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Effects of feeding on thermoregulatory behaviours and gut blood flow in white sturgeon (*Acipenser transmontanus*) using biotelemetry in combination with standard techniques

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SUMMARY

The effects of thermoregulatory behaviours on gut blood flow in white sturgeon *Acipenser transmontanus* before and after feeding was studied using a blood flow biotelemetry system in combination with a temperature preference chamber. This is the first study to look at cardiovascular responses to feeding in white sturgeon, and also the first time behavioural tests in fish have been combined with recordings of cardiac output, heart rate, cardiac stroke volume and gut blood flow. The results showed strong correlations between gut blood flow and temperature choice after feeding (R^2=0.88±0.03, 6–8 h postprandially and R^2=0.89±0.04, 8–10 h postprandially) but not prior to feeding (R^2=0.11±0.05). Feeding did not affect the actual temperature preference (18.4±0.7°C before feeding, 18.1±0.7°C, 6–8 h postprandially and 17.5±0.5°C, 8–10 h postprandially). Fish instrumented with a blood flow biotelemetry device, and allowed to move freely in the water, had a significantly lower resting heart rate (37.3±0.26 beats min^{-1}) compared with the control group that was traditionally instrumented with transit-time blood flow probes and kept in a confined area in accordance with the standard procedure (43.2±2.1 beats min^{-1}). This study shows, for the first time in fish, the correlation between body temperature and gut blood flow during behavioural thermoregulation.

Key words: gastrointestinal blood supply, postprandial, temperature preference, chronic measurements.

INTRODUCTION

The majority of all living vertebrates are ectotherms and their main mean of controlling their body temperature is through behaviour. Thermoregulation in ectotherms is shown to be extremely complex, and numerous reviews have discussed the potential costs and benefits associated with these behaviours (Huey and Slatkin, 1976; Reynolds and Casterlin, 1979; Seebacher, 2005; Seebacher and Franklin, 2005; Golovanov, 2006). It is generally believed that ectothermic animals can benefit from behavioural thermoregulation by avoiding harmful temperatures and by getting some control over metabolic processes, but these behaviours are associated with costs, which in some situations outweigh the benefits making thermoregulation impractical.

Most studies on thermoregulatory behaviour have been conducted on reptiles and show that most species will regulate their body temperature when given the opportunity (Bogert, 1949; Seebacher and Franklin, 2005). Thermoregulation in ectotherms is believed to be most pronounced in land-living species where the thermoregulatory behaviour to a large extent involves basking in, or avoiding, the sun. In an aquatic environment, the high thermal conductivity of water leads to most aquatic ectotherms having the same temperature as the surrounding water. The preferred temperature in water is therefore mainly achieved by seeking out areas with more beneficial (warmer or colder) temperatures. This temperature-seeking behaviour has mainly been studied in different species of fish but has also been observed in the amphibian Danube crested newts *Triturus dobbrogicus* (Reynolds and Casterlin, 1979; Gvozdik, 2003).

The temperature preferred by a fish, in a stable environment when food is abundant, tends to be centred around the temperature that provides conditions optimal for physiological activity, e.g. maximum growth (Magnuson et al., 1979; Reynolds and Casterlin, 1979; Jobling, 1981). The choice of an individual fish can however be influenced by a range of different external and internal factors, e.g. ontogeny (Lafrance et al., 2005), time of the day (Matern et al., 2000), season (Mortensen et al., 2007), intra- and inter-specific interactions (Magnuson et al., 1979), feeding status (Wallman and Bennett, 2006), parturition (Wallman and Bennett, 2006) and infection (Reynolds et al., 1976). Sometimes a combination of several factors may trigger individuals to change their temperature preference. For example, feeding status in combination with time of day and perhaps also with ontogeny influence temperature preference in larvae of Bear Lake sculpin *Cottus extensus* (Wurtsbaugh and Neverman, 1988) and adult roach *Rutilus rutilus* (van Dijk et al., 2002).

So far, studies linking the complex thermoregulatory behaviours in fish to physiological parameters are sparse. One reason has been the difficulty in measuring physiological parameters like metabolic rate and cardiovascular variables *in vivo* without heavily constraining the mobility of the studied animal. Traditional methods to measure, e.g. blood flow involve hard-wired animals, restricted to relatively small experimental chambers and thus limit the ability of the fish to behavioural thermoregulation. In a recent study, we successfully used a fully implantable dual-channel radio-based Doppler blood flow biotelemetry system to record cardiac output and gut blood in fish (Gräns et al., 2009b). This biotelemetry system has opened up the possibility to combine measurements of blood flow with behaviour by allowing the animal under study more freedom.

In the present study, juvenile white sturgeon *Acipenser transmontanus* (Richardson) were allowed to move freely in a temperature gradient while cardiovascular variables were measured. The effects of feeding on temperature preference were examined. To measure temperature preference, a horizontal gradient was used. The setup consists of an annular-shaped chamber designed to minimise the effects of external influences while maximising the span of the gradient. The design was first presented by Myrick and colleagues (Myrick et al., 2004) and has later been used by other research groups (Chen et al., 2008; McMahon et al., 2008).

Because we suspect that it could be beneficial for juvenile white sturgeon to grow fast, our initial hypothesis was that sturgeon would select a higher temperature after feeding. By selecting a higher temperature the juvenile fish could potentially increase the rate of digestion, absorption efficiency and ultimately enhance growth.

We also wanted to investigate how the cardiovascular variables (with the main focus on gut blood) would respond to voluntary changes in body temperature. Linking blood flow regulation, feeding and temperature preference is a continuation of the extensive work previously devoted to understanding each of these variables on their own.

MATERIALS AND METHODS

Animals used

Sixteen white sturgeon were used in this study (body mass 2.53±0.11 kg). They were kept at the Center for Aquatic Biology and Aquaculture (CABA) Davis California, CA, USA, in 12,800-l tanks until needed for experiments. The tanks received a continuous flow of air-equilibrated, 19°C well water. The fish were fed commercial trout pellets (Silver Cup, Murray, UT, USA) at 0.5% body mass day^{-1}. The fish were kept under natural photoperiod.

Preoperative care

The animals were fasted for approximately 72 h before surgery. The sturgeon were anaesthetised by placing them in a 40-l water tank with well water containing 0.2 g l^{-1} 3-aminobenzoic acid ethyl ester (MS-222) and 10 g l^{-1} NaCl, and buffered to pH 7.0 with 4.2 g l^{-1} $NaHCO_3$. The sturgeon were kept in the anaesthetics until ventilatory movements ceased, and they were then transferred to the operating table. The fish were positioned with the ventral side facing up and the eyes protected from the bright light and from evaporation by a wet towel. Anaesthesia was maintained by pumping oxygenated, buffered MS-222 (0.075 g l^{-1}) over the gills during surgery. Prior to the surgery, the sturgeon were injected with 2.5 mg kg^{-1} (1 mol l^{-1}) Enrofloxacin (Baytrilo®, Bayer, KS, USA). The surgery was performed under semi-sterile conditions. A sterile surgical drape (1051 Incise Drape, 3M, St Paul, MN, USA) was placed over the fish to keep the surgical surface sterile. Sterile gloves (Ansell Healthcare Products Inc., Red Bank, NJ, USA) were used throughout the surgery to minimise contamination, and all of the surgical instruments, the implant, all associated electrical leads and probes were sterilised by the use of Cidex (Johnson & Johnson Company, New Brunswick, NJ, USA). The surgical precautions taken have been stressed in several reviews on fish surgery (Butcher and Wildgoose, 2001; Fontenot and Neiffer, 2004) and were decided together with the veterinary staff at the University of California (UC), Davis. All animal experiments were performed in accordance with national and local ethical guidelines (UC Davis Animal Care and Use Protocol Number: 07-12677).

Surgeries and preoperative care

Half of the animals (N=8) were instrumented with a fully implantable dual-channel Doppler blood flow biotelemetric system (Endosomatic Systems, Inc., Davis, CA, USA). White sturgeon has one single large celiacomesenteric artery that supplies the gastrointestinal tract with blood. To expose the celiacomesenteric artery, a 5 cm mid-ventral incision was made posterior to the pectoral girdle and the liver was carefully moved aside. Gut blood flow was measured using a silicon cuff transducer probe with two parallel canals with silk sutures for closing the probes (ES-2.5, Iowa Doppler Products, Iowa City, IA, USA) that were placed around the celiacomesenteric artery. The probe cuffs were tied down onto the vessels using the two silk sutures. When the first flow probe was in place, a second 4 cm ventral incision was made posterior to the gill juncture and carefully, without disrupting the pericardium or damaging any vessels, the dermal and sub-dermal musculature and connective tissue were separated using blunt dissection tools in order to expose the ventral aorta. To fully internalise the lead from the ventral aortic flow probe, the probe was tunnelled under the skin from the ventral incision to the mid-ventral incision. Thereafter a second, larger silicon cuff-type Doppler blood flow transducer (ES-4.0, Iowa Doppler Products) was placed around the ventral aorta posterior to the first two anterior pairs of branchial arteries as described above. The wires from the probes were anchored with a single stitch of 3/0 silk suture in the sub-dermal muscle tissue. The implant and battery were then carefully placed in the abdominal cavity and the retracted organs restored to their original places. The two incisions were closed using sterile 3/0 nylon monofilament suture. The locations of the probes and the implant are illustrated in Fig. 1.

In order to study the absolute cardiovascular effects (Doppler flow probes will only give relative values) of feeding in white sturgeon, a second group of animals were instrumented using a Transonic transit-time blood flow probe (2S or 4S, Transonic Systems, Ithaca, NY, USA). In order to minimise biases due to surgery, the incisions and surgical procedure followed the protocol described above. The only major difference was that the leads from the transit-time flow probe were secured on the outside of the animals, using single silk sutures in the skin and connected to a

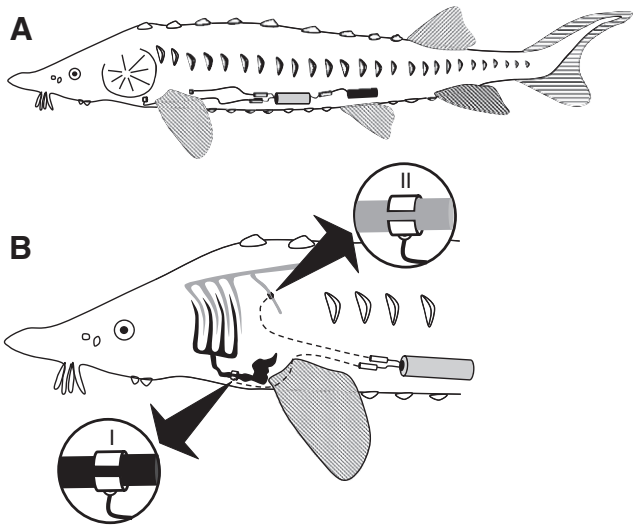

Fig. 1. (A) Schematic picture showing the position of the implant (grey cylinder) and battery pack (black cylinder) placed in the abdominal cavity of the white sturgeon *(Acipenser transmontanus)*. (B) The Doppler flow probe positioned on the ventral aorta (I) and the celiacomesenteric artery (II).

Transonic flowmeter (T206, Transonic Systems). The line-powered flowmeter automatically identifies the scaling factor and individual calibration factor of the flow probe connected to it, and thus gives the absolute flow in $cm\,s^{-1}$.

Data were digitally stored for subsequent analysis using a PowerLab unit (AD Instruments Pty Ltd, Castle Hill, Australia) connected to a commercial data acquisition system (ML865 PowerLab 4/25T Data Acquisition System, AD Instruments Pty Ltd) running the software Chart 5 (AD Instruments Pty Ltd) for Windows on a PC-computer (Dell Latitude 820, Austin, TX, USA).

The surgical procedure including anaesthesia and awakening took between 60 and 90 min. Postoperatively, all animals were given a subcutaneous injection of $0.4\,mg\,kg^{-1}$ Torbugesic (butorphanol, Fort Dodge, Iowa, IA, USA) for postoperative pain relief.

Temperature preference chamber

The temperature preference chamber is a larger version of the setup described in detail by Myrick and colleagues (Myrick et al., 2004). The total diameter was 3 m and the swimming channel was 30 cm wide with a water depth of 15 cm. Water was distributed from 16 reservoirs containing either cooled (11.5°C), ambient (19°C) or warm (24°C) water. The cool water was obtained using two custom made 15-horsepower chillers; the warm water was obtained using two Mobius gas boilers (T-M1 Takagi, Irvine, CA, USA). Thirty-two pairs of calibrated thermistors (YSI 400-series, Advanced Industrial Systems, Inc., Harrods Creek, KY, USA; accuracy ±0.1°C) were coupled to the inner and outer walls of the swimming channel at mid-water depth to measure temperature. A schematic figure of the swimming channel and the temperature gradient retained in it during the temperature preference trials are shown in Fig. 2.

The perimeter of the temperature preference chamber was surrounded by a 2.5 m high curtain in order to diffuse the natural light from the building's translucent ceiling. In order to minimise the disturbance from the observer, a camera connected to a video monitor was positioned overhead the fish. The swimming channel was divided into 32 sections, each about 11.25 deg arc and labelled with numbers visible on the monitor's screen. Each individual fish was released into the chamber at a randomly selected location. At the start, all 32 sections of the swimming channel received water of ambient temperature, keeping the temperature stable at 19°C throughout the chamber.

Experimental protocol

The fish instrumented with Doppler-based blood flow biotelemetry system (N=8) were used in the temperature preference chamber while the group instrumented with traditional benchtop transit-time (hard-wired) flow probes (N=8) was used as a control group in the laboratory.

All fish used in the temperature preference setup were kept in the chamber overnight after surgery, in order to acclimate prior to the first preference test. After turning on the cold and warm water flow, the temperature gradient stabilised within 5 min. Fish could swim around the swimming channel in <15 s, which minimises possible space and time autocorrelations. It was also noted that all sturgeon turned during their time in the temperature preference chamber.

Each fish was exposed to the temperature gradient for 2 h immediately before feeding, during which time the location of the snout of the fish and the corresponding temperature (mean value from the closest pair of thermistors) were recorded every 4 min. After 2 h, the temperature gradient was turned off and the animal was netted and anaesthetised until the righting reflex was lost. The sturgeon was fed its daily ratio (0.5% body mass day^{-1}) of Silvercup trout pellets by gavage. This is a standard method to control for meal size and exact time of feeding.

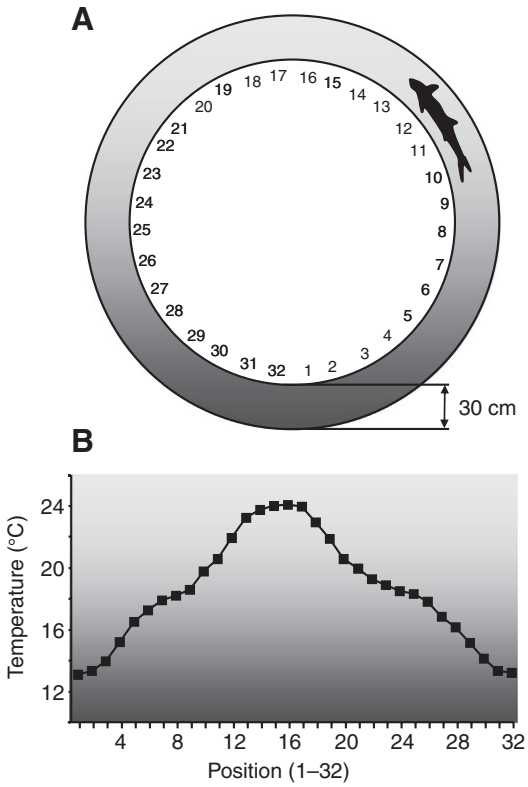

Fig. 2. Schematic drawing of the temperature preference chamber with its 32 locations for temperature measurements (A). Representation of the temperature gradient and the 32 areas (B). The silhouette of a sturgeon illustrated in the figure is according to scale and is drawn from the mean tail-to-head length of the animals used (78.5±0.6 cm).

After the meal, the fish was again put into the ambient temperate water at a randomly selected location, and left to recover and start digesting the meal. Six hours after the meal the temperature gradient was turned on again and the location of the snout and the corresponding temperature were recorded for another 4 h (6–10 h postprandially).

During the experiment, starting with a 30-min control period before the animals were fed and ending 10 h postprandially, cardiac output, gut blood flow and temperature were continuously recorded. Heart rate was calculated from the phasic cardiac output signal using the cyclic measurement feature in Chart 5 (AD Instruments Pty Ltd) while cardiac stroke volume was calculated as cardiac output divided by heart rate.

The fish from the control group, instrumented with traditional bench-top transit-time flow probes, were after surgery moved to a 100 cm-long and 30 cm-wide transparent chamber for recovery. The chamber received a continuous flow of air-equilibrated, 19°C well water. Fish were allowed to recover overnight before the experiment started.

The same cardiovascular parameters were recorded as in the first experimental group (starting 2 h before feeding). After the first two hours, the animal was netted and fed as described above. Thereafter the sturgeon was carefully returned to its chamber and left alone for the rest of the experiment.

Data analysis

The recorded variables were analysed for three 2-h periods, including 2 h before feeding, 6–8 h after feeding and 8–10 h after feeding. When possible, comparisons were made also between the two groups (biotelemetry and hard-wired).

All data are presented as means ± s.e.m. if nothing else is stated. Statistical analyses were conducted in SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). A linear mixed model was used for comparisons between and within the experimental groups. Individuals were set as subjects and the three time points as repeated measures. Compound symmetry was used as type of covariance. The cardiovascular variables (cardiac output, gut blood flow, heart rate and cardiac stroke volume) were set as dependent variables and treatment group (when possible) together with the three time points as factors. The factors and the interactions between them were compared using a Sidak confidence-interval adjustment. For comparison of routine heart rate between the two groups a two-tailed *t*-test assuming equal variances was used. Differences where $P<0.05$ were regarded as statistically significant. Due to the relative values obtained from a Doppler flow probe, for the comparison between the two groups the cardiovascular variables were calculated as a percentage, defining the control period before feeding as 100%.

In the biotelemetry group the temperature preference was calculated individually using SPSS 12.0.1. The median, of all of the temperatures a sturgeon was observed in, was defined as the temperature preference, and the 1st and 3rd quartiles represent the upper and lower limits for its temperature preference interval. For each animal a complete temperature preference (including the median and the 1st and 3rd quartiles) was calculated for each of the three 2-h periods (before feeding as well as 6–8 h and 8–10 h postprandially).

Three coefficients of determination of a linear regression (R^2-values), each representing the possible correlation between temperature and the corresponding gut blood flow for one of the three 2-h periods (before feeding as well as 6–8 h and 8–10 h postprandially), were calculated for each animal. In the raw data trace, a 3–5-min period from when the fish stayed in the temperatures representing that individual's median, 1st and 3rd quartiles was located. Gut blood flow from these 3–5-min periods was observed and the correlation between gut blood flow and temperature was calculated from this data. Temperatures only within that individual's temperature preference interval were used for the correlation analysis in order to minimise bias from gastric stress responses. Consequently, if the animal was seen recovering from a stress response that time period was not used in the analysis.

The swimming activity of the sturgeon in the temperature preference setup was calculated as the mean swimming speed ($cm\,s^{-1}$) during the three 2-h periods (before feeding as well as 6–8 h and 8–10 h postprandially).

RESULTS

Temperature preference

Most fish found an area in which they ended up staying for the most part of the trial. Although the warmest area in the temperature preference chamber (24°C) was avoided by all individuals, the relative proximity to this area where the avoidance behaviour started varied greatly. When observing the fish exploring different temperatures in the chamber, it was striking how differently individuals behaved. When approaching the extreme temperatures (mostly the warmest) many individuals showed signs of discomfort. While some individuals just gently increased their swimming speed and cruised through the warm/cold areas, others made drastic movements or turned around in the chamber. These sudden movements often resulted in a dramatic reduction in gut blood flow.

The temperature preference (median, 1st and 3rd quartiles) for each fish is presented in Table 1. The median of temperatures preferred of unfed white sturgeon acclimated to 19°C, ranged from 15.0 to 21.8°C with a mean of 18.4±0.7°C (N=8). After feeding, the median temperature was 18.1±0.7°C (at 6–8 h post-feeding) and 17.5±0.5°C (8–10 h post-feeding). There were no significant differences between pre- and post-feeding median temperatures. Neither the temperatures of the 1st nor of the 3rd quartile were significantly changed after feeding, and no apparent negative skewness could be observed in the temperature profiles. On average, white sturgeon stayed within a range of 3–4°C around 18°C for 50% of the time independent of feeding status.

Effects of feeding

In both the hard-wired group (kept constant at 19°C) and the biotelemetry group (moving freely in the temperature gradient), the cardiovascular variables were affected by feeding. The general trend was a slow increase in cardiac output, heart rate and gut blood flow over the first 10 h after feeding while stroke volume did not change postprandially (Fig. 3).

All 16 animals showed one or several irregularities that could have been caused by external factors. The individual trace in Fig. 4 shows three such events that drastically interrupt the general trends of all cardiovascular variables. Note how the gut blood flow is dramatically reduced during these events. The first interruption was caused by the gavage feeding process but for

Table 1. Preferred temperatures and temperature preference intervals for white sturgeon

2 h before feeding	T1	T2	T3	T4	T5	T6	T7	T8	Mean	s.e.m.
1st quartile	15.0	16.9	13.0	17.0	16.2	17.2	17.5	16.8	16.2	0.5
Median	21.8	18.1	15.0	19.3	17.8	18.0	18.6	18.7	18.4	0.7
3rd quartile	23.1	19.6	16.4	20.7	18.6	18.8	20.9	21.7	20.0	0.7
6–8 h after feeding										
1st quartile	14.6	17.6	19.4	16.4	16.4	14.3	16.5	16.3	16.4	0.6
Median	16.5	18.6	20.9	19.0	18.6	14.6	18.3	18.4	18.1	0.7
3rd quartile	16.9	19.8	22.6	20.0	18.9	17.4	18.8	20.2	19.3	0.6
8–10 h after feeding										
1st quartile	13.2	14.0	19.4	16.0	18.0	17.5	16.8	13.8	16.1	0.8
Median	16.2	15.3	20.2	18.3	18.3	17.8	17.9	16.3	17.5	0.5
3rd quartile	17.7	16.9	21.0	19.5	18.3	19.0	22.0	19.1	19.2	0.6

Data from all individual sturgeon (T1–T8) instrumented with biotelemetry (N=8). For each animal the median (preferred temperature) and 1st and 3rd quartiles (the upper and lower limits for its temperature preference interval) are shown.

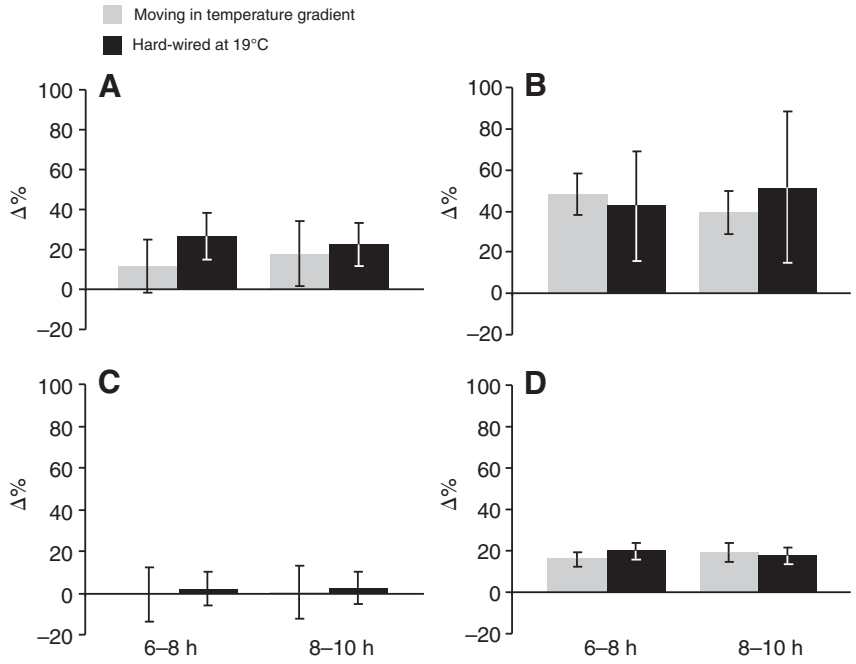

Fig. 3. Comparison between post-feeding, cardiovascular variables in white sturgeon (*Acipenser transmontanus*) in the biotelemetry (grey) and hard-wired (black) groups. Cardiac output (A), gut blood flow (B), cardiac stroke volume (C) and heart rate (D). All variables are presented as the percentage change between the values obtained 2 h before feeding and the values at either 6–8 h or 8–10 h after feeding. Data are presented as means (±s.e.m., *N*=8).

the second and third events there were no known external interferences (Fig. 4).

From the absolute measurements it is shown that 2 h before feeding cardiac output was 25.8±1.5 ml min^{-1} kg^{-1}, and 20.5% of this blood (5.3±0.8 ml min^{-1} kg^{-1}) was directed into the celiacomesenteric artery to the gastrointestinal tract. Heart rate was 43.2 beats min^{-1}, giving a cardiac stroke volume of 1.4±0.2 ml beat^{-1} kg^{-1} (Fig. 4). After feeding, the mean cardiac output was 32.3±3.5 ml min^{-1} kg^{-1} at 6–8 h and 30.9±2.8 ml min^{-1} kg^{-1} at 8–10 h postprandially. The distribution between the gastrointestinal tract and the rest of the systemic circulation remained similar with 21.3% (6.9±1.1 ml min^{-1} kg^{-1}) and 24.0% (7.5±1.4 ml min^{-1} kg^{-1}) of the blood distributed to the gastrointestinal tract 6–8 h and 8–10 h postprandially, respectively. Heart rate increased to 51.3±1.3 beats min^{-1} at 6–8 h and 50.3±0.9 beats min^{-1} at 8–10 h, with cardiac stroke volume unchanged (1.4±0.2 ml beat^{-1} kg^{-1}) at both 6–8 h and 8–10 h (Fig. 4).

Routine heart rate, defined as the mean heart rate recorded before feeding (without exposure to the temperature gradient for the biotelemetry group) was significantly lower (P<0.05) in the biotelemetry group, 37.3±0.3 beats min^{-1}, compared with the hard-wired group, 43.2±2.1 beats min^{-1} (Fig. 5).

There was no difference in the effects of feeding on cardiovascular variables between the telemetry group and the hard-wired group (Fig. 3). In comparison with routine values, heart rate had increased by approximately 20% in both groups at 6–8 h and 8–10 h after feeding. Gut blood flow increased on average with 50% in both groups (Fig. 3).

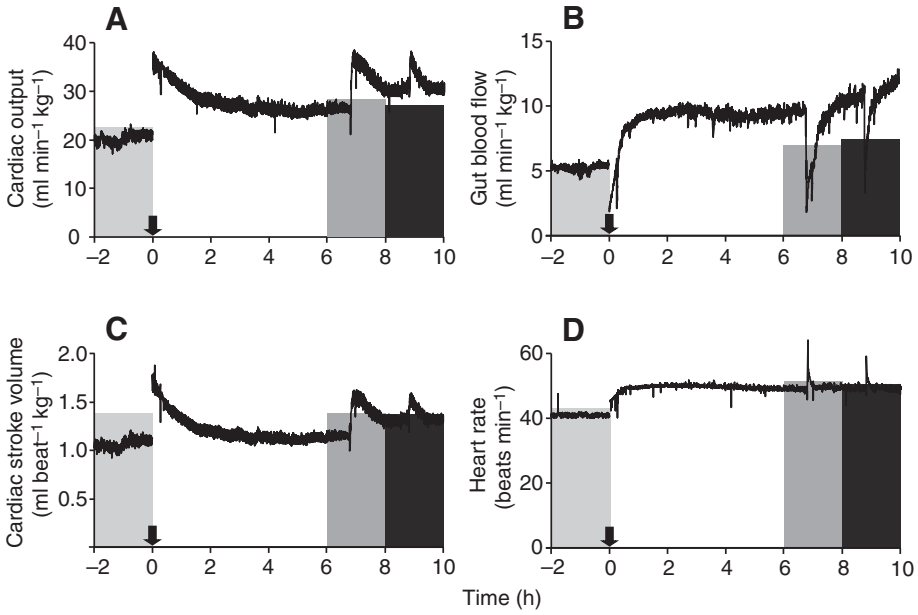

Fig. 4. Cardiovascular variables in white sturgeon (*Acipenser transmontanus*) before feeding and 6–8 h and 8–10 h after feeding. Cardiac output (A), gut blood flow (B), cardiac stroke volume (C) and heart rate (D). The grey bars are mean values from all hard-wired animals (*N*=8) and the black lines represent a representative trace from one sturgeon. The black arrow indicates time of feeding.

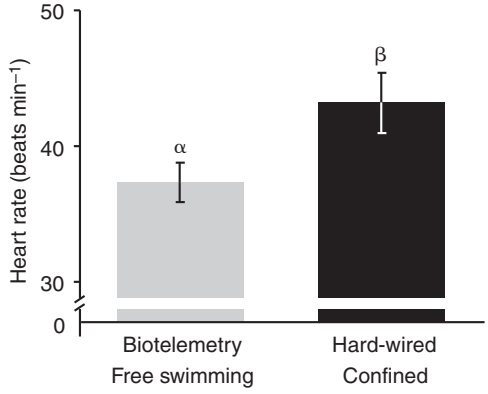

Fig. 5. Routine heart rate (beats min^{-1}) for white sturgeon (*Acipenser transmontanus*) from the biotelemetry (grey) and hard-wired (black) groups. Data are presented as means (±s.e.m., *N*=8). Different Greek letters indicate a significant difference between times (P<0.05).

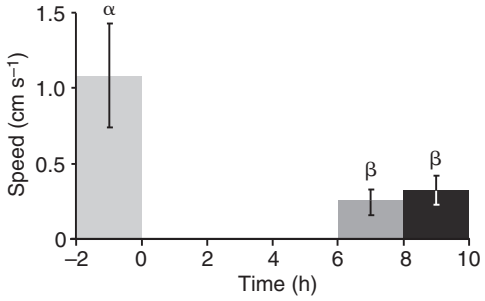

Fig. 6. Mean swimming speed before feeding and 6–8 h and 8–10 h after feeding for white sturgeon (*Acipenser transmontanus*) instrumented with the biotelemetry system. Data are presented as means (±s.e.m., *N*=8). Different Greek letters indicate a significant difference between times (P<0.05).

Feeding significantly decreased swimming activity (P<0.05). Before feeding, the sturgeon on average moved 1.07±0.34 cm s^{-1}, while 6–8 h and 8–10 h after feeding, the sturgeon on average only moved 0.25±0.07 and 0.32±0.10 cm s^{-1}, respectively, as shown in Fig. 6.

Temperature and gut blood flow

Fig. 7 shows original traces of heart rate, external temperature and gut blood flow from one individual fish during recovery, the first 2 h of temperature exposure (2 h before feeding) and 8–10 h after feeding. Although few individuals moved this extensively between temperatures, the trace illustrates an important observation. When the fish moved between temperatures, heart rate correlated to temperature regardless of the fish being fed (R^2=0.85) or not (R^2=0.76). By contrast, whereas gut blood flow was unaffected by the temperature fluctuations before feeding (R^2=0.09), it was affected 8–10 h after feeding (R^2=0.54) (Fig. 7). A similar trend was seen in all individuals.

The correlation between temperature and gut blood flow is presented in Table 2, showing that gut blood flow is linked to temperature after ingesting a meal (R^2=0.88±0.03 at 6–8 h and R^2=0.89±0.04 at 8–10 h) but not prior to a meal (R^2=0.11±0.05). When the sturgeon moved into warmer water there was a rapid increase in gut blood flow. The magnitude of the increased varied between individuals but was, in the postprandial period, on average 15% $°C^{-1}$.

DISCUSSION

This is the first study to combine temperature preference, feeding and cardiovascular variables in fish. With the use of a dual-channel biotelemetric system we recorded cardiac output and gut blood flow in white sturgeon, allowed to swim freely in a temperature gradient. Although our initial hypothesis that the white sturgeon would change temperature preference after feeding was not supported, our findings show how the cardiovascular system in fish is directly affected by short-term voluntary variations in temperature.

Temperature preference

When ectotherm species are allowed to thermoregulate in a wide range of environmental temperatures they generally do not select one single temperature but rather an interval of temperatures. This interval is usually negatively skewed, meaning that the animal selects a wider range of temperatures below the median than above it (Dewitt and Friedman, 1979; Martin and Huey, 2008). In order to include this potential skewness in the analysis, temperature preference should include the median and two additional parameters. The most appropriate statistical approach for describing a temperature preference is therefore to use the median in combination with the 1st and 3rd quartiles (Magnuson et al., 1979).

From the present study it is clear that white sturgeon can thermoregulate behaviourally. All individuals showed a clear pattern of temperature preference. When the data (times recorded at different temperatures) collected from each individual were plotted (not shown), the histograms displayed distinct peaks (represented by the median) and gradually declining frequency when approaching the extremes. Overall no apparent sign of skewness (represented by the 1st and 3rd quartiles) towards neither colder nor warmer temperatures was seen, instead most individuals showed a binomial distribution of selected temperatures. The shape of the temperature preference profile in fish has been shown to differ between both species and acclimation temperatures (McMahon et al., 2008). The large individual variations in preferred temperature, ranging from 15.0 to 21.8°C before feeding, indicate that white sturgeon as a species tolerate a large temperature range. However, the mechanisms controlling temperature preferences and the significance of the different shapes of the temperature profiles are still largely unknown.

Routine cardiovascular measurements

The routine values obtained using standard techniques are comparable with previously reported for white sturgeon, of similar size and at the same temperature, using the same kind of Transonic transit-time blood flow probe (Crocker et al., 2000).

In our study, the group instrumented with the biotelemetric system, moving freely in the temperature preference chamber, had a lower resting heart rate, compared with the hard-wired, confined group as well as with previous values reported for white sturgeon (Crocker et al., 2000). This is probably an indication that our animals were not as stressed due to confinement and the use of ‘hard-wired’ techniques. A lower heart rate is usually indicative of lower stress levels, and the level of stress has been shown to be extremely important when interpreting cardiovascular responses (Webber et al., 1998; Altimiras and Larsen, 2000). For instance, when trying to estimate oxygen consumption from heart rate in Atlantic cod, *Gadus morhua*, Webber and co-workers showed that if using stressed animals, oxygen consumption could be overestimated by as much as 100% (Webber et al., 1998). It is likely that a number

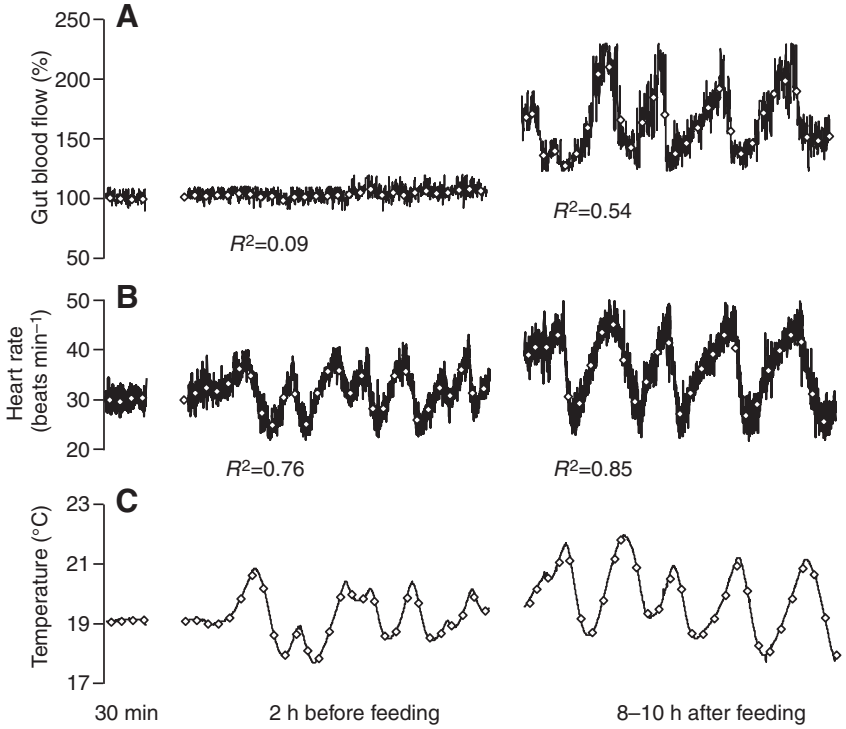

Fig. 7. Original traces of gut blood flow (presented as a percentage of the routine value), heart rate (beats min^{-1}) and implant temperature (°C) from one white sturgeon (*Acipenser transmontanus*) instrumented with the biotelemetry, 30 min before exposure to the temperature gradient, the 2-h temperature exposure before feeding and the temperature exposure 8–10 h after feeding. Dots in the trace indicate the mean values from 4 min sections from which R^2-values are calculated.

of both older and recent studies are heavily biased by post-surgical and/or confinement stress, thus casting some doubt on the conclusions drawn. Using biotelemetry to measure different variables from more or less free-ranging animals will cause less stress and should provide more accurate data.

Effects of feeding

Increased blood flow (hyperemia) to the gastrointestinal tract is expected after feeding when activities such as gut motility, intracellular biochemical activity and membrane transport all are enhanced (McCue, 2006). The increase in postprandial gut blood flow has been described in several groups of ectothermic vertebrates including snakes (Secor and White, 2010), crocodiles (Axelsson et al., 1991) and fish (Axelsson et al., 1989; Gräns et al., 2009a). In fish, the portion of cardiac output distributed to the gut after a meal ranges from 20% in European seabass *Dicentrarchus labrax* (Dupont-Prinet et al., 2009) to 52% in Atlantic cod (Axelsson and Fritsche, 1991). With ~24% of cardiac output distributed to the gut after a meal, the white sturgeon ends up in the lower part of this range.

The most common way for fish, unlike most other vertebrates, to elevate cardiac output is to increase cardiac stroke volume to a greater extent than heart rate (Farrell, 1991). This does however not seem to be the case for white sturgeon which, after a meal, increases cardiac output through a rise in heart rate rather than an increased cardiac stroke volume.

A rapid reduction in gut blood flow during bursts of activity as reported here has been described before in fish, including sturgeons and salmonids (Thorarensen et al., 1993; Crocker et al., 2000; Gräns et al., 2009a; Gräns et al., 2009b). The obvious benefits of this response would involve the redistribution of blood towards muscles used in fight-or-flight situations and then to resume digestion after the disturbance.

A reduction in postprandial gut blood flow during forced exercise has been reported in European sea bass (Altimiras et al., 2008) and lately also in Burmese python *Phyton molurus* (Secor and White, 2010). Prioritising gut blood flow after a meal is thus a likely explanation for the reduced swimming activity after feeding observed in the present study. Reduced locomotor activity after feeding has been shown before in juveniles of Siberian sturgeon *Acipenser baeri*, sterlet *Acipense ruthenus* and goldfish *Carassius auratus* (Zdanovich, 2006).

Changes in temperature preference associated with feeding have been reported in all groups of ectothermic vertebrates. Selecting a different temperature is an effective way for an ectothermic animal to regulate its energetic balance (Reynolds, 1979; Angilletta et al., 2002). However, the exact postprandial responses are unpredictable, and in fish as in most studied ectotherms, large variations occur

Table 2. Coefficient of determination of a linear regression between temperature and gut blood flow

	T1	T2	T3	T4	T5	T6	T7	T8	Means ± s.e.m.
2 h before feeding	0.12	0.02	0.01	0.23	0.01	0.38	0.03	0.10	$0.11\pm0.05^{\alpha}$
6–8 h	0.98	0.93	0.92	0.98	0.88	0.82	0.73	0.84	$0.88\pm0.03^{\beta}$
8–10 h	0.96	0.85	0.99	0.98	0.85	0.89	0.89	0.98	$0.89\pm0.04^{\beta}$

R^2-values from the correlation between temperature and gut blood flow at the three periods (before feeding, 6–8 h and 8–10 h after feeding) form the group of white sturgeon (T1–T8) (*Acipenser transmontanus*) instrumented with biotelemetry (N=8). Values are obtained from the correlation between the temperature preference median, 1st and 3rd quartiles and gut blood flows from the associated times. Different Greek letters indicate differences between periods (P<0.05).

among species (Javaid and Anderson, 1967). Bear Lake sculpin move into warmer water during the night, after a day of feeding at the bottom of the lake (Wurtsbaugh and Neverman, 1988). The temperature difference is up to 10°C and this postprandial/diurnal migration increases both the gut passage time and growth rate threefold for this species (Wurtsbaugh and Neverman, 1988). Moving into warmer water after feeding also characterises Atlantic stingray *Dasyatis sabina* (Wallman and Bennett, 2006) and *Doydixodon laevifrons* (Pulgar et al., 2003).

In other species, the thermoregulatory response is associated with deprivation of food rather than with feeding as such. These species seek colder temperatures when food is scarce. This has been observed in roach and is thought to minimise metabolic rate (van Dijk et al., 2002). When able to select temperatures, roach, starved for three weeks, showed a pattern of moving into cooler waters during night hours. A similar pattern has also been seen in brook trout *Salvelinus fontinalis*, rainbow trout (Javaid and Anderson, 1967), juveniles of Siberian sturgeon, sterlet and goldfish (Zdanovich, 2006). Also, it has recently been suggested that much of the seasonal differences in temperature preference in, e.g. Arctic charr *Salvelinus alpinus*, depend on changes in food availability (Mortensen et al., 2007).

In white sturgeon, we found no change in temperature preference after feeding. The mean selected temperature both before and after feeding was close to 19°C, the temperature to which the fish were acclimatised. These results suggest either that this temperature is the most profitable, independent of the fish being feed or not, or that the pre-experimental 72-h food deprivation was insufficient to allow for a clear difference between fasted and postprandial states. Several other parameters may have influenced the results, like size of the fish, season and effects of surgery. It is also possible that the food given was not sufficient, either in quality or quantity, to induce a change in thermal preference in the white sturgeon. Pulgar and colleagues showed that *Girella laevifrons* preferred higher temperatures if the quality of the food was high (Pulgar et al., 2003). This indicates that there is a cost associated with the thermoregulatory behaviour and only when fed a high quality diet did the energetic return outweigh the cost.

Temperature and gut blood flow

By using thermoregulatory behaviour, ectothermic vertebrates have great possibilities to influence their physiological status. In reptiles it has been shown that the cardiovascular system plays an important role in these thermoregulatory behaviours. By changes in heart rate and peripheral circulation, reptiles can increase or decrease their heat transfer, and to some extent, control the rate of which heat is gained or lost (Seebacher, 2000; Seebacher and Franklin, 2007). Many reptiles increase their body temperature postprandially but distributing blood to peripheral tissues when heating up might then be in conflict with the increased demand of blood to the gut. In unfed saltwater crocodile *Crocodylus porosus* the blood flow distributed to the duodenum is decreased during heating (Seebacher and Franklin, 2007). This conflict of interest is probably one reason why some reptiles cease active thermoregulatory behaviour after feeding (Hammerson, 1987). Other species, like the Savannah monitor lizard *Varanus exanthematicus*, seem to have the possibility to postprandially elevate heart rate both to meet the increased demand of the gut and to efficiently heat up (Zaar et al., 2004).

Fish do not have the possibility to ‘conserve’ the body temperature when moving into a cooler ambient environment as 80–90% of the heat contained in the blood is lost when passing the gills (Stevens and Sutterlin, 1976). While the rapid heat transfer might act negatively when moving out of the preferred temperature, it minimises the time lag when moving into a favourable temperature. In a previous study, we demonstrated how acute changes in temperature affect gut blood flow in green sturgeon (Gräns et al., 2009b). In the current study, it is clear that this correlation between gut blood flow and temperature is also present when white sturgeon move voluntarily between temperatures, and postprandially this can happen instantaneously. Voluntary movements between temperatures created fluctuations in gut blood flow that far exceeded the flow induced by feeding only. There are no reasons to believe that this response is exclusive for white sturgeon but this may be true for all species of fish.

Whether there is a strong correlation between body temperature and gut blood flow also in other ectotherms remains to be shown. However, the possibility that this correlation is found also in other ectothermic groups seems very likely because many of the postprandial effects of increased temperature reported are linked to gut blood flow, e.g. gut passage rate (Dorcas et al., 1997), absorption efficiency (Hailey and Davies, 1987) and metabolic profile (Secor and Faulkner, 2002).

Because the variation in gut blood flow caused by different temperatures was so much higher than the increase initiated by feeding, it is somewhat surprising that there was no correlation between gut blood flow and temperature prior to feeding. The results suggest the presence of a control mechanism, suppressing gut blood fluctuations when the gut is empty. How this control would work is, however, beyond the scope of this study.

Because no correlation was found between temperature and gut blood flow when flow was low (before feeding), the energetic benefits of moving into cold water during starvation (discussed above) are probably not due to a decline in gut blood flow but to a reduced overall metabolic rate at lower temperatures (Beitinger and Fitzpatrick, 1979).

Future perspectives

The present findings add new insights to the link between environmental temperature, the physiology of an animal and ultimately its fitness. In light of the ongoing climate change, this knowledge might be important to predict the effects on different animals and eventually populations. Populations of marine fishes have been shown to move northward as the ocean temperatures increase (Roessig et al., 2004; Perry et al., 2005). This may not be an option for fish living in smaller bodies of water such as streams and lakes, or in the more stable marine environments in tropical waters or in the polar areas. Increased temperatures in streams and lakes may reduce the areas in which thermoregulatory behaviours are possible. This restriction may lead to reduced scope for growth, and foraging will be reduced, potentially jeopardising the survival of the fish. Optimal processing of the food is an important factor for survival. Although we have demonstrated the close link between postprandial gut blood flow and temperature, we need a deeper knowledge of how other aspects of gut activity such as gut motility patterns, intracellular biochemical activity and membrane transport are affected when species move voluntarily between different temperatures. How do naturally occurring changes in temperature preference affect homeostasis, and what ways do fish have to cope with the physiological stress that different temperatures inevitably impose upon the animals?

Conclusion

This is the first study to look at cardiovascular responses to feeding in white sturgeon, using animals instrumented with a dual-channel

blood flow biotelemetric system. This allowed the animals to move freely in the temperature preference chamber, making it for the first time possible to combine a behavioural test in fish with recordings of cardiac output and gut blood flow. That lower routine heart rate in this group compared with a hard-wired control group, further supports the idea of using telemetric devices to record from freely moving animals.

No change in temperature preference was seen in white sturgeon after feeding. Both 2 h before feeding and 6–10 h postprandially, the mean selected temperature was close to 19°C, i.e. the acclimatisation temperature. The present study, however, shows how the cardiovascular system of white sturgeon is directly affected by the temperature of the animal. There is a positive correlation between temperature and gut blood flow, which can partly explain why postprandial changes in temperature preference can in some species be an extremely efficient way to increase gut-passage time and growth.

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