Arch. Pol. Fish. (2011) 19: 59-67
DOI 10.2478/v10086-011-0007-6

RESEARCH ARTICLE

# Impact of higher stocking density of juvenile Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, on fish growth, oxygen consumption, and ammonia excretion

*Received – 12 August 2010/Accepted – 16 November 2010. Published online: 30 June 2011; ©Inland Fisheries Institute in Olsztyn, Poland*

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**Abstract**. The impact of stocking density on the growth, feed conversion, oxygen consumption, and ammonia excretion of juvenile Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill) was studied. Three initial stocking densities were used in the experiment: 1.27, 2.49, and 3.80 kg $m^{-2}$. The length of the experiment was 64 days. The mean water temperature was 15.0°C. The fish were fed commercial feed Nutra T 1.9 (Trouv, France). Atlantic sturgeon body weight growth was the highest in the group stocked at the lowest density. The fish from this group attained a final body weight of 130.1 g, which was statistically significantly higher than that of the groups stocked at densities of 2.49 kg $m^{-2}$ ($P < 0.05$) or 3.80 kg $m^{-2}$ ($P > 0.01$). Stocking density had a substantial impact on the value of the feed conversion ratio (FCR) in the different groups. The lowest FCR was noted at the density of 1.27 kg $m^{-2}$ at 0.67. This value differed statistically significantly from group 2.49 kg $m^{-2}$, in which it was 0.74 ($P < 0.05$), and it differed highly statistically significantly from group 3.80 kg $m^{-2}$ at 0.83 ($P < 0.001$). The differences in the FCR value between groups 2.49 and 3.80 kg $m^{-2}$ were also highly statistically significant. Stocking density was not noted to impact oxygen consumption or ammonia excretion. Mean daily ammonia excretion fluctuated from 2.09 to 2.39 mg $kg^{-1}$ $h^{-1}$, and oxygen consumption ranged from 90.5 to 93.8 mg $O_2$ $kg^{-1}$ $h^{-1}$.

**Keywords**: Atlantic sturgeon, stocking density, growth, oxygen consumption, ammonia excretion

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## Introduction

The Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill, inhabits the waters of the east coast of North America, and it spawns in numerous rivers of the continent from the St. Lawrence River in the north to the rivers of the Florida peninsula and the Gulf of Mexico in the south (Leim and Scott 1966, Huff 1975, Smith and Clugston 1997). Within its area of occurrence there are two sub-species: the Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, from the north, and the Gulf sturgeon, *Acipenser oxyrinchus desotoi*, from the south (Kolman et al. 2008a). Genetic testing has provided evidence that the Atlantic sturgeon also inhabited the waters of Europe (Ludwig et al. 2002, Stankovič et al. 2007, Desse-Berset 2009), and currently, the sub-species *Acipenser oxyrinchus oxyrinchus* is being restored to the Baltic Sea (Gessner et al. 2005, Kolman et al. 2008a). Re-establishing the sturgeon population requires the production of stocking material that can be released into rivers. Rearing larval and juvenile stages of Atlantic sturgeon is, however, a formidable task. Little information is available in the scientific literature on this topic, and the data that are available usually refer to the sturgeon inhabiting the southern area of occurrence in North America, which is mainly the gulf sturgeon (Bardi et al. 1998, Mohler et al. 2000). The information available indicates that the Atlantic sturgeon has specific requirements for both food and environmental conditions (Mohler 2003), which renders the rearing of this fish exceptionally difficult.

Improving rearing results, especially of the most sensitive larval and juvenile stages, can be achieved in recirculating aquaculture systems because they permit maintaining rearing conditions at the optimal levels for a given species (Helfrich and Libey 1991). Stocking density is one of the important factors impacting rearing results, and it has been confirmed that this factor plays a key role in the rearing of larval Atlantic sturgeon (Mohler et al. 2000). Using recirculating aquaculture systems to rear fish allows applying substantially higher stocking densities than are possible with extensive rearing, and this lowers unit rearing costs; however, the issue of water purification arises since oxygen depleted by the fish has to be replaced and waste products must be removed. Safe stocking levels can only be determined when the impact the fish have on the closed system, specifically on the quantities of oxygen consumed and ammonia excreted, is known. Such studies have not yet been conducted with regard to the Atlantic sturgeon.

The aim of the current study was to determine the impact stocking density of juvenile Atlantic sturgeon, *A. oxyrinchus*, had on the results of rearing this species and to study the impact it had on the environment of the closed system by measuring oxygen consumption and ammonia excretion.

## Materials and methods

The material used in the study was Atlantic sturgeon fry aged 168 post-hatch that originated from the population inhabiting the St. John River (Canada). Larvae were obtained through artificial reproduction and incubation, and these were transported to the Department of Sturgeon Fish Breeding, Inland Fisheries Institute in Pieczarki where the larvae and fry were reared using methods described previously (Kolman et al. 2008b). For the experiment the fish were stocked into rectangular plastic tanks (2.50 x 0.62 x 0.35 m) that were part of a recirculating system with a total volume of 11.5 m$^3$. The water was purified with an SDK CN 3.2 bio-filter (SDK Poland) with a volume of 3.2 m$^3$ and filled with plastic Light Bioelements (RK Plast A/S, Denmark) with an total volume of 1.5 m$^3$. Three initial stocking densities were applied: 1.27 kg m$^{-2}$ (group L); 2.49 kg m$^{-2}$ (group M); 3.80 kg m$^{-2}$ (group H). The stocks in the tanks comprised 30 (group L), 60 (group M), or 90 (group H) individuals with mean body weights of 65.3 g, body lengths (SL) of 21.1 cm, and total lengths of (TL) 26.3 cm. Each variant was conducted in three replicates.

The length of the experiment was 64 days. The mean water temperature was 15.0°C, and pH ranged from 7.97 to 8.29. The content of total ammonium nitrogen did not exceed 0.32 mg l$^{-1}$, and that of nitrate did not exceed 0.30 mg l$^{-1}$. The water flow rate through all the tanks was identical at 7.1 dm$^3$ min$^{-1}$, which ensured full water exchange in the tanks every 77 minutes. At the beginning, on day 28, and at the end of the experiment, 20 fish from each tank were measured to determine their body weight W (to the nearest 0.1g), total length TL, and body length SL (to the nearest 0.1 cm). The total fish biomass B was also determined for each tank (to the nearest 0.1 g). The following indicators were calculated using these measurements:

$$FCR = F \times B^{-1}$$

where: FCR – feed conversion ratio, F – quantity of feed supplied (kg), B – fish biomass growth (kg);

$$CF = 100 \times W \times SL^{-3}$$

where: CF – Fulton's condition factor, W – body weight (g), SL – body length (cm);

$$SGR = (ln\ (W_2) - ln\ (W_1))\ D^{-1} \times 100$$

where: SGR – specific growth rate (% day$^{-1}$), $BW_2$ and $BW_1$ – mean body weight at the end and the beginning of the experiment (g), D – number of rearing days.

During the experiment the feed was supplied by an automatic band feeder for a period of 18 h per day (from 08:00 to 02:00). The feed supplied was Nutra T 1.9 (Trouv, France) with a protein content of 52%, fat

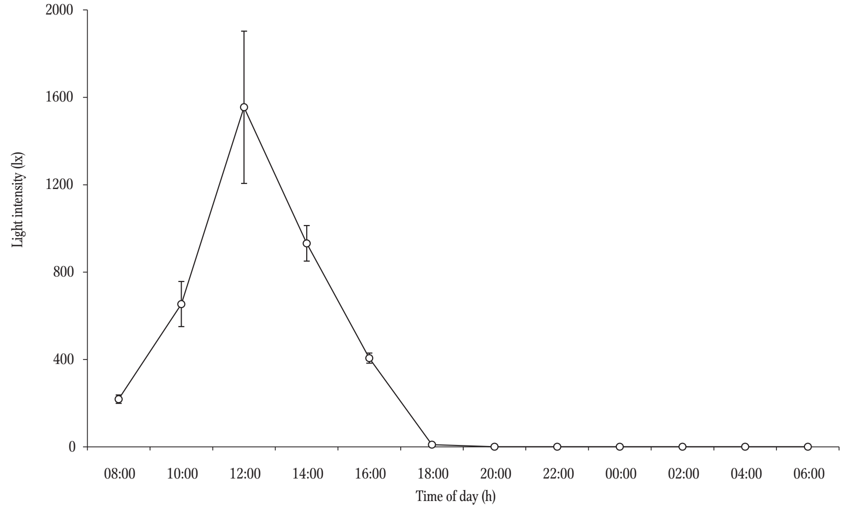


Figure 1. Changes in light intensity (lx) at the surface of rearing tanks during Atlantic sturgeon fry rearing.

content of 20%, and metabolic energy of 19.9 MJ kg$^{-1}$. The daily feed ration was 1% of the fish biomass (D169 – D176), and then 0.8% of the fish biomass until the end of the experiment. The fish were not fed immediately after being stocked into the tanks (D168).

Measurements to determine fry oxygen consumption and ammonia excretion were performed on day 63 of the experiment (fish aged 231 days post-hatch). On the same day, measurements were taken to determine the light intensity at the surface of the rearing tanks using an L-100 lux meter (Sonopan, Poland) (Fig. 1). Measurements of oxygen consumption, ammonia excretion, and light intensity were taken throughout the day at intervals of 2 h from 08:00 to 06:00 the following day. The light intensity among the tanks did not differ significantly statistically ($P > 0.05$).

Juvenile Atlantic sturgeon oxygen consumption and ammonia excretion were calculated based on the difference between the content of oxygen and ammonia at the water inflows and outflows of the tanks. The following formulas were used:

$$OC = (O_{in} - O_{out})\ Q \times B^{-1}$$

where:

OC – oxygen consumption (mg kg$^{-1}$ h$^{-1}$);

$O_{in}$ and $O_{out}$ – oxygen content at the inflow and outflow (mg dm$^{-3}$);

Q – water flow rate (dm$^3$ h$^{-1}$);

B – fish biomass (kg).

$$AE = (A_{out} - A_{in}) \times Q\ B^{-1}$$

where:

AE – ammonia excretion (mg kg$^{-1}$ h$^{-1}$);

$A_{in}$ and $A_{out}$ – ammonia content at the inflow and outflow.

Oxygen content was measured with a PCD 5500 meter (Eutech Instruments) to the nearest 0.01 mg dm$^{-3}$. The quantity of ammonia in the water was determined with direct nesslerization (Hermanowicz et al. 1999) with a Carl Zeiss 11 spectrocolorimeter. Immediately after completing the measurements, the body weight and length of the fry was determined as was the total biomass of the fish in each of the tanks.

Statistical calculations were performed with Statistica 7.1 (Stat Soft Inc.). The mean values of the indexes analyzed were compared using single-factor analysis of variance (ANOVA) (Tukey's range test). Differences were considered statistically significant at $P \leq 0.05$.

## Results

Atlantic sturgeon body weight growth was the highest at the lowest stocking density (group L, Fig. 2). At the end of the experiment the fish from this group

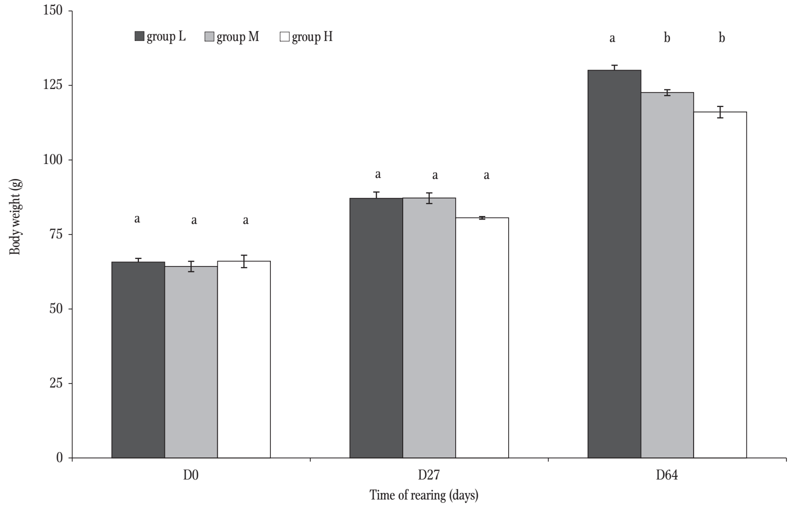


Figure 2. Body weight growth of juvenile Atlantic sturgeon reared at different stocking densities. Group L – 1.27 kg $m^{-2}$, group M – 2.49 kg $m^{-2}$, group H – 3.80 kg $m^{-2}$ (mean values ± SD; N = 3). Groups with the same letter index on the same day do not differ significantly statistically ($P > 0.05$).

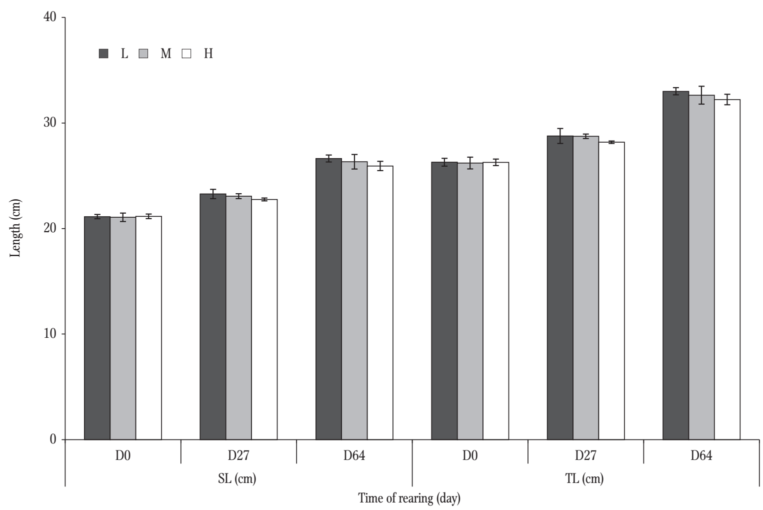


Figure 3. Growth in body length SL and total length TL of juvenile Atlantic sturgeon reared at different stocking densities. Group L – 1.27 kg $m^{-2}$, group M – 2.49 kg $m^{-2}$, group H – 3.80 kg $m^{-2}$ (mean values ± SD; N = 3). No statistically significantly difference was found between the groups ($P > 0.05$).

had achieved a body weight of 130.1 g, which was statistically significantly higher than in group M ($P < 0.05$) and in group H ($P < 0.01$). The differences in body weight were noted after 64 days of rearing since it was similar in all groups in the first period of the experiment (day 27) ($P > 0.05$, Fig. 2). No statistically significant differences ($P > 0.05$) were noted with regard to SL or TL, although the values of these decreased as stocking density increased (Fig. 3). Statistically significant differences were noted in the specific growth rate (SGR) throughout the rearing period between groups L and H. The value of SGR in the group with the lowest stocking density (group L) was 1.07, while in that with the highest density it was 0.88 ($P > 0.05$). The SGR value in group M was 1.01, which did not differ significantly from the values in the other groups ($P > 0.05$). Stocking density had a substantial impact on the values of FCR in the different groups. The lowest value was noted in group L at 0.67, and this differed statistically significantly from that of group M at 0.74 ($P < 0.05$), and highly statistically significantly from that of group H at 0.83

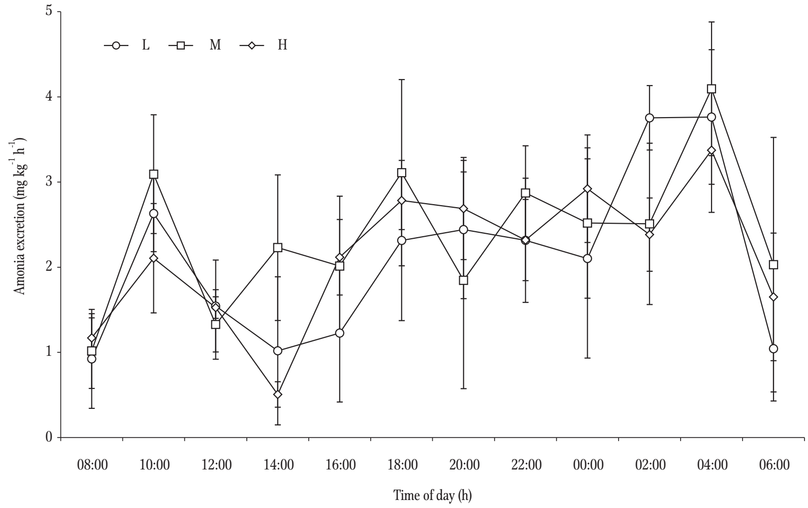


Figure 4. Daily changes in ammonia excretion of juvenile Atlantic sturgeon reared at different stocking densities. Group L – 1.27 kg m$^{-2}$, group M – 2.49 kg m$^{-2}$, group H – 3.80 kg m$^{-2}$ (mean values ± SD; N = 3).

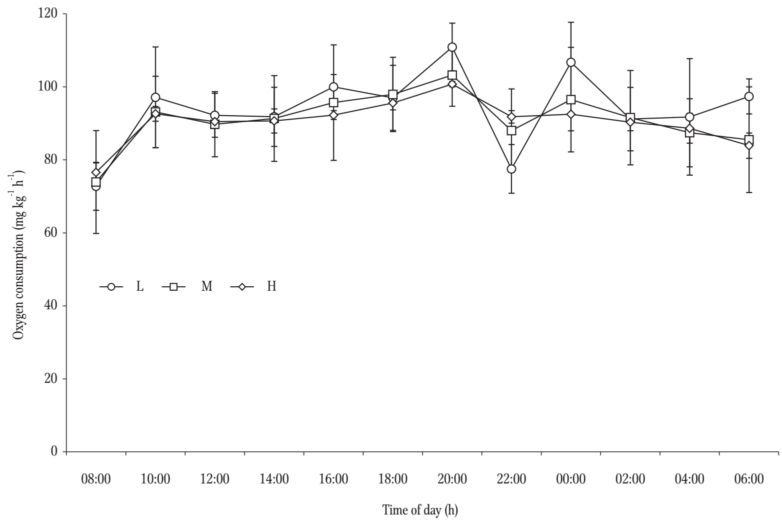


Figure 5. Daily changes in oxygen consumption of juvenile Atlantic sturgeon reared at different stocking densities. Group L – 1.27 kg m$^{-2}$, group M – 2.49 kg m$^{-2}$, group H – 3.80 kg m$^{-2}$ (mean values ± SD; N = 3).

($P < 0.001$). Differences in FCR values between groups M and H were also statistically significant ($P > 0.05$). The values of Fulton's condition factor (CF) remained at similar levels throughout the rearing period and did not differ among groups ($P > 0.05$). At the beginning of the experiment the CF value for all the groups was 0.69, while at the end of the experiment it was 0.68 in groups L and M and 0.67 in group H. Juvenile Atlantic sturgeon survival was similar in all the groups at 98.9% in groups L and M and 97.4% in group H ($P > 0.05$). Fish deaths were recorded only during the initial period following stocking during the first ten days of rearing.

Mean daily ammonia excretion was 2.09 mg kg$^{-1}$ h$^{-1}$ in group L, 2.13 mg kg$^{-1}$ h$^{-1}$ in group H, and 2.39 mg kg$^{-1}$ h$^{-1}$ in group M. Differences in the quantities of ammonia excreted in the different groups were not statistically significant ($P > 0.05$), and fluctuations in ammonia excretion were similar in all the groups (Fig. 4). Two hours after the beginning of feeding there was a distinct increase in ammonia excretion. During the feeding period (08:00-02:00), the quantity of ammonia excreted increased progressively, and the highest values were noted in all groups just after feeding had finished at 04:00. These were 3.76,

**Table 1**
Rearing indexes of juvenile Atlantic sturgeon reared at different stocking densities 1.27 kg $m^{-2}$ (group L), 2.49 kg $m^{-2}$ (group M), 3.80 kg $m^{-2}$ (group H) (mean values ± SD; N = 3). Groups with the same letter index in the same row do not differ significantly statistically (P > 0.05).

| | Stocking density | | |
|---|---|---|---|
| Index | Group L | Group M | Group H |
| Initial body weight (g) | 65.7 ± $1.3^{a}$ | 64.3 ± $1.7^{a}$ | 66.0 ± $2.1^{a}$ |
| Final body weight (g) | 130.1 ± $1.7^{a}$ | 122.6 ± $1.0^{b}$ | 116.1 ± $1.9^{b}$ |
| Specific growth rate SGR (% $d^{-1}$) | 1.07 ± $0.02^{a}$ | 1.01 ± $0.05^{ab}$ | 0.88 ± $0.02^{b}$ |
| Feed conversion ratio FCR | 0.67 ± $0.00^{c}$ | 0.74 ± $0.02^{b}$ | 0.83 ± $0.01^{a}$ |
| Condition coefficient K | 0.68 ± $0.01^{a}$ | 0.68 ± $0.01^{a}$ | 0.67 ± $0.02^{a}$ |
| Survival (%) | 98.9 ± $1.1^{a}$ | 98.9 ± $1.1^{a}$ | 97.4 ± $0.6^{a}$ |
| Final stocking density (kg $m^{-2}$) | 2.49 ± 0.06 | 4.69 ± 0.10 | 6.57 ± 0.12 |

4.09, and 3.37 mg $kg^{-1}$ $h^{-1}$, respectively, in groups L, M, and H.

No statistically significant differences were noted in oxygen consumption in the groups at different stocking densities. The mean daily values of this index were 93.8 mg $O_2$ $kg^{-1}$ $h^{-1}$ in group L, 91.1 mg $O_2$ $kg^{-1}$ $h^{-1}$ in group M, and 90.5 mg $O_2$ $kg^{-1}$ $h^{-1}$ in group H (P > 0.05). After feeding began, there was a rapid increase in the quantity of oxygen consumption which lasted until 20:00, when the maximum values were noted in all groups (Fig. 5). Following this, oxygen consumption decreased progressively.

## Discussion

Stocking density is one of the factors that has a strong impact on the final results of rearing fish. It can lead to restricted access to feed, size disparity among fish, and increased cannibalism (Folkvord 1997, Fessehaye et al. 2006) resulting in lowered fish growth rates and survival (Haylor 1992). The effects of applying different stocking densities are not the same for all fish species; for example, some species reared at higher stocking densities exhibit better growth, which might be linked to greater competition for feed that increases appetites (Potthoff and Christman 2006). Stocking density can also cause stress and decrease the ability of the fish to react to it. Although this is usually noted when stocking density is too high (Caipang et al. 2008), sometimes this occurs when stocking densities are too low, as has been observed in species that employ a shoal life strategy such as perch, *Perca fluviatilis* (L.) (Strand et al. 2007). The initial stocking densities of 1.27 to 3.80 kg $m^{-2}$ applied in the current study had a significant impact on the results of rearing juvenile Atlantic sturgeon. Increasing the stocking density to 3.80 kg $m^{-2}$ resulted in lowered daily body growth rates and the final body weights of these fish in comparison to those reared at lower stocking densities. Similar results were obtained by Jodun et al. (2002) in an experiment with larger Atlantic sturgeon (368 g) reared at increasing stocking densities from 3.6 to 16.3 kg $m^{-2}$, and by Rafatnezhad et al. (2008) in an experiment with juvenile beluga sturgeon, *Huso huso* (L.), in which growth rates decreased as stocking densities increased from 1 to 8 kg $m^{-2}$. These authors also reported that the analysis of blood parameters did not indicate that this was caused by increasing levels of stress. However, in another experiment with lake sturgeon, *Acipenser fulvescens* Rafinesque, reared at similar stocking densities (from 1.35 to 3.75 kg $m^{-2}$) to those of the current study, Fajfer et al. (1999) reported that this factor had no impact on the final results of rearing.

The impact of different numbers of fish in groups can vary at different stages of fish development. This refers particularly to species in which pronounced cannibalism can occur. A strong correlation between stocking density and cannibalism was noted for larval pikeperch, but no such correlation was noted at higher stocking densities among juvenile stages (Szkudlarek and Zakęś 2002, 2007). While cannibalism among sturgeon juveniles and older stages is extremely rare, aggressive behavior in which the fish attack each other has been noted. For example, Rafatnezhad et al. (2008) reported increased incidences of tail fin wounds among juvenile beluga sturgeon as stocking density increased. This phenomenon was not noted in the current study, and it appears that juvenile Atlantic sturgeon do not exhibit aggressive behavior towards each other. Mohler (2003) reported similar observations on the rearing of this species.

Although oxygen consumption and ammonia excretion are key factors when rearing Atlantic sturgeon in closed recirculating aquaculture systems, there is no data available in the literature on this issue. The values of these parameters obtained in the current study are much lower than those reported for Siberian sturgeon, *Acipenser baerii* Brandt, with mean body weights of 104 and 167g that consumed oxygen at rates from 228 to 187 mg $kg^{-1}$ $h^{-1}$ and excreted 3.5 mg $kg^{-1}$ $h^{-1}$ ammonia (Szczepkowski et al. 2000), and for those of white sturgeon, *Acipenser transmontanus* Richardson, with body weights of 90 g that consumed oxygen at 140 mg $kg^{-1}$ $h^{-1}$ (Thomas and Piedrahita 1997). These differences could have been due largely to the different water temperatures at which the experiments were conducted. In the current experiment, the water temperature was lower (15$^{o}$C) than it was in the experiments with Siberian (20$^{o}$C) or white (21.1$^{o}$C) sturgeon, and it is widely known that increased water temperatures cause increased oxygen consumption and ammonia excretion (Kamler 1992). Water temperature in the current experiment was close to the optimal temperature for Atlantic sturgeon from the northern part of its area of occurrence and that which is applied in the rearing of this fish (Kelly and Arnold 1999, Giberson and Litvak 2003). It was also adjusted to correspond to the season of the year (winter) in which the experiment was conducted. This last factor could have had a certain impact on the values of the indicators of metabolism since seasonality has been confirmed in some species; for example, muskellunge, *Esox masquinongy* Mitchill, consumed more oxygen in summer than it did in winter at the same water temperatures (Chipps et al. 2000).

Stocking density can impact the indexes of metabolism. It was confirmed, for example, that at higher stocking densities routine metabolism of white sturgeon increased (Ruer et al. 1987), and the ammonia excretion of *Morone saxatilis* × *M. chrysops* increased (Liu et al. 2009). Increased Atlantic sturgeon stocking density did not impact either oxygen consumption, ammonia excretion, or fluctuations in these indexes during the daily cycle. However, the daily profile of fluctuations in oxygen consumption and ammonia excretion was varied. Two hours after feeding commenced, increased values of both of these indexes were noted. Throughout the feeding period the amount of ammonia excreted increased, while oxygen consumption increased only during the first part of this period until 20:00. The fluctuations in indexes of metabolism can be linked to various factors; however, they are most dependent on feeding, including the type of feed supplied, the ration, and the feeding schedule (Dąbrowski et al. 1987, Zakęś 1999). When feed is supplied in portions, sharp increases in oxygen consumption were noted for Siberian sturgeon a few hours following feeding, and the type of feed supplied determined when maximum oxygen consumption occurred (Dąbrowski et al. 1987). A similar dependence was noted with regard to ammonia excretion. Increased ammonia excretion was noted 3 h after feeding was begun irrespectively of the size of the fish (Jatteau 1997). The decrease in oxygen consumption after 20:00 noted in the current study was not linked to the feed since the fish were still being fed continually at this time. This was probably linked to changes in light intensity, because this is when the lighting shifted from daytime to night (Fig. 1) during which the fish became less physically active. Although precise information regarding Atlantic sturgeon light preferences is lacking, it is known that larval stages initially exhibit negative phototaxis (Mohler 2003, Kolman et al. 2008a), and that fry weighing about 4 g attained similar growth during rearing in illuminated and dark tanks, which indicates that light is of little significance to feeding in this species (Szczepkowska, unpublished data). Nevertheless, juvenile stages of the closely-related European sturgeon, *Acipenser sturio* L., exhibited greater activity at night (Staaks et al. 1999). The current results on metabolism might indicate that juvenile Atlantic sturgeon are more active during the day, but this requires further study.

The results of the current study indicate that increasing stocking density above 3.80 kg $m^{-2}$ limits growth and lowers the feed conversion ratio of juvenile Atlantic sturgeon. The occurrence of negative effects at relatively low stocking densities (3.80 kg $m^{-2}$) indicates that Atlantic sturgeon is highly sensitive to this factor, which is disadvantageous in terms of the rearing bio-techniques of this species in recirculating aquaculture systems. Increased stocking density did not, however, have an impact on oxygen consumption or ammonia excretion or on the fluctuations of these indexes during the daily cycle.

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## Streszczenie

### Wpływ zagęszczenia obsady juwenalnego jesiotra ostronosego, *Acipenser oxyrinchus* Mitchill na wzrost ryb, konsumpcję tlenu i wydalanie amoniaku

Celem przeprowadzonych badań było określenie wpływu różnych zagęszczeń obsady juwenalnego jesiotra ostronosego na wyniki chowu oraz konsumpcję tlenu i wydalanie amoniaku. Materiałem do badań były juwenalne jesiotry ostronose (średnia masa ciała 65,3 g, długości ciała SL 21,1 cm i długości całkowitej TL 26,3 cm). Zastosowano trzy początkowe zagęszczenia obsady: 1,27 kg $m^{-2}$ (grupa L), 2,49 kg $m^{-2}$ (grupa M) i 3,80 kg $m^{-2}$ (grupa H). W 63 dniu eksperymentu (wiek ryb 231 dni po wykluciu) przeprowadzono pomiary konsumpcji tlenu i wydalania amoniaku narybku oraz natężenia oświetlenia. Wzrost masy ciała był najszybszy w grupie z najniższym zagęszczeniem obsady. Na koniec eksperymentu ryby w tej grupie osiągnęły masę ciała 130,1 g i była ona istotnie statystycznie wyższa niż w grupach M i H. Stwierdzono istotne statystycznie różnice wartości dobowych przyrostów masy ciała (SGR) w całym okresie chowu pomiędzy grupami L i H. Zagęszczenie obsady wpłynęło w dużym stopniu na wartość współczynników pokarmowych (FCR) w poszczególnych grupach. Wartość FCR uzyskano w grupie L wynosiła 0,67 i różniła się istotnie statystycznie od grupy M i H (odpowiednio: 0,74 i 0,83). Nie stwierdzono wpływu zagęszczenia obsady na wielkość konsumpcji tlenu i wydalania amoniaku. Średnia dobowa wielkość wydalania amoniaku wahała się od 2,09 do 2,39 mg $kg^{-1}$ $h^{-1}$, a wielkość konsumpcji tlenu od 90,5 do 93,8 mg $O_2$ $kg^{-1}$ $h^{-1}$. Uzyskane wyniki wskazują, że zwiększenie zagęszczenia obsady powyżej 3,80 kg $m^{-2}$ ogranicza wzrost i znacznie obniża efektywność wykorzystania paszy u juwenalnego jesiotra ostronosego, nie ma natomiast wpływu na wielkość zapotrzebowania tlenowego i wydalania amoniaku oraz zmiany tych wskaźników w cyklu dobowym.