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Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results

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ABSTRACT

Dissolved oxygen and salinity are relevant structuring factors which should be incorporated into habitat and bioenergetic models for estuarine fishes. We measured growth, food consumption, routine and postprandial metabolism, egestion and survival responses of juvenile Atlantic sturgeon (young-of-the-year: YOY, 6–48 g) in an incomplete factorial array of temperature, salinity and dissolved oxygen levels. Complementary measures were also conducted on yearlings (1 year-old, 70–300 g) to evaluate size and age effects upon food consumption and growth. All three factors had a significant effect on major bioenergetic responses, as well as several of their first order interactions. Maximum growth and food consumption rates were observed above 70% dissolved oxygen saturation, at 20 °C, and between salinities of 8 and 15. Postprandial metabolism was reduced and egestion increased under hypoxia (50% DO saturation), suggesting compensatory mechanisms aimed to reduce assimilation rates. A significant shift in growth responses with age indicated higher tolerance to salinity in yearlings than in YOY. No other size dependent changes were significant, either for hypoxia or for temperature effects. Survival tended to increase with dissolved oxygen saturation, and decreased at the highest temperature and salinity levels. Our results indicated both additive and synergistic effects of tested environmental factors upon ecophysiological responses and highlighted the need to consider these in new bioenergetic models.

1. Introduction

Estuarine fish habitats are structured spatially by strong gradients in salinity, temperature and dissolved oxygen. While salinity and temperature variability are inherent features of most estuaries worldwide, hypoxia in shallow estuaries is a seasonal phenomenon, which has become more pervasive during the past century in populated areas, the likely consequence of anthropogenic organic enrichment (Officer et al., 1984; Cooper and Brush, 1991a). Although high physiological plasticity is expected in estuarine species, strong effects of temperature, salinity and dissolved oxygen have been reported upon behavior, distribution, physiology, energetics, growth and survival (Fry, 1971; Brett, 1979; Brandt, 1993; Boeuf and Payan, 2001).

The importance of hypoxia curtailments on estuarine fish habitats is supported by a rich literature, which relates fish distribution, survival, growth and behavior responses to water quality in estuarine habitats, and particularly to dissolved oxygen levels (Cech et al., 1984; Susanto and Peterson, 1996; Secor and Gunderson, 1998; Breitburg et al., 1999; Pichavant et al., 2001; Marcus et al., 2004; Stierhoff et al., 2006). Substantial literature has also assessed critical (lethal) hypoxia levels, such as LC_{50} (Scholz and Waller, 1992; Jenkins et al., 1993; Plante et al., 1998; Miller et al., 2002; Landman et al., 2005; Shimps et al., 2005; Goodman and Campbell, 2007). A relevant part of this body of information has been compiled and used for defining management goals and regulatory thresholds in highly impacted estuaries such as the Chesapeake Bay (US-EPA, 2003).

Although there is strong evidence that temperature, salinity and other environmental factors may interact with dissolved oxygen, modifying its effects upon growth and survival of estuarine species (Fry, 1971; Claireaux and Lagardere, 1999; Yamashita et al., 2001; Niklitschek and Secor, 2005), most dissolved oxygen goals and thresholds have been established as fixed criteria, neglecting such interactions. More integral approaches are restrained by a limited availability of multivariable response models for estuarine species.

In the present work we measured and modeled (Niklitschek and Secor, 2009-this issue) the effects of dissolved oxygen, temperature, salinity and their interactions upon growth, survival and other major bioenergetic responses in Atlantic sturgeon *Acipenser oxyrinchus*. Sturgeons have been considered as indicator species due to their particularly low tolerance to hypoxia, which can exceed well known oxyphilic species such as Oncorhynchus mykiss (Klyashtorin, 1982). This tolerance has been shown to be reduced at high temperature (Secor and Gunderson, 1998) and salinity levels (Niklitschek, 2001), suggesting that the areas of suitable nursery habitats could be largely reduced in summer months (Collins et al., 2000; Niklitschek and Secor, 2005).

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Atlantic sturgeon once inhabited a wide geographic range along the Atlantic coast of North America (Evermann and Bean, 1898; Leim and Day, 1959; Vladykov and Greeley, 1963). After initial depletion by severe overfishing in the late 1900s (Secor and Waldman, 1999), most Atlantic sturgeon populations have not recovered despite decades of strict fishing regulations and a fishing moratorium since 1998 (ASMFC, 1998). Hence, reduced suitability of spawning and nursery habitats have been identified as key issues in sturgeon recovery (ASMFC, 1998; Atlantic Sturgeon Status Review Team, 2007). Nursery habitats in fresh and brackish waters, where Atlantic sturgeon juveniles spend their first 3–5 years of life, have been seriously affected by increasing pollution, nutrient uploads and hypoxia during the 20th century (Officer et al., 1984; Cooper and Brush, 1991b). Regulated flow regimes in dammed rivers and global warming trends (Najjar et al., 2000; Preston, 2004) would further affect suitable nursery areas for Atlantic sturgeon and other sturgeons (Niklitschek and Secor, 2005).

Our investigations on ecophysiological responses of juvenile sturgeons to estuarine conditions have been divided in two companion papers: in the present one we measure and test the main effects and interactions between dissolved oxygen, temperature, salinity and size/age-class (age 0 and age 1 juveniles) on metabolic, growth, and survival responses. The companion paper (Niklitschek and Secor, 2009-this issue) uses these ecophysiological responses in the development of a mass-balance predictive bioenergetics model, suitable for Atlantic sturgeon and other estuarine species.

2. Materials and methods

Survival, growth and main bioenergetic responses to dissolved oxygen saturation (DO_{SAT}), salinity and temperature were measured in microcosm studies, conducted on hatchery-produced Atlantic sturgeon juveniles, offspring from Hudson River parents, spawned in 1996. Fish were made available by the US Fish and Wildlife Service, Northeast Fisheries Science Center (Mohler, 2000). Because fish were of limited supply, the experimental design was accommodated to gain maximum use from a few hundred individuals. Two age classes were used in the present work: young-of-the-year (YOY, 6–48 g) and yearlings (100–300 g).

All fish were tagged and identified through subcutaneous visible elastomer inclusions (Northwest Marine Technologies) injected in the pectoral fins, several weeks before starting the experiments. Fish were acclimated to experimental conditions at transition rates no greater than 1 (salinity), 1 °C and 5% DO_{SAT} per day. Salinity was maintained within ±5% of targeted levels by mixing local well water with filtered ambient brackish water (salinity 3–18, Patuxent River, Chesapeake Bay). Artificial salt (Instant Ocean®) was added when necessary. Dissolved oxygen was kept within ±10% of targeted levels by mixing nitrogen with ambient air. Temperature was regulated within ±8% of targeted levels by using a combination of room-temperature control, water baths, and individual heaters. Water quality parameters were checked at least 3 times each day unless otherwise stated.

2.1. Growth and food consumption

Temperature, salinity and DO effects on food consumption and growth rates were tested on YOY via an incomplete factorial design (Mead, 1988) that included 23 combinations of four temperatures: 6, 12, 20 and 28 °C; four levels of dissolved oxygen: 30, 40, 70 and 100% DO_{SAT}; and five salinity levels: 1, 8, 15, 22 and 29 (Table 1). This design allowed us to evaluate responses to main effects and first order interactions between temperature, salinity and dissolved oxygen.

To assess size or age effects upon functional responses to hypoxia, temperature and salinity, we conducted an additional set of food consumption and growth experiments on yearlings. In this case we followed a simplified multi-axial design aimed to test only main effects at three levels of temperature: 12, 20 and 28 °C; three dissolved oxygen treatments: 40, 70 and 100% DO_{SAT}; and four levels of salinity: 1, 15, 22 and 29 (Table 1).

Table 1
Summary of the incomplete experimental designs used to evaluate bioenergetics and survival effects of dissolved oxygen saturation, temperature and salinity upon juvenile Atlantic sturgeon.

Temperature	Dissolved oxygen saturation	Salinity				
		1	8	15	22	29
6	70		3			
12	40		3			
	70	3	6	3(3)		
	100		3			
20	30		3			
	40	3	5	4(3)		
	70	3(3)	6	3(3)	4(3)	2(3)
	100	2	5	2(3)		
28	40		2			
	70	4	5	4(3)		
	100		3			

Number of replicates corresponding to each growth-consumption experiments indicated for YOY and yearlings (in parenthesis).

Fish were fasted for 24 h, measured, weighed and left to recover for 12 h before each experiment. During the following 10 days, fish were fed *ad libitum* 3-mm Biokyowa® pellets thrice a day, and measured and weighed again 12 h after the last feeding. Non-consumed food was removed 30–40 min after each feeding and dried to constant weight (≥48 h) at 60 °C. Apparent consumption was reduced by an empirically derived factor of 5% to account for pellet losses in the tank. Average energy-density in Biokyowa pellets, estimated by wet digestion (Maciolek, 1962), was equal to 21.9 kJ g^{-1}. Daily consumption rates were calculated for each tank by dividing total consumed food by the expected tank biomass, back-calculated from observed growth.

We planned to obtain six replicates for the treatment representing center conditions for YOY trials (20 °C, 70% DO_{SAT}, salinity 8), and at least three replicates for all the remaining treatments and yearlings. However, low survival occurred at high salinity, high temperature and/or low DO_{SAT}. To minimize harm to individual fish and preserve sufficient experimental animals, lethal treatments were not always replicated, causing some unequal replication (Table 1).

A total of 81 experiments were successfully completed for YOY in seven sequential runs, using 34-l glass aquaria with either one or two fish per tank. Preliminary experiments showed no difference in growth or food consumption between fish reared individually and in pairs (Niklitschek, 2001). Individuals were sequentially allocated to a maximum of three experiments, using a semi-random balanced design. Yearlings were individually reared in 70-l tanks, conducting a total of 63 experiments, distributed along three sequential runs.

Daily instantaneous growth rates (G) were calculated for each individual, and averaged within each tank, using the relationship: $G = [\log_e(W_t) - \log_e(W_0)]/(t - t_0)$, where, W_t is final weight at time t; and W_0 is initial weight at time t_0.

2.2. Respirometry

Energy costs of both routine metabolism and postprandial metabolism were estimated by respirometry. In routine metabolism trials we aimed to measure the energy consumption of fasted fish at rest. Although noise and light stimuli where minimized, some spontaneous activity occurred and obvious activity spikes were deleted from the data set. Oxygen consumption was measured for individual fish using either 2-l flow-through or 15.4-l static respirometers. The smaller flow-through units (0.35 l min^{-1}) were used for fish up to 18 g, and the larger respirometers for fish between 14 and 37 g. Previous experiments indicated oxygen consumption results

were not significantly affected by respirometer type (Niklitschek, 2001). In each trial 6 to 8 respirometers were run simultaneously, including 1 or 2 respirometers without fish as blanks to correct final results for microbial oxygen consumption. Fish were placed in respirometers for at least 12 h prior to any respiration measures. Salinity, oxygen and temperature levels were maintained within ± 10% of targeted values; any respiration reading outside those limits were excluded. All temperature, dissolved oxygen and salinity measurements were made using a YSI-85 sensor, re-calibrated in saturated water every time a new sequence of readings at a given temperature was initiated. Apparent oxygen consumption values from flow-through respirometers were corrected later for hydraulic residence time (Niklitschek, 2001). Measured consumed oxygen was then transformed into consumed energy using an oxycalorific coefficient of 13.55 kJ $g\text{-}O_2^{-1}$ (Brett and Groves, 1979).

Routine metabolism respirometry experiments were done immediately before or after growth-consumption experiments, for the same fish and under the same 23 combinations of temperature, salinity and dissolved oxygen conditions (Table 1). Due to the use of small and static respirometers, targeted DO_{SAT} for normoxia treatments was defined to be 90% rather than the 100% DO_{SAT} used in food consumption and growth experiments. Two to six replicates were obtained for each treatment, depending on fish availability. We tested a total of 128 fish, which were randomly allocated into 17 respirometry experiments. Oxygen consumption rates were measured every 45 min for at least 4 h or until 4 stable readings were obtained.

Respirometry experiments for measuring postprandial metabolism (classically named specific dynamic action, SDA) were based upon a factorial design with three replicates at three temperatures: 12, 20 and 28 °C and two levels of oxygen saturation: 50 and 100%. Salinity was 8 across all experiments. Actual conditions during SDA experiments were kept within 1 ºC, 5% DO_{SAT} and 1 salinity unit from prescribed values. All experiments were conducted in 15.4-l flow-through respirometers, suitable for long-term rearing and retrieval of non-consumed food. To increase precision in dissolved oxygen measurements, hermetic probe holders with magnetic stirring systems were used at tank inflows and outflows.

After measuring routine oxygen consumption for about 4 h, flow was interrupted and fish were allowed to eat Biokyowa® pellets for 30 min. After that period, excess food was removed, flow restored ($\leq 0.6\ l\,min^{-1}$) and oxygen consumption measured every 1–2 h during the first 16 h, and every 2–4 h thereafter (48–60 h) until oxygen consumption was observed to decline to the previously observed routine metabolism levels. A noticeable peak in metabolism, observed within 0.5–1 h after feeding, was interpreted as the result of feeding activity and/or manipulation stress. Consequently, data between 0 and 2 h post-feeding were excluded and substituted by extrapolated values. Postprandial respiration was calculated as the area under the curve, decremented by routine respiration for each trial. The curve was fit using non linear regression and Niklitschek's (2001) modified version of Thornton and Lessem's (1978) algorithm, which facilitates both computing total oxygen consumption (area under the curve) and comparing the shape of SDA responses between treatments.

2.3. Egestion

Egestion experiments were conducted with the same fish and under the same combination of temperature and DO_{SAT} we used in SDA experiments. In addition to these abiotic variables, we tested 3 ration sizes: 10, 55 and 100% of the mean consumption per meal observed when fish were fed *ad libitum*, during the acclimation period. Before each experiment, fish were fasted for 60 h, weighed, rinsed and transferred to a clean 38-l tank with filtered (5 μm) and salinity = 8 water, and left to recover overnight (12–14 h). Three tanks with one fish per tank were used as replicates for all treatment levels.

Fish were then fed *ad libitum* three times at 2.5-h intervals. Non-consumed food was removed after 30 min, dried (24 h at 60 °C) and weighed. Observed feces were pipetted out three times a day and then frozen for further analysis. After 60 h the experiments were concluded, fish were rinsed, removed and weighed. Two control tanks were included in each run, using a randomly selected set of environmental conditions. To estimate non-fecal contributions of energy from the fish to the water, one of these control tanks contained one fish, but did not receive any food during the experiment. To estimate energy leakages from the food to the water, the other tank contained no fish but was "fed" the average amount of food given to the other tanks. Water contained in all experimental and control tanks was filtered (polycarbonate 35 μm) for further analysis. Feces and filters were dried at 60 °C for 48 h or until weight was stable. All feces samples were weighed and divided into 1–3 sub-samples (~10 mg each) for energy content analysis through a colorimetric variant of Maciolek's (1962) technique. Here, closed reflux digested samples were analyzed using a standard spectrophotometer at 600 nm wavelength, and compared with a standard potassium hydrogen phthalate solution, which had a theoretical chemical oxygen demand of 9408 μg O_2 ml^{-1}. Organic matter in fasted fish control tanks accounted for an average of 0.024 kJ ± 0.0070 (SE) per gram of fish (wet weight), which was subtracted from the fecal energy content estimated from experimental tanks. Energy content in filtrates from water corresponding to experimental and no fish control tanks was below the sensitivity of the dichromate technique.

2.4. Survival

The same YOY individuals and experimental design used for growth and food consumption experiments (Table 1) were used to evaluate the sub-acute effects of temperature, dissolved oxygen and salinity on fish survival. Fish were kept under the same conditions described in growth/food consumption experiments (see above) for at least 21 d after growth experiments were concluded. Thus, survival was monitored on 172 fish, which were randomly distributed among 23 treatments and six consecutive experimental runs.

2.5. Hypotheses testing

We tested the main linear and quadratic effects, and first order interactions between temperature, DO_{SAT} and salinity; and the $\log_e$-transformed fish weight effect using a mixed model regression approach (MIXED Procedure; SAS-Institute, 1997). This framework, based in maximum likelihood theory, allows means, residuals and standard errors to be adjusted for the effect of covariates (random effects) that are beyond the inference scope of the analysis (Hinkelmann and Kempthorne, 2005). The linear mixed model we used to test null hypotheses about measured bioenergetics responses followed the general form,

$$Y = X\beta + Zu + e$$

where

- Y — Measured response (instantaneous growth rate, food consumption, routine metabolism, SDA, or $\log_e$-transformed egestion ratio).
- X — Design matrix for fixed effects, including $\log_e$-transformed weight, linear and quadratic effects for temperature, dissolved oxygen, salinity, ration size and/or interactions between linear components. For exploring age-class effects, a categorical "dummy" variable was added to this matrix with values 0 and 1, for YOY and yearlings, respectively.
- β — Vector of coefficients for each of the fixed effects (included the intercept)

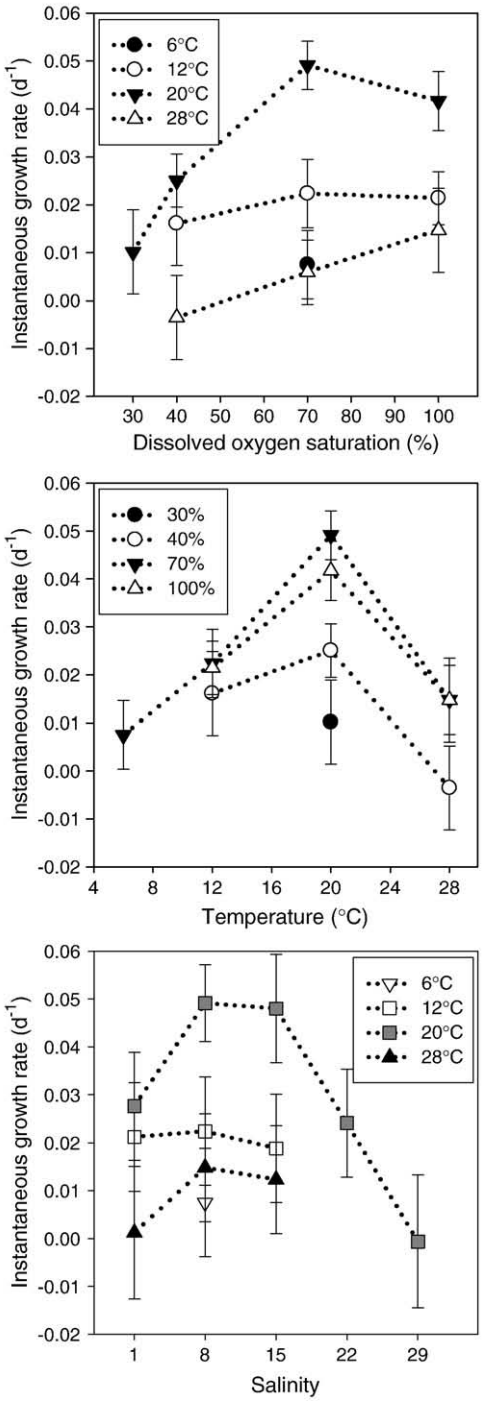

Fig. 1. Effects of dissolved oxygen saturation, temperature, and salinity on instantaneous growth rates of juvenile Atlantic sturgeon (means ± SE). Figures show variability caused by two factors at the time, while keeping the third one at fixed conditions (salinity = 8 for the top and middle panels, $DO_{SAT} \geq 70\%$ for the bottom panel). Means are connected by lines to facilitate interpretation.

Z Design matrix for random effects (experimental set).
μ Vector of coefficients for the random effects ~$N(0,G)$; G = matrix of variance–covariance for the random effects
e Vector of random errors ~$N(0,R)$; R = matrix of variance–covariance for the random errors

Log-transformation of egestion observations was required to meet the assumptions regarding homogeneity of variances and normality in error distribution. Original scales were used for all other response variables. To avoid colinearity derived from polynomials and interactions, all explanatory variables were centered by subtracting their respective means.

Survival results were analyzed using a non parametric maximum likelihood version of Cox proportional hazard regression (Allison, 1995) implemented in SAS, PHREG procedure (SAS-Institute, 1997). Assuming no-time dependence in the effect of the covariates (i.e., temperature, dissolved oxygen level, and salinity) on survivorship, we simplified the Cox survivor function to the expression,

$$S(t) = S_0(t)^{\mathrm{EXP}(\beta X)}$$

where,

$S(t)$ Probability of surviving beyond time t.
$S_0(t)$ Baseline survivorship function when all covariates are zero.
EXP Exponential function
β Vector of regression coefficients for explanatory variables
X Matrix of covariates: dissolved oxygen, temperature and salinity treatments.

A significance threshold of alpha = 0.1 was used for all tests, unless otherwise stated. Cases where the probability dropped below 0.05 were regarded as highly significant.

3. Results

3.1. Growth

Instantaneous growth rate was strongly affected by temperature, salinity and dissolved oxygen, as well as by fish weight (Table 2). Significant linear and quadratic effects, identified for all three environmental factors, suggested asymptotic patterns for dissolved oxygen and dome-shaped patterns for temperature and salinity, with maximum growth rates at 70–100% DO_{SAT}, 20 °C and salinity 8–15. We found a significant interaction between temperature and dissolved oxygen ($p<0.05$), which was reflected in very different slopes in the response of growth rates to dissolved oxygen between high and low temperatures. While at 28 °C growth rates increased continuously along the DO_{SAT} gradient, at 12 and 20 °C growth remained relatively constant above 70% DO_{SAT} (Fig. 1). These 70% DO_{SAT} levels are equivalent to 7.9 mg l^{-1} at 12 °C, and 6.7 mg l^{-1} at 20 °C. Temperature–salinity interaction was significant only at $p = 0.1$. No evidence of salinity-dissolved oxygen interaction was found.

Table 2
Linear regression analysis of dissolved oxygen (DO), temperature (T), salinity (SAL) and individual weight (W) effects upon instantaneous growth rate in juvenile Atlantic sturgeon.

Effect	Estimate ± SE	DDF	*F*-value	$p>F$
Intercept	0.043 ± 0.009	13.4	22.5	<0.001
T	0.047 ± 0.01	103.7	23.2	<0.0001
T^2	−0.048 ± 0.01	103.6	23.0	<0.0001
DO	0.029 ± 0.011	105.0	6.8	<0.05
DO^2	−0.024 ± 0.011	105.4	4.5	<0.05
SAL	0.016 ± 0.004	101.0	13.6	<0.001
SAL^2	−0.027 ± 0.007	105.9	15.7	<0.001
Ln(W)	−0.005 ± 0.002	18.0	4.4	<0.05
DO·T	0.003 ± 0.002	96.4	4.6	<0.05
Ln(W)·SAL^2	0.002 ± 0.001	105.7	4.0	<0.05
Ln(W)·DO	−0.001 ± 0.002	95.2	0.7	>0.1
Ln(W)·T^2	0.014 ± 0.011	98.5	1.6	>0.1
SAL·lnW	−0.006 ± 0.004	100.0	2.9	>0.05
T·SAL	0.005 ± 0.002	97.5	3.7	>0.05

Backward selection procedure on centered variables; alpha = 0.05. Results for regressors with $p>0.5$ are omitted. Numerator degrees of freedom = 1; DDF = denominator degrees of freedom (Satterthwaite's approximation).

Although different in magnitude, similar shapes were observed in the response of growth to temperature and dissolved oxygen between YOY and yearlings (Fig. 2). No significant differences were found between linear or quadratic coefficients for temperature or dissolved oxygen effects between age classes ($p>0.1$). Salinity effects, on the other hand, were significantly different between them, with maximum growth rates shifted from salinity = 15 for YOY to salinity = 22 for yearlings (Fig. 2).

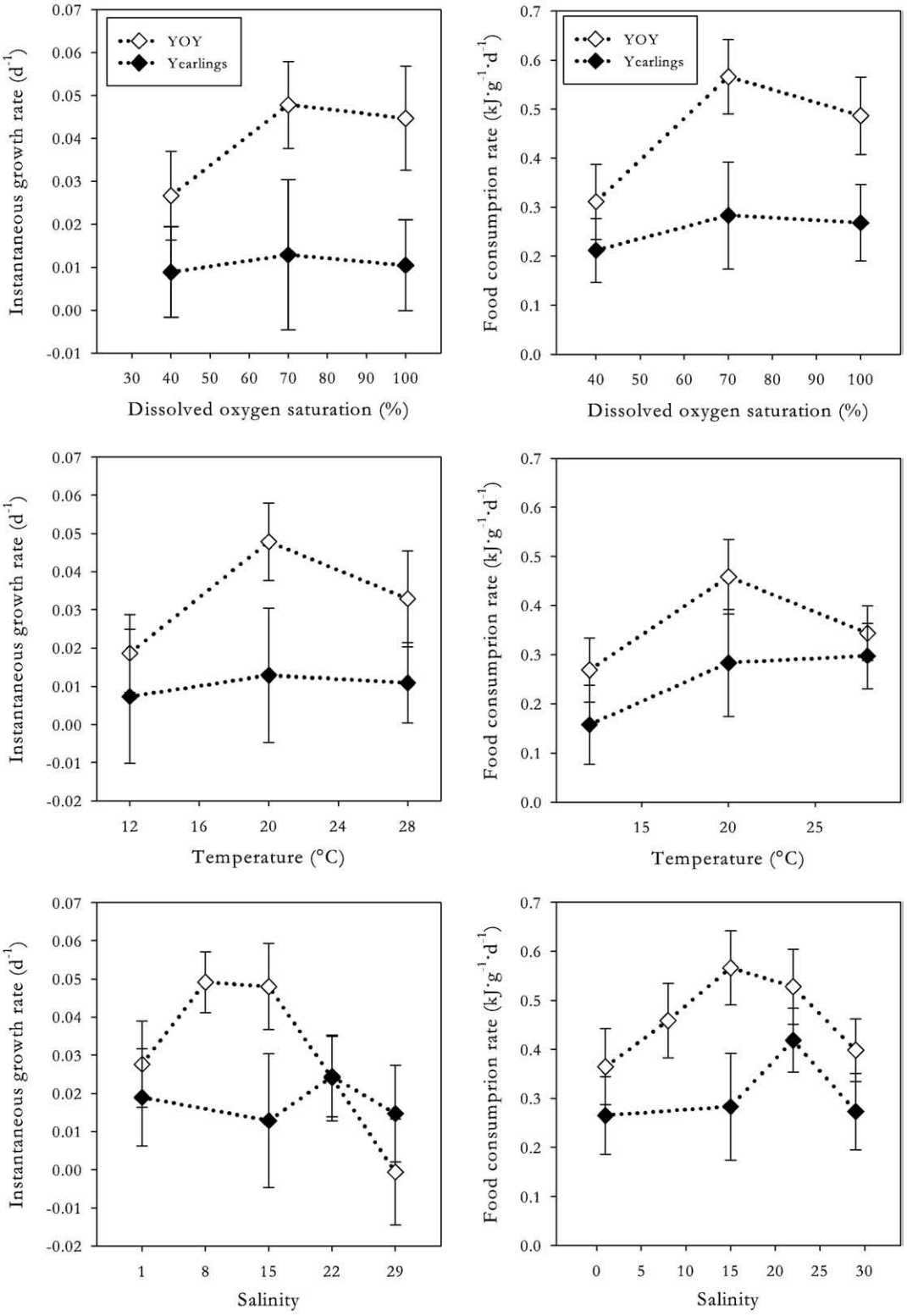

Fig. 2. Comparative effects of temperature and salinity upon instantaneous growth (left pane) and food consumption (right panel) rates in YOY (6–64 g) and yearlings (100–300 g) Atlantic sturgeon. Figures show variability caused by dissolved oxygen, temperature, and salinity (top, middle and lower panel, respectively), while keeping the remaining two factors at fixed conditions (≥70% DO_{SAT}, 20 °C, and/or salinity = 8). Means are connected by lines to facilitate interpretation.

3.2. Food consumption

Mean daily consumption rates measured in YOY showed a range between 0.3 and 0.7 kJ g^{-1} d^{-1} across all treatments (Fig. 3). Linear regression analysis indicated that food consumption was significantly affected by fish weight and by both main and quadratic components of dissolved oxygen, temperature and salinity (Table 3). After a sharp increase between 30 and 40% DO_{SAT}, food consumption continued to increase with oxygen at a smaller and rather continuous rate at 12 °C and 20 °C (Fig. 3). Although steeper DO responses were suggested by results at 28 °C, no significant interactions between explanatory variables DO and temperature were detected.

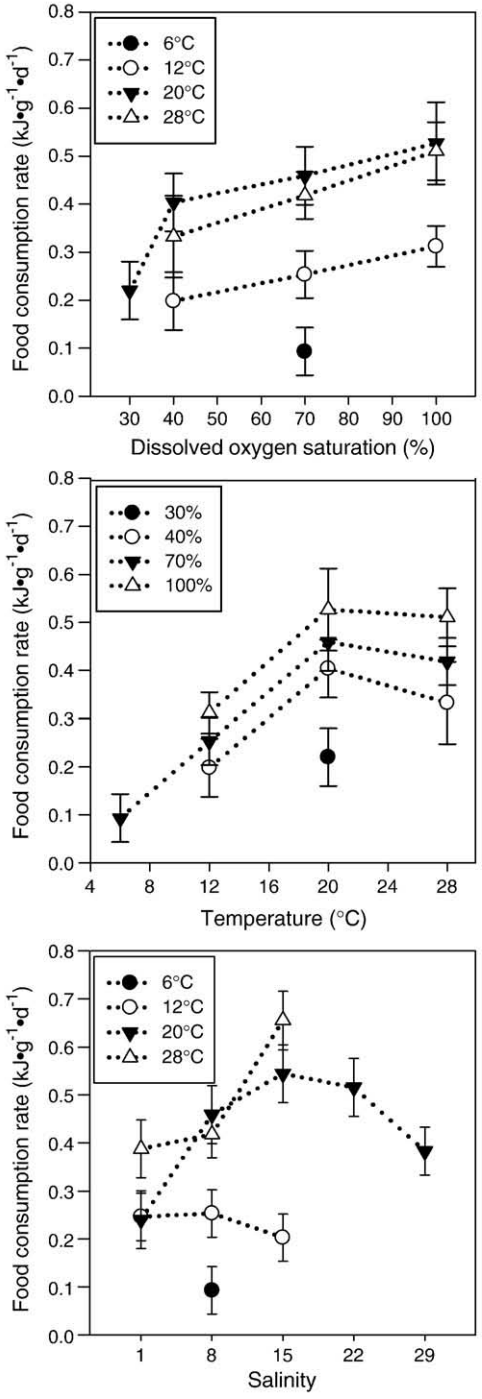

Fig. 3. Effects of dissolved oxygen saturation, temperature, and salinity on food consumption rates of YOY Atlantic sturgeon (means ± SE). Figures show variability caused by two factors at the time, while keeping the third at fixed conditions (salinity = 8 for the top and middle panels, $DO_{SAT} \geq 70\%$ for the bottom panel). Means are connected by lines to facilitate interpretation.

Table 3
Linear regression analysis of dissolved oxygen (DO), temperature (T), salinity (SAL) and individual weight (W) effects upon food consumption in juvenile Atlantic sturgeon (kJ g^{-1} d^{-1}).

Effect	Estimate ± SE	DDF	*F*-value	*p*>*F*
Intercept	0.63 ± 0.077	5.4	67.0	<0.001
T	0.421 ± 0.089	34.3	22.5	<0.0001
T^2	−0.367 ± 0.09	32.9	16.7	<0.001
DO_{SAT}	0.287 ± 0.077	68.2	13.7	<0.001
DO^2_{SAT}	−0.247 ± 0.076	67.7	10.6	<0.01
SAL	0.099 ± 0.035	66.0	8.1	<0.01
SAL^2	−0.094 ± 0.036	69.2	6.8	<0.05
$\log_e(W)$	−0.071 ± 0.02	6.4	13.3	<0.01
$\log_e(W) \cdot DO^2_{SAT}$	0.059 ± 0.083	68.3	0.5	>0.1
$\log_e(W) \cdot T$	−0.079 ± 0.095	35.4	0.7	>0.1
$\log_e(W) \cdot SAL^2$	0.006 ± 0.008	67.1	0.5	>0.1

Backward selection procedure on centered variables; alpha = 0.05. Results for regressors with $p > 0.5$ are omitted. Numerator degrees of freedom = 1; DDF = denominator degrees of freedom (Satterthwaite's approximation).

Food consumption responses to temperature showed a maximum at 20 °C for all treatments except at 100% DO_{SAT} where food consumption was similar at 20 and 28 °C (Fig. 3). Responses to salinity suggested a dome-shaped response with maximum values at intermediate salinities (8–22) and lowest values at salinity 29, the highest tested level (Fig. 3).

Analysis of food consumption responses to environmental factors by age classes showed similar patterns to those observed for growth responses. While parallel curves were observed in response to temperature and dissolved oxygen, a shift in salinity responses was suggested, where maximum consumption rates occurred at salinity 15 and 22 in YOY and yearlings, respectively (Fig. 2). This difference between age classes was only significant at the alpha = 10% level.

3.3. Routine and postprandial metabolism

Routine oxygen consumption was significantly affected by dissolved oxygen, temperature, salinity and fish mass ($p < 0.05$; Table 4). The only significant interaction detected was a positive interaction between dissolved oxygen and temperature. Thus, although routine metabolism increased with dissolved oxygen saturation at all tested temperatures, effects were larger as temperature increased (Fig. 4).

Table 4
Linear regression analysis of dissolved oxygen (DO), temperature (*T*), salinity (SAL) and individual weight (*W*) effects upon juvenile Atlantic sturgeon routine metabolism (mg O_2 g^{-1} h^{-1}).

Effect	Estimate ± SE	DDF	F-value	p>F
T	0.100 ± 0.019	83.6	27.6	<0.0001
T^2	−0.033 ± 0.018	83.5	3.4	<0.1
DO	0.095 ± 0.032	87.6	8.7	<0.01
DO^2	−0.054 ± 0.026	88.7	4.3	<0.05
SAL	−0.023 ± 0.011	80.9	4.2	<0.05
SAL^2	0.028 ± 0.010	84.2	7.6	<0.01
DO*T	0.021 ± 0.008	90.4	6.6	<0.05
$Log_e(W)$	−0.025 ± 0.011	90.6	4.9	<0.05
$Log_e(W) \cdot DO_{SAT}$	0.132 ± 0.128	82.8	1.1	>0.1
$Log_e(W) \cdot T^2$	−0.124 ± 0.094	79.9	1.7	>0.1
$Log_e(W) \cdot T$	0.014 ± 0.019	84.4	0.5	>0.1
T·SAL	−0.011 ± 0.008	84.6	1.8	>0.1
$DO_{SAT} \cdot SAL$	−0.012 ± 0.009	88	1.6	>0.1
$Log_e(W) \cdot SAL$	0.017 ± 0.011	88	2.1	>0.1

Backward selection procedure on centered variables; alpha = 0.05. Results for regressors with $p > 0.5$ are omitted. Numerator degrees of freedom = 1; DDF = denominator degrees of freedom (Satterthwaite's approximation).

For instance, differences between DO_{SAT} levels were minor at 12 °C, but circa 2-fold between 40% and 70% DO_{SAT} at 28 °C (Fig. 4).

Salinity effects upon routine metabolism followed an inverse dome-shaped response, reflected in significantly negative linear and positive quadratic coefficients for this factor. Higher oxygen consumption rates were observed at the most extreme tested salinities (1 and 29), while the minimum oxygen consumption rate was measured at salinity 8. This overall pattern was consistent across temperature and DO_{SAT} tested levels (Fig. 4), with no significant interactions estimated between these factors (Table 4).

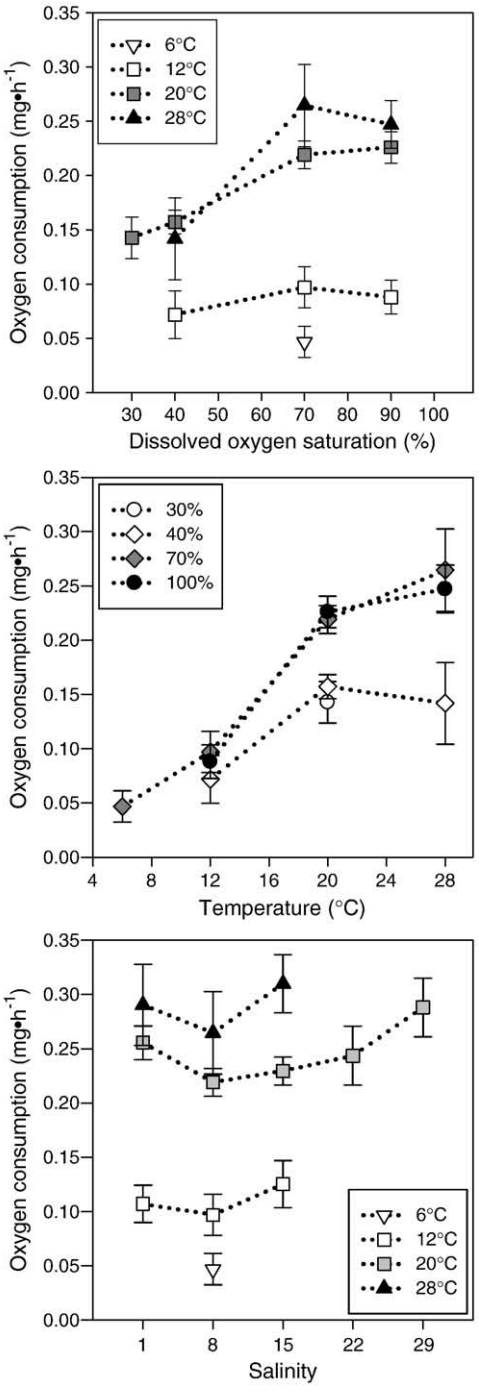

Fig. 4. Effects of dissolved oxygen saturation, temperature and salinity on routine metabolic rates of YOY Atlantic sturgeon (means ± SE). Figures show variability caused by two factors at the time, while keeping the third at fixed conditions (salinity = 8 for the top and middle panels, $DO_{SAT} \geq 70\%$ for the bottom panel). Means are connected by lines to facilitate interpretation.

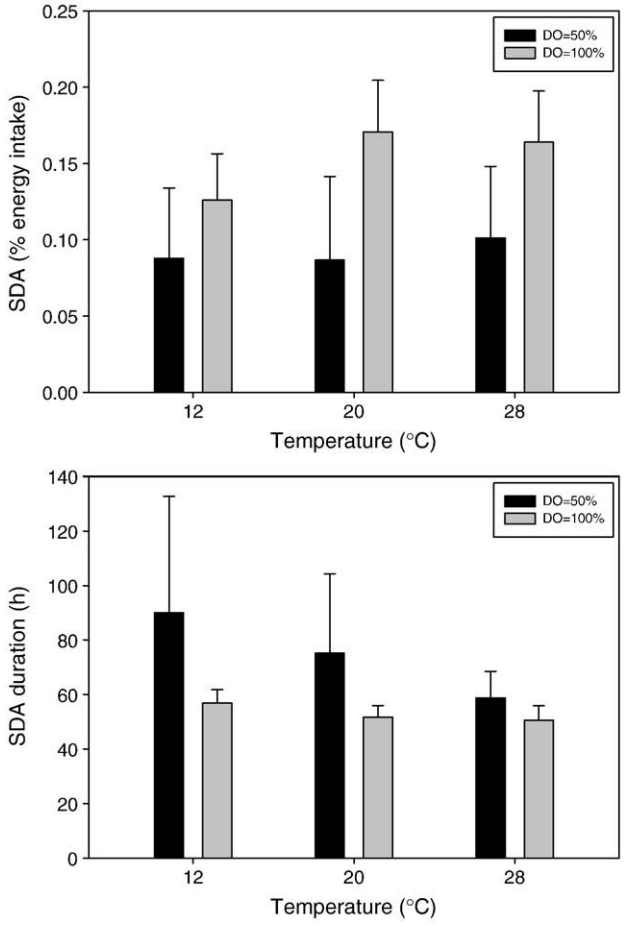

Fig. 5. Postprandial metabolism (SDA) of YOY Atlantic sturgeon at three temperatures (12, 20 and 28 °C) and two dissolved oxygen treatments: 50 and 100% DO_{SAT}. Bars represent mean SDA cost, as a proportion of energy intake (upper panel), and mean SDA duration (lower panel). Error whiskers represent standard errors in both panels.

Postprandial metabolism (SDA) ranged across treatments between 0.010 and 0.034 kJ g^{-1} $meal^{-1}$, with an average value of 0.024 kJ g^{-1} $meal^{-1} \pm 0.0025$ (SE), equivalent to 13% ± 1.3 (SE) of total energy intake. Mixed model regression analysis showed absolute SDA was affected by energy intake (ration size), temperature, DO_{SAT} and fish weight ($p<0.05$). However, when postprandial metabolism was expressed as a proportion of total energy intake ($SDA_{\%}$), only DO_{SAT} was found to have a significant and positive effect upon $SDA_{\%}$ (Fig. 5). While temperature showed a marginally positive effect ($0.05<p<0.1$), ration size and fish weight showed p-values>0.1. Mean $SDA_{\%}$ increased from 11% ± 2.9 (SE) at 50% DO_{SAT} to 16% ± 2.3 (SE) at 100% DO_{SAT} Mean temperature effects were of similar magnitude to DO_{SAT} in the tested range, where $SDA_{\%}$ increased from 12% ± 2.6 (SE) at 12 °C to 14% ± 2.8 (SE) at 28 °C (Fig. 5).

Postprandial metabolism tended to be lower in magnitude, but longer in duration under limited oxygen saturation conditions. Duration appeared longer at lower temperatures (Fig. 5), although both DO_{SAT} linear effects and its interaction with temperature resulted in significance only at the 10% level ($p = 0.09$). As an example, at 12 °C, mean postprandial duration increased from 57 h under normoxia to 90 h under hypoxia (50% DO_{SAT}). At 28 °C, a marginal change in duration from 56 to 60 h occurred over this same change in DO_{SAT} (Fig. 5).

3.4. Egestion

Mean egestion ratio across all treatments was 18% ± 2.3 (SE) of total energy intake, being significantly affected by temperature and

dissolved oxygen and ration size ($p<0.05$). Salinity effects were not tested in this case. Egestion ratio was inversely related to temperature ($p<0.05$) and dissolved oxygen saturation ($p<0.1$), with no conclusive evidence of ration size effects (Fig. 6). Mean egestion ratio at 100% DO_{SAT} (across all temperatures) was estimated to be 11% ± 5.6 (SE) of ingested energy, which increased to 19% ± 9.6 (SE) at 50% DO_{SAT}. Mean egestion ratio at both DO_{SAT} levels decreased with temperature from 21% ± 10.6 (SE) at 12 °C to 11% ± 5.5 (SE) at 28 °C. Regression analysis detected no significant interaction between dissolved oxygen and temperature ($p>0.1$), or significant effects due to fish weight.

3.5. Survival

YOY Atlantic sturgeon survival was significantly affected by dissolved oxygen, temperature and salinity (Cox' survival analysis, $p<0.01$). While low to nil mortality was observed at 100% DO_{SAT}, regardless of the corresponding salinity or temperature treatment, it increased sharply at hypoxic conditions. At 30% DO_{SAT}, estimated mortality (0.041 d^{-1} ± 0.0090 SE) was two-fold higher than that estimated at 40% DO_{SAT}, and was 4-fold higher than that estimated at 70% DO_{SAT} (Fig. 7).

While no mortality was observed at 6 °C, mortality increased with temperature at 40 and 70% DO_{SAT} (Fig. 7). A non linear u-shaped mortality response to salinity was apparent at 20 °C and 70% DO_{SAT}, where the wider range of salinity treatments was applied (Fig. 7). Here, the lowest mortality was observed at salinity 15 (100% survival), increasing toward both extremes, particularly toward salinity 29, where it reached a maximum of 0.033 d^{-1} ± 0.0048 SE. Despite apparent interactions between dissolved oxygen, temperature and salinity, these crossed relationships were not significant under the Cox model ($p>0.1$).

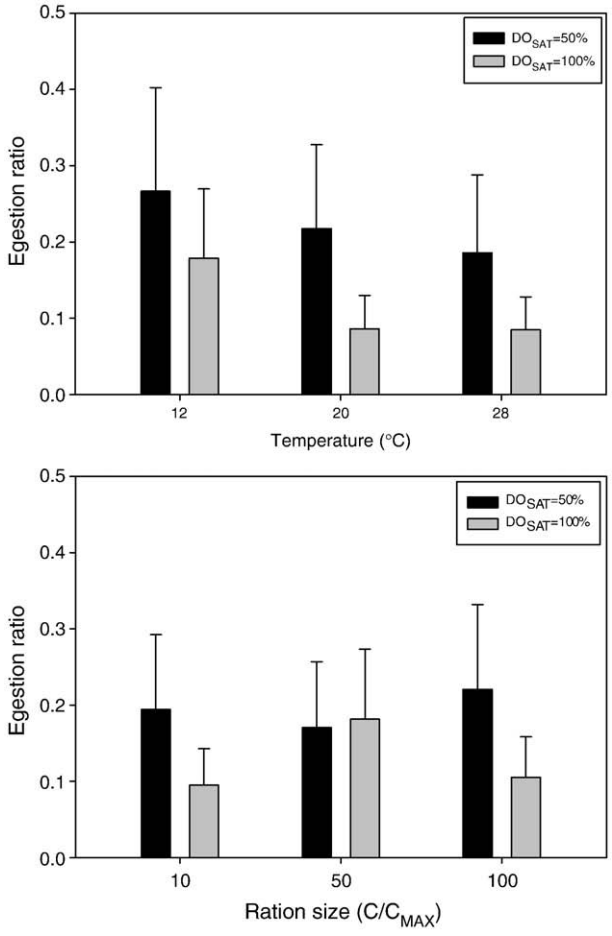

Fig. 6. Effects of temperature (upper panel) and ration size (lower panel) on egestion rates of YOY Atlantic sturgeon (mean ± SE) at 50% and 100% DO_{SAT}.

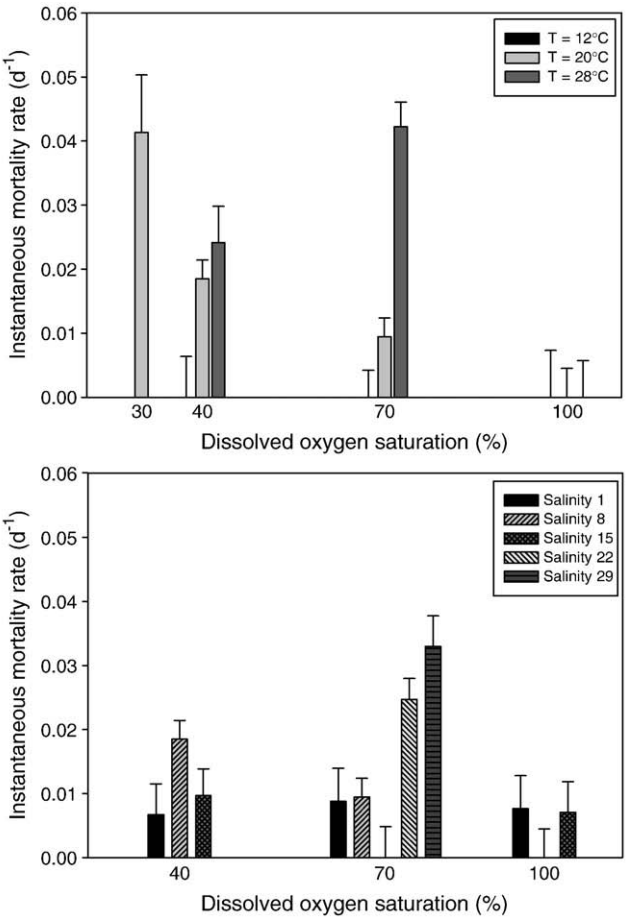

Fig. 7. Instantaneous mortality rate (d^{-1}) for YOY Atlantic sturgeon, previously acclimated to an incomplete factorial combination of dissolved oxygen saturation, temperature and salinity conditions. Upper panel shows dissolved oxygen and temperature effects at a fixed salinity of 8. Lower panel shows dissolved oxygen saturation and salinity effects at 20 °C.

4. Discussion

4.1. Sensitivity of sturgeons to hypoxia

Our results support the idea that average sensitivity of sturgeons to hypoxia is higher than in other fishes, exceeding well known oxyphyllic species, such as rainbow trout *Oncorynchus mykiss* (Klyashtorin, 1976; Secor and Niklitschek, 2002). A heightened sensitivity of sturgeons to hypoxia has been ascribed to an inefficient oxyregulatory system associated with ancestral morphological and

Table 5
Type I deviance explained by temperature (T), dissolved oxygen saturation (DO) and salinity (SAL) in mixed regression models for tested responses, expressed both as absolute and relative quantities.

	Type I deviance				Relative contribution			
	T	DO	SAL	$T\times$DO	T	DO	SAL	$T\times$DO
Growth	12.0	9.3	12.1	5.9	31%	24%	30%	15%
Food consumption	22.9	11.9	13.8	0	47%	25%	28%	0
Routine metabolism	94.5	29.5	10.1	4.1	68%	22%	7%	3%
Postprandial metabolism	1.7	2.9	n.t.	n.t.	37%	63%	n.t.	n.t.
Egestion	5.1	6.3	n.t.	n.t.	45%	55%	n.t.	n.t.
Survival	32.8	3.1	12.4	0	68%	6%	26%	0

Explanatory variables added following the order T, DO, SAL, $T\times$DO; n.t. = non-tested.

physiological traits (Klyashtorin, 1976, 1982). These traits include less efficient gill ventilation, low cardiac performance (Agnisola et al., 1999), and lower affinity of hemoglobin to oxygen (Baker et al., 2005). This overall pattern of especially high sensitivity of sturgeons to hypoxia seems counterintuitive. Many sturgeon populations may have historically used shallow warm estuaries, where hypoxia occurred naturally in the deepest waters before recent anthropogenic effects (Burggren and Randall, 1978; Crocker and Cech, 1997). At the same time, this limited ability to adapt to hypoxia could explain the lack of recovery observed for sturgeon populations inhabiting heavily eutrophied estuaries along the East Coast of the United States (Collins et al., 2000).

Scarce literature has focused on estimating lethal hypoxia effects on sturgeon species. Here, we provided new information on sub-acute lethal DO levels, although we avoided short-term lethal treatments to conserve a limited supply of experimental animals and to prevent undue animal suffering. Accumulated mortality (21 d) in our experiments reached 30% under hypoxia (40% DO_{SAT}) at 20 °C (salinity = 8), increasing to nearly 50% at 28 °C. Secor and Gunderson (1998) found much higher mortality rates (>85%) under similar hypoxia levels (37%–44% DO saturation) after 10-d exposure experiments conducted at 26 °C. Different laboratory settings or source of juveniles (different Hudson River parentage) may have contributed to these differences, but they suggest that survival responses reported here may be conservative. Jenkins et al. (1993) conducted acute mortality (6 h) experiments on YOY Atlantic sturgeon and observed 86–100% mortality for 25–64 d old fish at 30% DO saturation and 22.5 °C, while older juveniles (100–310 d old) experienced only 12–20% mortality under the same conditions. The latter mortality rates are consistent with results reported here for older YOY.

4.2. Ecophysiological responses of Atlantic sturgeon to hypoxia, temperature, and salinity

Our experiments focused on multiple ecophysiological responses to hypoxia, salinity and temperature. Although interactive effects of these three variables were suggested for most responses, such interactions were only significant for growth and routine metabolism under the applied experimental design (Tables 2 and 4). Comparing the deviance explained by each of these three environmental factors across measured responses (Table 5) we observed that temperature was the most important explanatory factor for most responses, except SDA and egestion, where DO_{SAT} was the most influential factor. Dissolved oxygen and salinity explained similar proportions (24–30%) of observed variability in food consumption and growth responses. The effects of salinity upon routine metabolism were rather limited, explaining < 10% of model deviance. The much larger effects of salinity upon food consumption and growth are in agreement with the idea that salinity effects upon fish bioenergetics can exceed what would be expected due to osmoregulation costs alone (Boeuf and Payan, 2001).

The lack of an age-class effect on food consumption and growth responses to hypoxia was somewhat unexpected. We expected tolerance to sub-lethal hypoxia to increase, as mass-specific oxygen demand by metabolism decreases with size (Ishibashi et al., 2005). Although this effect could be mitigated as oxygen delivery rates also decrease with size (Pauly, 1981). Hence, observed higher tolerance of larger organisms to hypoxia in the wild might be related to the ability to escape and/or to endure unfavorable conditions for longer periods (Breitburg, 1992), rather than to a higher physiological tolerance.

The influence of hypoxia on respiration in sturgeons has received large scientific attention (e.g., Klyashtorin, 1976). The most sensitive response has been reported by Burggren and Randall (1978), where movement-restrained white sturgeon *A. transmontanus* exhibited reduced respiration at experimental DO_{SAT} conditions <90% (8.1 mg l^{-1}, 18 ºC). At the other extreme, Nonnotte et al. (1993) observed that Siberian sturgeon *A. baeri* maintained standard metabolism down to 25% DO_{SAT} (2.4 mg l^{-1}) at 15 ºC. The effects of DO_{SAT} on Atlantic sturgeon that we observed were intermediate to these previous studies, and highly temperature-dependent. We observed similar metabolic rates at 70 and 100% DO_{SAT}, followed by a strong reduction in routine metabolism when dissolved oxygen saturation was lowered from 70% to 40%. Such saturation values are equivalent to DO concentrations of 5.24 and 2.99 mg l^{-1}, respectively, at 28 °C and salinity 8. Milder responses to low DO_{SAT} were observed as temperature decreased to 20 °C and, then, to 12 °C (Fig. 4). Beyond possible species-specific differences (Taylor et al., 1999), discrepancy among previous results could be related to routine metabolism being more responsive to hypoxia than standard metabolism. In fact, a reduction in locomotor activity might be a primary reaction to hypoxia (Nilsson et al., 1993; Crocker and Cech, 1997; Taylor et al., 1999).

We found hypoxia effects were also evident for egestion and postprandial metabolism, which actually showed much larger relative responses to hypoxia than food consumption and routine metabolism. The average egestion ratio we estimated (18% ± 2.3 SE of the energy intake) was somewhat lower than egestion levels reported for teleosts, which average 20% (Brett and Groves, 1979), but higher than the 15% assumed for white sturgeon by Bevelhimer (2002). Although our results might be biased by the use of commercial food pellets, the estimate for mean egestion ratio at normoxia (11%) was quite close to equivalent egestion ratios found in other Acipenserids fed natural food (Dawrowski et al., 1987; Gershanovich and Pototskij, 1992; Cui et al., 1996). High assimilation efficiency (and low egestion rates) might be expected in sturgeons due to the high absorptive surface provided by the presence of the spiral valve in the intestine (Buddington and Christofferson, 1985).

While egestion ratio increased ca. 2-fold, postprandial metabolism decreased by half under hypoxia. These results are consistent with recent findings in cod (Jordan and Steffensen, 2007), and support the idea that fish reduce not only food consumption, but also assimilation rate when metabolic scope is limited by hypoxia (Jobling, 1981; Pauly, 1981). In this way, sturgeons might avoid toxic concentrations of unstable amino acids accumulating in the blood when catabolism is limited (van Dam and Pauly, 1995). It is not clear how fishes might reduce assimilation rates under hypoxia, but it has been hypothesized that fish might increase egestion by reducing irrigation of absorptive intestinal tissues (Brett, 1979; Jobling, 1981).

4.3. Size and age responses to salinity and temperature

We observed a size (and age) dependent shift in growth and food consumption responses to salinity, reaching maximum rates at higher salinities for yearlings (salinity = 22) than for YOY (salinity = 15) Atlantic sturgeon. This shift is consistent with their life cycle and also with physiological expectations from fish allometric growth. In terms of their life cycle, YOY Atlantic sturgeon are restricted to upstream estuarine sections and do not migrate to more coastal saline waters until their second year or later (Secor et al., 2000). The change of influence of salinity with size is consistent with what Fry (1971) termed a directive factor: an ontogenetic physiological adaptation well known in smoltification or anadromous salmons. Alternatively, it could reflect the reduction of the exposed gill surface area relative to body biomass (Pauly, 1981). Using similar isometric considerations, a larger body biomass might increase thermo-regulatory capabilities in larger sturgeons dampening their responsiveness to temperature. Although this was not evident in our results, an increase temperature tolerance in larger fish has been found in walleye *Stizostedion vitreum* and mullet *Liza aurata* (Shekk et al., 1990; Clapp et al., 1997).

4.4. Defining hypoxia thresholds for juvenile Atlantic sturgeon and other estuarine fishes

The additive and interactive effects we found for DO, temperature and salinity should have major consequences for juvenile Atlantic

sturgeon in the wild. In historical nursery areas, such as the Chesapeake Bay and other U.S. southeastern estuaries, high temperatures coincide with hypoxia every summer (Collins et al., 2000; Niklitschek and Secor, 2005). Under this scenario the limiting effects of hypoxia would reduce physiological scopes to a point where the relative importance of salinity effects becomes critical. For instance, in Virginia estuaries that support juvenile Atlantic sturgeon, summer temperatures become super-optimal in freshwater, but brackish oligohaline bottom waters are often hypoxic (<40% DO_{sat}). A potential refuge from sub-lethal conditions of high temperature and hypoxia exists in the lower Chesapeake Bay (due to marine influence), which is normoxic and cooler, but here salinity is super-optimal (Niklitschek and Secor, 2005). Hence a three-way "habitat squeeze" (sensu Coutant, 1987) can be envisaged, which could be further reduced by anthropogenic perturbations such us pollution, freshwater flow reductions and global warming (Niklitschek and Secor, 2005).

Our results show the importance of considering temperature and salinity as relevant covariates for hypoxia criteria definitions: considering both their effects upon physiological rates and upon oxygen solubility in water and blood (Holeton and Randall, 1967). For illustration purposes, if optimal growth or survival rates were used as criteria to set a hypoxia threshold for juvenile Atlantic sturgeon, that value would rise from 40 to 70% DO_{SAT} if temperature increased from 12 to 20 °C. At salinity 1 these values would correspond to concentrations of 4.3 and 6.3 mg l^{-1}, respectively. At salinity 29, on the other hand, the same thresholds would correspond to concentrations of 3.6 and 5.4 mg l^{-1}, respectively. At this point, it must be emphasized that "percent DO saturation" or "partial pressure of DO" are the biologically relevant factors for hypoxia, since these, rather than oxygen concentration, represent what physically determines fish oxygen uptake from the surrounding water (Cech, 1990; Kiceniuk and Colbourne, 1997).

Hypoxia has been frequently defined by fixed oxygen criteria, commonly at either 2 ml l^{-1} or 2 mg l^{-1} (Diaz and Rosenberg, 1995; Diaz, 2001; Wu, 2002; US-EPA, 2003). A broader operational definition considers hypoxia as any oxygen concentration reduced from full saturation that impairs living (US-EPA, 2003). To expand this definition further, we propose to define hypoxia as any oxygen concentration reduced from full saturation that produces measurable negative effects upon physiological and survival rates of a given species. Under this conceptual approach, hypoxia thresholds should be species-specific (Klyashtorin, 1976; Goodman and Campbell, 2007), and incorporate those significant environmental and biological covariates known to affect fish responses to hypoxia. In a companion paper (Niklitschek and Secor, 2009-this issue), we develop and test a model that can generate predictions of ecophysiological response based upon multiple environmental and biological variables.

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