/Modification to the Personnel List referenced above by Administrative Review.

Received:	October 29, 2018
Approval Date:	November 6, 2018
Initial Approval Date:	December 6, 2010
Expiration Date:	December 6, 2019

This action is included on the November 8, 2018 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
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