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## THE INFLUENCE OF FEEDING FREQUENCY ON THE METABOLIC RATE OF PERCH PERCA FLUVIATILIS L.

Zdzisław Zakęś, Krystyna Demska-Zakęś\*

The Stanisław Sakowicz Inland Fisheries Institute in Olsztyn, Poland \* University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. The aim of the study was to determine the impact of feeding frequency (one, two or three feeds and continuous feeding) on the magnitude of oxygen consumption (OC, mg  $O_2$  kg<sup>-1</sup> h<sup>-1</sup>) and ammonia excretion (AE, mg TAN kg<sup>-1</sup> h<sup>-1</sup>) by juvenile perch. The study was conducted during routine rearing in a recirculating system. No significant impact of the applied feeding schedules (P > 0.05) on the average diurnal values of OC and AE was confirmed. The values ranged from 165.88 mg  $O_2$  kg<sup>-1</sup> h<sup>-1</sup> (one feed) to 188.48 mg  $O_2$  kg<sup>-1</sup> h<sup>-1</sup> (continuous feeding) and from 5.51 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (two feeds) to  $6.44 \text{ mg TAN kg}^{-1} \text{ h}^{-1}$  (continuous feeding). The feeding schedule had a significant influence on the diurnal fluctuations of oxygen consumption and ammonia excretion and was especially evident in the latter. The  $AE_{max}/AE_{average}$  ratio in the group of fish fed once was 2.46, but the  $AE_{max}/AE_{min}$  ratio was 19.01. AE variability over a twenty-four hour cycle was much lower in other feeding schedules. The course of the diurnal profiles of OC and AE was characteristic for each of the applied experiment variants. Statistically significant dependencies were confirmed between ammonia excretion and oxygen consumption (P < 0.001) and were specific to each experiment group. Correlation coefficients of the linear regression equation that describes the AE - OC relation ranged from 0.57 (three feeds) to 0.94 (two feeds). The values of OFR (kg  $O_2$  kg<sup>-1</sup> feed fed day<sup>-1</sup>) and AFR (kg TAN kg<sup>-1</sup> feed fed day<sup>-1</sup>) were not determined by the applied feeding schedule (P > 0.05).

Key words: PERCA FLUVIATILIS, FEEDING FREQUENCY, OXYGEN CONSUMPTION, AMMONIA EXCRETION, RECIRCULATING SYSTEMS

### INTRODUCTION

The study revealed that feeding frequency may determine the growth of fish, their body composition and the effectiveness of food utilization (Boujard and Leatherland 1992, Hayward et al. 1997, Jarboe and Grant 1997, Lee et al. 2000). It may also influence the creation of a social hierarchy in the stock or the variability of fish sizes within groups (Jobling 1994). By applying the appropriate feeding frequency it is possible to minimize differences in the amounts of food consumed by particular specimens, and thus increase the effectiveness of fish production, decrease feed loses and increase water quality (Jobling and Baardvik 1994). Therefore, determining the optimum feeding schedule has important commercial and economic implications. It is not, however, a constant value even for a given species and depends on the degree

of ontogenetic development, food quality and environmental conditions (Goddard 1996). In order to achieve an optimum growth rate and a high survival rate in larvae and juvenile stages, it is usually recommended to provide feed more frequently than is done for older specimens (Folkvord and Otterå 1993).

Feeding frequency influences water quality (Philips et al. 1998) and the diurnal variability of oxygen consumption (OC) and ammonia excretion by fish (AE) (Colt 1991). This is especially important when fish are cultivated in recirculation systems. Determining the impact of feeding frequency OC and AE for fish cultivated under such conditions is especially important. Studies of this type concern species that are of significant, commercial importance, as well as those which are currently being introduced into aquaculture. One such species is certainly perch *Perca fluviatilis* L. The production of stocking material and commercial-sized perch is becoming more common in western European countries (Fontaine et al. 2000). Recirculation systems are especially useful for this purpose (Fontaine et al. 1996). Naturally, the most important part of the system, that which guarantees the safety of the cultivated fish, is maintaining water quality, i.e. the appropriate concentrations of oxygen and total ammonia nitrogen (Westers and Pratt 1977). Therefore, it is crucial to learn about the impact of the more influential factors which determine OC and AE values.

The aim of this study was to determine the impact of feeding frequency on the magnitude of oxygen consumption and ammonia excretion by juvenile perch and the diurnal variability of these parameters.

### MATERIAL AND METHODS

The study materials were obtained from the Dgał Experimental Hatchery of the Inland Fisheries Institute in Olsztyn in northeastern Poland. A pond with an area of  $100 \text{ m}^2$  was stocked on 30 April 1999 with fertilized perch eggs. On 25 June 1999, following 55 days of cultivation, part of the material was fished out (1,672 specimens with an average body weight of 0.35 g) and transferred to the hatchery. The fish were placed in two 2 × 1× 0.35 m fiberglass tanks which were a part of a recirculation system and were fed only artificial feed. The methodology is described in detail in Szczepkowski et al. (1999).

The main aim of the introductory phase of rearing was to teach the fish to feed on artificial feed. Following this period, on 27 September 1999, some of the fish, with an average total length (TL) of 10.56 cm and average body weight of 12.40 g, were trans-

ported in plastic bags to the Inland Fisheries Institute in Olsztyn. The water temperature during transportation was 20°C, and the transportation time was one hour. About 1.2 kg of fish were placed in each bag (20 l water and 20 l oxygen). The fish were stocked in six experimental tanks with a volume of 200 l each. The tanks were part of two independent recirculation systems, and the amount of fish in each was 1.2 kg. The systems were equipped with water treatment facilities, which included mechanical and biological filters. The water temperature in the systems was maintained at a fixed level ( $\pm 0.2^{\circ}$ C) and was 20°C in both systems on the day of stock. After four days, it was increased in one of the systems to 23°C at a rate of 1°C d<sup>-1</sup>. The acclimation time of the fish to the higher temperature was two weeks. The oxygen concentration at 20 and 23°C did not fall below 7.90 and 7.40 mg  $O_2 l^{-1}$  (input) and 5.3 and 4.8 mg  $O_2 l^{-1}$  (output), respectively. The concentration of total ammonia nitrogen (TAN =  $NH_4^+$ -N + NH<sub>3</sub>-N) in both temperature variants did not exceed 0.03 mg TAN l<sup>-1</sup> (input) and 0.25 mg TAN  $l^{-1}$  (output). The water pH at input and output was 7.74 (± 0.05) and 7.91 (± 0.03), respectively. Water flow was maintained at a level of 4 l min<sup>-1</sup>; this allowed water exchange in the tanks with a frequency of 1.2 exchange h<sup>-1</sup> and maintained a difference of oxygen concentration between input and output waters of above 2 mg l<sup>-1</sup>. Twenty-four hour illumination was applied, and the light intensity above the surface of the experimental tanks was 50 - 80 lx.

During routine perch cultivation from 28 September to 21 December 1999, studies were conducted to determine the impact of water temperature (20 and 23°C), fish size and feeding on the metabolic rate of this species (Zakęś et al. unpublished data). When that experiment had concluded, the temperature in the 20°C system was increased to 23°C, the temperature of the second system. The temperature was increased over three days, at a rate of 1.0°C per 24 hours. The measurements of the oxygen consumption (OC, in mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) and total nitrogen ammonia excretion (AE, in mg TAN kg<sup>-1</sup> h<sup>-1</sup>) by perch fed at different frequencies were taken from 7 to 29 January 2000.

#### **EXPERIMENTAL PROCEDURE**

The fish were fed with a commercial trout feed produced by Aller Mølle (2 mm PL SAFIR with a content of 45% protein, 20% fat, 16% carbohydrates and an energetic value of 21.6 MJ kg<sup>-1</sup> gross energy and 17.3 MJ kg<sup>-1</sup> digestible energy). The fish were fed using 4035 FIAP type automatic band feeders manufactured by Fish Technic, Gmbh,

Germany. Four fish feeding schedules were applied – one, two, or three feeds and continuous feeding. The fish that were fed one, two or three times were fed for three hours daily at the following times: one feed at 12.30-15.30; two feeds at 12.00-13.30 and 23.00-00.30; three feeds at 10.30-11.30, 18.30-19.30 and 02.30-03.30. The continuously fed fish were given feed for 18 hours daily from 09.30-03.30. A sub-maximum feed dose (Mélard et al. 1996) was applied at 0.9% of the stock biomass.

Twenty-four hours after checking the metabolic rate, the fish were weighed  $(W \pm 0.1 \text{ g})$  and measured (TL  $\pm 0.1 \text{ cm}$ ). The average length and weight of the fish were determined from 20 specimens randomly fished out from each tank. The stock biomass was determined by weighing all the fish from each tank. All of these procedures were carried out after the specimens had been anesthetized using PROPISCIN (1.5 ml I<sup>-1</sup>) (Kazuń and Siwicki 2001). The average total length and weight of fish on the first day of the experiment were 17.4 cm and 69.1 g, and after three weeks of the experiment these figures were 18.3 cm and 80.5 g. The average fish biomass was 15.4 kg m<sup>-3</sup> at the beginning of the experiment and 17.2 kg m<sup>-3</sup> at its conclusion.

# PROCEDURE OF OXYGEN CONSUMPTION AND AMMONIA EXCRETION MEASUREMENTS

The procedure for measuring the perch metabolic rate was the same as in previously described experiments (Zakeś et al. 2001). During the experiment, two diurnal measurements of perch metabolic rate were taken for each feeding schedule and each was repeated twice.

At the beginning, oxygen consumption (OC', mg  $O_2$  kg<sup>-1</sup> h<sup>-1</sup>) was calculated for each of the experimental tanks using the following formula:

$$OC' = ((DO_{in} - DO_{out}) \times Q) / B,$$
(1)

where:

 $DO_{in}$  – input oxygen concentration (mg O<sub>2</sub> l<sup>-1</sup>);

 $DO_{out}$  – output oxygen concentration (mg O<sub>2</sub> l<sup>-1</sup>);

Q – water flow through the tanks (l h<sup>-1</sup>);

*B* – fish biomass (kg).

In order to include oxygen diffusion, the diffusion rate ( $r_{diff}$ ) was calculated for all the tanks (Metcalf and Eddy Inc. 1991):

$$r_{diff} = K \left( DO_{sat} - DO \right) \tag{2}$$

where:

K – diffusion constant;

 $DO_{sat}$  – oxygen concentration at 100% water saturation (mg O<sub>2</sub> l<sup>-1</sup>);

DO – current oxygen concentration in water (mg O<sub>2</sub> l<sup>-1</sup>).

The corrected value of OC was calculated using the following formula:

$$OC = ((DO_{in} - DO_{out}) \times Q) / B + r_{diff} V / B$$
(3)

where:

V – tank volume (l).

The remaining notation is as in formulae (1) and (2).

In order to determine the magnitude of total nitrogen ammonia excretion (AE -  $mg TAN kg^{-1} h^{-1}$ ), the following formula was applied:

$$AE = ((TAN_{out} - TAN_{in}) \times Q) / B,$$
(4)

where:

 $TAN_{in}$  – input TAN concentration (mg TAN l<sup>-1</sup>);

 $TAN_{out}$  – output TAN concentration (mg TAN l<sup>-1</sup>).

The remaining notation is as in formula (1).

The amount of oxygen needed to metabolize 1 kg of feed (parameter OFR, kg O<sub>2</sub> kg<sup>-1</sup> feed fed day<sup>-1</sup>) and the amount of ammonia created as the result of consuming this amount of feed (parameter AFR, kg TAN kg<sup>-1</sup> feed fed day<sup>-1</sup>) were calculated using the following formula:

 $OFR \text{ or } AFR = [(OC (mg O_2 kg^{-1} h^{-1}) \text{ or } AE (mg TAN kg^{-1} h^{-1}) \times 24 h day^{-1}) \times 1,000$ g kg^{-1}] / [feed dosage (g day^{-1}) × 1,000,000 mg kg^{-1}] (5)

#### STATISTICAL ANALYSES

Data regarding the metabolic rate (OC, AE, OFR and AFR – daily averages, average maximum and minimum values of these parameters in diurnal cycles) were analyzed using the STATISTICA program. One-way variance analysis (ANOVA) was applied. When statistically significant differences ( $P \le 0.05$ ) were detected between the average values of the analyzed parameters in subsequent experiment variants, further statistical analysis was conducted (Scheffé test). Linear regression was used to determine the dependence between AE and OC in each of the experiment variants.

#### RESULTS

#### FISH METABOLIC RATE

The perch feeding frequencies used in the experiment did not have a statistical impact on the average daily values of oxygen consumption and ammonia excretion (P > 0.05). The values of OC ranged from 165.88 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (one feed) to 188.48 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (continuous feeding), and AE ranged from 5.51 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (two feeds) to 6.44 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (continuous feeding - Table 1).

TABLE 1

Oxygen consumption (mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) and ammonia excretion (mg TAN kg<sup>-1</sup> h<sup>-1</sup>) by juvenile perch fed daily at various frequencies (values are the averages from four repeated variants of the experiment)

Feeding frequency	Metabolic rate					
(number daily – feeds)	average*	maximum*	minimum*	- maximum/average	maximum/minimum	
		Oxygen co	onsumption			
1	165.88 <sup>A</sup>	210.33 <sup>A</sup>	116.30 <sup>A</sup>	1.27	1.81	
2	179.28 <sup>A</sup>	$206.98^{\mathrm{A}}$	137.53 <sup>A</sup>	1.15	1.50	
3	185.08 <sup>A</sup>	215.85 <sup>A</sup>	153.75 <sup>B</sup>	1.17	1.40	
Continuous feeding	188.48 <sup>A</sup>	209.93 <sup>A</sup>	164.60 <sup>B</sup>	1.11	1.28	
Value of P	0.1005	0.8817	0.0001			
		Ammonia	a excretion			
1	5.57 <sup>A</sup>	13.69 <sup>A</sup>	0.72 <sup>A</sup>	2.46	19.01	
2	5.71 <sup>A</sup>	10.62 <sup>AB</sup>	1.61 <sup>AB</sup>	1.86	6.60	
3	5.51 <sup>A</sup>	8.94 <sup>B</sup>	2.62 <sup>B</sup>	1.62	3.41	
Continuous feeding	6.44 <sup>A</sup>	9.19 <sup>B</sup>	3.09 <sup>B</sup>	1.43	2.97	
Value of P	0.4130	0.0012	0.0038			

\* - values denoted with the same letter index in the same column do not vary significantly statistically (P > 0.05)

The maximum values of oxygen consumption (OC<sub>max</sub>) in a twenty-four hour cycle did not statistically vary and were approximately 210 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> for all the experiment variants. Statistical analysis of the data revealed the existence of similar differences between the average minimum values of OC (OC<sub>min</sub>). These values in the groups fed once or twice were significantly lower than those for fish which were fed three times or continuously (P = 0.0001; Table 1).

The feeding frequency significantly influenced the average maximum and minimum values of ammonia excretion (AE<sub>max</sub> and AE<sub>min</sub>) (P < 0.01). The highest level of AE<sub>max</sub> was observed in the group fed once, while the lowest was in the group fed three times. The difference between the two groups was 4.75 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (Table 1). The lowest value of AE<sub>min</sub> was noted in the group of fish fed once and was four-fold lower than in the group fed continuously (Table 1).

The  $OC_{max}/OC_{average}$  ratio had similar values ranging from 1.11 (continuous feeding) to 1.27 (one feed), while the  $OC_{max}/OC_{min}$  ratio ranged more significantly

from 1.28 (continuous feeding) to 1.81 (one feed). The values of  $AE_{max}/AE_{average}$  and  $AE_{max}/AE_{min}$  were much higher and ranged from 1.43-2.46 and 2.97-19.01, respectively (Table 1).

# DEPENDENCE BETWEEN AMMONIA EXCRETION AND OXYGEN CONSUMPTION

The dependence between oxygen consumption and ammonia excretion in all the feeding schedules was statistically highly significant (P < 0.01) and specific to each experimental group. The correlation coefficients ranged from 0.57 (three feeds daily) to 0.94 (two feeds daily - Table 2).

TABLE 2

Correlation between ammonia excretion (AE) and oxygen consumption (OC) in subsequent experiment variants and regression equations which determine the value of AE (mg TAN kg<sup>-1</sup> h<sup>-1</sup>) using OC (mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>): AE = *a* (intercept) + *b* (slope) OC

Feeding frequency (number of daily feeds)	Number of observations	Correlation coefficient –	Regression equation y = a + bx		Significance of
			а	b	slope b, P
1	96	0.71	-12.93	0.118	0.0001
2	96	0.94	-16.28	0.123	0.0001
3	96	0.57	-6.79	0.071	0.0039
Continuous feeding	96	0.91	-20.86	0.145	0.0001

# DIURNAL VARIABILITY OF OXYGEN CONSUMPTION AND AMMONIA EXCRETION

The plots of OC and AE curves are characteristic for each of the applied feeding schedules.

#### ONE FEED

The most dynamic increase during feeding of OC and AE was observed in this group. During the three hours of feeding levels of OE and AE increased by 47 and 606%, respectively (Fig. 1). The maximum level of OC was observed at 16.00 and of AE at 18.00, i.e. 3.5 and 5.5 h after feeding had begun. After the maximum values had been reached, a rapid decrease in the ammonia excretion rate was observed and between 18.00 (AE<sub>max</sub>) and 09.00 (AE<sub>min</sub>) and was 0.8 mg TAN kg<sup>-1</sup> h<sup>-1</sup>. Oxygen consumption remained on a relatively high level until 02.00 (Fig. 1).



Fig. 1. Diurnal fluctuations of OC and AE values (average from four measurements) of juvenile perch fed once daily.

#### **TWO FEEDS**

An increase in oxygen consumption and ammonia excretion was observed at the start of feeding. Values of OC and AE reached high levels four hours after the first and second feeds at 16.00 and 03.00. The growth rates of both OC and AE were higher after the first feed. The minimum values of OC and AE were reported after seven (first feed) and eight hours (second feed) after the maximum values had been noted (Fig. 2).

#### THREE FEEDS

In this variant, three clear maxima and minima of OC and AE are distinguishable and related to feeding (Fig. 3). The observed increase of oxygen consumption and ammonia excretion, as with the previous groups, began directly after feeding started and lasted for a period of three to four hours following its completion. Then the OC and AE values decreased until the next feed. The maximum values of OC and AE were recorded at 14.00, 23.00 and 06.00 (Fig. 3). The differences between the maximum and minimum values were less significant than those of the single and double feeds and ranged from 30 to 37 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> and 3 – 5 mg TAN kg<sup>-1</sup> h<sup>-1</sup>. The most dynamic increase of OC was observed after the third feed was given, while that for AE occurred after the second feed (Fig. 3).

#### **CONTINUOUS FEEDING**

In this experiment variant the diurnal profiles of OC and AE were different than those in the other variants. An increase of OC and AE was observed two hours after the start of feeding. After five (AE) or eight hours (OC) of dynamic increase, these parameters stabilized at a relatively high level (Fig. 4). Maximum levels of OC and AE were observed 13 hours after the start of feeding. Following the completion of feeding at 03.30, a decrease in OC and AE was observed until 11.00 (Fig. 4).

#### FEEDING FREQUENCY AND OFR AND AFR VALUES

OFR parameters, calculated using both the average OC values and the maximum OC values in all the experiment groups, were similar, and differences among them were statistically insignificant (P > 0.05; Table 3). Different feeding frequencies did not significantly influence the AFR values calculated using the average diurnal values of AE (P = 0.1206). When AE<sub>max</sub> was used to derive this parameter, the AFR value estimated for the group that was fed once was significantly higher than that for the other groups (P < 0.0001; Table 3).



Fig. 2. Diurnal fluctuations of OC and AE values (average from four measurements) of juvenile perch fed twice daily.



Fig. 3. Diurnal fluctuations of OC and AE values (average from four measurements) of juvenile perch fed three times daily.



Fig. 4. Diurnal fluctuations of OC and AE values (average from four measurements) of juvenile perch fed continuously.

**TABLE 3** 

Feeding frequency	OFR (kg O <sub>2</sub> kg	<sup>-1</sup> feed fed day <sup>-1</sup> )	AFR (kg TAN kg <sup>-1</sup> feed fed day <sup>-1</sup> )	
(number of daily feeds)	average*	maximum*	average*	maximum*
1	0.151 <sup>A</sup>	0.191 <sup>A</sup>	0.0050 <sup>A</sup>	0.0124 <sup>A</sup>
2	0.162 <sup>A</sup>	0.187 <sup>A</sup>	$0.0052^{A}$	0.0096 <sup>B</sup>
3	0.168 <sup>A</sup>	0.196 <sup>A</sup>	$0.0050^{A}$	0.0081 <sup>B</sup>
Continuous feeding	0.171 <sup>A</sup>	0.191 <sup>A</sup>	$0.0058^{A}$	0.0083 <sup>B</sup>
Value of P	0.1291	0.8826	0.1206	0.0000

Values of OFR and AFR estimated for juvenile perch fed at different frequencies
(the values of the averages from four repeats of each experiment variant)

\* - values denoted with the same letter index in the same column do not vary significantly statistically (P > 0.05)

## DISCUSSION

Studies focused on determining the impact of feeding frequency on the metabolic rate of fish are not very numerous, and the results presented in them are not unequivocal. No significant impact on the average metabolic rate of fish was observed by Beamish and MacMahon (1988) for walleye *Stizostedion vitreum* Mitchill, Zakęś (1999) for pikeperch *Sander lucioperca* (L.) or Hamada and Maeda (1983) for carp *Cyprinus carpio* L. These studies also did not indicate any significant impact of the applied feeding frequencies on the average diurnal values of oxygen consumption and ammonia excretion by perch. Yager and Summerfelt (1994) confirmed that the average rate of oxygen consumption (mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) of juvenile *S. vitreum* which were fed once a day was significantly higher than for fish which were fed two, three or fifteen times daily. The differences between the extreme values of OC between single and 15 feeds reached 50%. It was symptomatic that the highest AE (mg TAN kg<sup>-1</sup> h<sup>-1</sup>) value was confirmed, in contrast to that of OC, for the group which was fed once daily.

The frequency of feeding fish has a significant impact on diurnal fluctuations of oxygen consumption and ammonia excretion. The maximum values of these metabolic parameters in a twenty-four hour cycle are the most objective criterion for determining the highest possible level of intense fish cultivation in production facilities (Fivelstad et al. 1990, Colt 1991). It appears that the course of diurnal profiles depends on the applied fish feeding procedure (frequency of feeding), and it may be of a unimodal or polymodal character (Kaushik 1980). In the majority of studies the

increase of fish metabolism is observed directly after the start of feeding (Kaushik 1980, Forsberg 1997). Values of OC and AE of perch that were fed once, two times and three times also increased significantly directly after the start of feeding and reached maximum values after three to four hours. Two or three periods of intense OC and AE increase were observed in the fish that were fed two or three times a day. There are data in the literature regarding the so-called accumulation feeding effect which results in the increase of maximum OC and AE values after each periodically given feed and reaches the maximum value following the last feed (Hamada and Maeda 1983, Yager and Summerfelt 1994, Zakęś 1999). The results of the current study do not fully confirm this effect in perch; however, it should be kept in mind that many phenomena or regularities may be masked during intensive fish production. It was noted in this study that the course of diurnal profiles of OC and AE for perch fed once, twice or three times are different from those for fish fed continuously. An increase of OC and AE was observed two hours after the start of feeding in the last group. After the maximum values had been reached, the levels of oxygen consumption and ammonia production remained high until the end of feeding when they dropped to values observed before feeding. It appears that this diurnal rhythm is characteristic for continuous fish feeding. Similar diurnal profiles were observed in Siberian sturgeon Acipenser baeri Brandt and pikeperch cultivated in recirculation systems and fed continuously (Jatteau 1997, Zakęś 1999).

Feeding frequency plays a significant role in determining the amplitude of variations in OC and AE. In this study, the ratio of maximum and minimum values of these parameters were 1.81 and 19.01 for fish fed once and only 1.28 and 2.97 for fish fed continuously. These data reveal that the diurnal variability of the ammonia excretion level is much higher than that of oxygen consumption. They also prove that increasing perch feeding frequency significantly decreases diurnal fluctuations in metabolism and lowers the risk of critical oxygen and ammonia concentrations occurring. The decrease of oxygen concentration below the optimum level may lead to a decrease in fish growth rate (Jobling 1994); however, short term hypoxia does not necessarily result in this phenomenon (Forsberg and Bergheim 1996). It must be borne in mind, though, that such changes are a significant stress factor, and they cause mobilization in fish that influences many physiological processes. In order to guarantee safe fish cultivation, especially during intensive production, it is advisable to determine the fish oxygen demand using the maximum diurnal oxygen consumption values. Due to OC fluctuation in a diurnal cycle, Westers (1981) suggests that values of OFR (kg  $O_2$  kg<sup>-1</sup> feed fed day<sup>-1</sup>), usually calculated using the average, diurnal oxygen consumption values, should be increased by 1.44 (the factor which includes the  $OC_{max}$  /  $OC_{average}$  ratio). This ratio was lower in the present study and ranged from 1.11 to 1.27; thus, the factor suggested by Westers for salmonids can safely be applied to perch. Similar values of the  $OC_{max}$  /  $OC_{average}$  ratio were confirmed for other percids in intensive production. These values ranged from 1.11 to 1.18 (Zakęś 1999) and from 1.14 to 1.22 (Yager and Summerfelt 1994) for *S. lucioperca* and *S. vitreum* that were fed at different frequencies. In the latter work, a significant impact of feeding frequency on the average values of OFR were observed for *S. vitreum*. These values were higher for fish fed once than for fish fed two, three or fifteen times daily. Neither in this research nor in earlier studies (Zakęś 1999) was such a significant impact of feeding frequency observed.

The AFR values (kg TAN kg<sup>-1</sup> feed fed day<sup>-1</sup>) calculated for perch using the maximum AE values were significantly higher than those estimated from average values of AE. The AE<sub>max</sub> / AE<sub>average</sub> ratio was 2.46 for the fish fed once and only 1.43 for the fish fed continuously. Therefore, the results presented in this paper confirm the opinion presented by Meade (1985), who confirmed that, in addition to fish size, water temperature and feed dose, feeding pattern is one of the key elements to be considered when calculating the amount of ammonia produced by fish. Information regarding the excretion level of this metabolite is very important while calculating the stock size or in designing biological filters to be used in recirculation systems.

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### STRESZCZENIE

WPŁYW CZĘSTOTLIWOŚCI ŻYWIENIA NA TEMPO METABOLIZMU OKONIA PERCA FLUVIATILIS L.

Celem badań było określenie wpływu częstotliwości żywienia (jedna, dwie i trzy dawki paszy oraz karmienie ciągłe) na wielkość konsumpcji tlenu (OC, mg O2 kg<sup>-1</sup> h<sup>-1</sup>) i wydalania amoniaku (AE, mg TAN kg<sup>-1</sup> h<sup>-1</sup>) przez juwenalnego okonia. Badania prowadzono w czasie rutynowego podchowu ryb w obiegu recyrkulacyjnym. Nie stwierdzono istotnego wpływu zastosowanych wariantów żywieniowych (P > 0,05) na średnie dobowe wartości OC i AE, które mieściły się w przedziale od 165,88 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (jedna dawka paszy) do 188,48 mg O<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup> (karmienie ciagłe) oraz od 5,51 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (dwie dawki paszy) do 6,44 mg TAN kg<sup>-1</sup> h<sup>-1</sup> (karmienie ciągłe; tab. 1). Częstotliwość żywienia wpłynęła istotnie na fluktuacje dobowe konsumpcji tlenu i wydalania amoniaku. Było to szczególnie widoczne w przypadku AE. Iloraz AEmax/AEsrednia w grupie ryb żywionych jedną dawką osiągnął wartość 2,46, a w przypadku  $AE_{max}/AE_{min}$  aż 19,01. W pozostałych wariantach zmienność AE w cyklu dobowym była istotnie niższa (tab. 1). Przebieg dobowych profilów OC i AE był charakterystyczny dla każdego z zastosowanych wariantów doświadczalnych (rys. 1 - 4). Stwierdzono wysoce istotne statystycznie zależności między wydalaniem amoniaku i konsumpcją tlenu (P < 0,001), specyficzne dla każdej grupy doświadczalnej. Współczynniki korelacji równań regresji prostoliniowych, opisujących związek AE - OC, przyjęły wartości od 0,57 (trzy dawki paszy dziennie) do 0,94 (dwie dawki dziennie; tab. 2). Wartości OFR i AFR nie były determinowane przez zastosowany harmonogram karmienia i przyjęły wartości, odpowiednio około  $0,160 \text{ kg O}_2 \text{ kg paszy}^{-1} \text{ dzien}^{-1} \text{ i } 0,0050 \text{ kg TAN kg paszy}^{-1} \text{ dzien}^{-1} (P > 0.05; \text{ tab. 3}).$ 

#### CORRESPONDING AUTHOR:

Doc. dr hab. Zdzisław Zakęś Instytut Rybactwa Śródlądowego ul. Oczapowskiego 10 10-719 Olsztyn Tel./Fax: +48 89 5240171, +48 89 5240505; e-mail: zakes@infish.com.pl