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**Investigating the roles of temperature
and exercise in the development of
chalkiness in Pacific halibut**

by

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Abstract

The incidence of chalky muscle occurs in a small portion of commercially landed Pacific halibut affecting its marketability. Numerous studies have determined that the chalky condition is associated with increased lactic acid and subsequent lower pH in fish muscle but have yet to elucidate the biochemical processes responsible for the condition. The goal of this project was to reproduce the chalky condition in the laboratory while monitoring multiple biochemical properties of the fish. Fish were exposed to three durations of stress in a controlled experiment to simulate longline hooking to produce chalky muscle and quantify the biochemical changes in muscle and blood chemistry. Biochemical parameters determined in muscle were proximate composition, lactate, glucose, nucleotides, pH, and color. Lactate and glucose were also determined for blood samples. Based on the three-, six-, and twelve-hour stress periods, we were unable to produce the chalky condition in most of the fish. We found little or no statistical difference in the biochemical properties of experimental groups that were stressed versus controls. However, three fish that did produce chalky muscle post mortem had muscle with lower pH, higher lactate and more opacity than non-chalky muscle. Based on low initial glucose, protein and lipid levels, we concluded that the limited chalky development in this study may have been due to the condition of the fish just prior to experimentation. Future studies should address the importance of halibut condition and glucose in particular to provide a substrate for lactic acid buildup and subsequent chalky development. Also, changing the timing of capture and limiting the duration of acclimation time prior to experimentation may render more specific results.

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Introduction

The chalky condition experienced by Pacific halibut (*Hippoglossus stenolepis*) is described as a result of decreased pH due to lactic acid accumulation in the muscle often brought about by struggle on the longline during capture. The decrease in pH causes the muscle to lose its translucence, increase drip loss and change texture resulting in what is considered a less desirable cooked product (Tomlinson et al. 1965, 1966a, 1966b). Many factors appear to affect the development of chalky halibut including amount of struggle during capture, season, condition factor, water temperature, post-mortem handling and chilling, onset of rigor, and possibly differences among geographical locations (Kaimmer 2000). A better understanding of the onset of chalkiness and conditions that occur is necessary to develop practices that might prevent the condition from occurring.

Review of selected papers relating to stress and food quality

Chalky halibut is described as occurring when muscle pH drops below 6.0, denaturing proteins and resulting in the opaque appearance of the fillet (Tomlinson et al. 1966a). This change of muscle pH is often brought about by capture stress (Tomlinson et al. 1966b). Capture stress is a very complex set of reactions not well understood for its effect on muscle tissue. Love (1988) tabulated the level of various chemicals which change as a result of stress. Compounds storing energy such as glycogen and ATP decrease in the muscle, while blood glucose and adrenaline increase. Hormones from the adrenal cortex sometimes increase, but this is species dependant. Lactic acid is released into the muscle in acute stress and seems to be retained there, rather than released into the blood stream (Wardle 1978).

This relationship between stress levels in animals prior to processing and the subsequent food quality problems has been investigated for many years. Besides Pacific halibut, most species of tuna (Watson et al. 1988), pork (Lee and Choi 1998), and poultry (Owens et al. 1998) suffer from stress-related quality problems. This research is now extending to aquaculture with investigations of farmed Atlantic salmon (Sigholt et al. 1997; Skjervold et al. 1999).

Fish are very sensitive to capture and handling stress and producing high quality seafood requires understanding of the origins of stress responses and their subsequent effects. The response to fish capture and handling is primarily hormonal (Wells 1987), which increases the metabolic and energy rates leading to changes in muscle tissue. Adrenalin and cortisol are released into the blood during stress to prepare the fish for “fight or flight”. Metabolic production resulting from stress influences the muscle tissue. Accumulation of lactic acid, loss of ATP and breakdown of histidine-containing compounds may have a detrimental effect on fish muscle quality.

Basic knowledge of the effects of stress comes from biological studies focusing on the ability of an animal to recover from the effect of stress, such as measuring stress response through changes in blood plasma, acid-base balance, osmotic ions and hormonal levels. Changes in flatfish as a result of stress have been examined by several researchers (Wood et al. 1977; Wardle, 1978; Turner et al. 1983b). Analyzing changes in blood pH from exhausted starry flounder, Wood et al. (1977) found that a 10 minute stress period produced an immediate drop in blood pH caused by elevated carbon dioxide levels rather than lactic acid. Blood lactate levels increased slowly and only affected the animals late in the recovery period. This stress response is very different from mammals where lactic acid plays the primary role in changing blood pH. This late increase in blood lactate levels prompted the researchers to suggest that lactic acid was retained in the muscle to prevent a potentially fatal blood pH level. Wardle (1978) stressed plaice under laboratory conditions to examine levels of lactic acid in muscle. He also concluded that plaice retained lactic acid in their muscle and relied on gluconeogenesis for its recovery. A comparison between flathead sole and rainbow trout showed several differences between inactive and active fish (Turner et al. 1983a). While many of the ionic and blood changes were similar between the species, exercised flathead sole had much lower muscle lactate than trout, but stayed at high levels through the recovery period. Again, this phenomenon was determined to be a strategy for the sole to maintain acid-base balance in its blood and to use muscle lactate in recovering glycogen. The effects of lactic acid accumulation and fish death were examined by Wood et al. (1983). Post-exercise death had been attributed to excess lactic acid in the blood. Wood measured blood lactate levels in stressed fish that recovered and those that died as a result of excessive exercise. Blood lactate levels were 50% higher in fish that died, but well within the normal range of tolerance. However, intracellular lactic acid built up to very high levels in white muscle, which he suggested was the probable cause of mortality. This buildup of lactic acid in the muscle of flatfish has consequences on quality of muscle tissue. Wood's research also implies that measurement of blood parameters in stressed fish may not be an acceptable indicator of fish quality.

Correlating stress responses to the quality of processed animal muscle has been investigated in several commercially important species. The most researched stress and quality relationships have been with pork and halibut. Pale, soft, exudative or PSE pork is meat that has lost its water holding capacity and is unable to be used in most meat products (Warner et al. 2001). It was first described by Briskey (1964) and has been a significant problem in the pork industry for almost 40 years. PSE pork is the result of increased aerobic and anaerobic metabolic rates induced by pre-slaughter stress (Lee and Choi 1998). Heat, carbon dioxide, and lactic acid are produced in high quantities with substantial muscle contraction. The lactic acid reaction is similar to that of chalky halibut where muscle pH denatures proteins causing the tissue to become opaque. This is a severe condition, where pH can drop below 5.8 in 30 to 45 minutes after death (Honkel and Kim 1986). PSE pork exhibits post mortem glycolysis rates twice that of non-PSE pork. Another factor contributing to the exudative or loss of water binding capacity is the high body temperature of the carcass. The carcass will also reach temperatures above 38°C. The denaturation of myosin irreversibly expels water from cells, producing poor texture.

PSE pork has developed as a result of genetic selection. The economics of the industry has dictated that pigs must be grown quickly and cheaply. Selecting animals that produce lean muscle mass rapidly means shorter rearing periods and more muscle. This biochemical/biophysical change has resulted in larger muscle fibers or fast muscle that burn glycogen at a rapid rate post-mortem and a higher sensitivity to stress (Solomon et al. 1998). In addition to genetic pre-disposition to PSE, pre-slaughter handling and the accelerated metabolism also contribute to the condition (Warner et al. 1997). The accelerated metabolism allows a rapid onset of rigor mortis leading to some of the texture problems associated with PSE pork. Preventing or reducing PSE in pork requires a careful analysis of the amounts and types of muscle in the pig. Three

types of muscle fibers can be found in all animals - "white", "red" and "intermediate." Each has properties related to size, energy metabolism and ATP turnover. The "white" muscle is the one most associated with PSE development. Sensitivity to stress is also genetically determined by evaluating genes responsible for muscle contraction. During slaughter, keeping the animals calm, avoiding even mild exercise and reducing processing room temperature helps reduce incidence of PSE pork even in genetically susceptible animals.

Conditions similar to PSE pork have been found in poultry, especially turkey (Owens et al. 1998). PSE turkey is characterized by rapid post mortem pH decline and loss of protein functionality. The desire for large breasted animals has led to a genetic breeding program not unlike that of pigs. Similar conditions exist in many turkeys, where they have large amounts of "white" muscle prone to rapid glycolysis post mortem. Rapid breast muscle development has resulted in reduced capillary density, calcium loading during contraction and other structural irregularities. In addition, the change from marketing whole birds to further processed products has made the condition more apparent. However, PSE turkey may be the result of the same types of stress that can cause rapid rigor development. Pre-slaughter heat stress accelerated pH decline after death and led to paler meat and higher drip loss in cooked meats (McKee and Sams 1997). Slow chilling of turkey carcasses following a hot boning process has also accelerated the incidence of PSE turkey (McKee and Sams 1998). Detecting PSE turkey by measuring L values was evaluated by Owens et al. (1998). The L value in color scale measures the degree of whiteness. It was found that L value measurement was more effective in detecting PSE turkey than either determining pH or water holding capacity. Again, lowering pre-slaughter stress and rapidly chilling carcasses were processes needed to lower the incidence of this defect.

The causes of chalky halibut have been investigated since 1950. Bailey (1950) was interested in the differences between chalky and normal halibut and measured oil and moisture contents. He found that moisture content increased with increasing chalkiness, i.e., very chalky halibut had higher moisture content than normal halibut. The best understanding of the condition came from the research of Tomlinson, Geiger, and Dollinger (1965, 1966a, 1966b). In their 1965 paper, Tomlinson et al. identified low muscle pH as the main factor in chalky halibut. Using both trawl and longline caught halibut; they identified chalkiness in animals with pH less than 6.0. In several trawl caught fish, it took up to six days for chalkiness to appear. Associated with low muscle pH was decreased protein solubility. The authors suggested that the loss of protein solubility contributed to the opaque appearance of the fillet as well as a reduced water holding capacity resulting in a softer meat. This is very similar to what occurs in PSE pork. The authors also pointed out that the presence of lactic acid responsible for muscle pH drop was related to glycogen levels in the animal. As a consequence, well fed and active animals were more likely to become chalky than those less well fed. In their second paper, Tomlinson et al. (1966a) investigated the relation between drip loss, muscle pH and chalkiness in halibut. From trawl caught halibut, they measured pH variation within a fish and found slightly higher pH near the head. The time series showed a rapid pH drop within the first two days postmortem with a slow rise over the remaining nine days of storage. Drip loss was greatly influenced by muscle pH. The lower the muscle pH, the higher was the amount of drip loss. This loss of moisture would affect the texture and eating quality of the fish, suggesting that chalky halibut may be less "juicy". In their final paper, Tomlinson et al. (1966b) evaluated fish handling techniques and their relation to development of chalkiness. Beginning with trawl caught halibut, fish were handled in different ways. Some fish were immediately killed, gutted and iced. Others were hooked and put into a seawater tank to recover from capture stress, and imitate the effects of longline capture. These fish were held for 9 to 13 hours before they were processed. Those fish allowed a recovery period had higher mean muscle pH and significantly lower incidence of chalkiness. This suggested that the amount of time between capture and death could influence the development of chalkiness. Patashnik (1966) offered his view on chalky halibut in a general paper on halibut quality. He

noted that chalkiness is not readily apparent in fresh fish, but often requires as long as two days to develop. He noted a reduced protein solubility and lower protein content in free and cook drip. This also produced a dry and tough cooked fillet. Finally, Patashnik (1966) points to four conditions that can cause chalkiness: high glycogen in muscle tissue, an extremely exhausted animal, an inability to remove lactic acid from its body, and high storage temperature.

Fish species other than halibut where the relationships between stress and quality have been investigated include tuna, salmon and snapper. The incidence of "burnt" tuna has often been described as a consequence of low pH and poor storage conditions (Cramer et al. 1981). Tuna harvested in tropical and subtropical waters sometimes exhibit PSE like conditions. Burnt tuna are pale muddy brown, exude a clear fluid, and have a soft texture and slightly sour taste. Although these fish are acceptable as canned and cooked product, they have no value as sashimi. Initial research indicated high muscle temperature and low pH caused myofibrillar denaturation. The muddy brown color may be a result of metmyoglobin production under anaerobic conditions while the fluid is the result of protein denaturation (Davie and Sparksman 1986). "Burnt" tuna were also found at moderate storage temperatures. Tuna physiology tended to support the accumulation of lactic acid and temperature abuse as the cause of "burnt" tuna. It is known that tuna are capable of producing muscle temperatures significantly above ambient. Davie and Sparksman (1986) conducted a microscopy study to evaluate postmortem changes occurring in tuna caught by longline, handline, and rod and reel. Burning within three hours of landing was associated high storage temperatures. When comparing fishing methods, longlining produced the least amount of burning followed by rod and reel and handline. Short capture times resulted in higher levels of burnt tuna. To explain this occurrence, the researchers suggested that tuna have the ability to remove lactic acid from the muscle through transfer to the blood and excretion through the gills. This is in direct opposition to earlier research conducted on other species such as plaice and trout where this transport mechanism does not exist. A new hypothesis on "burnt" tuna has been proposed that suggests an enzymatic process may be responsible (Watson et al. 1988). Extracellular pH drop (similar to that in halibut) would denature muscle protein resulting in the defects, but it has been shown that intracellular fluid is well buffered and often does not reach that low pH. Experiments indicate that for every 1 pH unit drop in extracellular fluid, intracellular fluid has a 0.4 pH drop (Hagberg 1985). Low levels of lactic acid were insufficient to denature myosin, suggesting decomposition of other muscle proteins, possibly enzyme induced. The nature of muscle damage where the Z discs, tropomyosin, and troponin components underwent rapid deterioration suggested that calcium activated neutral proteases could be responsible for "burnt" tuna.

Stresses on farmed salmon and their effects on quality have recently been conducted by several researchers. Berg et al. (1997) evaluated the effect of stress on the development of rigor mortis in Atlantic salmon (*Salmo* sp.). Unstressed fish (individually handled and killed immediately) had higher levels of phosphocreatine, ATP, and IMP than stressed fish (normal slaughter procedure that included pumping, stunning, and bleeding). Rigor mortis developed more slowly with unstressed fish and also was resolved more slowly. Stressed fish with very low ATP levels went into rigor almost immediately. The authors concluded that the standard handling procedures produced considerable stress, although the effect on meat quality was not evaluated. Sigholt et al. (1997) found that handling stress and storage temperature adversely affected meat quality of farmed Atlantic salmon. Standard handling procedures were compared with confining animals for 10 minutes prior to processing. As expected, the confined animals exhibited stress as indicated by lower phosphocreatine and ATP levels. Shorter pre-rigor periods were also observed for stressed salmon. Holding these fish at either 0.3°C or 3.3°C also affected meat quality. Holding fillets from stressed salmon at 3.3°C resulted in slightly lower muscle pH and water holding capacity in the early stages of storage. After six days, no differences could be detected between stressed and unstressed fish. Sensory evaluation indicated stressed fish had a

softer texture although flavor and color were unaffected. Skjervold et al. (1999) noted stress on Atlantic salmon due to crowding. High density (300 kg/m²) crowding prior to slaughter resulted in higher levels of cortisol, lactate, and osmolality than low density (30 kg/m²) crowding. Glucose levels in stressed fish were significantly lower than those with less stress. These conditions caused an earlier onset and resolution of rigor. Gomez et al. (2000) examined biological stress on the quality of smoked Atlantic salmon fillets. Stressed fish (induced by 30 days starvation) produced a slight decrease in protein solubility making smoked muscle slightly softer than the unstressed controls.

Quality changes in New Zealand snapper as affected by capture stress was investigated by Lowe et al. (1993). Stress was induced by chasing snapper with a pole for one hour. When compared to resting fish, stressed snapper went into rigor almost immediately after death. Muscle ATP levels decreased immediately for stressed fish while rested fish required almost 16 hours to lose its ATP. This corresponded with the onset of rigor. As ATP levels decreased, lactate levels increased. K values, a general measure of quality calculated by ratios of nucleotides, remained low for all fish indicating a high quality even after 72 hrs storage. The authors suggest that better handling and reducing stress in snapper produced fish with higher quality meat as evaluated by the onset of rigor and reduced changes in muscle biochemistry. While stressed snapper showed chemical indices indicating lower quality, the actual effect of stress on the cooked quality of the fish was not investigated.

“Chalkiness” occurs in many species as a result of changes in muscle pH or the accumulation of lactic acid and other mitigating factors. Lactic acid appears to contribute to the genetic predisposition of pork and poultry to produce PSE meat. The pH decline in tuna would appear to activate specific enzymes responsible for the “burnt” condition. The pH drop in halibut muscle is the cause of chalkiness. Preventing these conditions involves reducing the stress in the animal prior to slaughter as well as appropriate post mortem handling techniques.

Materials and methods

Fish collection and holding system

Seventy-five halibut were collected by longline (Table 1) in the waters off the northeast side of Afognak Island in the Kodiak Archipelago (151° 55'W and 58° 27' N) on November 15, 2002 aboard the *F/V Alaskan*. Three longline skates soaked at 72 m depth for two hours were baited with 50 hooks per skate. Halibut between 9 and 14 kg were selected, placed in totes with circulating seawater pumped from approximately 2 m, brought to Kodiak within 24 h and transferred to three circular tanks at the Kodiak Fisheries Research Center. Forty five fish were placed in a 4,500 l (4.5 m diameter) holding tank with circulated seawater replenished at a rate of 48.6 l/min. Two 3,000 l tanks (3.3 m diameter) with 24.3 l/min inflow each held 15 fish.

Halibut were originally acclimated to the holding tanks for approximately 30 days. Fish were assumed to be acclimated when daily feeding rates and swimming patterns were constant on December 15, 2002. During the acclimation period halibut were observed daily and fed herring every other day until the experiments began. Food consumption was recorded and any distressed fish noted. Consumption among halibut was variable and noted when possible. Fish were observed daily for infections or lethargic behavior. Five fish were removed and euthanized during this period due to lingering effects of capture (hook wounds) or lack of swimming. The fish remaining after the acclimation period had a wide range of conditions. Most fish appeared very healthy, swam periodically and reacted to people standing over the tanks. Some halibut remained lethargic and developed some sores. Toward the end of the experiments, increased parasitic isopods were seen on many animals. The specific condition of each fish used in the experiments was noted to ensure that fish condition did not bias the results.

Table 1. Summary of sample sizes of halibut collected, used for experiments and mortalities.

Summary	Count
Halibut collected and brought to Kodiak Fisheries Research Center	75
Halibut mortalities during acclimation period November 15, 2002 to December 15, 2002	5
Halibut sacrificed for baseline data to reduce tank density	23
Halibut mortalities during acclimation period December 15, 2002 to January 28, 2003	5
Halibut treated for stress experiments	36 (3 controls & 9 experimental fish per 3 treatments)
Halibut treated for temperature experiments (three were euthanized after tank failure)	6

At the beginning of each experiment, halibut were videotaped to determine the amount of struggle time. Seawater temperature, oxygen, and salinity were monitored daily with a YSI O₂/temp/salinity meter. Oxygen levels were not allowed to go below 80%. Water temperature varied between 4.5°C and 5.5°C throughout the acclimation and experimentation periods.

Analyses

Proximate composition

Moisture and ash were determined in triplicate using standard drying and ashing procedures (AOAC 1999). Protein was measured in triplicate using a Leco Model 1000 nitrogen analyzer (LECO Inc. 2000). Lipids were extracted in duplicate using an acid hydrolysis, ether/ethanol extraction, and ambient temperature evaporation (Lindahl 1983).

Lactate and glucose

Lactate was determined for both the blood and muscle samples using methods described by Barker and Summerson (1941) and Taylor (1996). Glycogen was measured using a modification of the Keppler and Dekker method (Keppler and Dekker 1974; Cappelin and Jessen 2002). Glycogen was hydrolyzed by adding a PCA extract to a glucoamylase solution, incubated for two hours, neutralized, and centrifuged. Supernatant was reacted with a buffer and then hexokinase added and absorbance read at 339 nm. Glucose was then calculated from a standard curve.

Nucleotides

Nucleotides were analyzed using reverse phase chromatography in Waters Model 1000 HPLC system (Cappelin and Jessen 2002). A reverse phase column operating with an isocratic elution of potassium phosphate, tetrabutylammonium hydrogen sulfate, and methanol separated all nucleotides. Nucleotides are sometimes used as an indicator of quality when expressed in a quantity called K value. K value is the ratio of IMP, inosine, and hypoxanthine to ATP,

ADP, and AMP. Typically, as quality declines, K values become higher. K values can also be used as a measure of the depletion of the high energy compounds driving glycolysis. K values were calculated as a measure of quality and indication of remaining high energy phosphate compounds.

pH

pH was measured from ten equally spaced points on each fillet using a Corning Model 200 pH meter with a penetrating probe glass electrode (Corning Inc. 2000; AOAC 1999).

Color

Muscle color was measured in triplicate using a Minolta Model 300 Chromameter set to measure L, *a*, and *b* values (Minolta 2001). These values are used to describe any color in a three dimensional plot. The *a* value, scaling from -60 to +60, denotes color range between red and green. The *b* value, scaling between -60 and +60, denotes a range between yellow and blue. The L value, scaling between 0 and 100, describes lightness, from black to white.

Baseline data

A total of 23 halibut were sacrificed (Table 1) between December 16 and 19, 2002 to reduce the density of fish in the holding tanks, to perfect analytical methods, and to develop baseline data on muscle conditions and quality prior to experimentation. Fish were removed from the tanks one at a time, immediately stunned, weighed and measured. Blood for lactate analysis was collected into vials from ten of the 23 fish, immediately frozen to -60°C using an ethanol/dry ice solution, and then placed in a -80°C freezer. Dorsal and ventral muscle from fifteen of the twenty three halibut was filleted from the light side of the halibut for proximate composition. Dorsal and ventral fillets of the dark side were sampled from ten of the halibut for muscle lactate analysis. Dorsal and ventral fillets of the dark side were sampled from five of the halibut for glucose and pH. Initial samples for lactate and glucose were taken within two minutes of filleting, immediately frozen to -60°C, and stored at -80°C until analyzed. Samples were taken along the lateral line, close to the middle of the fillet. A series of pH samples were randomly taken from the whole fillet and then averaged.

Stress experiments

As a result of the baseline data (low lipid content), we decided to continue feeding the remaining 47 halibut for 40 more days, for a total of 70 days for acclimatization prior to conducting experiments. During this time, five more fish died due to wounds associated with capture.

To reproduce hooking stress and produce chalkiness in halibut muscle, longline operations were simulated in the holding tanks. Treatments of three, six, and twelve hours were defined as the amount of time left on the hook prior to retrieval. Three groups of three halibut were hooked with a circle hook through the jaw, tied off to a line with their tail touching the bottom of the tank and allowed to struggle. This is a total of nine halibut for each of three treatments. A single control fish from each group was left off the hook but in the same tank for each treatment. This is a total of 3 control fish per treatment.

At the end of each treatment, halibut were removed from the tanks, allowed to rest for five minutes before being stunned, weighed and measured. Dorsal and ventral muscle was filleted from the light side of the halibut for proximate composition. Dorsal and ventral fillets of the dark side were sampled for muscle lactate, glucose, nucleotides (K), pH, and color (L). Initial samples were taken within two minutes of filleting, immediately frozen to -60°C and stored at -80°C until analyzed. The remainder of each fillet was poly bagged and stored at 4°C for up to six days to observe post mortem changes. Every day additional samples were cut from the fillets,

immediately frozen to -80°C, and held for biochemical analyses. Samples were taken along the lateral line close to the middle of the fillet but were progressively taken from the tail towards the head. pH samples were randomly taken from the whole fillet and then averaged.

Temperature experiments

A temperature experiment was conducted to simulate stress caused by high temperature which may increase the occurrence of chalky muscle. Three halibut were held in a circulating 3,000 l tank (3.3 m diameter) at 12°C for three days. Daily observations were made to assess behavior during the treatment. At the end of the test period, fish were removed from the tanks, processed and sampled as described above (see Baseline Analysis). Muscle lactate, nucleotides (K), pH, and color (L) were analyzed for a period of six days in this experiment. As stated in the original statement of work, additional temperature related experiments were planned. A failure in the circulating holding tanks prior to these experiments caused us to prematurely euthanize three experimental fish.

Results and discussion

Baseline analysis

Fish sacrificed in late December were evaluated for proximate composition, blood and muscle lactate, glucose, and pH (Table 2).

T-tests showed no significant differences in lipid, protein, water, or ash content between the two light side fillets removed from each fish, so those data were therefore averaged \pm SD for subsequent discussion. The baseline proximate composition (Table 2) indicated higher than expected moisture content, slightly less lipid and protein than unpublished data (R. J. Foy, University of Alaska Fairbanks, 118 Trident Way, Kodiak, AK 99615, unpub. data) and published

Table 2. Proximate composition, muscle and blood lactate, and pH for a subset of halibut that were sacrificed on December 19, 2002 for baseline data.

Proximate Composition [mean% (sd)]					
	n	Moisture	Ash	Lipid	Protein
	15	79.5 (1.42)	1.3 (0.06)	1.6 (1.10)	16.1 (1.73)
Muscle Lactate (mg/kg)					
	n	mean (sd)			
	10	0.95 (0.270)			
Blood Lactate (mg/kg)					
	n	mean (sd)			
	10	0.65 (0.097)			
Glucose					
	n	mean (sd)			
	5	0.008 (0.0057)			
Muscle pH					
	n	mean (sd)			
	5	6.8 (0.18)			

data which averaged a number of data points across seasons and areas (Sidwell 1981). Lipid content (\pm SD) averaged $1.6\% \pm 1.10$ which is less than the 2.0-2.5% reported by Sidwell and the 4.0% found in November 2002 halibut by Foy, perhaps indicating some lipid deposits had been used during the early part of the fall. Lower lipid content and higher moisture content ($79.5\% \pm 1.42$) suggested that fish used for these experiments may have had lower initial energy density due to low in situ prey availability. Many of the female fish also had well developed roe perhaps adding to physiological stress.

T-tests showed no significant differences in muscle lactate, glucose, or pH between the two dark side fillets removed from each fish; these data were averaged \pm SD for subsequent discussion. Muscle pH was consistent along the fillet so measurements were also averaged across each fish. Blood and muscle lactate were at low levels (<1.0 mg/kg) indicating that these fish were not stressed. Glucose levels were very low at 0.008 g/100 g tissue. Muscle pH averaged almost 7.2, which was slightly higher or at the maximum of published values (Tomlinson et al. 1966a, Kaimmer 2001).

Stress experiments

By mid-January, 2003, the halibut appeared to be in their best condition since capture, based on food consumption and general activity in each tank. Over a three day period, between January 20 and 22, 2003, experiments simulating longline hooking were performed during which the fish were monitored for struggling. Struggling was defined as persistent tugging against the line or violent thrashes that wound the line into tight coils and suspended the fish farther off the bottom of the tank. In general, most fish struggled a total of 37 to 46 min/h, in two to four min periods of activity. Fish would typically rest following a period of struggle. After the prescribed treatment time, the fish were removed from the water and left on the floor for five minutes before stunning. Approximately 90% of the fish did not move or moved very little before stunning, which was indicative of exhaustion.

Proximate composition

The proximate composition of experimental halibut was significantly different than the baseline fish (Table 3). Lipid and protein content were both lower in experimental fish while water content was higher. This suggests that the halibut were not gaining storage lipids nor putting on growth during the 70 day acclimation period. One group of fish, twelve-hour controls, had very low protein and the highest moisture content. The lipid content of all these fish was 0.5 to 0.25 of what was expected for a fish in generally good condition. This is partially due to the initial condition of the fish at capture and the apparent low intake of energy prior to experimentation. It was also noted that many of the females had fairly well developed roe sacs that could further reduce lipid levels in the muscle if energy was being diverted to gonad development.

Table 3. Proximate composition of control and stressed halibut from three treatment groups. Data presented as mean % (sd).

Analysis	3 hours		6 hours		12 hours	
	control n=3	stress n=9	control n=3	stress n=9	control n=3	stress n=9
Moisture	80.0 (1.60)	79.9 (1.40)	79.7 (1.96)	80.6 (1.06)	81.3 (2.04)	80.6 (1.08)
Ash	1.2 (0.11)	1.3 (0.06)	1.3 (0.03)	1.2 (0.03)	1.2 (0.09)	1.2 (0.17)
Lipid	1.2 (0.05)	0.5 (0.20)	0.6 (0.44)	0.6 (0.19)	1.2 (0.62)	0.6 (0.19)
Protein	16.9 (1.68)	15.1 (2.06)	15.2 (1.06)	13.1 (1.57)	9.9 (3.07)	14.1 (1.05)

Muscle lactate and glucose

The accumulation of lactic acid causing the decrease in the fish muscle pH has been cited to cause the development of chalky halibut (Tomlinson 1966a). There were no differences among the control and three- and six-hour treatment groups. The high lactate values of the twelve-hour treatment group were due to a single male halibut which showed significant lethargy. There was no change in lactate over time in any treatment group, although there were differences among treatment groups (Table 4). There appeared to be a decrease in lactate between the three- and six-hour treatments, while the twelve-hour treatment had higher lactate levels suggesting increased stress with longer duration on the hook. The control values were similar to the baseline values determined in December, suggesting little change in stress over this period.

Table 4. Muscle lactate (mg/kg) of control and stressed halibut from three treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments. Data presented as mean (sd).

Day	3 hours		6 hours		12 hours	
	control n=3	stress n=9	control n=3	stress n=9	control n=3	stress n=9
0	1.73 (0.653)	1.52 (0.288)	1.26 (0.500)	1.34 (0.281)	2.57 (0.283)	1.36 (0.381)
2	1.58 (0.362)	1.44 (0.130)			2.71 (0.407)	1.67 (0.647)
3	1.55 (0.278)	1.47 (0.279)	1.22 (0.393)	1.30 (0.075)	2.76 (0.687)	1.38 (0.443)
4	1.79 (0.492)	1.48 (0.306)		1.67 (0.034)	2.56 (0.233)	1.47 (0.444)
5	1.50 (0.481)	1.50 (0.511)	1.24 (0.260)	1.17 (0.170)	2.34 (0.361)	1.83 (0.790)

The low lactate levels encountered were insufficient to produce chalky halibut in an entire group, but one fish from each treatment was found to be chalky. Values cited by other researchers (Turner et al. 1983b; Wardle 1978; Love 1988) are much higher than those measured on average, ranging between five and ten mg/kg muscle tissue. Lactic acid is a byproduct of anaerobic glycolysis where glycogen is consumed. The fact that no additional lactic acid was produced from initial values may suggest that glycogen reserves within the fish muscle were very low and perhaps limited the production of lactic acid. This may be a reflection of the initial condition of the fish.

Glucose was analyzed for control and stressed fish from the three-hour and twelve-hour treatments only (Table 5). Glucose levels in all halibut sampled were very low and perhaps were the limiting factor in lactic acid production. The fish used for baseline data averaged 0.008 g glucose/100 g tissue, significantly below values reported for other species in good biological condition (Cappelin and Jessen 2002). In all tests, glucose levels varied from a high of 0.0536 g

Table 5. Results from muscle glucose test of control and stressed halibut from two treatment groups. Data (g/100 g tissue) represent the mean value of measurements taken from two fillets each day after treatments. Data presented as mean (sd).

Glucose	3 hours		12 hours	
	control	stress	control	stress
n	3	7	3	2
g/100g tissue	0.008 (0.0043)	0.004 (0.0039)	0.008 (0.0037)	0.005 (0.0073)

glucose/100 g fish to lows of 0.0014 g glucose/100 g fish. Cappelin and Jessen (2002) reported glucose levels ten times higher in their investigation of cod muscle. Stressed fish had even less muscle glycogen. Three-hour fish contained 0.004 g glucose / 100 g tissue, while twelve-hour fish contained 0.005 g glucose / 100 g tissue, not enough substrate to produce sufficient amounts of lactic acid necessary for the onset of chalkiness.

Nucleotides

There were no significant differences between the control and treatment values and there were no differences among the treatments at the initial measurement (time = 0). The K values calculated for both control and treated halibut showed a steady increase over the storage period (Fig. 1). These values are an indication that the high energy phosphates diminished during the storage period and degradation compounds increased, independent of treatment.

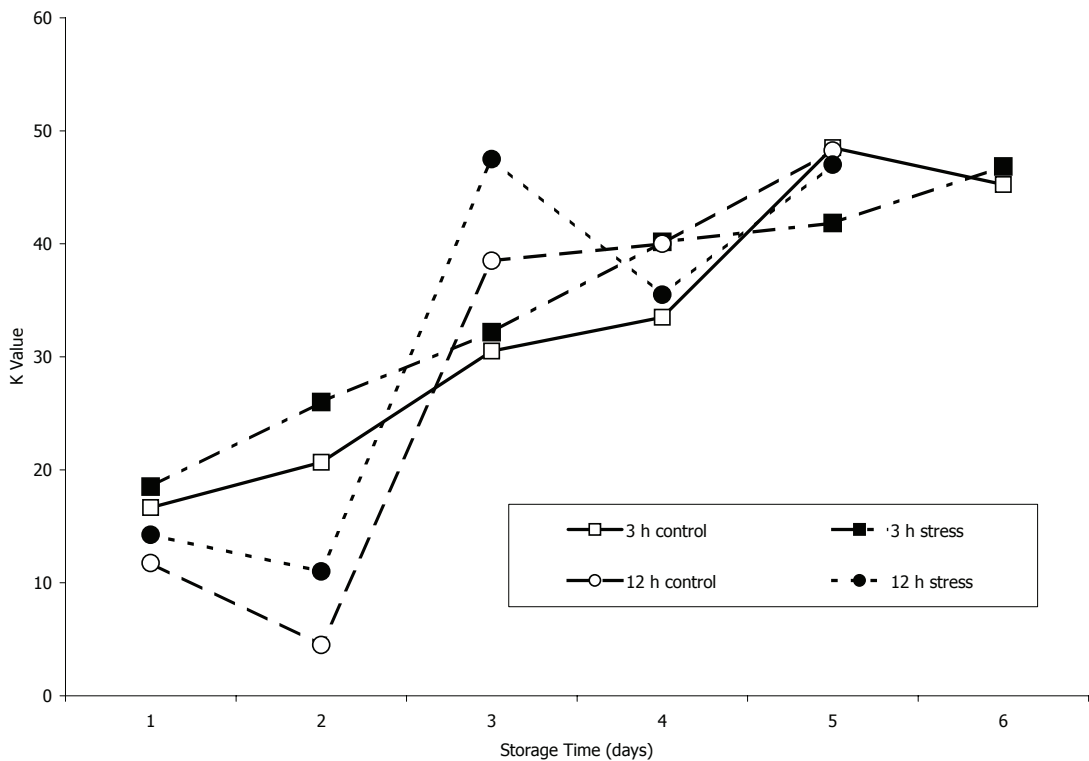


Figure 1. K values of control and stressed halibut from two treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments.

pH

The typical determinants of chalky halibut are pH and the opaque color of the fillet. Average pH measurements for all three stress trials indicated no treatment group of fish became chalky (Table 6). However, one fish from the three and one from the six h treatment had low enough pH to be considered chalky during the post stress period (see section below). At time zero, all stressed fish had pH values 0.5 to 0.6 units lower than the control fish. This would be expected since stressed fish had been exercised. The control fish for all treatments had a high initial pH similar to that of the baseline studies one month prior. This was 0.5 to 0.7 pH units higher than expected from other studies. This initial high pH could have been a factor in the failure to

Table 6. pH of control and stressed halibut from three treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments. Data presented as mean (sd).

Day	3 hours		6 hours		12 hours	
	control n=3	stress n=9	control n=3	stress n=9	control n=3	Stress n=9
0	7.4 (0.27)	6.8 (0.18)	7.2 (0.22)	6.8 (0.19)	7.5 (0.29)	6.9 (0.25)
2	6.8 (0.31)	6.5 (0.20)	6.9 (0.29)	6.7 (0.21)	7.0 (0.20)	6.8 (0.21)
3	6.5 (0.31)	6.5 (0.18)	6.5 (0.23)	6.5 (0.15)	6.8 (0.30)	6.7 (0.18)
4	6.3 (0.20)	6.4 (0.17)	6.5 (0.20)	6.5 (0.14)	6.3 (0.26)	6.6 (0.17)
5	6.3 (0.17)	6.4 (0.15)	6.5 (0.20)	6.5 (0.14)	6.4 (0.24)	6.6 (0.17)
6	6.3 (0.18)	6.4 (0.15)	6.6 (0.18)	6.6 (0.14)		

produce chalkiness in any of the three treatments. During the five and six day holding period, the pH of control and stressed fish declined steadily. On day 4, the difference between control and stressed fish were not significant. This observation follows that of Tomlinson (1966a) and other researchers where over time differences between stressed fish and unstressed fish diminish. This result is expected, as the control fish would produce lactic acid at a slower rate until the glycogen was consumed while treated fish would produce lactic acid faster.

Color

Color changes in the halibut flesh were determined using a colorimeter that measured L, a, and b values. The L value was the parameter chosen to determine development of chalkiness. When halibut flesh goes from translucent to opaque, it becomes lighter and the L value increases. In the simulated capture stress trials, the average color changes as measured by L did not change significantly (Table 7). The typical translucent color of halibut ranges between 40 and 50. Initial values for all halibut, control and treatment, were within this range. During the subsequent storage period, these values only increased slightly. Previous work on chalky halibut (J. Babbitt, National Marine Fisheries Service, 118 Trident Way, Kodiak, AK 99615, unpub. data) showed that L values of >70 would indicate chalkiness, whereas values between 65 and 75 may indicate chalkiness.

Table 7. Color (L) of control and stressed halibut from three treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments. Data presented as mean (sd).

Day	3 hours		6 hours		12 hours	
	control n=3	stress n=9	control n=3	stress n=9	control n=3	Stress n=9
0	47.0 (2.58)	44.3 (5.42)	44.4 (4.71)	51.8 (5.46)	51.0 (11.1)	44.8 (7.63)
2	49.3 (0.90)	49.0 (4.07)	48.0 (3.87)	52.9 (3.23)	51.4 (10.45)	45.2 (8.44)
3	53.3 (1.86)	52.0 (4.62)	50.8 (1.72)	53.5 (3.36)	55.8 (11.25)	46.8 (8.68)
4	55.6 (0.66)	52.6 (4.53)	53.1 (0.75)	53.6 (2.77)	57.1 (10.53)	49.2 (6.81)
5	56.3 (0.72)	55.7 (4.57)	53.8 (2.15)	55.6 (3.16)	58.2 (11.04)	50.1 (6.55)
6	55.8 (2.23)	53.6 (3.94)	55.1 (3.82)	56.4 (2.44)		

Temperature experiments

Three fish were held for three days at 12°C before sampling. During the test, halibut were observed for one hour each day. During those periods, the halibut were kept in constant movement. This was in contrast to the acclimation temperature of 5.5°C, where fish remained motionless for long periods of time on the bottom of the tank. During the typical hour, the fish swam around the tank for 45 to 50 min, only resting about 10 to 15 min. At the end of the storage period, the fish were removed from the tank, set on the floor for five minutes, and stunned. During the time on the floor, all fish thrashed vigorously and did not stop struggling until stunned.

pH and color

The pH of halibut held for three days was similar to the controls for the stress tests (Table 8). After three days at 12°C, the initial pH was 7.3. Over the six-day refrigerated storage, pH dropped to 6.7, far above the critical pH of 6.1 typically found in chalky halibut. Again, the initial pH value of the fish was 0.4 to 0.5 unit above values expected for vigorous fish. The L value of the flesh changed only slightly over the storage period, again not reaching the value that denoted chalk.

Lactate and nucleotides in muscle

As with the previous fish, lactic acid levels increased slightly over the storage period (Table 8). Between days 2 and 3, a large jump in lactic acid was noted that corresponded to a noticeable drop in pH during that period. Again, the levels of lactic acid found in the muscle were not sufficient to produce chalkiness. Lactate levels were still very low compared to values published for other stressed fish (Lowe et al. 1993; Turner et al. 1983b).

Table 8. pH, color (L), muscle lactate (mg/kg), and nucleotides (K) of three stressed halibut from one 12°C treatment group. Data represent the mean value of measurements taken from two fillets each day after the treatment. Data presented as mean (sd).

Day	pH n=3	L Value n=3	Lactic Acid n=3	K value n=3
0	7.3 (2.24)	47.5 (0.28)	1.0 (0.18)	30.50
2	7.1 (1.73)	48.9 (0.28)	0.9 (0.38)	29.67
3	6.8 (2.55)	49.2 (0.22)	1.4 (0.35)	38.50
4	6.7 (1.02)	49.9 (0.18)	1.4 (0.35)	40.17
5	6.7 (1.78)	52.0 (0.19)	1.3 (0.28)	46.50
6	6.7 (2.04)	54.2 (0.19)		

Evaluation of chalky halibut

One fish from each of the three treatments became chalky following the treatment and subsequent storage period. pH measurements of these fish showed a steady decline to numbers consistent with the development of chalky muscle (Fig. 2). Fish from the three and twelve-hour stress trials started at very high initial pH of 7.5. The six-hour fish was considerably more acidic at pH 6.7. Chalkiness in the twelve-hour fish, as indicated by pH and L value (Fig. 3), had developed by day four. The pH of halibut from the three-hour stress was slightly higher, perhaps at the borderline of chalkiness in the same time period. The one fish from the six-hour test was chalky after day three, reaching the lowest muscle pH of any animal in the experiments.

L values for the three halibut suggested the fish from the twelve-hour test was chalky from day one (Fig. 3), although this was not supported by the pH measurements. This fish had an

initial L value of 65 that increased to 72 after five days of storage. The other two chalky fish had significantly lower initial L values around 45. The six-hour fish reached an L value of 63 on day five, while opaqueness in the three-hour fish peaked at 57 on day four. In the case of the three and six-hour stressed fish, the L value did not suggest chalkiness had developed while the pH did.

Lactate levels in the three-hour fish (1.65 mg/kg tissue) and six-hour fish (1.78 mg/kg tissue) were slightly higher than the average lactate levels in the other fish from the same treatment group (1.47 and 1.67 mg/kg tissue, respectively). The twelve-hour fish had much higher lactate levels of 2.58 mg/kg tissue compared to the average 1.38 mg/kg tissue of the remaining fish in that treatment.

Observation indicated that all fillets from these three halibut had some level of chalkiness. The three- and twelve-hour fish had pockets of opaque flesh surrounded by unaffected muscle. It was estimated that 40% of the fillets were chalky. The six-hour fillet exhibited a chalkier condition, with 80% of the surface opaque. Comparing the visual examination and the chemical and physical data shows a confusing picture of the development of chalkiness in these fish. L values did not indicate chalkiness in three- and six-hour fish, while pH levels did. Twelve-hour fish had a high muscle lactate but did not appear to be very chalky. The high initial pH of the three- and twelve-hour fish affected the development of chalkiness.

Since these fish developed chalkiness between 72 and 96 hours post mortem, histological samples were not collected. Meaningful histological samples would require differentiation between normal cell degradation and that caused by the chalky condition. The general organ structure may be interpretable with low power magnification, but the cellular and subcellular details of tissue chalkiness would not be visible at higher magnifications. Future studies should observe

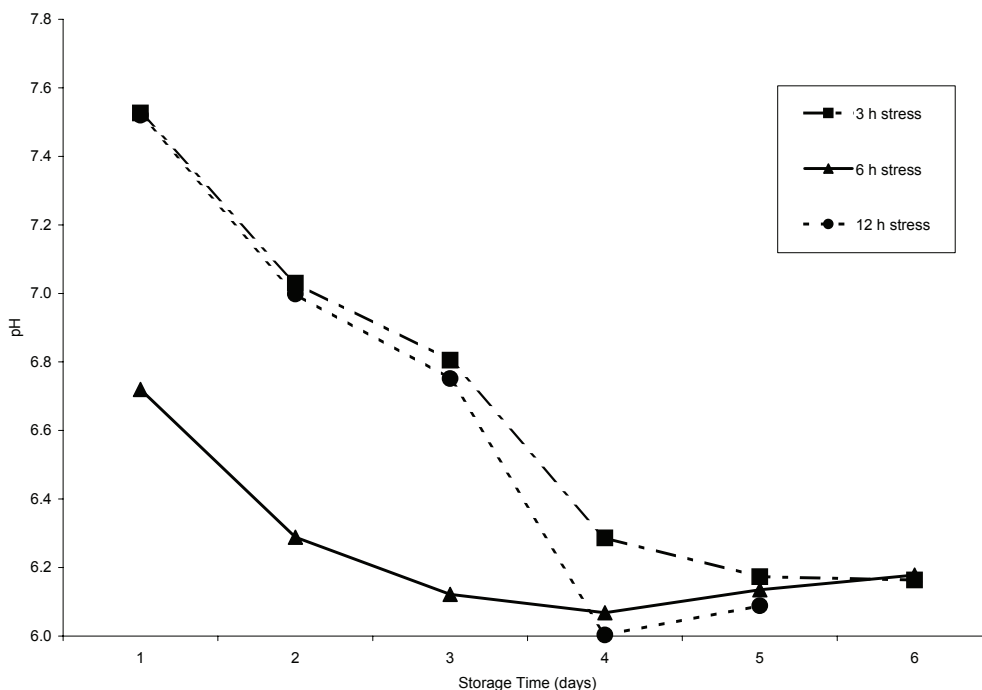


Figure 2. pH values of control and stressed halibut from one fish from each of three treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments. The muscle from these three fish achieved a chalky state after the stress experiments.

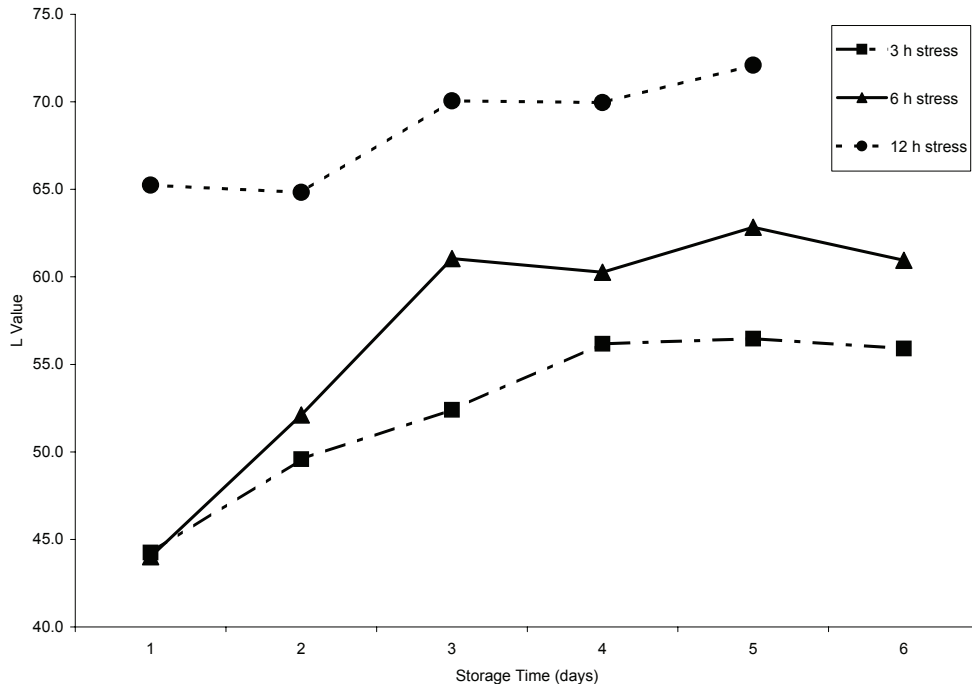


Figure 3. Color (L) values of control and stressed halibut from one fish from each of three treatment groups. Data represent the mean value of measurements taken from two fillets each day after treatments. The muscle from these three fish achieved a chalky state after the stress experiments.

cellular structure during the onset of chalky condition while assessing the normal degradation of a control group over time. Understanding the changes in postmortem halibut muscle structure during chalky development may elucidate the process of chalky development.

Conclusions and recommendations

The failure of the experimental design to produce chalkiness in most experimental halibut was unexpected. No differences existed between control and treated fish. Of the 36 fish treated by simulation of capture stress or temperature abuse, only three developed chalkiness. During the acclimation period, animals appeared healthy, swam frequently, and ate food when it was provided. The capture stress tests were conducted as described, with the fish exhibiting exhaustion after all treatment durations.

The cause for limited chalky development in halibut muscle may be due to the underlying condition of the fish. While the fish appeared in good shape, the proximate analysis revealed a higher moisture content and lower protein and lipid levels when compared to published values. The lower lipid content in the muscle may indicate that the fish were utilizing lipid stores due to low prey resources. The low muscle glucose would also limit the potential for chalkiness. Tomlinson (1966a) and Patashnik (1966) both suggest that glycogen levels must be high or chalkiness is less likely to occur. Lactic acid is formed as the result of anaerobic metabolism of glucose. Lower amounts of glucose do not allow for high levels of lactic acid and, therefore, a reduced chance of chalkiness.

The timing of chalky muscle in halibut caught around Kodiak is not well described. Chalky muscle in halibut has been known to show up in fish around Kodiak Island in September (Kaimmer

2000) but it is not well known how long this persists. Fish caught later in the fall (as in this study) may have other physiological conditions that could confound an understanding of chalky development. These may include changing prey resources as fish migrations are affected by the onset of winter and reproductive development. Several of the female fish in this study had well developed roe sacs indicating a move to spawning conditions and the associated physiological changes typical during the winter.

The initial high muscle pH of most of the fish is not unusual but suggests that chalkiness would be more difficult to attain in these fish. While typical halibut should have pH of 6.7, some of our control fish measured up to pH 7.5. This is almost a ten-fold decrease in the muscle acids. This also provided considerable buffering capacity for any acid that was produced during our stress tests. Had the pH been within expected ranges, more fish might have entered the range where chalkiness could develop. A possible reason for the high pH was that these fish had indeed recently eaten and their pH increased as a result.

While we observed considerable struggle during the simulated capture stress tests, perhaps there was not sufficient stress to force the fish into anaerobic glycolysis. Many of the fish appeared to be totally exhausted when removed from the tanks after tests, but it may have been the result of aerobic exercise only.

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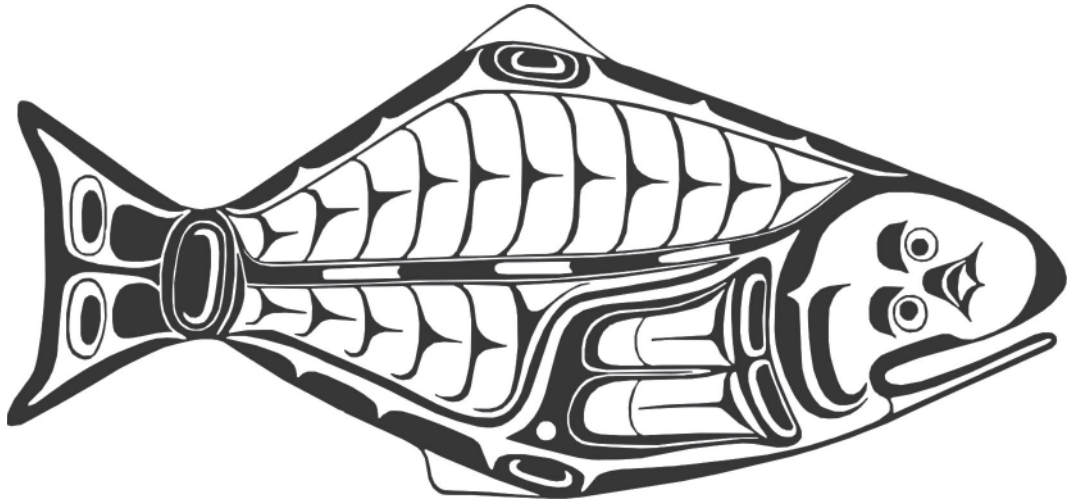
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HALIBUT CREST - adapted from designs used by Tlingit, Tsimshian and Haida Indians