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Live chilling of turbot and subsequent effect on behaviour, muscle stiffness, muscle quality, blood gases and chemistry

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Abstract

*During the commercial slaughter of farmed turbot (*Scophthalmus maximus*), a total of 67 fish were, on six occasions, removed from their rearing conditions at 14°C and put, as is standard commercial practice, into chilled seawater (–1.5 to –0.8°C) to monitor behavioural, muscular, osmoregulatory and respiratory responses during chilling time (90 min). Results show that a thermal insult alters the iso-osmotic balance, leading not only to an Na^+ influx and an intracellular release of Ca^{2+} and K^+, but also to a disturbance of respiratory function, leading to acidosis as a result of H^+ and CO_2 accumulation, increased pCO_2 and reduced HCO_3^- in the blood. Once the internal temperature dropped below 1°C, the muscles contracted (cold shortening) and, although the fish were still alive, they reverted to a state of rigor, leading to a complete breakdown in their ability to move or ventilate and resembling an unconscious condition or death. Remarkably, the fish were able to prevent themselves undergoing hypoxia as pO_2 remained within acceptable limits. No changes in muscle pH were observed and, thus, no noted effects on textural properties. We conclude that live chilling from 14°C to approximately –1°C is a highly questionable practice. It causes physical and physiological changes that are generally associated with stress and, in the case of observed forced muscle contractions, could lead to severe pain. Furthermore, we conclude that cold shortening associated with chilling can be easily mistaken for* rigor mortis *and, as such, should be subject to further attention in future research on quality.*

Keywords: *animal welfare, hypothermia, live chilling, quality, stunning, turbot*

Introduction

Fish generally tend to be immobilised when exposed to ice water, with death being ensured by asphyxiation on ice (Robb & Kestin 2002). The impact of this process on the welfare of the fish is yet to be fully documented but it is generally considered to have a detrimental effect (van de Vis *et al* 2003). Turbot are known to be extremely tolerant of thermal insult and, in commercial practice, are often chilled, transported live on ice and sold live in, for example, Asian markets. A number of authors have dismissed this method of stunning on welfare grounds as it does not act immediately, it appears to cause the animals great stress and provokes an earlier onset of *rigor mortis* (Ruff *et al* 2002; Morzel *et al* 2003; Roth *et al* 2007). Previous studies on stunning with ice using EEG have shown that gilthead seabream (*Sparus aurata*) lose consciousness within 5 min (van de Vis *et al* 2003) and eel (*Anguilla anguilla*) exposed to –9°C heavily salted water (salt brine) lose consciousness within 1 min (Lambooij *et al* 2002). Studying behavioural responses, Morzel *et al* (2003) reported that turbot, exsangiunated in ice slurry, lost consciousness at between 15–90 min. However, previous studies on cold shocking of sub-tropical and temperate species, such as Red Sea bream (*Pagrus major*), bighead (*Aristychthys nobilis*) and Japanese flounder (*Paralichthys olivaceus*) have shown that muscles contract in direct response to low temperature; also known as cold shortening (Curran *et al* 1986; Parry *et al* 1987; Lee *et al* 1998). It has been well documented that a thermal shock alters the homeostatic balance within the fish, either by affecting gill membranes directly or having an indirect effect via a temperature-dependent elevation of physiological plasma cortisol (Tanck *et al* 2000; Rorvik *et al* 2001). The question that then arises is how exactly a thermal insult affects respiratory function and whether or not fish undergo respiratory failure, followed by hypoxia or hypercapnia which ultimately impacts upon the quality of the end product. Thus, this study sought to investigate the effect of subzero temperatures on behaviour and respiratory function in turbot farmed in two different temperature zones (Iceland and Portugal) and consider the effects of the associated stress on meat quality.

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Table 1 Behavioural and muscle responses in turbot during chilling.

Chilling time (min)	Internal temperature (°C)	Behavioural responses			Muscle responses		
		Handling (0–2)	Eye (0–2)	Gill (0–2)	Reflex (0–2)	Rigor (0–2)	Gaping (0–1)
0	14.1	2/2	2/2	2/2	2/2	0/0	0/0
10	4.05	1/1	1/1	1/1	1/1	0/0	0/0
20	2.85	1/1/1	1/0/1	0/0/0	1/1/1	0/0/1	0/0/0
30	1.60	1/1/1	0/1/0	0/0/0	0/1/1	1/1/0	1/0/0
40	0.75	0/0	0/0	0/0	1/1	1/1	0/1
50	0.73	0/1/1	0/0/0	0/0/1	0/0/1	1/1/1	1/1/0
60	0.35	0/1	0/0	0/0	0/0	1/1	1/1
70	0.63	0/0/1	0/0/0	0/0/0	0/0/1	2/2/1	1/1/0
80	0.25	0/0	0/0	0/0	0/0	2/2	1/1
90	0.27	0/0/0	0/0	0/0/0	0/0/0	2/2/2	1/1/1

For behaviour responses, 2 represents normal responses for handling, eyes and gill ventilation, while 0 represents no response at all. For muscle responses, 2 represents normal reflex while 0 no reflex at all. For rigor, 0 represents flexible muscle, while 2 represents full rigor. Zero in gaping represents flexible jaws, while 1 represents full muscle contraction of the jaws and gaping. Each number represents 1 fish.

Figure 1

Materials and methods

A total of 67 farmed turbot (*Scophthalmus maximus*) were, on six occasions, removed from rearing tanks at either Silfurstjarnan, Iceland (mean weight 1107 [± 123] g; n = 25) or A Coelho e Castro, Portugal (1298 [± 298] g; n = 42) and placed into ice-water bins at temperatures of –1.4 to –0.8°C, respectively. These bins consisted of a tank (1.0 × 0.8 × 0.8 m; length × width × height) filled with seawater and (freshwater) ice, maintaining a salinity that ranged from 24% (Portugal) to 31% (Iceland) and temperature and oxygen levels as seen in Table 1. The water in the rearing tank at the Icelandic site consisted of thermal ground heated seawater holding a temperature of 14.1°C, mixed with freshwater, providing salinity equal to 22.5%. The pH of the water entering the tank was 7.72 and exiting, 7.14. In the Portuguese site, seawater was pumped from a depth of 40 m, holding 34% salinity, and the temperature was 13.9°C. The water pH was 7.6.

The experimental procedure

On each occasion, 9–14 fish were placed into the ice-water bins and at 10 min intervals, one fish was removed from the tank and behavioural responses measured, before blood sampling occurred. The total duration of the experiment was 90 min. Internal temperature was measured by placing a soft probe into the oesophagus, and, once blood sampling had been carried out, fish were returned to water equal to rearing conditions, in order to ensure any mortality observed was due to thermal insult. In Portugal, all fish were euthanased with a blow to the head, prior to the measurement of muscle pH, and packed in ice in polystyrene boxes before shipping to Norconserv, Stavanger, Norway for texture analysis, seven days later.

Behavioural analysis

Behavioural analysis was carried out in accordance with Morzel *et al* (2003), whereby eye, muscle and gill responses were determined on a scale from 0 to 2 to describe the level of consciousness. Due to the fact that muscles can be affected by low temperature, leading to stiffness, it was decided that mouth gaping and body stiffness would also be evaluated. Mouth gaping (see Figure 1) was rated as 0 (no gaping) or 1 (gaping), while stiffness fell into one of three categories: 0 (no rigor); 1 (partially in rigor) and 2 (full rigor).

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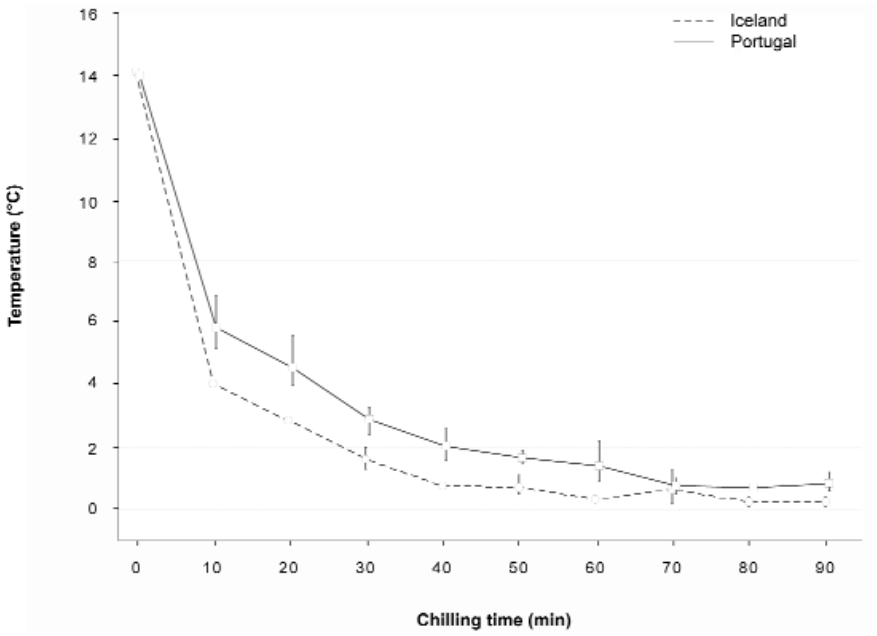
Figure 2

Mean (± SE) internal temperature as a function of chilling. Fish from Iceland were exposed for –1.5°C water while fish in Portugal were exposed to –0.8°C.



Blood analysis

Blood was extracted from fishes' caudal vessels and analysed using an i-STAT® Portable Clinical Analyzer (Abbot Inc, NJ, USA). Blood was heated automatically to 37°C before undergoing analysis for pH, Na^+, K^+, haematocrit (Hct), haemoglobin (Hb), glucose (Glu), pCO_2, HCO_3^- and total CO_2, (TCO_2). In addition, pO_2, SO_2 and ionised Ca^{2+} levels were ascertained from the experiments in Portugal.

Extensive literature reviews were unable to provide any reliable sources for calculating real time blood gas pressures and H^+ concentrations from 37°C down to subzero temperatures, therefore blood gas results are presented as raw data.

Muscle pH

For measuring muscle pH, an X-Mate portable meter and Inlab 489 pH probe (Mettler Toledo Inc, NY, USA) were used. Measurements were taken in white muscle tissue at the cranial part of the dorsal backs, perpendicular to the abdominal cavity.

Measurement of meat texture

For measurement of texture, both dorsal backs, ie the white and dark side, were filleted off the fish and used for measuring shear force (blade) and hardness (puncture), separately, using an TA-XT2® Texture Analyzer (Stable Micro Systems, London, UK) with a load cell of 50 kg. For shear force measurements, standard muscle samples (69 × 26 mm) were cut both anterior and posterior to the midpoint of the upper dorsal fillet (Roth *et al* 2007). Each muscle sample was sliced in two separate locations, providing a total of four shear force samples per fillet; anterior to posterior (1–4). The muscle samples were sliced with a 3 × 70 mm flat-bedded blade with a 60º knife edge.

Measurements of texture hardness were carried out in accordance with Roth *et al* (2007), compressing the fillets at a constant speed of 1 mm s^{-1} with a flat bedded cylinder (20 mm in diameter). Penetration depth was set at 80% of fillet height and breaking and maximum force (N·m) were recorded to define hardness. Texture profile was sampled at four separate locations (1–4) directly on the lower dorsal back, beginning anteriorly and moving backwards with a distance of 1 cm between each puncture test (Roth *et al* 2007).

Statistical analysis

A general linear model (GLM) was used for analysing the continuous and dependent variables such as blood pH, sodium, potassium, Hct and glucose against independent variables including both categorical (Iceland versus Portugal) and continuous (chilling time), testing both similarities and differences in the slope using a separate slope model. To measure continuous dependent variables originating from one experiment, such as Ca^{2+}, muscle pH, pO_2, SO_2, shear force and hardness, linear regression was used as the statistical model.

Results

Since the experimental water in Iceland was colder than in Portugal and fish were on average 180 g lighter ($P < 0.05$), it was unsurprising that the chilling rate for fish in Iceland was faster than in Portugal ($P < 0.05$) (see Figure 2).

Immediately after being placed in ice water, turbot performed a flight reaction for a few seconds, before quickly settling at the bottom. One fish died during the thermal treatment and blood analysis showed pO_2, pCO_2, pH and K^+ levels significantly out of the range seen in live counterparts: pO_2 = 4 mm Hg, $pCO2$ = 63.9 mm Hg, pH = 7.14 and K^+ = 8.6 ppt.

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Table 2 Mean (± SE) weight, ionised calcium (i Ca) and haematocrit (Hct) for turbot taken from 14°C seawater and placed in subzero temperatures.

Country	Chilling time (min)	Weight (g)	i Ca (mmol l^{-1})	Hct (%)	n
Iceland	0	1,115 (± 130)	–	9.50 (± 1.50)	2
	10	1,117 (± 12)	–	9.50 (± 1.50)	2
	20	1,067 (± 22)	–	10.00 (± 1.00)	2
	30	1,158 (± 108)	–	11.33 (± 0.33)	3
	40	1,150 (± 20)	–	15.00 (± 1.00)	2
	50	1,150 (± 58)	–	13.67 (± 2.60)	3
	60	1,061 (± 135)	–	13.00 (± 0.00)	2
	70	1,226 (± 60)	–	12.33 (± 1.20)	3
	80	1,103 (± 72)	–	9.50 (± 0.50)	2
	90	1,138 (± 16)	–	13.00 (± 1.53)	3
Portugal	0	1,138 (± 60)	1.8 (± 0.06)	11.86 (± 0.49)	14
	10	1,383 (± 248)	2.0 (± 0.06)	13.33 (± 1.20)	3
	20	1,434 (± 14)	2.1 (± 0.05)	12.33 (± 1.20)	3
	30	1,453 (± 243)	2.2 (± 0.14)	13.33 (± 0.88)	3
	40	1,248 (± 160)	1.8 (± 0.08)	12.00 (± 1.15)	3
	50	1,366 (± 193)	1.9 (± 0.04)	12.00 (± 0.58)	3
	60	1,403 (± 69)	1.9 (± 0.06)	14.67 (± 0.88)	3
	70	1,274 (± 92)	2.0 (± 0.14)	12.33 (± 0.88)	3
	80	1,529 (± 291)	2.1 (± 0.12)	12.00 (± 0.00)	3
	90	1,278 (± 203)	1.8 (± 0.14)	12.00 (± 2.31)	3
Regression analysis: Iceland vs Portugal		$P < 0.0005$	–	$P > 0.19$	
Regression analysis: chilling rate		$P < 0.05$	$P > 0.76$	$P > 0.15$	

Behavioural analysis

Behavioural responses, such as respiratory ventilation, reflexes and responses to tactile sensation were easy to determine, whereas eye responses were far more difficult to record due to incomplete eye migration in many of the individuals.

Within 10 min of chilling, fish appeared sedated with only minor responses to handling and/or blood sampling being demonstrated (Table 2). At the 30-min mark, fish responses were barely detectable and by 40–60 min, some fish appeared more dead than immobile. Once the internal temperature dropped below 2°C, stiffening of fish started to appear (see Table 2) and at or below 1°C we observed full muscle contraction with fish becoming totally stiff and often showing mouth gaping (Figure 1). Naturally, it was impossible to perform further observations once the muscles were fully contracted. All those fish placed back into 14°C water were seen to recover within 30 min.

Blood chemistry

Blood analysis showed that fish from Portugal had higher plasma sodium levels than those from Iceland ($P < 0.0005$, Figure 3) but that, irrespective of location, Na^+ accumulated with time in ice water ($P < 0.0005$, Figure 3). Similarly, potassium levels also accumulated with chilling time ($P < 0.0005$, Figure 4), and did so at a greater rate in Portugal compared to Iceland ($P < 0.05$). The thermal insult caused an increase in ionised blood Ca^{2+} levels during the first 10 min, and remained at a constant, high level throughout the experiment ($P > 0.76$, Table 1).

Live chilling lead to elevated plasma glucose levels in the Portuguese sample over time ($P < 0.005$, Figure 5) but no differences were found between fish used in Iceland or Portugal ($P > 0.38$). Regarding Hb or Hct, no significant differences were observed between the two groups of fish ($P > 0.74$) and no changes were found during the chilling process ($P > 0.81$).

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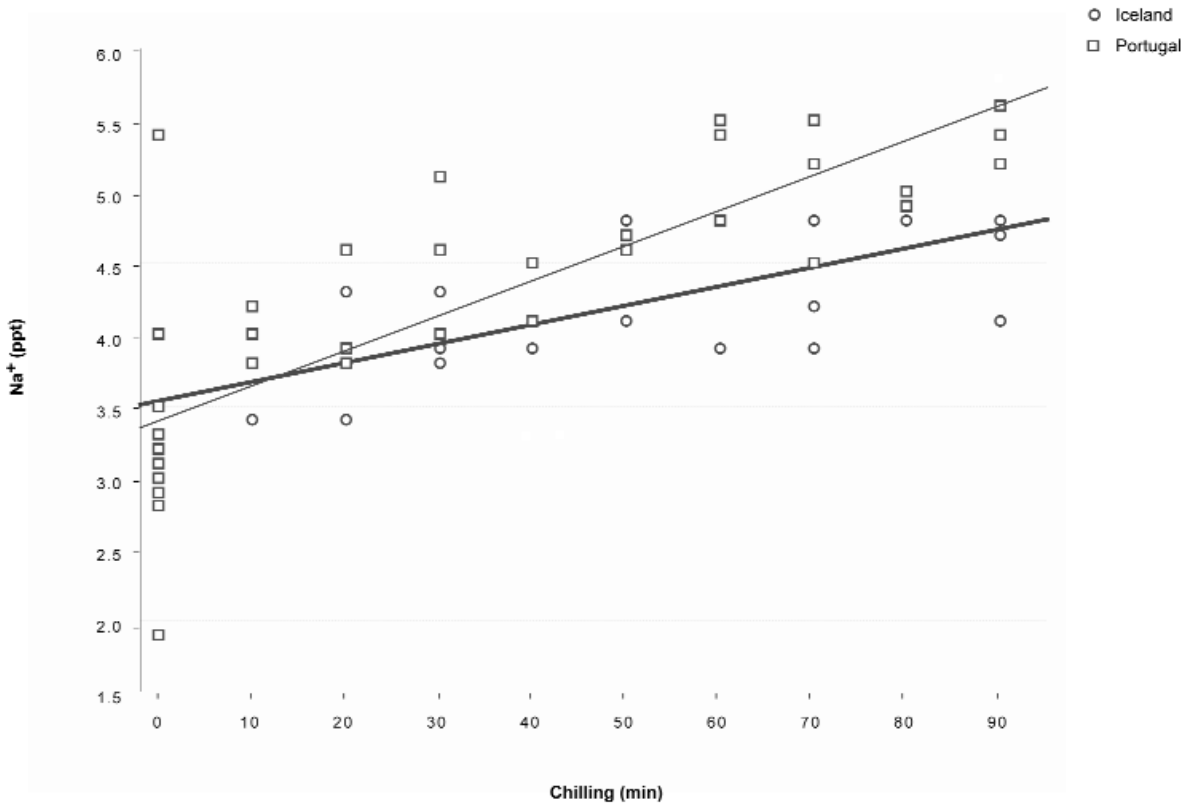
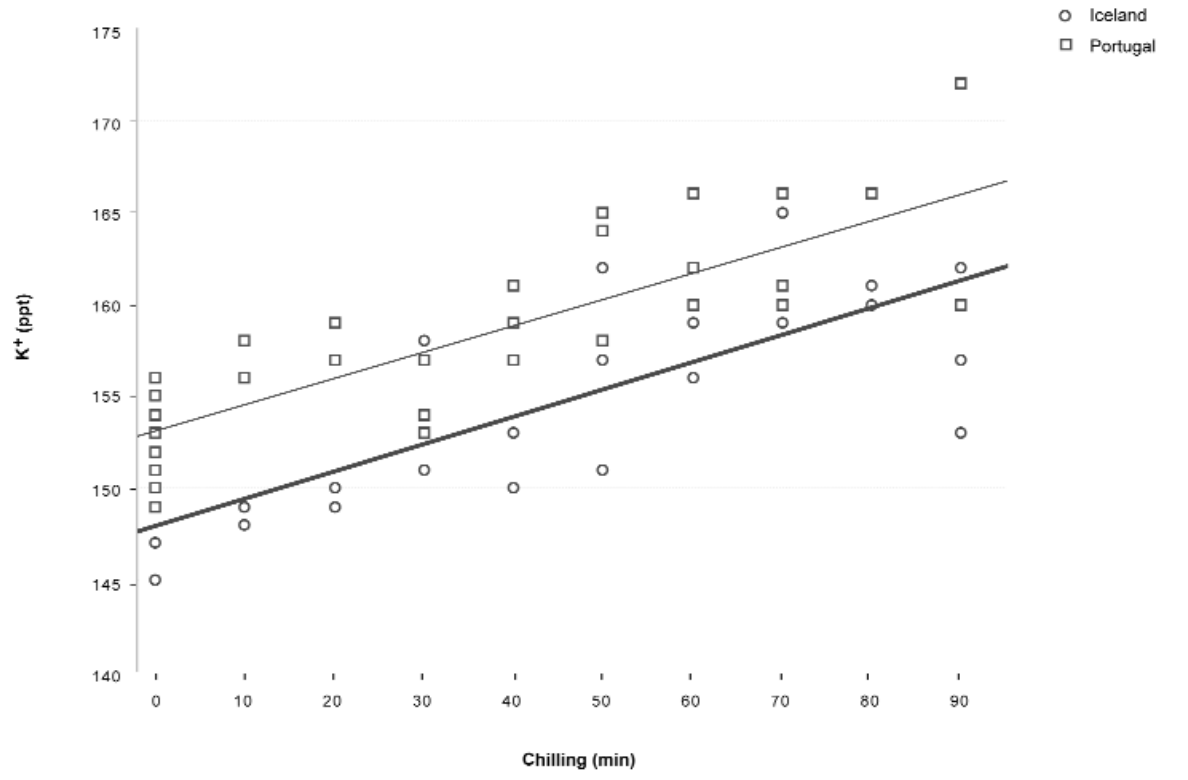
Figure 3



Mean (± SE) sodium levels for turbot taken from 14°C seawater and placed in subzero temperatures.

Figure 4



Mean (± SE) potassium levels for turbot taken from 14°C seawater and placed in subzero temperatures.

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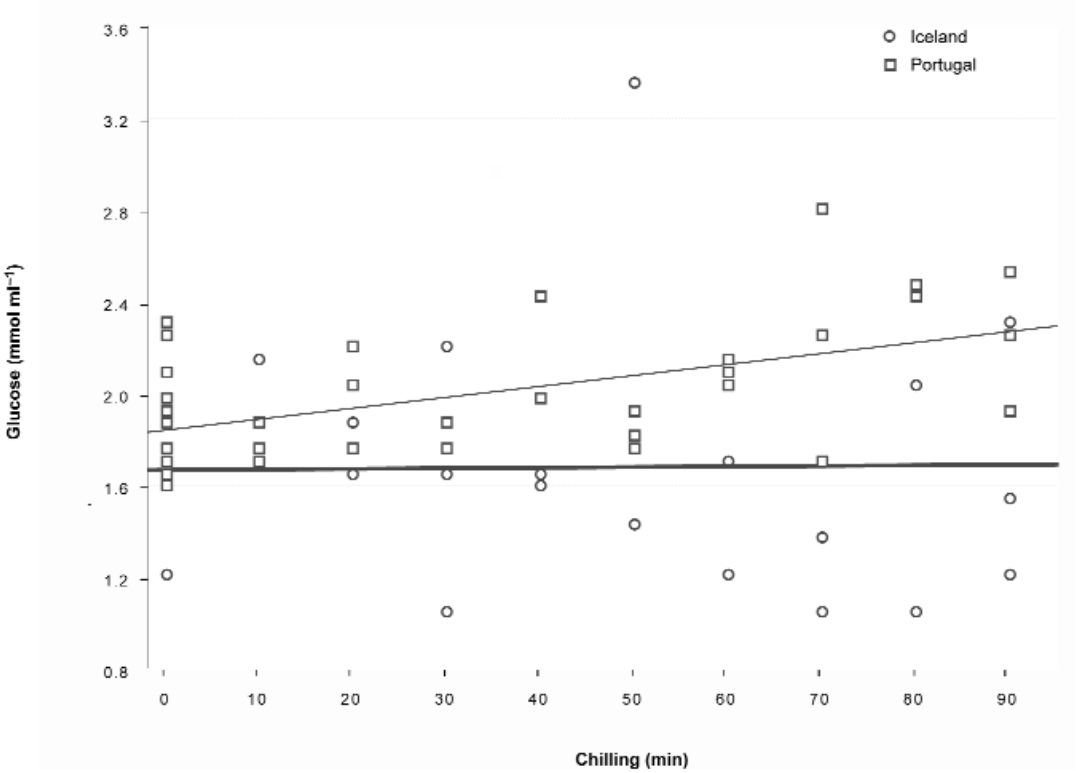
Figure 5



Mean (± SE) glucose levels for turbot taken from 14°C seawater and placed in subzero temperatures.

Muscle and blood pH

Blood pH dropped significantly over time during chilling for both experiments ($P < 0.0005$, Table 3), but the decrease was more rapid in fish from Iceland, compared to those in Portugal ($P < 0.05$). Muscle pH appeared unaffected by the chilling process ($R = 0.14$, n = 40, $P > 0.38$, Table 4).

Blood gases

During chilling, pCO_2 was seen to increase ($P < 0.0005$, Table 3), and this occurred to a greater extent in fish from Portugal, compared to Iceland ($P < 0.05$). Icelandic fish displayed a significant drop in bicarbonate levels after only 10 min of hypothermic exposure ($P < 0.005$), whilst in Portugal only a minor reduction in HCO_3^- was observed during chilling ($P < 0.05$). Total CO_2 levels remained more or less constant during the entire chilling period ($P > 0.13$), as did oxygen levels ($P > 0.45$, n = 34).

Flesh quality

Table 4 shows that both shear force ($P > 0.90$, n = 160) and hardness ($P > 0.41$, n = 158) were unaffected by duration of chilling.

Discussion

The monitoring of physical and physiological responses of turbot to extremely cold temperatures sheds no light on whether the animal is merely stunned or dying. In keeping with previous findings in Atlantic salmon (*Salmo salar*) (Skjervold *et al* 2001), turbot display stress responses, followed by elevated glucose and difficulties maintaining the ionic balance. Despite these effects, it appears that turbot handle these extreme conditions rather well from a physiological perspective. Although the fish were stiffened physically and prohibited from ventilating, no evidence of hypoxia was observed and only temporary problems in maintaining ionic balance. A mild case of hypercapnia and acidosis was also observed. The actual cause of this remarkable ability to withstand these extremes is unclear. To the authors' knowledge, there have been no such studies on fish, but research into other exothermic animals, such as frogs, show that these animals can suppress aerobic metabolism in skeletal muscle in order to conserve energy (Boutilier *et al* 2000; Donohoe *et al* 2000). In general terms, short-time mechanisms which facilitate survival of hypothermic and/or

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Table 3 Mean (± SE) blood pH, pCO_2, pO_2, HCO_3^- and total CO_2 of turbot taken fron 14°C seawater and placed in sub-zero temperatures.

Country	Chilling time (min)	pH	pO_2 (mm Hg)	pCO_2 (mm Hg)	HCO_3^-(mmol l^{-1})	TCO_2 (mmol l^{-1})	n
Iceland	0	7.46 (± 0.02)	–	27.8 (± 1.9)	19.7 (± 0.6)	20.5 (± 0.5)	2
	10	7.29 (±0.07)	–	27.0 (± 0.9)	13.0 (± 1.6)	13.5 (± 1.5)	2
	20	7.31 (± 0.09)	–	30.1 (± 6.1)	14.9 (± 0.1)	16.0 (± 0.0)	2
	30	7.10 (± 0.02)	–	34.2 (± 5.3)	10.4 (± 1.1)	11.7 (± 1.2)	3
	40	7.21 (± 0.08)	–	31.5 (± 0.2)	12.9 (± 2.4)	13.5 (± 2.5)	2
	50	7.16 (± 0.06)	–	36.2 (± 4.4)	13.0 (± 1.7)	14.0 (± 1.5)	3
	60	7.15 (± 0.03)	–	31.3 (± 3.8)	11.1 (± 2.0)	12.0 (± 2.0)	2
	70	7.03 (± 0.02)	–	37.0 (± 9.2)	9.6 (± 1.9)	10.7 (± 2.2)	3
	80	7.09 (± 0.01)	–	35.0 (± 4.7)	11.6 (± 2.6)	12.5 (± 2.5)	2
	90	7.10 (± 0.07)	–	38.8 (± 4.3)	11.9 (± 1.2)	13.3 (± 1.2)	3
Regression analysis		$P < 0.0005$		$P < 0.05$	$P < 0.05$	$P < 0.05$	
Portugal	0	7.46 (± 0.01)	35.3 (± 2.7)	28.0 (± 0.6)	20.0 (± 0.4)	20.8 (± 0.5)	14
	10	7.32 (± 0.00)	37.0 (± 15.0)	38.1 (± 2.6)	19.7 (± 1.3)	20.7 (± 1.2)	3
	20	7.28 (± 0.01)	40.0 (± 3.8)	42.1 (± 4.5)	19.7 (± 1.6)	21.0 (± 1.7)	3
	30	7.36 (± 0.03)	–	35.8 (± 4.0)	19.9 (± 1.3)	20.7 (± 1.5)	3
	40	7.35 (± 0.01)	27.5 (± 5.5)	40.5 (± 4.8)	21.8 (± 1.7)	23.3 (± 1.9)	3
	50	7.42 (± 0.15)	44.0 (± 1.7)	38.2 (± 3.3)	18.3 (± 0.4)	19.3 (± 0.3)	3
	60	7.26 (± 0.04)	30.0 (± 1.5)	42.5 (± 4.2)	18.9 (± 0.4)	20.3 (± 0.3)	3
	70	7.29 (± 0.04)	32.0 (± 7.0)	39.7 (± 4.6)	18.7 (± 0.8)	20.0 (± 1.2)	3
	80	7.26 (± 0.03)	29.0 (± 6.0)	45.5 (± 2.9)	20.3 (± 2.7)	21.5 (± 2.5)	3
	90	7.28 (± 0.04)	31.5 (± 0.5)	44.0 (± 2.8)	20.8 (± 0.8)	21.7 (± 0.7)	3
Regression analysis		$P < 0.0005$	$P > 0.45$	$P < 0.0005$	$P > 0.82$	$P > 0.84$	

Values are given as raw data, measured with I-stat portable meter at 37°C.

Table 4 Mean (± SE) muscle pH, hardness, fracture strength and shear forces of turbot taken from 14°C seawater and placed in subzero temperatures.

Chilling time (min)	Muscle pH*	Hardness (N)	Fracture (N)	Shear force (N)	n
0	7.10 (± 0.03)	111 (± 4.4)	84 (± 3.8)	72 (± 2.9)	32
10	7.20 (± 0.06)	115 (± 9.9)	92 (± 6.0)	78 (± 9.6)	12
20	6.90 (± 0.10)	138 (± 12.7)	119 (± 6.3)	86 (± 4.3)	12
30	7.20 (± 0.02)	138 (± 11.2)	120 (± 6.0)	85 (± 7.2)	12
40	7.10 (± 0.10)	107 (± 6.7)	98 (± 6.1)	73 (± 4.6)	12
50	7.10 (± 0.09)	112 (± 10.2)	98 (± 6.0)	67 (± 3.1)	12
60	7.10 (± 0.13)	126 (± 9.2)	107 (± 6.0)	90 (± 6.3)	12
70	7.10 (± 0.12)	106 (± 6.6)	91 (± 6.0)	66 (± 2.3)	12
80	7.10 (± 0.09)	105 (± 4.7)	99 (± 6.1)	71 (± 3.9)	12
90	7.00 (± 0.14)	109 (± 8.6)	92 (± 6.0)	72 (± 3.6)	12

* Muscle pH was measured during the experiment while texture properties were measured after seven days storage in ice.

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hypoxic environments seem to be based upon the ability to switch to anaerobic respiration, whilst longer-term strategies, as seen in hibernating animals, rely on combining a suppression of aerobic respiration with hypothermia. It is a reasonable assumption that turbot, a marine species, do not possess the same capability to hibernate as frogs and other hibernating fish species such as carp (*Cyprinus carpio*) and tench (*Tinca tinca*), yet, despite this, turbot are still sold live after several days being transported in air and on ice; a highly unlikely scenario for other commonly cultured species, for example, salmon and sea bass (*Sparus aurata*). Although our research into blood gas and muscle pH demonstrate that turbot are capable of suppressing anaerobic metabolism, it remains unknown whether this mechanism occurs as a direct result of a physiological response or simply that the temperature within the fish has become so low that any metabolic activity is inhibited.

Apparently, seroplasmic reticulum membrane loses the ability to withhold Ca^{2+} and this, combined with the inhibition of both Mg^{2+}/Ca^{2+} and Na^{+}/Ca^{2+} ATPases, leads to the formation of actin and myosin bonds — causing muscle contraction (Ushio *et al* 1991). This may explain why levels of intracellular ions, such as K^{+} and Ca^{2+} increased in the blood during chilling. Although a thermal insult would be expected to cause a physiological stress response, from a welfare perspective, it is of considerable concern that muscle begins contracting while the fish are still alive. Although forced muscle contractions and a cold shock can be regarded as being painful for humans, it remains a matter of great debate whether fish can feel pain and thus suffer (Rose 2002, 2007; Braithwaite & Boulcott 2007). If we assume that they do feel pain, that they are conscious, and that both nociception and nerve signaling to the brain are intact during the period of forced muscle contraction, live chilling — as practiced here — can be considered to impact grossly on welfare. However, for ectothermic animals, such as fish, pain, related to a 15°C temperature drop, may be difficult to assess since it has been shown that fish have a thermal nociception for heat (Sneddon 2003), but not for chilling (Ashley *et al* 2007).

Regarding the live chilling of Atlantic salmon, several authors have described a positive correlation between muscle pH and meat quality, thereby indirectly recommending this practice from a welfare perspective (Skjervold *et al* 1999, 2001; Erikson *et al* 2006). However, practices vary widely between facilities, including differences in seawater temperature, chilling temperatures ranging from –0.5 to 2°C (Skjervold *et al* 2001) and whether or not oxygen or CO_2 are added to the process (Skjervold *et al* 2001; Erikson *et al* 2006; Roth *et al* 2006). As this study demonstrates, the use of quality as a welfare indicator can be highly misleading when determining fish well-being at the point of death. In fact, results in Table 1 clearly suggest that temperate species, such as turbot, can undergo cold shortening similar to that reported in warm water species (Curran *et al* 1986). This evidence should be considered in quality research as well since an early onset of *rigor mortis* might occur as a result of chilling temperatures rather than low energy levels at the point of death.

In terms of welfare, we conclude that dropping 15°C to subzero temperatures is a highly questionable practice which involves the release of primary and secondary stress responses, as well as forcing the muscles to contract which can be associated with severe pain. Muscle pH and pO_2 levels revealed that the fish did not undergo hypoxia and the flesh quality remained unaffected. However, there was clear evidence of cold shortening in turbot that can easily be mistaken for *rigor mortis*. More effort is needed therefore to help understand and map the extent to which different fish species are able to withstand ranges in temperature, before they are subjected to extreme practices such as live chilling.

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