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Environment affects stress in exercised turbot

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Abstract

We investigated the interaction of water temperature (10, 18 and 22 °C) and salinity (33.5 and 15‰) on the stress response of juvenile turbot. At each temperature/salinity combination, fish were subjected to 10 min enforced exercise. This induced a moderate stress response, which differed at the various temperature and salinity combinations. High temperatures caused more rapid increases in plasma cortisol and glucose, larger and more rapid increases in plasma lactate levels, which were also influenced by body weight, and a faster recovery in plasma Na^+ and Cl^-. Low salinity ameliorated cortisol responses at low but not at high temperatures. The magnitude of ionic disturbance was reduced at 15‰. Plasma K^+ did not change at any temperature or salinity. The stress response involved activation of the brain–pituitary–interrenal axis, as indicated by the cortisol elevations. The low magnitude of glucose responses, the mild Na^+ and Cl^- disturbances, and the lacking K^+-responses indicated mild activation of the brain–sympathetic-chromaffin cell axis, and hence a low release of catecholamines, which seemed though to occur to a higher extent at higher temperatures. The relatively low catecholaminergic response of turbot may be linked to their inactive sedentary lifestyle. The higher responsiveness at higher water temperatures may reflect a higher overall adaptive capacity.

Keywords: Cortisol; Enforced exercise; Glucose; Osmoregulation; Salinity; Stress response; Temperature; Turbot

1. Introduction

Stress in teleost fish is generally characterized by a set of changes in the normal metabolism, the stress response, thought to be compensatory and/or adaptive and enabling the animal to cope with stressors. The stress response in fish is usually divided into primary, secondary and tertiary responses (Schreck, 1982; Barton and Iwama, 1991). Primary responses include the activation of the brain–sympathetic-chromaffin cell axis and the brain–pituitary–interrenal axis, resulting in the release of stress hormones (catecholamines, or CAs, and corticosteroids). Secondary responses are the actions and effects of these hormones at blood and tissue levels, including mobilization of energy substrates and disturbance of hydromineral bal-

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ance. Tertiary stress responses are on the level of whole organisms and/or populations, and include inhibition of growth and changes in metabolic rate (Specker and Schreck, 1980; Barton and Iwama, 1991; Wendelaar Bonga, 1997).

A large part of research on stress in fish has been performed on salmonids, tilapia species and carp, and other teleosts are often assumed to respond in a similar way to stressors. However, the validity of this assumption can be challenged, since even closely related species may show differences in their responses (Pankhurst et al., 1992; Pickering, 1998). Until now, only few papers have described stressor-effects in turbot, a demersal, euryhaline and eurythermal flatfish with an inactive and sedentary lifestyle (e.g. Staurnes, 1994, 2001; Waring et al., 1996, 1997; Mugnier et al., 1998; Person Le Ruyet et al., 1998). These studies were performed under various conditions, i.e. with cannulated or uncannulated fish after exposure to different stressors in a temperature range of 10–20 °C. The water temperature is an important modifying factor of the stress response in fish, but reported temperature effects are not consistent. At higher water temperatures, both higher and lower resting levels of stress parameters have been reported, as well as higher rates of increase and recovery, and/or higher magnitudes of stress responses (e.g. Barton and Schreck, 1987; Davis and Parker, 1990; Ryan, 1995; Pottinger et al., 1999). Apart from the different water temperatures applied in the various studies on this subject, differences in species and applied stressors may also account for some of the reported differences in temperature effects.

None of the studies on stress in turbot has examined the possible influence of water salinity on the stress response of this species. In several fish species hydromineral disturbance during stress conditions may be reduced when the external salinity is nearly iso-osmotic with the blood plasma of the fish (e.g. Redding and Schreck, 1983; Reubush and Heath, 1997). This has been attributed to a lower energetic cost of ion regulation in iso-osmotic environments, where the ionic gradients between blood and water are usually minimal (Morgan et al., 1997). It has been hypothesized that this energy saving may be substantial enough to increase growth (Morgan and Iwama, 1991; Likongwe et al., 1996; Imsland et al., 2001). Lower water salinity does indeed ameliorate growth performance of turbot. Imsland et al. (2001) demonstrated that the optimal growth temperature for turbot varies with water salinity: growth, feed consumption and feed conversion efficiency were higher at 15‰ than at full salinity, and an interactive temperature–salinity effect was found at temperatures between 18 and 22 °C. It was concluded that growth and feed conversion of turbot juveniles is better at intermediate salinities in the upper temperature range.

In this paper we present a follow-up study of the experiments described by Imsland et al. (2001). The objectives were to investigate (i) whether different combinations of water temperature and salinity would influence the stress response of the fish, and (ii) if different stress parameters are influenced differentially by different combinations of water temperature and salinity. We used enforced exercise to evaluate the stress response in juvenile turbot, which is a handling stressor that has not been studied in this species before. We characterized the rate, magnitude and recovery of the adaptive stress response of the fish by measuring survival as well as several well-established primary and secondary stress parameters (cortisol, glucose, lactate and ions) in blood plasma.

2. Materials and methods

2.1. Animals

Juvenile 0-group turbot (*Scophthalmus maximus* Rafinesque), which were the offspring of wild-caught parents of Norwegian origin, were obtained from Stolt Seafarm in Kvinesdal, Norway. The animals had an initial body mass approximately 10 g and were kept in 1-m^2 square, grey, covered fiberglass tanks (400-l water volume, oxygen saturation >80%, 62–63 fish per tank) at the High Technology Center in Bergen, Norway, with flow-through seawater and a light regime of 18 h light and 6 h darkness (L:D=18:6). Prior to the stress experiments described here, the fish were used in a 3-month growth study. The fish were reared at temperatures of 10 ± 0.2, 18 ± 0.3 and 22 ± 0.2 °C (means±S.D.), and salinities of 15 ± 0.4 and 33.5 ± 0.1‰ at each temperature. All treatment groups consisted of two replicate tanks. In this period, water flow was initially set to 4 l/min for all experimental tanks and increased gradually up to 10 l/min at the end of the experiment. Throughout the growth period the fish were left undisturbed

apart from daily routine husbandry and monthly weightings to monitor the growth. During the growth period the fish were fed ad libitum with a commercial formulated feed (Supra Marin, Nor-Aqua A/S, Bergen, Norway) containing 55% protein, 12% fat and 15% carbohydrate. For more details, see Imsland et al. (2001).

2.2. Experimental setup

After the last growth measurements the fish were left undisturbed for another 10 days under the same rearing conditions and feeding regime. By that time the fish had reached the following mean weights (±S.D.): (i) 10 °C-33.5‰: 33.9±7.9 g, (ii) 10 °C-15‰: 36.6±7.4 g, (iii) 18 °C-33.5‰: 111.9±60.6 g, (iv) 18 °C-15‰: 141.1±58.0 g, (v) 22 °C-33.5‰: 189.9±24.7 g, and (vi) 18 °C-15‰: 107.3±21.2 g.

Prior to the start of the stress experiment, pre-stress (PS) samples were taken from 8 fish at each temperature/salinity combination. At the start of the experiment, all fish in one of each replicate tank were forced to swim actively in their tank for 10 min by chasing them manually with a dip net. At the end of this period all the fish had lost equilibrium and did not longer respond to the manual chasing. The fish from the other replicate tank of each temperature/salinity combination were used as non-stressed controls. In line with studies by Kieffer et al. (1994, 2001), the amount of work done by the exercised fish was not quantified and this may have been influenced by temperature and/or salinity. Instead, we have exercised turbot to a behavioral state of exhaustion at each temperature/salinity combination, and examined response magnitudes and recovery of physiological disturbances. Samples were taken from both control (CO) and stressed (EX) fish at four subsequent time-points: $t=0.5$, 2, 5 and 24 h ($n=6–8$), in order to measure plasma cortisol, glucose, lactate, osmolality, Cl^--ions, and at 10 and 22 °C also Na^+ and K^+-ions. Survival of the remaining fish was monitored until 4 days post-stress. The animals were not fed 24 h prior to and during the stress experiments.

2.3. Sampling methods and analytical techniques

Fish were removed from the tanks and immediately killed with a blow to the head before sampling. Blood samples were collected from the caudodorsal blood vessels, just below the spine at the right (dorsal) side, with cooled 1-ml syringes flushed with heparin (Leo 5000 IE). Plasma samples were obtained by centrifugation of the blood for 10 min at 3000 rpm and 5 °C, and stored at −80 °C until further analysis. Plasma cortisol concentrations were measured with a specific rabbit-anti-cortisol antibody (Klinger, St. Albans, Herts., England). Radioactivity of the [^{3}H]-labeled cortisol tracer was quantified using a Wallac 1410 Liquid Scintillation Counter (Pharmacia). Plasma glucose and lactate were measured with commercial enzymatic assays from Sigma. Plasma osmolality levels were measured with an Osmomat 030 osmometer (Gonotech, Germany). Plasma Na^+- and K^+-levels were measured with an IL 943 flame photometer (Instrumentation Laboratories, Italy), whereas Cl^--levels were measured with a CMT10 chloride meter (Radiometer, Denmark).

2.4. Statistical analyses

Statistical analyses were performed with STATISTICA 5.5 for Windows (StatSoft Inc., 2000). The assumption of homogeneity of variances was tested for all data, which were log-transformed if necessary. Data were initially subjected to regression analyses according to Packard and Boardman (1999) for determination of possible body size effects. Subsequently, data were subjected to two-way ANOVA or, when a significant and moderate-to-high correlation was found, two-way ANCOVA with body weight as a covariate to remove body size effects (Packard and Boardman, 1999). Two-way ANOVAs and ANCOVAs were used (a) to test for temporal differences between CO and EX-fish for each parameter at each temperature/salinity combination, and (b) to test for the interaction between temperature and salinity separately on CO and EX-fish for each parameter at each time-point. All ANOVAs and ANCOVAs that showed significant differences were followed by a Tukey HSD post hoc test. Significance was accepted when $P<0.05$ (Zar, 1984).

3. Results

No mortalities occurred at any temperature/salinity combination during the experiment.

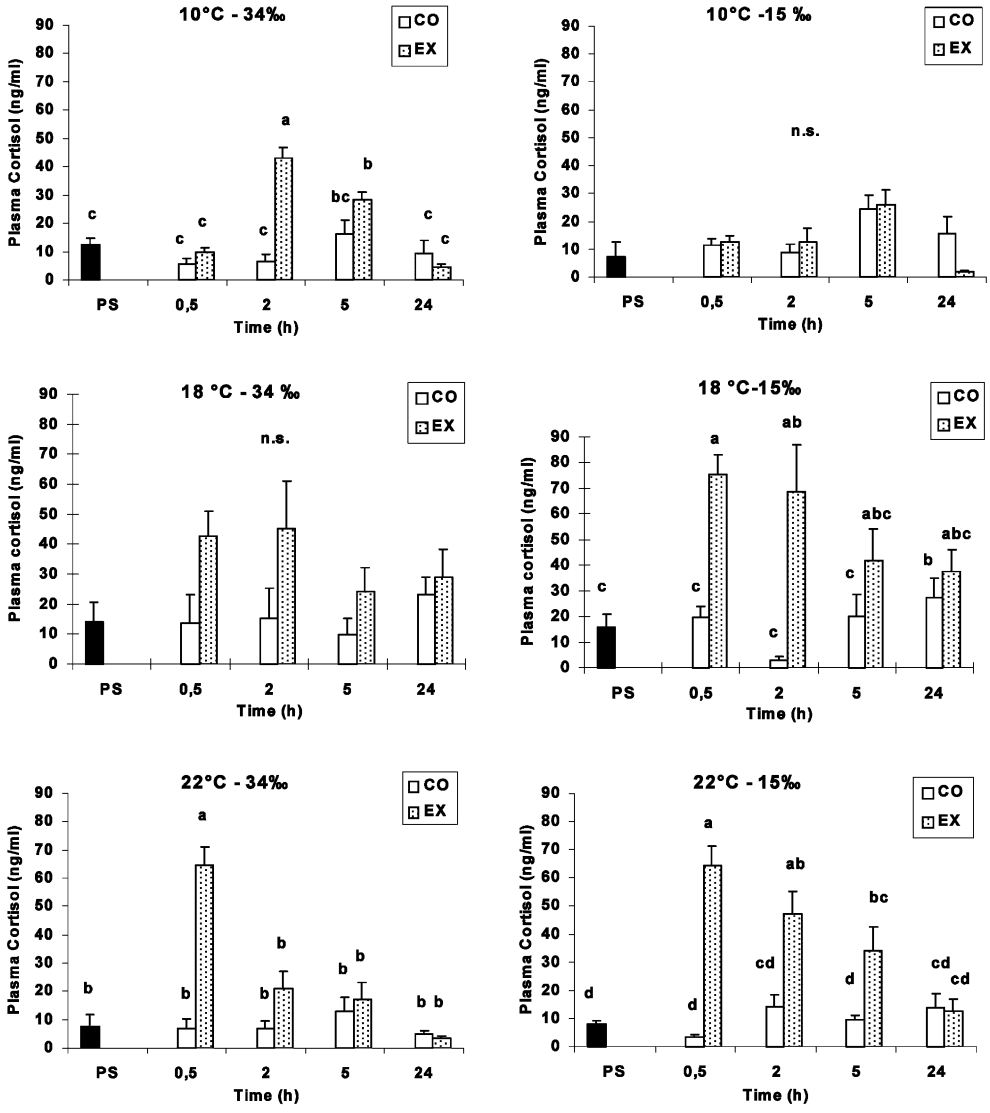

Fig. 1. Plasma cortisol concentrations (ng/ml) before (PS) and after stress-treatment (exercise) in turbot reared at 10, 18 and 22 °C and 33.5 and 15‰. Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values with different letters are significantly different ($P < 0.05$). n.s., no significant differences.

Mean plasma cortisol values before stress (PS) ranged from 7 to 16 ng/ml and were not significantly different between groups ($P > 0.3$, Fig. 1). Levels in CO-fish did not change significantly over time at any temperature/salinity combination. In EX-fish, plasma cortisol increased transiently between 2 and 5 h at 10 °C-33.5‰. No significant changes were found at 10 °C-15‰. At 18 and 22 °C, cortisol levels already increased at $t = 0.5$ h, and declined between $t = 2$ and 5 h. Similar trends were found at both salinities, but at 18 °C-33.5‰ changes were not significant. Statistical analyses for the interaction between temperature and salinity in EX-fish revealed significant temperature ($P < 0.0001$) and salinity ($P < 0.05$) effects at $t =$ 0.5 h, an interaction effect at $t = 2$ h ($P < 0.001$), and temperature ($P < 0.0001$) and interaction ($P < 0.05$) effects at $t = 24$ h, reflecting the overall differences in cortisol responses at 10 °C compared to 18 and 22 °C.

Mean plasma glucose levels before stress (PS) ranged between 2.1 and 2.5 mmol/l and were not significantly different between groups ($P > 0.5$, Fig. 2). Levels in CO fish did not change signifi-

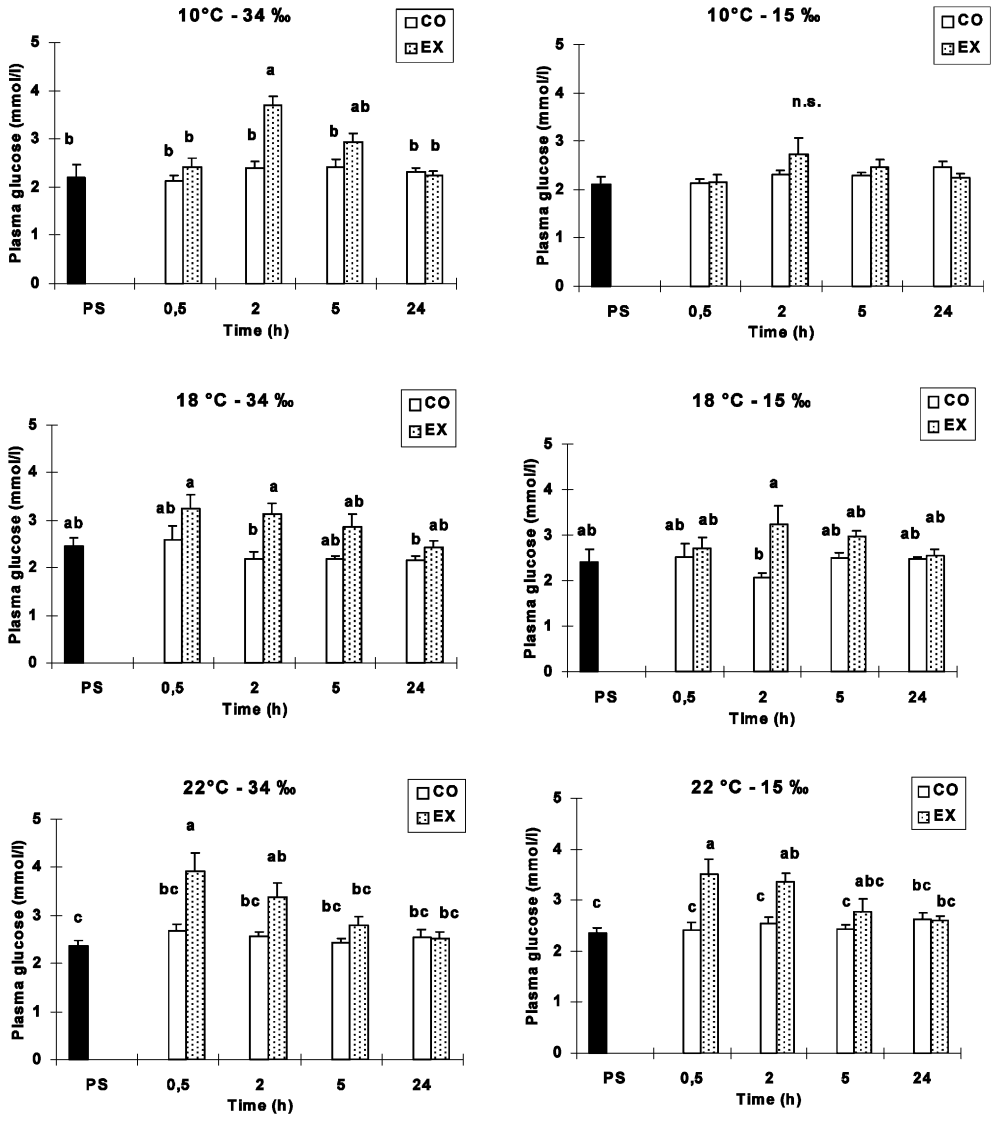

Fig. 2. Plasma glucose concentrations (mmol/l) before (PS) and after stress-treatment (exercise) in turbot reared at 10, 18 and 22 °C and 33.5 and 15‰. Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values with different letters are significantly different ($P < 0.05$). n.s., no significant differences.

cantly over time at any temperature/salinity combination. In EX-fish, plasma glucose increased significantly at 10 °C-33.5‰, whereas at 10 °C-15‰ no significant changes occurred. At 18 °C, plasma glucose increased transiently at both salinities. Glucose levels in EX-fish at 22 °C showed similar patterns, with significant increases at $t = 0.5$ h, and at 15‰ also at $t = 2$ h. Statistical analyses revealed a significant temperature effect on plasma glucose in EX-fish at $t = 0.5$ h ($P < 0.001$).

Mean plasma lactate levels before stress (PS) were between 0.2 and 0.5 mmol/l and not significantly different between groups ($P > 0.2$, Fig. 3). Levels in CO fish did not change significantly over time at any temperature or salinity. In EX-fish at both salinities at 10 °C, levels were significantly higher at $t = 0.5$ and 2 h. Similar but more pronounced elevations were found in EX-fish at both salinities at 18 °C, with higher peak levels at 15 than at 34‰. At 22 °C, the pattern of changes in plasma lactate of EX-fish was similar at both

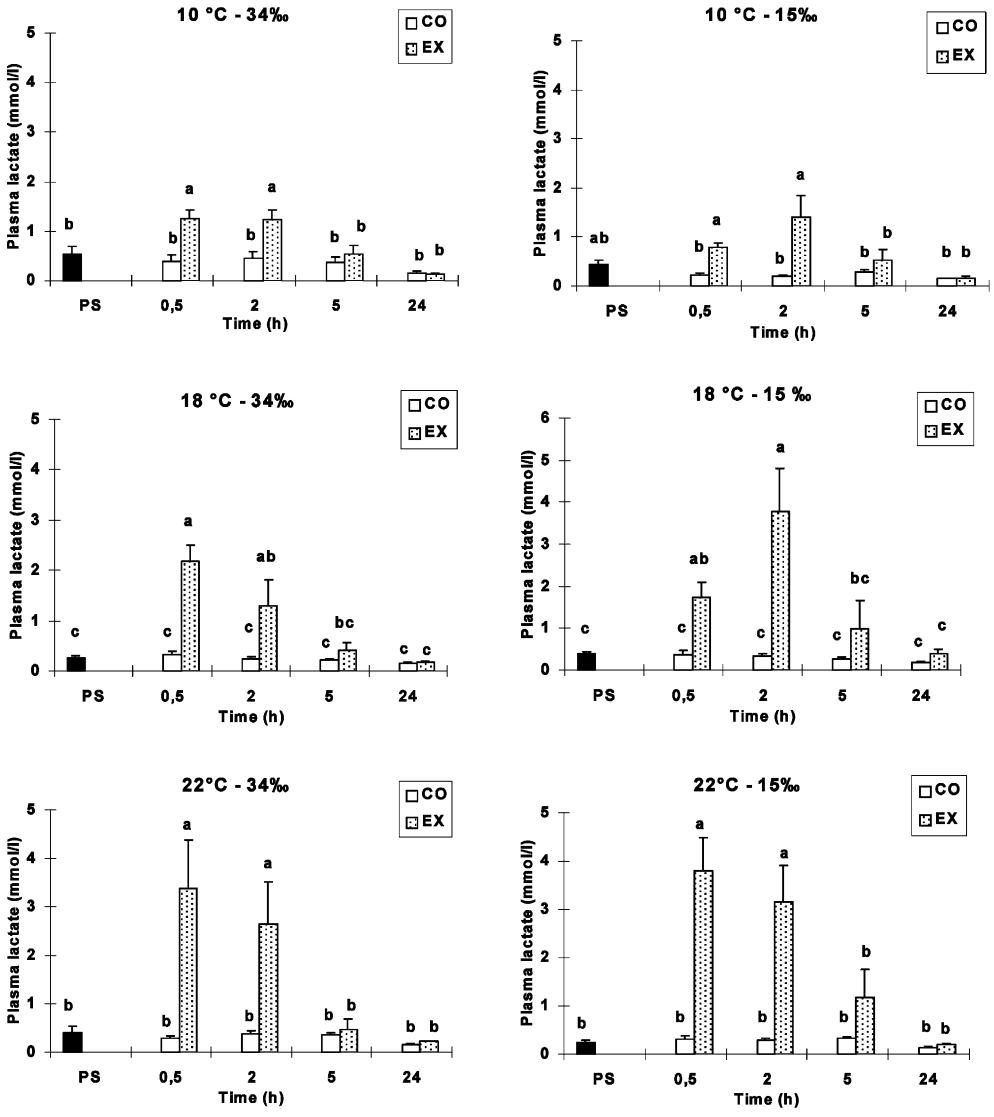

Fig. 3. Plasma lactate concentrations (mmol/l) before (PS) and after stress-treatment (exercise) in turbot reared at 10, 18 and 22 °C and 33.5 and 15‰. Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values with different letters are significantly different ($P < 0.05$).

salinities, with significantly higher levels at $t = 0.5$ and 2 h. Statistical analyses revealed a significant temperature effect on plasma lactate in EX-fish at $t = 0.5$ h ($P < 0.01$). Further, regression analyses revealed a significant positive correlation between body weight and plasma lactate of EX-fish at $t = 0.5$ h ($r^2 = 0.10$, $n = 48$, $P < 0.05$) and $t = 2$ h ($r^2 = 0.29$, $n = 45$, $P < 0.01$). Body weight did not correlate with any of the other parameters at any other time-point ($r^2 < 0.05$, $n = 43$ to 48, $P > 0.05$).

Mean plasma osmolality before stress (PS) were between 310 and 325 mOsmol/kg (Fig. 4), and lower at 18 °C than in the other groups ($P < 0.05$). Levels in CO fish did not change significantly over time at any temperature or salinity. In EX-fish, plasma osmolality showed a similar trend at all temperatures at 33.5‰, with a slight but significant increase at $t = 0.5$ h, which was diminished at $t = 2$ h. The same trend was found in EX-fish at 10 °C-15‰, whereas in EX-fish at the two higher temperatures and 15‰ levels were still elevated at $t = 2$ h. Statistical analyses in EX-fish revealed

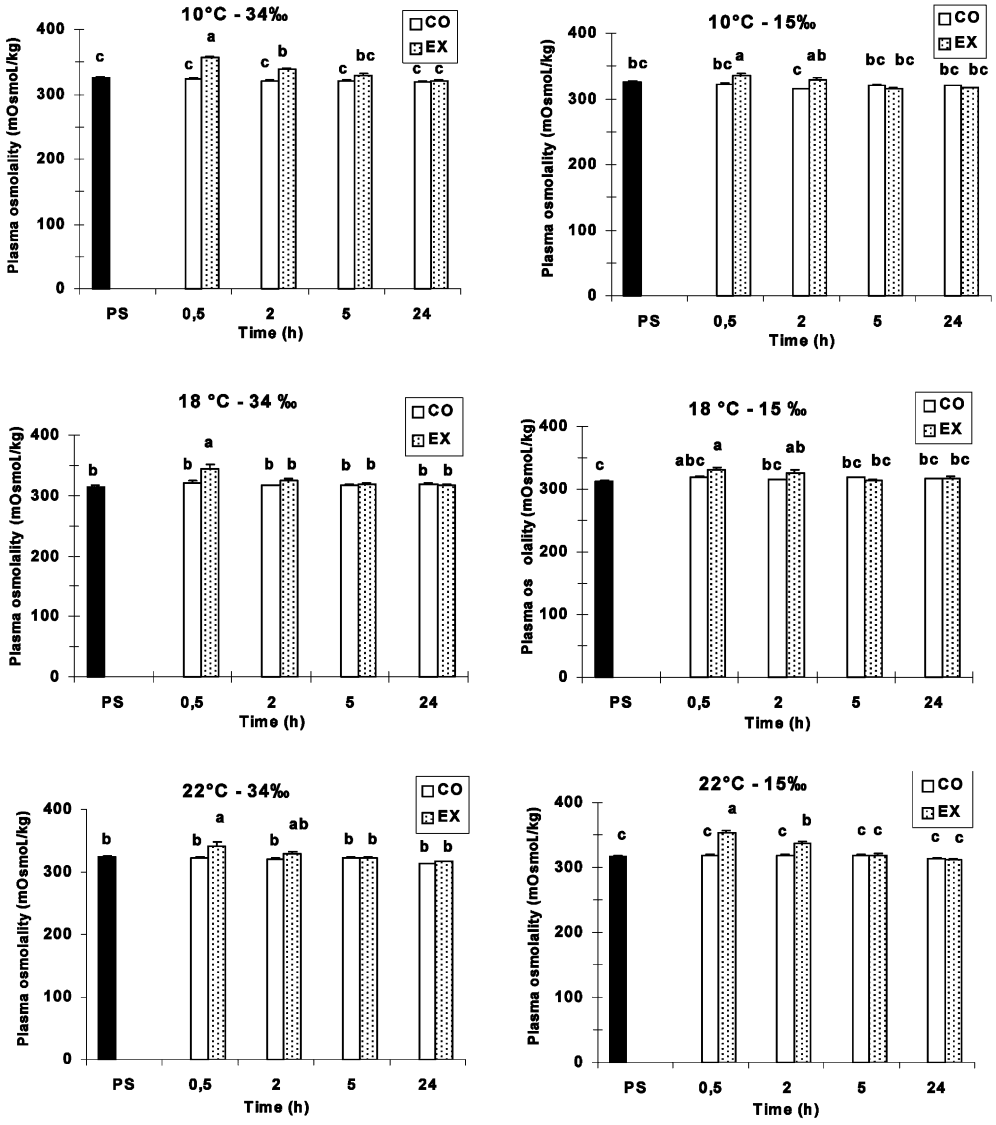

Fig. 4. Plasma osmolality (mOsmol/kg) before (PS) and after stress-treatment (exercise) in turbot reared at 10, 18 and 22 °C and 33.5 and 15‰. Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values with different letters are significantly different ($P < 0.05$).

significant salinity ($P < 0.05$) and interaction ($P < 0.01$) effects at $t = 0.5$ h, and a salinity effect at $t = 5$ h ($P < 0.01$).

Before stressor application, mean plasma Cl^- levels were between 130 and 140 mmol/l, and significantly lower in fish reared at 10 °C-15‰ compared to those at 22 °C-33.5‰ (Tables 1–3). In some groups, slight fluctuations in plasma Cl^- occurred over time in CO and EX-fish. In EX-fish at 10 and 18 °C and 33.5‰, plasma Cl^- was significantly higher at $t = 0.5$ h, compared to PS-levels. At the same temperatures, this elevation was not seen in EX-fish at 15‰, nor was it found at any salinity at 22 °C. Statistical analyses revealed a temperature effect on plasma Cl^- before stress ($P < 0.01$). In EX-fish, temperature ($P < 0.01$), salinity ($P < 0.001$), and interaction effects ($P < 0.0001$) were found at $t = 0.5$ h, whereas at $t = 2$ h both salinity ($P < 0.001$) and interaction ($P < 0.001$) effects were found. At $t = 5$ h temperature ($P < 0.01$), salinity ($P < 0.0001$), and interaction effects ($P < 0.01$) were found.

Before stressor application, mean plasma Na^+ levels were approximately 160 mmol/l at 10 and 22 °C and both salinities (Tables 1 and 3). In some groups, small fluctuations in plasma Na^+ levels occurred over time in CO and EX-fish. Plasma Na^+ levels at 10 °C were significantly higher at $t=0.5$ h in EX-fish at 33.5‰, compared to PS levels ($P<0.05$). At the same temperature, this elevation was not seen in EX-fish at 15‰, nor was it found at any salinity at 22 °C. Satistical analyses for the interaction between temperature and salinity on plasma Na^+ in EX-fish at 10 and 22 °C revealed temperature ($P<0.05$), salinity ($P<0.001$), and interaction effects ($P<0.0001$) at $t=0.5$ h, temperature and interaction effects at $t=2$ h ($P<0.05$), and a temperature effect at $t=5$ h ($P<0.01$).

Plasma K^+ levels showed minor fluctuations in some groups, but without any clear trend (Tables 1 and 3).

4. Discussion

In this study we demonstrated, for the first time in juvenile turbot, a moderate stress response after enforced exercise at various water temperature and salinity conditions. However, not all parameters were affected to the same extent: plasma cortisol, glucose, and lactate were more affected than iono-regulatory parameters. All physiological changes in blood plasma had disappeared 24 h after the exercise.

The results on plasma cortisol in our CO-fish demonstrate a relative insensitivity of turbot to repeated handling disturbance, which is in agreement with observations on turbot by Mugnier et al. (1998), but in contrast with several studies on salmonids (e.g. Pickering et al., 1982; Pottinger et al., 1994; Gerwick et al., 1999). The plasma cortisol response in EX-turbot was clearly affected by the water temperature, which caused a more rapid increase at 18 and 22 °C, compared to 10 °C. This temperature effect on plasma cortisol corresponds with observations in several other fish species subjected to handling stress (e.g. Sumpter et al., 1985; Pankhurst et al., 1992; Pottinger et al., 1999). Such an effect of high water temperature is usually attributed to an increase in rate of cortisol release, as a result of higher metabolic rates (e.g. Sumpter et al., 1985; Pottinger et al., 1999). In EX-fish, the magnitude of cortisol elevations was also higher at 22 than at 10 °C.

The influence of water salinity on the plasma cortisol response in EX-fish was less consistent. At 10 °C, changes in plasma cortisol levels were lower in EX-fish at 15 than at 33.5‰, whereas at 18 °C the response was lower in EX-fish at 33.5 than at 15‰. At 22 °C, the cortisol response in EX-fish was initially similar at both salinities, but the rate of recovery appeared to be faster in EX-fish at 33 than at 15‰. These results are difficult to compare with findings in other fish species, since hardly any study has examined the interaction of water temperature and salinity on the response of a marine species after exposure to an acute stressor. In several freshwater species, i.e. striped bass (*Morone saxatilis*; Mazik et al., 1991) and walleye (*Stizostedion vitreum*; Barton and Zitzow, 1995), an increase in water salinity decreased the magnitude of plasma cortisol elevations after acute stress. However, such an ameliorating effect of salt addition was not found for stressed paddlefish (*Polyodon spathula*; Barton et al., 1998). Nolan et al. (1999) did not find differences in cortisol responses of fresh- and seawater acclimated tilapia (*Oreochromis mossambicus*) after net-confinement. The overall impression emerging from our results is that low salinity may, to some extent, reduce the cortisol response of turbot at low temperatures, but this effect disappears at higher temperatures, perhaps from an overshadowing by temperature-related effects.

The water temperature appeared also to be the main factor influencing plasma glucose in exercised turbot, and again this became more evident from a faster increase at 22 °C than from the increase in absolute levels. The response in EX-fish at 18 °C was in between those found at 10 and 22 °C. The water salinity did not appear to be a major factor influencing plasma glucose of EX-fish. Both the magnitude and duration of the glucose response in EX-fish were in line with values found after exercise in other species. In rainbow trout (*Onchorhynchus mykiss*), basal levels are usually between 3 and 4 mmol/l, and may increase up to 6 mmol/l or higher within 2–6 h after 5 min of exhaustive exercise (Pagnotta and Milligan, 1991; Milligan and Girard, 1993). However, for the marine flatfish species starry flounder (*Platichthys stellatus*) and winter flounder (*Pseudopleuronectes americanus*), basal levels and max-

Table 1
Plasma ion concentrations (mmol/l) before stress (PS) and at various time-points (in hours) after exercise stress in turbot reared at 10 °C and 34 or 15‰

10 °C		Na^+		Cl^-		K^+	
Time ↓	Stressor ↓	33.5‰	15‰	33.5‰	15‰	33.5‰	15‰
PS		159.7 ± 1.8^{b}	163.8 ± 0.9^{a}	134.5 ± 0.6^{c}	133.9 ± 1.2^{bc}	4.3 ± 0.1	4.7 ± 0.3
0.5	CO	160.2 ± 1.1^{b}	154.9 ± 1.2^{c}	138.9 ± 1.4^{bc}	138.9 ± 1.2^{ab}	4.1 ± 0.3	4.0 ± 0.3
	EX	174.7 ± 1.7^{a}	161.7 ± 1.9^{a}	151.8 ± 1.3^{a}	137.1 ± 2.4^{ab}	4.2 ± 0.4	3.7 ± 0.3
2	CO	156.9 ± 1.4^{b}	154.1 ± 1.4^{c}	135.4 ± 1.1^{c}	134.5 ± 0.7^{ab}	4.1 ± 0.3	3.6 ± 0.1
	EX	164.9 ± 1.4^{b}	162.1 ± 1.0^{a}	139.0 ± 0.8^{bc}	129.9 ± 1.9^{c}	3.8 ± 0.3	4.1 ± 0.4
5	CO	162.9 ± 1.4^{b}	155.8 ± 0.1^{bc}	138.4 ± 0.5^{bc}	137.9 ± 1.3^{abc}	4.5 ± 0.3	4.2 ± 0.3
	EX	160.9 ± 2.4^{b}	160.4 ± 1.2^{ab}	138.6 ± 0.6^{bc}	135.9 ± 0.7^{abc}	3.3 ± 0.2	4.1 ± 0.3
24	CO	158.2 ± 1.4^{b}	158.3 ± 0.8^{abc}	140.6 ± 0.9^{b}	140.0 ± 0.6^{a}	3.7 ± 0.2	3.7 ± 0.1
	EX	154.7 ± 1.4^{b}	156.7 ± 0.9^{abc}	143.1 ± 1.5^{b}	137.7 ± 0.5^{ab}	3.4 ± 0.1	3.9 ± 0.2

Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values in the same column without letters or with the same added letter are not significantly different ($P > 0.05$).

imal responses after exercise were reported to be below 1.3 and 2.3 mmol/l, respectively (Milligan and Wood, 1987; Pagnotta and Milligan, 1991; Girard and Milligan, 1992).

A rapid initial hyperglycemic response after acute stress is usually interpreted as a result of liver glycogenolysis induced by CAs, whereas more prolonged hyperglycemia under (post-)stress conditions is thought to reflect hepatic gluconeogenesis mediated by cortisol (Vijayan et al., 1994, 1997; Fabbri et al., 1998). Plasma CAs were not directly measured in this study, since they are very sensitive to sampling disturbance (Barton and Iwama, 1991), and can only be measured reliably in cannulated fish, preferably kept under light-shielded conditions (McDonald and Milligan, 1997). Nevertheless, the trend of glucose elevations within the first hour in our study most likely reflects CA-induced glycogenolysis. Higher glucose responses to stressors at higher temperatures are considered to be a reflection of stimulated release of CAs due to a greater metabolic activity in fish acclimated to a higher temperature (Barton and Schreck, 1987; Davis and Parker, 1990).

Responses in plasma lactate after exercise were strongly affected by water temperature, which caused more rapid increases and higher peak levels at 18 and 22 °C, compared to 10 °C. Salinity did not have a major effect on plasma lactate in EX-fish, apart from the tendency of delayed recovery at low salinities at 18 and 22 °C. Muscle and plasma lactate levels increase mainly from increased anaerobic glycogenolysis, but in fish much of the produced lactate is retained within the white muscle tissue (Milligan and Girard, 1993; Sadler et al., 2000). Until recently, peak blood lactate levels after exercise in salmonids and other active pelagic species were usually between 15 and 20 mmol/l, in contrast with peak levels around 2 mmol/l usually found in Pleuronectidae and other relatively inactive benthic species. This difference was presumed to result from a higher muscle lactate retention in sluggish, inactive benthic species, which was attributed to their lifestyle

Table 2
Plasma Cl^- concentrations (mmol/l) before stress (PS) and at various time-points (in hours) after exercise stress in turbot reared at 18 °C and 33.5 or 15‰

18 °C		Cl^-	
Time ↓	Stressor ↓	33.5‰	15‰
PS		136.8 ± 1.4^{b}	134.3 ± 1.0^{ab}
0.5	CO	141.6 ± 1.1^{ab}	139.1 ± 0.9^{a}
	EX	146.0 ± 2.5^{a}	138.8 ± 0.8^{a}
2	CO	139.8 ± 1.0^{ab}	136.9 ± 1.0^{ab}
	EX	139.2 ± 1.3^{ab}	136.4 ± 1.1^{ab}
5	CO	141.5 ± 1.9^{ab}	137.5 ± 1.0^{ab}
	EX	139.4 ± 1.0^{ab}	133.0 ± 0.6^{b}
24	CO	141.9 ± 1.7^{ab}	139.3 ± 0.8^{a}
	EX	139.7 ± 1.3^{ab}	137.9 ± 1.2^{ab}

Mean values ± S.E., $n = 6–8$. CO, control fish; EX, exercised fish. Values in the same column with the same added letter are not significantly different ($P > 0.05$).

Table 3
Plasma ion concentrations (mmol/l) before stress (PS) and at various time-points (in hours) after exercise stress in turbot reared at 22 °C and 33.5 or 15‰

22 °C		Na^+		Cl^-		K^+	
Time ↓	Stressor ↓	33.5‰	15‰	33.5‰	15‰	33.5‰	15‰
PS		162.7±0.9	158.5±1.0^{ab}	138.9±0.9	138.3±0.8^{ab}	4.5±0.2	4.2±0.2^{ab}
0.5	CO	157.4±0.6	156.3±0.7^{b}	138.1±2.1^{b}	135.6±1.0^{b}	4.0±0.1	3.8±0.2^{b}
	EX	158.9±1.7	162.8±1.3^{a}	138.1±2.1	142.1±1.1^{a}	3.9±0.4	3.5±0.2^{b}
2	CO	153.8±1.4	153.0±0.8^{b}	137.8±1.2	136.7±0.8^{b}	4.5±0.4	4.0±0.2^{ab}
	EX	156.1±2.0	162.0±2.1^{a}	134.1±1.4	135.4±1.1^{b}	4.1±0.3	4.9±0.3^{a}
5	CO	156.9±1.3	155.3±0.9^{b}	138.0±0.7	138.9±0.9^{ab}	4.2±0.3	4.3±0.2^{ab}
	EX	157.0±1.2	154.0±0.9^{b}	138.9±0.9	128.9±1.4^{c}	3.7±0.1	4.2±0.1^{ab}
24	CO	156.2±0.8	154.8±0.6^{b}	139.1±0.9	137.6±0.7^{ab}	3.7±0.1	3.8±0.1^{ab}
	EX	153.3±0.9	153.0±1.0^{b}	138.8±0.7	139.0±0.6^{ab}	3.8±0.3	4.2±0.1^{ab}

Means±S.E., $n=6$–8. CO, control fish; EX, exercised fish. Values in the same column without letters or with the same added letter are not significantly different ($P>0.05$).

and swimming performance (Girard and Milligan, 1992; Milligan and Girard, 1993). However, in a recent study Milligan et al. (2000) measured blood lactate peaks as low as 5 mmol/l, as well as a faster recovery and no elevations in plasma cortisol in rainbow trout allowed to swim at low velocities following exhaustive exercise, compared to fish held in still water for recovery. The latter procedure is usually applied in exercise studies, but this might not be a natural situation for salmonids, which seek refuge in current and continue swimming after catch-and-release in the wild, rather than in still water (Milligan et al., 2000). However, for many flatfish species recovery in still water is probably more relevant to their natural life-style. Therefore, earlier reported major differences in lactate metabolism between salmonids and flatfish after exhaustive exercise might have been due to the imposition of post-exercise inactivity, causing a pronounced cortisol-mediated stress response and delayed recovery in salmonids but not in flatfish.

Our results demonstrate that enforced exercise can also induce a moderate lactate response in a benthic species with an inactive, sedentary life-style, and that this response is heavily influenced by the water temperature. Enhancement of lactacidosis after exhaustive exercise at higher temperatures has been reported for roach (*Rutilus rutilus*; Wieser et al., 1986), rainbow trout (Kieffer et al., 1994) and Atlantic salmon (*Salmo salar*; Wilkie et al., 1997), and may result from temperature-dependent changes in various metabolic processes, such as temperature-dependent increases in lactate diffusion rates out of the muscle, increased diffusion of small molecules within the muscle tissue, and higher perfusion rates of white muscle tissue (Kieffer et al., 1994; Wilkie et al., 1997). However, there is considerable evidence that also body size influences and/or limits the physiological post-exercise lactate response in fish (Kieffer, 2000). In salmonids, the anaerobic capacity generally increases with body size, and so does the cost of burst activity. In rainbow trout, Atlantic salmon and brook trout (*Salvelinus fontinalis*) the energy stores used to support burst activity also vary with body size, with higher muscle ATP and glycogen levels in larger fish (Ferguson et al., 1993; Kieffer et al., 1996; McDonald et al., 1998). Plasma lactate levels were not measured in these studies, but it seems reasonable to expect that a smaller anaerobic muscle capacity would also result in less lactate being released into the blood. Such a relation between muscle and plasma lactate levels was suggested by Kieffer et al. (2001) for shortnose sturgeon (*Acipenser brevirostrum*). In net-confined carp Pottinger (1998) found threefold higher plasma lactate peaks after 1 h in fish >500 g than in fish <50 g, despite a lower water temperature of 4 vs. 15 °C at which the large fish were kept. Childress and Somero (1990) and Kieffer et al. (1996) found that the relationship between body size and anaerobic capacity was less pronounced in inactive, benthic species than in active pelagic species. This was suggested to be related to the level of sprint performance capacity required for an animals predator–prey interactions. In our study EX-fish were considerably smaller at

10 °C than at 18 and 22 °C, and regression analyses showed a low to moderately significant correlation between plasma lactate and body weight at $t=0.5$ and 2 h. We, therefore, conclude that the lower magnitude of lactate responses in EX-fish at 10 °C might at least partly be the result of a smaller anaerobic muscle capacity, due to a smaller fish size.

In EX-fish, an initial rise in plasma osmolality occurred in all groups, whereas changes in plasma ions were more variable. In EX-fish at full salinities, an initial rise occurred in plasma Na^+ and Cl^- at 10 °C, and to a lesser extent in Cl^- at 18 °C. However, no elevations were found in EX-fish at 22 °C, which probably reflects a faster restoration of the hydromineral balance at higher water temperature, due to higher activity of ion transport mechanisms in gills, kidneys, intestine, and white muscle tissue (Davis and Parker, 1990; Wilkie et al., 1997). At 15‰ no changes occurred in plasma Na^+ and Cl^- of EX-fish at any temperature. A reduction of the magnitude of ionic disturbance after stress at low salinity has also been observed in other species, e.g. coho salmon (*Oncorhynchus kisutch*; Redding and Schreck, 1983), striped bass (Mazik et al., 1991), and pejerry (*Odontesthes bonariensis*; Tsuzuki et al., 2001), and reflects a facilitated maintenance and recovery of osmotic homeostasis in a more iso-osmotic environment (Redding and Schreck, 1983; Reubush and Heath, 1997). We conclude that both water temperature and salinity influenced the maintenance and recovery of the hydromineral balance in exercised turbot. The low magnitude and fast recovery of those disturbances that did occur point towards hemoconcentration, due to loss of plasma water moving out of the circulation into the tissues, as the primary cause of these changes (Wendelaar Bonga, 1997).

Pronounced changes in plasma K^+ did not occur in exercised turbot at any temperature or salinity. Elevated plasma K^+ are usually a sign of intracellular acidosis in arterial blood, which can be of mixed respiratory and metabolic origin and has been correlated with K^+ extrusion in mammalian skeletal muscle (Turner et al., 1983a,b; Knudsen and Jensen, 1998). Opdyke et al. (1982) suggested that elevations in plasma K^+ might be partly responsible for the release and maintenance of circulating CAs after exercise. The absence of a clear rise in plasma K^+ levels indicates that metabolic acidosis was limited in our EX-fish.

Overall, the present study demonstrates that enforced exercise induced a moderate stress response in juvenile turbot reared at various temperature and salinity combinations. Temperature was the dominating environmental factor modifying this response, causing more rapid increases in plasma cortisol and glucose levels, more rapid increases and response magnitudes of plasma lactate levels, and faster recovery of plasma Na^+ and Cl^- levels at high temperatures. Responses in plasma lactate were also dependent of the body weight of the fish. The influence of water salinity was less pronounced, with a moderating effect on the cortisol response at low temperatures, and a reduction of the magnitude of ionic disturbance after exercise at low salinities. The response of turbot to exercise involved activation of the BPI-axis, as indicated by the elevations in plasma cortisol. The modest responses in plasma glucose, the small changes Na^+ and Cl^-, and the absence of plasma K^+ elevation indicate at most a mild activation of the BSC-axis, and hence little release of CAs, at least at the lower water temperatures. This might be related to the ecological niche of this species. Turbot, living in estuarine and coastal areas, frequently experience low environmental oxygen conditions and fluctuating environmental temperatures (Maxime et al., 2000). They are very tolerant to O_2 deficiency and capable of maintaining a high O_2-tension in arterial blood, even during severe hypoxia, through mechanisms like hyperventilation, lack of metabolic depression and blood acidosis, and possibly cutaneous oxygen uptake (Boeuf et al., 1999; Maxime et al., 2000). In addition, Imsland et al. (1997) demonstrated the existence of three different hemoglobin types with different O_2-affinity properties in turbot. This so-called hemoglobin polymorphism results in an overall higher oxygen affinity capacity. Thanks to these adaptive features, the fish in our study may have been able to prevent severe blood hypoxemia during exercise, which is thought to be the ultimate stimulus for CA release (Perry and Bernier, 1999), and hence a massive CA-release did not occur.

When interpreting stress-induced physiological changes in fish, it is important to realize that a stress response has essentially an adaptive value for an animal. Higher short-term stressor responsiveness does not necessarily have to be disadvantageous for an animal, but may reflect a higher and overall beneficial capacity to adapt to different stress conditions (Pottinger and Pickering, 1997;

Pottinger and Carrick, 1999). Given the fact that the highest responses in the present study were found within the optimal temperature range for growth and feed conversion of turbot (Imsland et al., 2000, 2001), we conclude that this higher short-term responsiveness most likely reflects a higher and overall beneficial adaptive capacity.

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