Intercontinental transfer of adult Acipenser oxyrinchus – impact assessment of aviation transport conditions on blood parameters

By J. Gessner¹, A. Horvath², G.-M. Arndt³, B. Urbányi², E. Anders⁴, A. Hegyi² and S. Wuertz¹

¹Department of Biology and Ecology of Fishes, Leibniz Institute of Freshwater Ecology and Inland Fishery, Berlin, Germany; ²Department of Fish Culture, Faculty of Agriculture and Environmental Sciences, Szent István University, Gödöllő, Hungary; ³Fish and Environment Mecklenburg-Vorpommern, Rostock, Germany; ⁴Regional Research Board for Agriculture and Fisheries Mecklenburg-Vorpommern, Born/Darss, Germany

Summary
In order to facilitate intercontinental air transport of live sturgeon broodstock, a simulation test for an 8-h flight was performed in a pressure chamber (pressure profiles resembling conditions during trans-Atlantic cargo flights). Atlantic sturgeon (Acipenser oxyrinchus) were maintained in sealed polyethylene bags with water and an oxygen-enriched atmosphere at a ratio of 1:5:10 (fish:water:oxygen by volume) over a 10 h period at 15°C water temperature. Minimum pressure during the simulated flight was regulated at an elevation of 2600 m equalling 850 hPa. Decompression and compression phases to simulate takeoff and landing were set at 30 min each. Respiration frequency was recorded during flight simulation. Blood pH, blood pO2 and pCO2 as well as Ca²⁺, Na⁺, K⁺, Cl⁻, glucose and cortisol, cholesterol and triglycerides were also monitored prior to and after transport (at 12, 24, 36, 280 and 366 h). During exposure in the bags, blood pH decreased from a mean of 7.35 to 7.11 and blood pCO2 increased from 2.48 to 8.53 hPa. Both parameters revealed the most significant deviations from control levels immediately following the trial, returning to normal levels after 36 h. In contrast, the Na⁺²⁻, Ca²⁺ and Cl⁻ ion as well as glucose concentration required 72 h following the simulated transport until baseline levels were reached. During the subsequent transatlantic transport trials from Canada to Germany, blood parameters were utilized to assess fish recovery following transport. Additionally, testing of the transport water revealed that NH₃-N reached critical levels of 6 mg l⁻¹ within 16–18 h when the fish were kept in the sealed bags at 10°C. Following transport, adaptation of pH in the water of the rearing facility to levels of pH 6.9–7.0 for 20–28 h minimized toxic NH₃ concentrations and ensured recovery. Recovery times varied to a large extent, influenced by the condition factor of the fish. Fish survival was 100% for 2 months post-transport, indicating that the critical parameters were met during transport.

Introduction
The Baltic sturgeon was a widespread species reproducing in the rivers of the southern and eastern Baltic Sea as well as in Lake Ladoga and Lake Onega (Holck et al., 1989). Heavily exploited in coastal and inland fisheries until the end of the 19th century (Debus, 1995), it has been driven to the verge of extinction by a combination of anthropogenic activities resulting in habitat loss, pollution and overfishing (Mamcarcz, 2000; Gessner et al., 2006) during the first half of the 20th century. The last capture of a Baltic sturgeon documented in Estonia was in 1996 (Paaver, 1996).

With the decreasing pollution of historic sturgeon rivers, remediation of the species became a focus of conservation policies (Gessner, 2000). As a prerequisite for remediation, the historic sturgeon stocks from the North and Baltic Sea drainages were characterized using mitochondrial DNA. Based on these genetic results as well as on morphological data, it became evident that Acipenser oxyrinchus Mitchell 1815 was endemic to the Baltic Sea at least since the middle ages (Ludwig et al., 2002). Subsequently, the restoration strategy for the Baltic – previously focusing on Acipenser sturio L. 1758 – was altered (Kirschbaum et al., 2004) and the use of A. o. oxyrinchus from the most northern populations of North America (King et al., 2001) was recommended by international experts in a 2002 workshop (Gessner and Ritterhoff, 2004).

As a prerequisite re-establishment the transfer of adult fish of wild origin from Canada to Germany became necessary in order to develop a captive founder broodstock as a source for subsequent annual reproduction and restocking (Gessner et al., 2007). Mature or subadult fish from the St. John population were obtained from commercial fisheries. Considering the perennial reproductive cycle and the asynchronous gonad development, 100 individuals of different age-classes were considered necessary to provide an effective population size of 10 mature fish for each annual reproduction (St. Pierre, 1996).

A 1-year period of rearing under controlled conditions was performed upon catch in the country of origin. During this time, the fish were closely monitored for parasites and diseases (Arndt and Gessner, 2005). After transfer to Germany, the fish were held in a quarantine facility at the Regional Fisheries Research Institute of Mecklenburg-Vorpommern to comply with the ICES Code of Practice (ICES, 2005).

The first test airfreight shipment in 2005 resulted in substantial mortalities following transport. The causes were unknown. Therefore the current study was designed to verify the risks associated with air-transport of large size fish. Apart from water quality deterioration due to metabolic activity, the short-term changes in cabin pressure were anticipated to be among the most critical impacting factors upon the fish. Strong pressure changes during rapid changes in flight levels have been reported to cause decompression effects such as micro-gas bubble formation in the tissues. Decompression effects have been described in detail for altitude simulations by

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Whitaker et al. (1945) as potentially causing gas bubble trauma (Alderdice and Jensen, 1985; Counihan et al., 1998) and gas bubble disease (Schäperclaus et al., 1990). For this reason, the effects of the pressure changes were tested during a flight simulation experiment in October 2006 in a pressure chamber in the Federal Training Centre Kienbaum, Germany.

The aim of the study was to determine the effects of fish exposure to the confined rearing conditions on the water quality and physiological parameters as well as to determine potential effects of the pressure changes on the blood gases. The investigation aimed for the development of sound transport techniques that would allow safe long-distance (inter-continental) air transport of valuable broodstock.

Materials and methods

Test-trial

The impact of atmospheric pressure changes (simulated aerial transport) was tested in October 2006. For the test trial 20 A. oxyrinchus from controlled reproduction in 2001 were used with a wet weight 8.7 ± 1.5 kg, total length 117.0 ± 5.6 cm (mean and SD, respectively), and tagged individually with passive integrated transponders (Trovan™; Siemens, Germany). The fish were reared in ponds at the Institute of Freshwater Ecology and Inland Fisheries, Berlin at 15°C prior to transport. For the experiment the fish were taken 21 km to the pressure chamber by truck with a transport tank (1500 L) equipped with an oxygen diffuser. Oxygen content in the water was maintained at 120% saturation, temperature was 15°C upon arrival. Combined handling and transfer time was 60 min. For the test, the fish were placed individually in polyethylene bags (150 x 100 x 70 cm) filled with 36 L of transport water. Five fish served as negative control not being subjected to packing and pressure changes. Five bags received 50 g CaCO₃ to buffer a pH decrease in the water through accumulated respiratory CO₂ during the flight simulation (CaCO₃ group). In a second group of five fish, 0.5 ml clove oil was added to the water (Mohler; 2004) to slightly anaesthetize the fish during the simulated flight. A further five fish were transferred into water-filled bags without additives (flight control). After transfer of the fish 75 L O₂ gas (ratio fish : water : gas by volume = 1 : 4 : 8) was added. The water level was sufficient to cover 2/3 of the fish and provided a water-saturated atmosphere, allowing normal respiration (Burggren, 1978). The bags were scaled and placed in the pressure chamber at an air temperature of 15 ± 2°C. Two video cameras were installed in the chamber to observe and record fish behaviour during the experiment.

The flight simulation lasted 8 h, including 30 min takeoff and landing phase simulations, respectively. Total time fish were maintained in the bags was 10 h.

To determine fish response during transport, the respiration frequency was calculated as the number of opercular movements per 30 s. Ten counts were analysed per each 5 min observation period.

At the end of the experiment, the pH, O₂, and temperature of the water in each bag were determined. Individual blood samples were taken 12 h prior to the flight simulation, immediately after the simulation process, as well as 12, 36, 120 and 336 h following the simulation. The negative control underwent the same sampling procedure.

To verify the impact of the transport simulation as well as to monitor the dynamics of the recovery phase following transport, blood samples were used to determine the gas saturation, blood pH and stress-related parameters including blood ion contents, cholesterol, cortisol, glucose, and triglyceride concentrations.

Intercontinental transport

Intercontinental transfers were carried out three times: on 7, 14 and 21 November 2006. For each transport, 10 fish of 20-40 kg individual body mass with 1.6-2.1 m TL were transferred from the quarantine rearing unit at Huntsman Marine Science Centre, St. Andrews, NB, Canada to the Halifax International Airport, NS, Canada by commercial fish transport truck. For the transport 20 m³ of dechlorinated tap water at 10°C were used. Oxygen was adjusted to 120% saturation throughout transport. For the air shipments, the fish were packed individually in polyethylene bags (250 x 100 x 100 cm) according to IATA specifications (IATA, 2006). The bags were equipped with tarp inlays to protect their surface from mechanical damage by the scutes of the fish. The bags were placed in water-tight 220 x 60 x 60 cm aluminium containers (Brossbox™; Germany) insulated with a 2.5 cm thick Styrofoam layer. The ratio of fish to water gas volume was approximately 1 : 5 : 10. Oxygen was injected into the deflated bag through the water until a complete exchange of the gas atmosphere was achieved, ensuring initial O₂ supersaturation of transport water. The total box volume of 750-L comprised 33% water (fish + water) and 66% O₂ atmosphere. Due to the low total hardness of the dechlorinated tap water, which did not exceed 1.5 dH, 200 g CaCO₃ and 50 g NaHCO₃ were added per transport bag, resulting in a total hardness of > 6.5 dH at pH 8.0. Temperature and pressure were recorded in 30 s intervals in five of the 10 air transport boxes using temperature and pressure loggers (IFC 200; Madge Tech). Transport temperature was 10.5 ± 1.0°C. Atmospheric pressure during the flight did not drop below 80% of the initial surface level. Decompression took place over a 30–60 min period at a rate of 0.3-0.4% pressure change per minute, reflecting less extreme changes than during the simulated flight. Upon arrival at Frankfurt Airport, Germany, O₂ and pH were determined in the bags with Oxyguard MKIII and Oxysguard pH probes. NH₄-N was determined using the Aquamerck ammonium calorimetric test in a dilution series with distilled water (Merck, Germany).

Following air transport the water used for hauling the fish as well as for acclimatization in the rearing facility was adjusted for pH to levels similar to those in the bags by the application of 36% HCl. Oxygen was maintained at supersaturation levels of 160-200% in an attempt to compensate the root effect on O₂ affinity of haemoglobin at low pH levels. Upon arrival at the farm the fish were maintained individually in 3 × 1 × 0.6 m troughs to allow acclimation to water quality and permit blood sampling without further stressing the fish by frequent handling. Blood samples were collected upon arrival at the farm (12 h after arrival at the airport) as well as 5, 11 and 15 h thereafter.

Blood analysis

From the caudal vein 1.5 ml blood was sampled with a 2 ml heparinized syringe and analysed immediately. The blood gas contents and pH were determined with a gas analysis system (Rapidlab® 241; Bayer Healthcare) in two replicates using 0.15 ml of blood each. Gas content was determined at a reference temperature of 37°C to avoid temperature-related
variability according to the protocol of the producer and is given as partial pressure (kPa). Na⁺, K⁺, Ca²⁺, CI⁻, and glucose concentrations were determined in two replicates using 0.3 ml of blood using an automatic analysis system (Bayer Healthcare, Rapidlab 273). Between samples, the probes were rinsed and the analyser was calibrated every 10 measurements. Analyses for cortisol and cholesterol were limited to the test trials. Samples were centrifuged at 10,000 g for 2 min to separate the blood plasma, and alcohol extraction was carried out immediately (Wuertz et al., 2006). Plasma was frozen at −20°C until processed.

Blood plasma cortisol concentration was determined using an ELISA test kit (Human, Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) according to the manufacturer’s protocol. Absorbance was determined with a Humareader photometer (Germany) at 450 and 630 nm. Cortisol concentrations were calculated in nanogram per litre using a standard. Concentration of cholesterol was determined using the enzymatic colorimetric kit (CHOD-PAP; Reanal, Budapest, Hungary). Therefore, 0.01 ml of blood plasma was added to 1 ml of the reagent buffer (75 mmol l⁻¹ PIPES buffer, 5 mmol l⁻¹ phenol, 0.3 mmol l⁻¹ amino-antipyrine) and incubated for 5 min at 37°C. Cholesterol concentrations were determined with a photometer at 500 nm. Cholesterol levels were determined as millimole per litre from a standard curve. An enzymatic colorimetric kit (GPO-PAP; Reanal, Budapest, Hungary) was used for the quantification of triglycerides. Blood plasma (0.01 ml) was added to 1 ml of reagent buffer (50 mmol l⁻¹ PIPES, 5 mmol l⁻¹ 4-chlorophenol, 40 mmol l⁻¹ Mg²⁺, 1.4 mmol l⁻¹ ATP). After 5 min incubation at 37°C, absorbance was determined at 500 nm with an automatic photometer (UV mini-1240; Shimadzu) and content of triglycerides was determined with a standard dilution series.

Statistical analysis was performed using SPSS 10.0 for Windows, SIGMA STAT 2.0 for Windows and MS Excel (Office 2000). Data are presented as mean ± SD (standard deviation). Homogeneity of variance and normality was determined by Kruskal–Wallis and Kolmogorov–Smirnov test (passed if P < 0.05). Statistical comparisons were carried out using the t-test or the Mann–Whitney rank sum test. Multiple comparisons were carried out by either parametric Tukey or non-parametric Dunn’s posthoc test.

Data on concentrations of cortisol, cholesterol and triglycerides were subjected to two-way analysis of variance (ANOVA) at P < 0.05 to test the effects of treatments during transportation. Bonferroni’s post-test was conducted to investigate individual differences among blood plasma component concentrations at each sampling time. These statistical analyses were carried out using the statistical software GRAPHPAD PRISM version 4.0 for Windows.

Results

Initial test-trial

Baseline levels for the blood parameters (pO₂, pCO₂, pH) in *A. oxyrinchus* are given in Table 1. Significant differences between arterial and venal blood were observed with regard to gas pressure (pO₂ and pCO₂). Blood from the venous system had a mean pO₂ of 2.14 ± 0.765 kPa while arterial blood had a pO₂ = 5.86 ± 1.583 kPa. Corresponding pCO₂ values for venal and arterial blood were determined as 2.35 ± 0.135 kPa and 1.95 ± 0.157 kPa, respectively, being statistically different (P < 0.05, Tukey, n = 12). Blood pH revealed insignificant differences between both groups with means of 7.410 ± 0.0240 and 7.369 ± 0.0253 for arterial and venal blood, respectively.

Baseline levels for glucose (2.59 ± 0.17 mmol l⁻¹), cholesterol (2.65 ± 0.0 mmol l⁻¹), triglycerides (2.28 ± 0.17 mmol l⁻¹) and cortisol (84.2 ± 8.6 ng l⁻¹) were determined (n = 15). The respective data for the main ions K⁺, Na⁺, Ca²⁺ and Cl⁻ are given in Table 2.

The response to the pressure changes during the test trial in respiratory frequency, given as opercular movements, is shown in Fig. 1. The mean respiration frequency prior to the exposure was determined to be 30.6 ± 0.6 n/30 s. During the 30 min decompression phase the initial pressure dropped by 25%. During this initial pressure reduction and the first 120 min of hypobaric conditions the respiration frequency increased significantly to mean values of 34.0 ± 0.6 n/30 s (P < 0.05, Tukey, n = 10). During the following constant pressure conditions of 850 hPa, the respiration frequency during 200 min decreased significantly to an average of 31.9 ± 0.6 n/30 s (P < 0.001, Tukey, n = 40). This value still reveals statistically significant (P < 0.005, Tukey, n = 10) elevated respiration levels compared to pre-decompression conditions. During the subsequent increase in cabin pressure only an insignificant alteration of the respiration frequency to a mean of 31.7 ± 0.5 n/30 s was observed (P > 0.05, Tukey, n = 40).

Water quality analysis in the transport bags after the experimental exposition revealed an oxygen super-saturation of 187.1% at 15.3°C. The pH of the water significantly decreased over the 10 h exposure from a mean of 7.60 ± 0.24 to 7.05 ± 0.31 (n = 15, Tukey P < 0.01).

In venal blood, pCO₂ and pH revealed the most prominent changes for the first 12 h following exposure (Fig. 2). In pO₂ no significant changes were recorded prior to and after exposure in the test trial (n = 30, Tukey P > 0.05). The
The effects of adding either CaCO_3 or clove oil to the water did not result in significant differences in water or blood values between the treatments and the control, as demonstrated for pH in Fig. 3.

The data for sodium, potassium, and calcium contents in the blood prior to and after the flight simulation did not reveal any statistically significant differences (Table 2). Chloride contents in the blood decreased from 1192.4 ± 2.1 mmol l\(^{-1}\) to 119.0 ± 2.0 mmol l\(^{-1}\) (P < 0.001, Tukey, n = 15) following exposure. Recovery to the initial levels was completed after 4 days. A reciprocal trend was observed in glucose concentration (Fig. 4), which increased from a baseline value of 2.65 ± 0.18 mmol l\(^{-1}\) to 6.29 ± 2.39 mmol l\(^{-1}\) immediately following exposure. After 36 h, the blood glucose was still significantly higher than in the controls (3.06 ± 0.16 vs 2.84 ± 0.15, n = 5, Tukey, P < 0.05). Glucose levels in the blood recovered completely 72 h post-stress exposure. Cholesterol blood contents varied between groups and time after exposure in an undirected manner. Differences between the groups were insignificant throughout the test period. Triglyceride levels in all groups revealed mean values between 1.96 ± 0.23 and 2.57 ± 0.18 mmol l\(^{-1}\) without sig-

![Fig. 1. Respiration frequency as operculae movements (n = 10) of subadult A. oxyrinchus during simulated flight conditions. Exposure to fully hypobaric conditions (850 kPa) (arrows) with pressure change for 30 min, simulating takeoff and landing.](image1)

![Fig. 2. Changes in pO\(_2\) (white bars), pCO\(_2\) (grey bars), pH (black dashed line with triangles as data points) in venal blood of A. oxyrinchus following 10 h flight simulation experiment in a stagnant waterbody of 15°C at oxygen super-saturation. Vertical line separates ground time from flight simulation period. T –12 = sampling 12 h prior to experiment. T0–120 sampling times in hours after ‘landing’. Data represent means and standard deviations (bars); n = 20.](image2)

![Fig. 3. Water and blood pH values following 10 h exposure of A. oxyrinchus (mean body mass 8 kg) in 32-L H\(_2\)O at 15°C in control group, with addition of clove oil (0.2 ml/32-L H\(_2\)O) and 50 g CaCO\(_3\). Bars represent mean values, whiskers are standard deviations, n = 10.](image3)

![Fig. 4. Comparison of glucose blood content (mmol l\(^{-1}\)) in A. oxyrinchus following 10 h exposure of A. oxyrinchus (mean body mass 8 kg) in 32-L H\(_2\)O at 15°C at flight simulation in the containment (grey n = 15) and control (white n = 5). Vertical line separates ground time from flight simulation period. T –12 = sampling 12 h prior to experiment. T0–366 sampling times in hours after ‘landing’. Bars and whiskers represent means and standard deviations.](image4)

![Fig. 5. Comparison of cortisol blood content (ng*ml\(^{-1}\)) in A. oxyrinchus following 10 h exposure of A. oxyrinchus (mean body mass 8 kg) in 32-L H\(_2\)O at 15°C at flight simulation in the containment (grey n = 15) and control (white n = 5). Vertical line separates ground time from flight simulation period. T –12 = sampling 12 h prior to experiment. T0–366 sampling times in hours after ‘landing’. Bars and whiskers represent means and standard deviations.](image5)
significant differences between treatments and time after exposure. In cortisol (Fig. 5), the results revealed no significant differences among groups over time.

**Transatlantic transport**

Subsequent air transports of adult sturgeon were carried out at 10°C. The pressure profiles during transport were characterized by a mean decompression period from 1028 ± 0.3 hPa to 870 ± 0.4 hPa during 42.5 ± 12.8 min. The compression phase prior to landing lasted 20.6 ± 7.2 min on average, with pressure increasing to 999.5 ± 0.2 hPa. The saturation of the soluble gases in the transport bags resembled the experimental trials, with O₂ saturation ranging between 61 and 158%.

**Blood pH**

Blood pH upon arrival at the farm varied between 6.84 and 7.12 (mean = 6.99 ± 0.09). The pCO₂ levels were between 8.24 and 4.78 kPa (6.46 ± 1.17 kPa). The most prominent changes were observed in pO₂ values with mean partial pressures of 29.7 ± 20.1 kPa upon arrival and 45.6 ± 20.7 kPa after five hours of acclimatization at the hatchery. Recovery dynamics of the blood parameters are given in Fig. 6.

Increase of the water pH in the rearing facility was gradually facilitated with the increasing blood pH of the fish. Return to baseline levels of blood pH, pCO₂ and pO₂ were reached 11–24 h after arrival at the facility (e.g. 23–36 h following landing), with a pronounced individual variability allowing separation of fish into two distinct groups: recovery in the first group regardless of the initial pH value was rather constant, with an hourly pH increase of 0.029 and 0.023 for the first 6 h and subsequent 5 h period. This compares to increases in pH of 0.013 and 0.013 h⁻¹ in the second group, respectively. The last stage of recovery in the second group was characterized by an increase of 0.033 pH per hour. After blood pH levels and pCO₂ levels returned to baseline values, the fish were transferred into rearing tanks of the quarantine facility.

**Discussion**

Differences in arterial and venal blood parameters (pO₂, pCO₂, pH), as well as for a variety of blood ions, cholesterol, triglycerides and cortisol, were documented in A. oxyrinchus prior to and following the intercontinental air transport. Knowles et al. (2006) described reference values for blood ion concentrations in A. brevirostrum such as sodium 124–141 mmol l⁻¹, potassium 2.9–3.7 mmol l⁻¹, chloride 106–121 mmol l⁻¹, calcium 6.6–12.1 mg dl⁻¹, glucose 37–74 mg dl⁻¹, and cholesterol 42–133 mg l⁻¹. Congruently, the values observed in this study were in good agreement with these reference values.

**CO₂ accumulation**

CO₂ accumulation in the stagnant transport water resulted in a significant decrease of water pH. Blood pCO₂ is closely related to the pH via the root-effect. During transport, the blood pH decreased on average by 0.35 ± 0.08 to reach 7.0 ± 0.12. This is caused by the reduced elimination of the CO₂ through the gills and CO₂ accumulation in the stagnant transport water resulted in a slightly lower pH than in the control group (Fig. 3). This may be attributed to the elevated hardness of the water (GH 9°C) prior to CaCO₃ supplementation, which in turn increased the CO₂ in the water. Addition of clove oil to the water resulted in intermediate values for the blood pH between the CaCO₃ group and the control. Clove oil decreased the respiratory activity rather than the basic metabolic rate, thereby resulting in increased blood pCO₂ and pH.

Although glucose is commonly considered as a sensitive stress parameter (Webb et al., 2007), no significant differences were detected between flight treatment or the control after 12 h of recovery. After chronic stress exposure by two consecutive stressors for 10 min day⁻¹, the blood glucose levels in A. medirostris increased by 200% (Lankford et al., 2005). Significant increases were observed in glucose after 1 min air exposure. The time until return to baseline levels was significantly longer at lower rearing temperatures (Lankford et al., 2003), which could explain the relatively quick recovery in the test trial at 15°C.

A number of studies documented deviations from normal cortisol values under different stress conditions. Beyea et al.
(2005) reported 17 ng ml⁻¹ of cortisol at 6 h post-stress in *A. brevirostrum*, which resembles 25% of the minimum values observed in this study. Würtz et al. (2006) observed < 10 ng ml⁻¹ cortisol in *A. brevirostrum* in the control group and 25–40 ng ml⁻¹ in fish reared at high densities, which is considered as a moderate stressor. These values are in good agreement with the results of Bayunova et al. (2002) in *A. stellatus* and Lankford et al. (2003) in *A. medirostris*. In conclusion, levels above 20–30 ng ml⁻¹ can be considered as indicative of elevated stress. Cortisol values determined in tissue and blood levels in NH₄⁺ hatcheries by a factor of 10–20 (Conte et al., 1988), could have pressure-induced changes in Na⁺–K⁺ ATPase activity, as typical in ammoniotelic animals might become affected by the transport processes (Na⁺–K⁺ ATPase activity). As a result, pH values of the transport water decreased to pH levels at or below seven only. This reduction in pH was considered advantageous with regard to the high NH₄⁺–N concentrations observed after transport. Since dissociation of NH₄⁺ to toxic NH₃ is pH-dependent (Tomasso, 1994), low pH levels were applied for 24 h in the water used for acclimation upon arrival. This was also considered important since ammonium excretion is largely dependent upon the pH of the medium (Milligan et al., 1991) and requires adaptation of the fish to changing conditions. Furthermore, the high ammonium concentrations in the transport water, which exceeded the safeguard levels for hatcheries by a factor of 10–20 (Conte et al., 1988), could have adversely affected excretion efficiency and thereby increased tissue and blood levels in NH₄⁺. Additionally, a significant impact of pressure changes upon ionic and osmoregulation was described by Pequeux and Gilles (1986). Tissue concentrations of Na⁺, Cl⁻ and K⁺ in eel subjected to increased pressure indicated a physiological imbalance and a reduction in active transport processes (Na⁺–K⁺ ATPase activity). As a consequence, the co-transport of ammonium with anions typical in ammionotic animals might become affected by the pressure-induced changes in Na⁺−K⁺ ATPase activity, leading to increased tissue concentrations.

Transatlantic transport
In contrast to the experimental trials, the low hardness (<1.5°dH) of the dechlorinated tap water used for transport from Canada to Germany necessitated an increase buffer capacity. For this purpose, 200 g CaCO₃ and an additional 50 g NaHCO₃ were added to the transport water per bag to provide sufficient buffer capacity of the water during transport to prevent excessive acidification. As a result, pH values of the transport water decreased to pH levels at or below seven only. This reduction in pH was considered advantageous with regard to the high NH₄⁺–N concentrations observed after transport. Since dissociation of NH₄⁺ to toxic NH₃ is pH-dependent (Tomasso, 1994), low pH levels were applied for 24 h in the water used for acclimation upon arrival. This was also considered important since ammonium excretion is largely dependent upon the pH of the medium (Milligan et al., 1991) and requires adaptation of the fish to changing conditions. Furthermore, the high ammonium concentrations in the transport water, which exceeded the safeguard levels for hatcheries by a factor of 10–20 (Conte et al., 1988), could have adversely affected excretion efficiency and thereby increased tissue and blood levels in NH₄⁺. Additionally, a significant impact of pressure changes upon ionic and osmoregulation was described by Pequeux and Gilles (1986). Tissue concentrations of Na⁺, Cl⁻ and K⁺ in eel subjected to increased pressure indicated a physiological imbalance and a reduction in active transport processes (Na⁺–K⁺ ATPase activity). As a consequence, the co-transport of ammonium with anions typical in ammionotic animals might become affected by the pressure-induced changes in Na⁺−K⁺ ATPase activity, leading to increased tissue concentrations.

Conclusions
The experiment revealed that pressure changes within the tested limits do not result in negative effects in subadult and adult sturgeons in terms of blood gas concentration or gas bubble trauma and, as a result, survival.

Transport of the fish in a closed confinement with a water volume only five times their body weight for more than 14 h resulted in the accumulation of metabolites in the transport water. Carbon dioxide accumulated in the transport water as well as in fish blood and the tissues, resulting in significant decreases of the pH, causing acidosis. The resulting decrease in capacity of the blood to bind oxygen can be circumvented by oxygen supersaturation of the transport medium. The oxygen atmosphere supplied to the container prior to air transport allowed the maintaining of supersaturation in the water during and after transport. The observed increase in NH₄⁺ in the water as well as its accumulation in the blood is counterbalanced by CO₂ accumulation, gradually reducing the pH of the water, which circumvents the increase of unionized ammonia and thereby avoiding toxicity. The process of acclimation during an extended phase after transport was required to allow the blood pH and pCO₂ of the blood to gradually return to normal values with the progress of decreasing excessive total ammonia concentrations. Therefore, water pH, blood pH, pCO₂ and pO₂ should be closely monitored as these provide reliable indicators of the recovery phase.

Since metabolism is temperature-dependent, the accumulation of the metabolic end products over time depend mainly on the temperature. Lower temperatures increase the time available for the entire operation due to low metabolic rates, resulting in low excretion and accumulation rates. Monitoring and maintaining the critical parameters (e.g. temperature, oxygen contents, pressure changes over time, pH and NH₄⁺–N in the transport and the recipient water, blood pH and pCO₂) is essential for successful transfer.

Acknowledgements
This study was supported through the German Ministry for Education, Science and Technology under the grant FKZ 0330532. The authors cordially thank Andreas Rhode and Derek Burris of Air Canada as well as Connie McKinnon of Schenker Halifax for their support in transport logistics; Thane T. Jones and the Dartek crew for helping out during preparation of the fish for transport at Halifax airport; Jane Symonds and John O’Halloran for their numerous attempts to cope with administrative preparations in Canada; and special thanks are devoted to Erhard Gräbert of the Federal Training Centre, Kienbaum, Germany for his support during utilization of the pressure chamber as well as to all the technicians for handling and maintaining the fish during the experiments.

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Author's address: J. Gessner, Department of Biology and Ecology of Fishes, Leibniz Institute of Freshwater Ecology and Inland Fishery, Müggelseedamm 310, D-12587 Berlin, Germany.

E-mail: sturgeon@igb-berlin.de