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NOTE

Aplasia of the septum transversum has no effect on plasma biochemistry following an acute hypoxic event in Atlantic salmon

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ABSTRACT: Aplasia of the septum transversum (AST) is a malformation that results in alterations in ventricle morphology. The condition has been linked to increased mortality during periods of increased cardiac demand in Atlantic salmon *Salmo salar*. The blood plasma biochemical response to an acute hypoxic event (1 h at 31–39 % O_2 saturation) was investigated in fish with and without a septum transversum to assess levels of anaerobic respiration (lactate) and the stress response (cortisol, glucose, osmolality, Na^+, Cl^- and K^+). AST had no effect on body size parameters or relative ventricular mass. The hypoxic event increased the levels of anaerobic respiration and induced a typical stress response. Contrary to our expectations, AST had no effect on any plasma parameter in normoxia or following severe acute hypoxia. We conclude that in the current scenario, AST does not affect the levels of anaerobic respiration or the plasma stress response in Atlantic salmon.

KEY WORDS: Aquaculture · Fish welfare · Heart · Stress · Cardiovascular · Hypoxia · Oxygen saturation · *Salmo salar*

INTRODUCTION

Heart deformities are a concern within the salmon farming industry due to economic losses and fish wellbeing. One such condition is aplasia of the septum transversum (AST), whereby the tissue separating the abdominal and cardiac cavities is absent (Poppe et al. 1998). AST is associated with high incubation temperatures in Atlantic salmon *Salmo salar* (Ørnsrud et al. 2004, Takle et al. 2005) and mortalities during periods of excessive cardiac demand (Brocklebank & Raverty 2002, Rodger & Mitchell 2011). The latter is presumed to be a result of major alterations in ventricle morphology caused by AST. Specifically, the malformation results in a heart ventricle that has a flattened rounded appearance (Fig. 1; see Fraser et al. 2014 for a morphological description) that is in contrast to the pyramidal shape that is known to optimise cardiac performance in salmonids (Claireaux et al. 2005). Based on the latter, one would expect fish with AST to have a reduced cardiac output that would limit oxygen (O_2) delivery to the body during periods of high cardiac demand. As the majority of the salmon heart is dependent on venous O_2 supply (Davie & Farrell 1991), less circulating O_2 may also reduce heart efficiency, leading to a further reduction in O_2 delivery to the body.

Within sea cages, O_2 levels fluctuate naturally due to season, depth, time of day and environmental conditions (Johansson et al. 2006, 2007, Oppedal et al.

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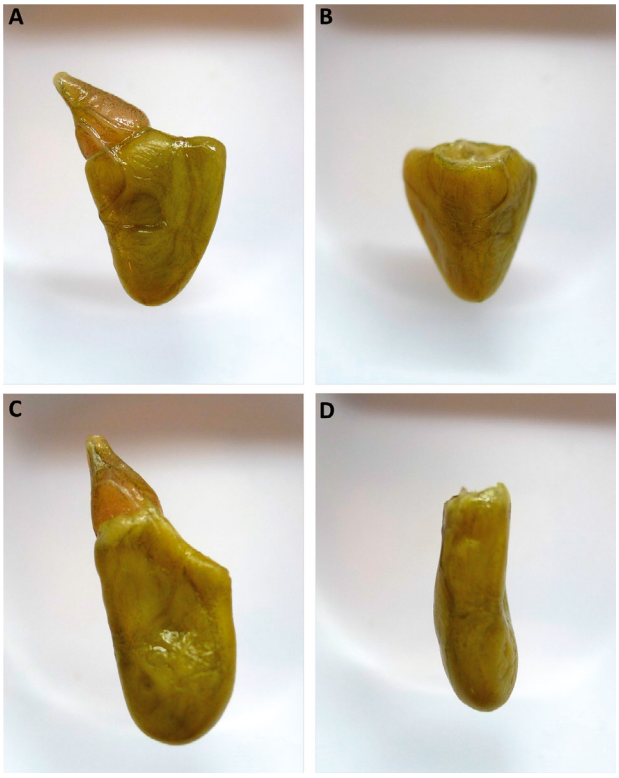

Fig. 1. (A,C) Lateral and (B,D) cranio-ventral view of formalin-fixed Atlantic salmon *Salmo salar* heart ventricles from ca. 800 g fish. Note the rounded and flattened appearance of the ventricle from a fish with aplasia of the septum transversum (C,D) compared to the more triangular ventricle of a fish with the septum transversum (A,B)

2011a, Burt et al. 2012, Stien & Kristiansen 2012), and low values may be seen during management practices such as handling, crowding, topical de-licing or anti-lice practices (e.g. Stien et al. 2012). In some instances, these fluctuations may reduce O_2 saturation levels within cages to values as low as 30% (Johansson et al. 2006, Oppedal et al. 2011b). In response to hypoxia, fish increase gill ventilation and perfusion (Randall 1982) in order to maintain aerobic respiration. However, below a certain O_2 threshold there is an increase in anaerobic respiration (Boutilier et al. 1988), anaerobic end-products accumulate (Dunn & Hochachka 1986), and a stress response is elicited (Remen et al. 2012). In addition, bradycardia (reduced heart rate and increased stroke volume) occurs as a method to increase O_2 loading at the gills (Farrell 2007). Survival is then dependent upon the ability of the fish to ferment available substrates, suppress metabolism, and manage anaerobic end-products (Nilsson & Östlund-Nilsson 2008).

The diverging heart morphology between fish with and without a septum transversum may impair heart function, reducing O_2 transfer and subsequently hypoxia tolerance in fish. This hypothesis was tested by subjecting fish with and without a septum transversum to 1 h of hypoxia (31–39% O_2 at 9°C) and comparing anaerobic metabolism and the stress response through plasma biochemistry. For this purpose, we used a population of fish that were incubated at 10°C between fertilisation and first feeding in which approximately 18% of the fish had AST at the parr stage.

METHODS AND RESULTS

All experimental work using fish was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulation on Animal Experimentation 1996. The experiment was approved by Forsøksdyrutvalget (FOTS id 5329).

The study population was described by Fraser et al. (2014), but only diploid fish incubated at 10°C were used in the current study. Briefly, Atlantic salmon ova and milt were provided by Aquagen (Trondheim, Norway), and the present experiment was carried out at the Institute of Marine Research, Matre Research Station, Norway. On 6 October 2011, 150 fish (mean body mass 114 g) per tank (n = 3) were vaccinated intraperitoneally (i.p.) with 0.1 ml of a multivalent oil-adjuvant vaccine (Norvax® Compact 6, Intervet Norge), and the light regime was changed to 24 h light:0 h dark. On 22 November 2011, the fish (mean body mass 138 g) in each tank were transferred to seawater tanks (34 ppt salinity, dimensions 1.5 × 1.5 m; water depth 45 cm, n = 3 tanks, ca. 20 kg fish m^{-3}). The number of fish in each tank was reduced to 75 in May 2012 to reduce stocking densities, and then the fish were redistributed among 6 tanks (37–39 fish $tank^{-1}$) on 3 June 2013 in order to acclimate the fish for the present study, which was conducted on 17 June 2013. In seawater, the temperature was maintained at 8.9°C, the photoperiod was simulated natural (60° N), and the oxygen saturation in the outlet water was always above 80%.

On 17 June 2013, fish in 3 tanks experienced decreasing oxygen levels over a 25 to 40 min period, down to hypoxic levels between 31 and 39% O_2 saturation (Fig. 2). This level of hypoxia is considered relatively severe and induces both anaerobic respiration and a stress response in Atlantic salmon (Remen et al. 2013). The fish were maintained in the hypoxic conditions for 1 h before sampling of blood and tissues (Fig. 2). Fish in the remaining 3 tanks (controls)

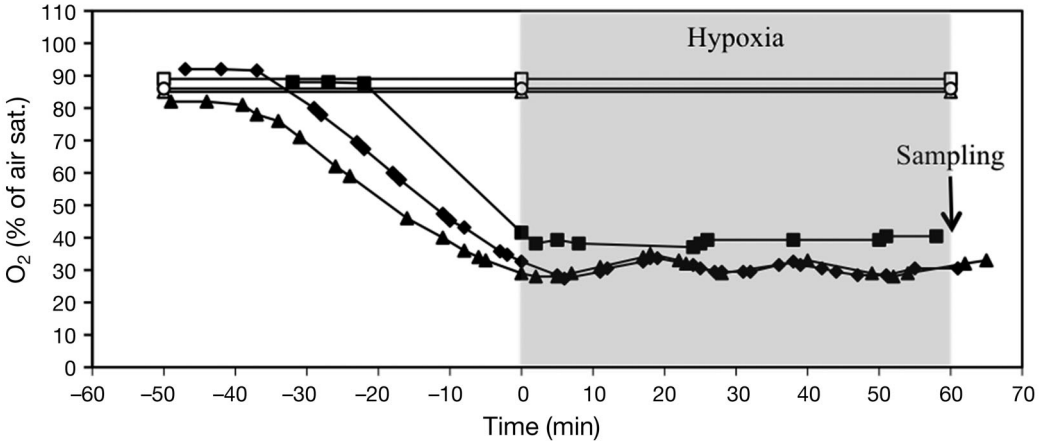

Fig. 2. Oxygen saturation (% of air saturation) in experimental tanks during the experimental period. Each point represents 1 O_2 measurement, using open symbols for control tanks and filled symbols for hypoxia tanks (n = 3, represented by different symbols). Shaded area represents the 60 min period of hypoxia after which sampling was performed

were kept in normoxic conditions (85–89% O_2, Fig. 2) before sampling. Hypoxic levels were established by closing the water inlet until 31 to 39% O_2 was reached as a result of fish O_2 consumption. After this, O_2 levels were kept stable for 1 h (SD = 1–2% O_2) by pumping 100% O_2 saturated water into the tanks through a supplementary inlet tube using a pump submerged in an adjacent tank (Universal pump 1250, EHEIM, 20 l min^{-1} flow-through rate). The pump was turned on and off according to the oxygen demand of the fish in order to maintain stable O_2 levels (13–20 l min^{-1}; 0.2–0.3 l kg^{-1} fish min^{-1}). In normoxic tanks, the water inlet was not touched (~60 l min^{-1}). It is accepted that this method may result in hypercapnia, but CO_2 measures were not taken. Previous experience indicates that CO_2 build-up is relatively minor (<2.5 mg l^{-1}) and with negligible physiological effects (Fivelstad et al. 1998) within the current setup, which mirrors the typical situation in sea cages (i.e. oxygen is removed from the water through its use by fish).

One tank was sampled at a time, with alternating hypoxic and normoxic tanks. In order to reduce sampling stress, all fish were anaesthetized in the tank by adding 1 l of Finquel® (ScanAqua) solution (4200 mg l^{-1}) into the 770 l tanks (water level reduced to 40 cm) through the supplementary inlet tube, resulting in a 55 mg l^{-1} solution in the tanks. Within 1.5 min, fish were anaesthetized, the lid was opened, and fish were rapidly netted into a transport tank. Within 6 to 7 min, all fish (37–39 ind.) were taken out, killed by a blow to the head and immediately sampled for blood (3–5 ml) from the caudal vein using heparinised 5 ml syringes (Heparin LEO; LEO Pharma). Blood was centrifuged at 16 000 × *g* (90 s at 4°C) for separation of plasma.

After blood sampling, all fish had their septum transversum examined by dissection and were measured for fork length and body mass. From each tank, plasma samples from the first 5 fish with a normal septum transversum and the first 5 fish with AST were frozen in liquid N_2, and the ventricle was weighed to the nearest 0.01 g. In total, 28% of the fish had AST.

Plasma lactate and glucose and the plasma electrolytes Na^+, Cl^-, and K^+ were analyzed using standard kits for the COBAS c111 autoanalyzer (Roche). Plasma cortisol levels were measured by ELISA (RE52061, IBL). Two fish (both subject to hypoxia, one with and one without the septum transversum) had cortisol values above the detection limit of the assay and were given the maximum value detected. Total plasma osmolality was measured using freeze point determination (Fiske micro-osmometer Mod 210).

Data were transferred to R version 2.15.2 (R Development Core Team, www.R-project.org/) for statistical analyses. All initial models described below contained all possible 3-way interactions between explanatory variables unless otherwise stated. Each model was then simplified by a hierarchical approach where non-significant terms were removed sequentially to arrive at the most parsimonious model. Significance was assigned at $p < 0.05$. All data were initially checked for homogeneity of variance using Bartlett's test. As such, lactate values were log transformed to achieve homogeneity of variance. Linear mixed effect models were used for all analyses.

For body size, body mass or fork length was included as the dependent variable, AST (yes/no) was set as a categorical independent variable, and tank was included as a random effect. For ventricle size and plasma analysis, ventricle mass or plasma lactate, cortisol, glucose, osmolality, Na^+, Cl^- or K^+ was the dependent variable, AST and O_2 conditions (normoxia/hypoxia) were categorical independent variables, body mass was a continuous independent variable, and tank was included as a random effect. One osmolality data point from a fish with AST maintained in normoxia was omitted from the statistical analysis as it was extremely high and was considered an outlier (458 mOsm kg^{-1} compared to the group mean of 331 mOsm kg^{-1}).

There was no effect of AST on body mass ($p = 0.69$; mean ± SE [n] 1597 ± 21 g [172] and 1617 ± 54 g [48] for fish with and without a septum transversum, respectively) or fork length ($p = 0.77$; mean ± SE [n] 49.0 ± 0.2 cm [172] and 49.1 ± 0.5 cm [48] for fish with and without a septum transversum, respectively). AST had a significant effect ($p < 0.01$) on the interaction between body mass and ventricle mass (Fig. 3).

Following hypoxia there were significant increases in plasma lactate, cortisol, glucose, osmolality and Na^+ (Table 1). We found no effect of hypoxia on plasma Cl^- or K^+. There was a significant ($p < 0.01$) positive correlation between body mass and plasma lactate and osmolality, and a non-significant ($p = 0.06$) trend of the same nature for plasma Cl^- (data not shown). AST had no effect on any plasma parameter and did not interact with any other independent variable.

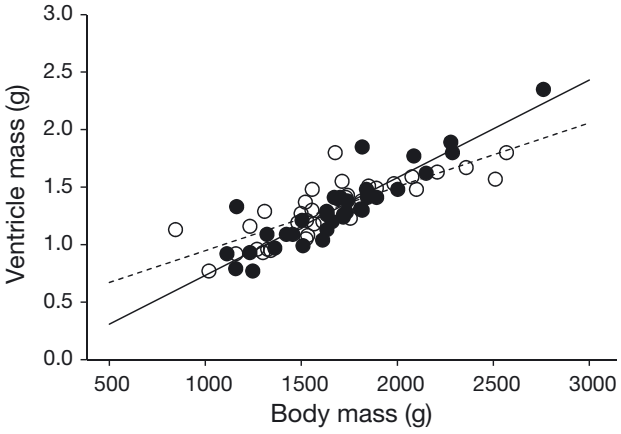

Fig. 3. Ventricle mass in relation to body mass in Atlantic salmon *Salmo salar* with (black, solid line) and without (white, dotted line) a septum transversum. There was a significant effect ($p < 0.01$) of aplasia of the septum transversum on the relationship between ventricle mass and body mass ($n = 35–36$ group^{-1})

DISCUSSION AND CONCLUSIONS

Our aim was to determine whether a developmental malformation that results in a significant alteration in heart ventricle morphology had any effect on the physiological response of Atlantic salmon to hypoxia. As expected, the severe acute hypoxic episode resulted in an increase in anaerobic respiration (lactate) and activation of the stress response (cortisol and glucose), but the response did not differ between controls and fish with AST.

In the current study, 28% of the fish had AST in comparison to only 18% in the same population at the parr stage (Fraser et al. 2014). If this condition results in a significant increase in mortality risk to the fish, one would expect this value to be lower over time as the only known risk factor for AST is high incubation temperature (Ørnsrud et al. 2004, Takle et al. 2005). We speculate that the favourable conditions within the research facility reduced periods of increased cardiac demand on these fish, reducing the mortality risk. Alternatively, high incubation temperatures may result in a septum transversum that is

Table 1. Plasma parameters (mean ± SE) and linear mixed effect model results in Atlantic salmon exposed to normoxia or a 1 h period of hypoxia. Aplasia of the septum transversum (AST) had no effect on any plasma parameter in comparison to fish with a septum transversum (Septum)

Parameter	Normoxia ($n = 14–16$ group^{-1})		Hypoxia ($n = 15$ group^{-1})		Model effects (hypoxia)	
	Septum	AST	Septum	AST	*t*(df)	p
Lactate (mmol l^{-1})	0.9 ± 0.1	0.9 ± 0.1	3.2 ± 0.2	3.8 ± 0.5	7.2(4)	0.002
Cortisol (ng ml^{-1})	62.7 ± 7.7	53.2 ± 8.5	577.8 ± 25	529.1 ± 38.1	20.6(4)	<0.001
Glucose (mmol l^{-1})	4.4 ± 0.1	4.3 ± 0.1	5.8 ± 0.2	6.1 ± 0.3	4.1(4)	0.015
Osmolality (mOsm kg^{-1})	331.4 ± 1.9	330.8 ± 1.1	349 ± 2.4	354.7 ± 3.4	3.8(4)	0.019
Na^+ (mmol l^{-1})	159.5 ± 0.9	160.4 ± 1.2	166.5 ± 1.2	166.6 ± 1.7	2.9(4)	0.042
Cl^- (mmol l^{-1})	139.6 ± 0.8	138.5 ± 1.0	142.4 ± 1.0	142.3 ± 1.3	2.0(4)	0.192
K^+ (mmol l^{-1})	2.9 ± 0.2	3.1 ± 0.2	2.7 ± 0.2	2.4 ± 0.1	−1.8(4)	0.146

weaker and more liable to rupture, but this would need to be confirmed by histological examination that was not undertaken in the present study. Nevertheless, AST had no effect on body size similar to results in parr (Fraser et al. 2014), suggesting that this condition does not impede growth.

AST results in major alterations to heart morphology, but there was no effect of this condition on any plasma parameter following hypoxia. For example, the round flattened profile of the heart from fish with AST is in direct contrast to the pyramidal shape that is optimum in salmonids (Claireaux et al. 2005). The ventricle mass to body mass relationship was affected by AST, but there was no increase in ventricle mass in fish with AST as reported in parr (Fraser et al. 2014). Therefore, an increase in heart mass cannot be counteracting any reduction in heart performance due to the morphological alterations. It may be that fish suffering from AST demonstrate changes in behaviour or have other methods of adaptation to their condition that were not measured (i.e. increased aerobic capacity, haematocrit or haemoglobin). For instance, when exposed to cyclic hypoxia, Atlantic salmon show a stress response (elevated cortisol) after the initial hypoxic event, but not after 1 wk of acclimation to such events (Remen et al. 2012). However, in the latter study, plasma lactate levels were always elevated following hypoxia irrespective of the number of previous hypoxic events. This suggests that fish with AST are unlikely to have adapted mechanisms to alter the anaerobic response to hypoxia over time. Future studies could determine the standard metabolic rates and response of fish with AST to swim challenges such as critical swimming speed (U_{crit}) or critical thermal maximum (CT_{max}) tests.

In conclusion, when using plasma values as indicators of anaerobic respiration and stress, AST had no effect on the physiological response to a severe acute hypoxic event. Therefore, it would seem unlikely that this condition will increase the risk of mortality during an acute period of low O_2 in sea cages.

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