

## Dynamics of nitrogen and phosphorus in closed and semi-closed recirculating aquaculture systems during the intensive culture of goldfish, *Carassius auratus auratus* (L.), juveniles

Received – 10 March 2010/Accepted – 27 August 2010. Published online: 30 September 2010; ©Inland Fisheries Institute in Olsztyn, Poland

Daniel Źarski, Dariusz Kucharczyk, Katarzyna Targońska, Sławomir Krejszeff,  
Tomasz Czarkowski, Ewelina Babiarz, Dorota B. Nowosielska

**Abstract.** The aim of the study was to compare the dynamics of nitrogen and phosphorus compounds in closed (cRAS) and semi-closed (scRAS) experimental recirculation systems during intensive culture of goldfish juveniles. The results obtained underscore the varied effectiveness of biological nitrification in recirculation systems, which is dependent on both the nitrogen compound loads and water exchange. Additionally, levels of nitrogen (22878.18 mg) and phosphorus (1878.55 mg) accumulation were high in the cRAS in comparison to those in the scRAS (maximum 3797.44 and 117.41 mg for nitrogen and phosphorus, respectively). This indicates that large quantities of nutrients are discharged into the natural environment as a consequence of water exchange. The data obtained from this study can be useful at the intensive aquaculture production design stage to minimize impacts on the natural environment. Based on the results obtained, the cRAS should be put into operation approximately ten days before any experimental or intensive culture is begun. With scRAS, the culture process can commence on the fourth day after disinfection. However, with scRAS the feeding rate has to be monitored closely because of the relatively low nitrification capability of this system.

**Keywords:** recirculating aquaculture system (RAS), nitrogen, phosphorus, nitrification, waste waters, goldfish

Aquaculture is one of the largest branches of food production. Intensive fish culture under controlled conditions is one of the areas of aquaculture that is developing dynamically since it allows limiting production costs and permits controlling culture conditions fully (Kolman 1999, Blancheton 2000, Remen et al. 2008). Nitrate nitrogen and phosphorus compounds accumulate in the water during intensive fish culture in recirculation systems (Rodehutsord and Pfeffer 1995, Barak and van Rijn 2000, Źarski et al. 2008). Low levels of these compounds, particularly ammonia nitrogen, have a direct negative impact on fish growth rate and wellness (Frances et al. 2000, Foss et al. 2003, Biswas et al. 2006, Remen et al. 2008). Filtration, including biological filtration, is used to limit the impact of these compounds on the effectiveness of production (Hargrove et al. 1996, Ridha and Cruz 2001). As a result, ammonia nitrogen is oxidized to nitrite nitrogen ( $\text{NO}_2$ ), and next to nitrate nitrogen ( $\text{NO}_3$ ) in the nitrification process (Kolman 1999, van Rijn et al. 2006).

Nitrate nitrogen is formed by the uninterrupted nitrification process of ammonia nitrogen, which is a metabolic product (Smutna et al. 2002). Nitrate usually does not reach levels lethal to fish during culture (Hamlin 2006). Phosphorus, on the other hand,

---

D. Źarski [✉], D. Kucharczyk, K. Targońska, S. Krejszeff,  
E. Babiarz, D.B. Nowosielska  
Department of Lake and River Fisheries  
University of Warmia and Mazury in Olsztyn  
Oczapowskiego 5, 10-957 Olsztyn, Poland  
Tel./Fax: +48 895234436, +48 895233969,  
e-mail: danielzarski@poczta.interia.pl

T. Czarkowski  
Warmia and Mazury Agriculture Consulting Centre in Olsztyn,  
Poland

is supplied together with feed, particularly compound feeds. Its accumulation in the water results from it not being fully assimilated by fish (Rodehutsord and Pfeffer 1995, Barak and van Rijn 2000). Although these two compounds decrease culture parameters only slightly, they are both undesirable elements in aquaculture production because they have significant negative impacts on the natural environment. Together with discharge waters, they contribute to increased eutrophication of open waters and, as a consequence, contribute to their degradation (Oliva-Teles et al. 1998, Barak and van Rijn 2000).

Many studies of the effectiveness of biological filtration have been published to date, and the majority have focused on commercial production. However, short-term rearing periods (usually 21 days followed by a two-day acclimation process) are commonly applied in scientific and commercial hatcheries. There are huge rotations of various species during the season at these facilities, which necessitates utilizing them several times per season. Thus, efforts are made to clean and disinfect rearing systems. Fish are usually stocked into such systems shortly after the disinfection process where biological filtration is ineffective. Because data regarding the short-term dynamics of these compounds is relatively limited, compiling it could be of practical importance in both scientific and commercial applications. The current study compared the dynamics of nitrogen and phosphorus compounds during short-term goldfish juvenile culture in closed and semi-closed recirculating aquaculture systems.

Two separate experiments were conducted within the framework of this study during which a hatchery-reared stock of goldfish with initial lengths of 5 to 7 cm and an average body weight of 5.4 g ( $\pm 1.7$ ) were reared. The larvae were obtained after mass spawning under controlled conditions. Spawners were cultured in 1000 dm<sup>3</sup> tanks with controlled environmental conditions (Kujawa et al. 1999). The larvae were fed *Artemia nauplii* initially (21 days) and later mixed live and compound feeds. During the experiments, the fish were placed in twelve 50 dm<sup>3</sup> glass tanks positioned in an

experimental closed water circuit (with a total volume of 1.2 m<sup>3</sup>) that allowed controlling the water temperature, photoperiod, and aeration, and allowed for partial water replacement. The temperature during the culture was set at 22°C ( $\pm 0.1$ ), and the photoperiod was 12 h (12L:12D). The fish density in each tank was 250 individuals (5 per 1 dm<sup>3</sup>). The fish biomass was 16.2 kg in the whole system. Each of the tanks was fed through the top water inlet and was also aerated. The water flow through the culture tanks was constant at 2 dm<sup>3</sup> min<sup>-1</sup>. From the culture tanks, the water was directed to a mechanical filter and next to a biological filter bed filled with polyethylene balls ( $\phi \sim 4$  mm) to a total volume of  $\sim 72$  dm<sup>3</sup>. Following biological filtration, the water was passed to the lower retention tank which also functioned as a tank for collecting the sediments that had not been separated in the mechanical filter. The waste water outflow was located in this tank. Next, water was pumped through a UV lamp to the head tank where the heater, make-up water inlet, and aerating diffuser were located. From the head tank the water was directed through PVC pipes to the culture tanks. For detailed information see Kujawa et al. (2000). Before the experiment began, the filtration medium was carefully flushed with clean water and dried. Water circulation began a week before the fish were stocked into the system. During each experiment, the fish were fed twice a day with commercial compound carp feed (feed composition: 62% protein, 11% fat, 0.8% hydrocarbons, 1.1% phosphorus, 10% ash; Skretting, Norway). The feed was distributed manually. The daily dose of the feed was 1.5% of the initial biomass for the duration of the experiment. Prior to the first daily feeding, residues of feed and excrements were removed only from the culture tanks.

During the experiments, ammonia nitrogen (N-NH<sub>4</sub>), nitrite nitrogen (N-NO<sub>2</sub>), nitrate nitrogen (N-NO<sub>3</sub>), and phosphates (P-PO<sub>4</sub>) were analyzed with a LF 205 photometer (Slandi, Poland). Samples were collected daily before the first feeding from the lower tank. If nitrate and phosphate contents exceeded the measurement range, the samples were diluted with water obtained from reverse osmosis (multiple analyses confirmed that it did not contain

nitrogen or phosphorus compounds). The results obtained were then converted to determine the actual concentrations of the compounds in the analyzed sample. All the analyses were conducted in two repetitions. Additionally, the content of dissolved oxygen in the water and pH were measured daily in the culture tanks using a multiparametric device (HI 9828, Hanna Instruments, Italy). Throughout the culture period in both experiments, the content of dissolved oxygen in the water did not drop below  $6 \text{ mg dm}^{-3}$ , while the pH value was within 7.3-7.6. No mortality was recorded among the fish.

During the first experiment, culture was conducted without water replacement (closed system – cRAS). In the second experiment (semi-closed system – scRAS), 20% of the water in circulation was replaced daily. Losses through evaporation in the cRAS were compensated daily with a small volume of water. The cultures in both experiments were conducted for 23 days.

The amounts of nitrogen and phosphorus were calculated for each day. Based on these results, regression analysis was completed for the values of compounds in the water and the time of the experiment. The cRAS and scRAS values were compared using the t-test ( $\alpha = 0.05$ ). Statistical analysis was performed using STATISTICA (9.0) software (StatSoft) and MS Excel for Windows.

Recirculation systems are used for intensive fish production and, in most cases, are equipped for partial water replacement, which ensures the removal from the system of nitrates produced during the nitrification process (van Rijn 1996). Because wastewaters are usually discharged into natural reservoirs, greater research efforts have recently been focused on eliminating the need for water replacement in fish culture systems (van Rijn et al. 2006). The intensity and character of the dynamics of nitrogen and phosphorus compounds are determined by water replacement frequency. These fluctuations also depend on culture procedures and conditions (Singh et al. 1999, Franco-Nava et al. 2004, Wolnicki 2005, Źarski et al. 2008).

The results obtained in this study indicated significant diversity in the levels of the compounds

analyzed depending on the system applied. In both cases, the highest concentrations of ammonia were found during the initial days of culture. During the first day after stocking the cRAS, the ammonia concentration reached  $0.5 \text{ mg dm}^{-3}$ . The maximum ( $0.6 \text{ mg dm}^{-3}$ ) was recorded on the second and third days of culture. Next, a gradual decrease in the content of ammonia was recorded until day 16 of culture, after which a small increase to the level of  $0.2 \text{ mg dm}^{-3}$  was recorded. A similar tendency was noted in the scRAS, where ammonia reached its maximum after the system was stocked with fish. Next, a rapid decrease was observed, and on day 13 another increase to the ultimate level of  $0.3 \text{ mg dm}^{-3}$  was observed (Fig. 1a). No statistical differences between treatments were recorded (t-test,  $P > 0.05$ ). Similar ammonia nitrogen dynamics were recorded by Hargrove et al. (1996) and Źarski et al. (2008). Faster ammonia removal during the initial days of culture in the scRAS probably resulted from water replacement. On the other hand, in the cRAS the notable decrease in ammonia nitrogen by day three was a consequence of the nitrification process. However, in the scRAS, the ammonia increase occurred earlier (on day 13) than in the cRAS (on day 16) although ammonia production in both cases was the same. This could have been caused by the different rates of increase in the biomass of the biological bed microorganisms which progressed slightly more slowly in the lower load of the scRAS (in which ammonia was removed through partial water replacement) (Parimala et al. 2007).

The highest nitrite concentration value in the closed system was noted at the beginning of the culture period (from  $0.24$  to  $0.3 \text{ mg dm}^{-3}$ ). Following a significant increase in N-NO<sub>2</sub> content during the initial four days, a decrease was noted in the water analyzed. By day 12, concentrations of this compound did not exceed  $0.14 \text{ mg dm}^{-3}$ . This was opposed to the system with partial water replacement, in which nitrite content over 23 days of culture exceeded  $0.1 \text{ mg dm}^{-3}$  only twice: on days 10 and 23 ( $0.12$  and  $0.13 \text{ mg dm}^{-3}$ , respectively). Following an initial increase, a slight decrease in N-NO<sub>2</sub> occurred in the analyzed water. Only on day 17 was another increase in the concentration of this compound

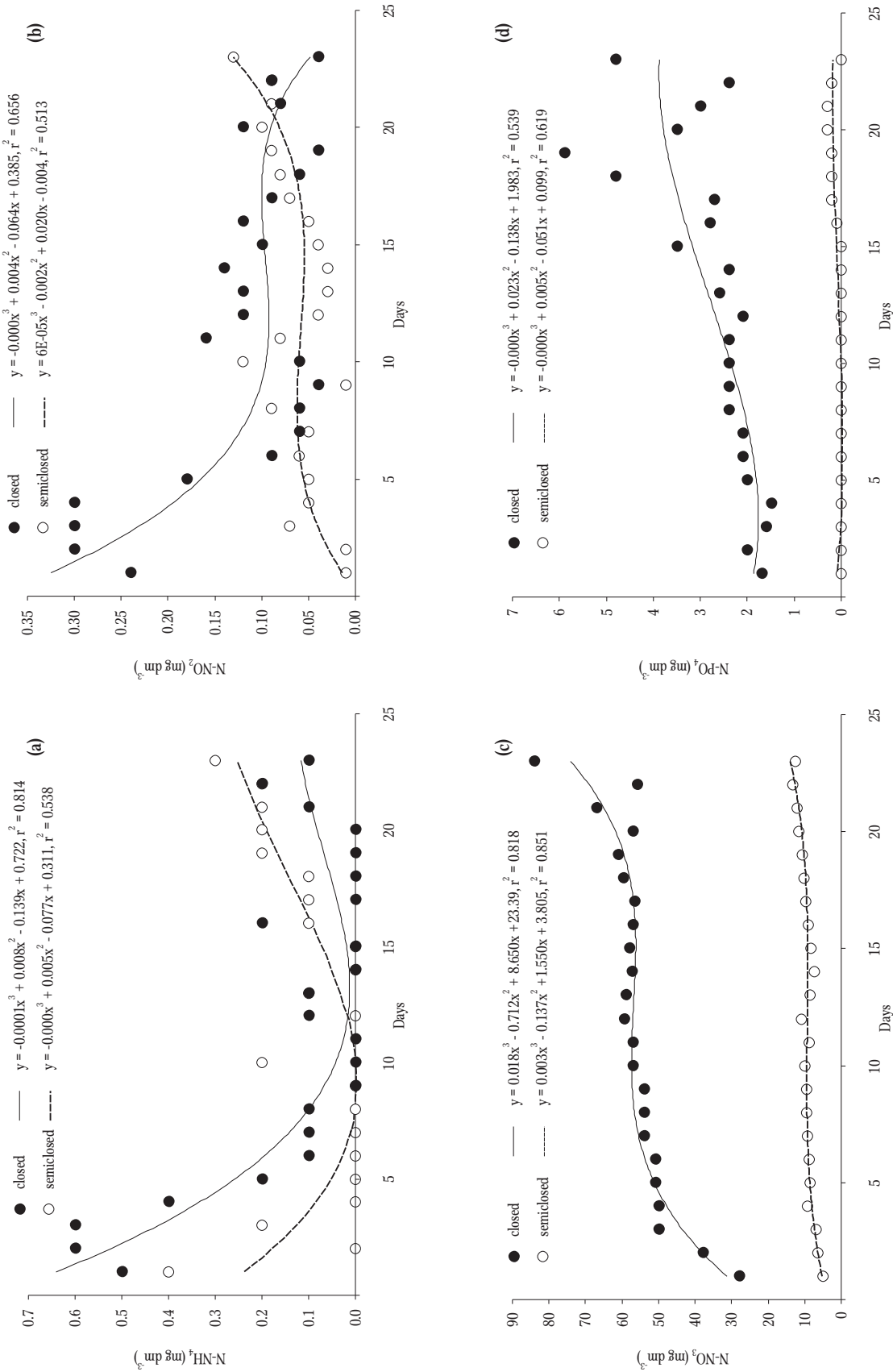


Figure 1. Water concentrations of the analyzed compounds during goldfish culture in closed and semi-closed recirculating systems.

detected (Fig. 1b). Statistical differences between treatments were recorded daily until the fifth day of culture and on days 13 and 14 of the experiment (t-test,  $P < 0.05$ ). These results indicate the nitrification process was effective in both cases (Rodehutsord and Pfeffer 1995, Barak and van Rijn 2000, Źarski et al. 2008). Nitrate concentrations in the cRAS increased throughout the culture period and ranged from 28 to  $135 \text{ mg dm}^{-3}$ . This differed from the situation in the scRAS, where the nitrate nitrogen concentration increased throughout the culture period, but without exceeding  $13.2 \text{ mg dm}^{-3}$

until day 23 of the experiment (Fig. 1c). These changes indicated a lower extent and rate of nitrification. The first disturbances were recorded on day 10 of the culture for ammonia and nitrites, and on day 16 for phosphates. The character of these changes was probably also the consequence of the smaller biomass increase of the biological bed bacteria and partial water replacement. As a consequence, the initial two stages of the nitrification process did not exhibit constant trends which, on the other hand, were observed in the cRAS. Changes in the content of phosphates in both cases were very similar in

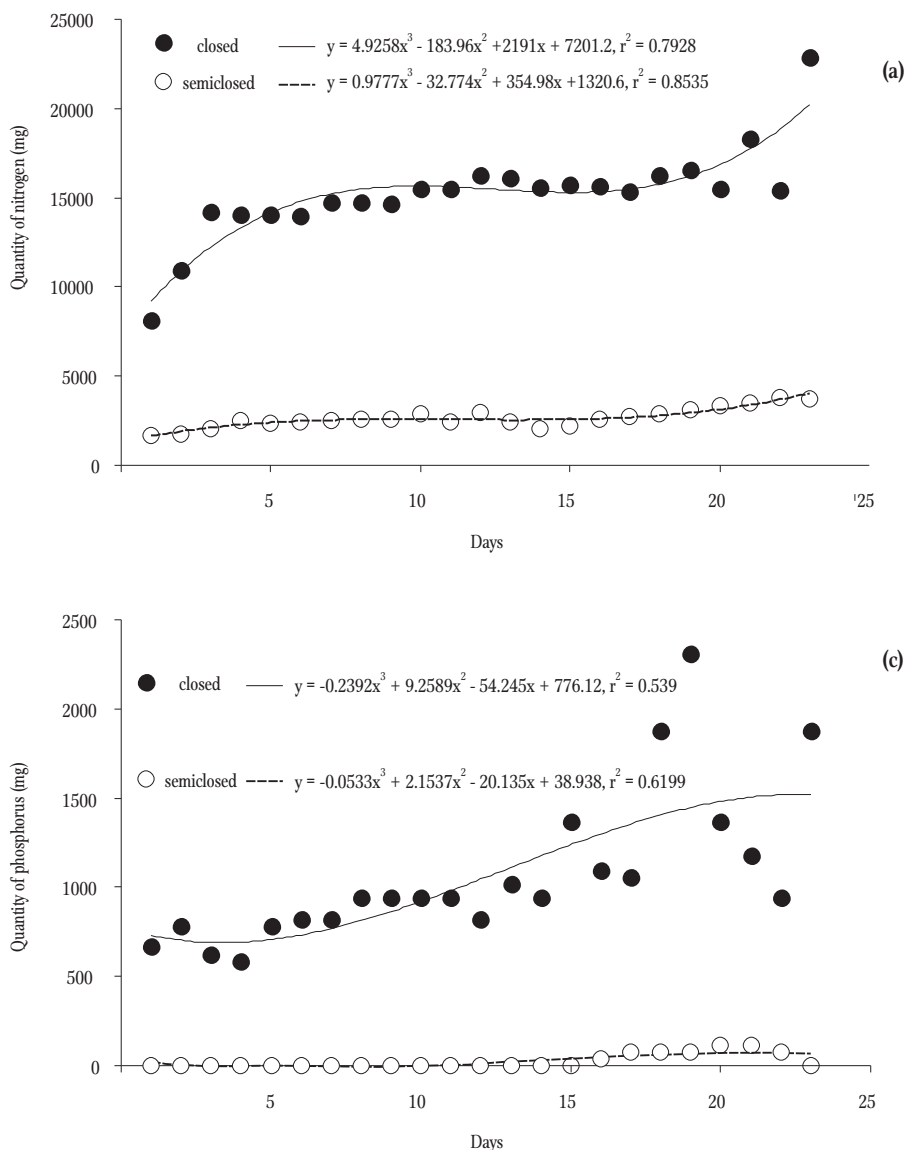


Figure 2. Quantity of nitrogen (a) and phosphorus (b) compounds in closed and semi-closed recirculating systems (total capacity  $1200 \text{ dm}^3$ ) during intensive goldfish culture. Data between closed and semi-closed systems differ significantly statistically (t-test,  $P < 0.05$ ).

character (Fig. 1d); however, the accumulation rate and the content of phosphates in the cRAS by the end of the experiment were several tens of times higher (t-test,  $P < 0.05$ ) than in the scRAS. This was likely linked to the removal of these compounds from the scRAS mainly by water replacement.

The results obtained in this study reflect quite characteristic fluctuations in the contents of compounds that are by-products of aquaculture production in both cRAS and scRAS. They highlight the different reaction times of the biological bed to the dynamics of individual compounds. Increasing the load of the scRAS with ammonia nitrogen immediately resulted in a higher nitrite content, which is toxic to fish. As a consequence, the lower load that resulted from water replacement meant that biological filtration was less effective. However, water replacement did not influence the potential increment of system capacity for fish load and higher feeding levels due to removal capability. The content of ammonia and nitrates was at a low level, which indicates the high effectiveness of filtration applied. Thus, it is assumed that the biomass growth rate of microorganisms in the biological bed increases as the loads of nitrogen and phosphorus compounds supplied increase. However, the amounts of nitrogen and phosphorus compounds calculated in the cRAS were statistically higher than those in the scRAS throughout the culture period (t-test,  $P < 0.05$ ) (Fig. 2). This is why research into the development of devices for denitrification are needed urgently (van Rijn et al. 2006), as is the implementation of such technologies in fish-culture farms since this will significantly limit the negative influence of aquaculture on open water ecosystems. Based on the results obtained in the current study, the cRAS should be in operation for ten days (at a low feeding level) before any experimental or intensive culture is performed. With scRAS, fish culture can be conducted beginning on the fourth day following biofilter media disinfection in rearing facilities. However, feeding rates must be monitored more closely in scRAS because of its relatively low nitrification capability.

## References

- Barak Y., van Rijn J. 2000 – Biological phosphate removal in a prototype recirculating aquaculture treatment system – *Aquacult. Eng.* 22: 121-136.
- Biswas J.K., Sarkar D., Chakraborty P., Bhakta J.N., Jana B.B. 2006 – Density dependent ambient ammonium as the key factor for optimization of stocking density of common carp in small holding tanks – *Aquaculture* 261: 952-959.
- Blancheton J.P. 2000 – Developments in recirculation systems for Mediterranean fish species – *Aquacult. Eng.* 22: 17-31.
- Foss A., Vollen T., Oiestad V. 2003 – Growth and oxygen consumption in normal and O<sub>2</sub> supersaturated water, and interactive effects of O<sub>2</sub> saturation and ammonia on growth in spotted wolffish (*Anarhichas minor* Olafsen) – *Aquaculture* 224: 105-116.
- Frances J., Nowak B.F., Allan G.L. 2000 – Effects of ammonia on juvenile silver perch (*Bidyanus bidyanus*) – *Aquaculture* 183: 95-103.
- Franco-Nava M.A., Blancheton J.P., Deviller G., Charrier A., Le-Gall J.Y. 2004 – Effect of fish size and hydraulic regime on particulate organic matter dynamics in a recirculating aquaculture system, elemental carbon and nitrogen approach – *Aquaculture* 239: 179-198.
- Hamlin H.J. 2006 – Nitrate toxicity in Siberian sturgeon (*Acipenser baeri*) – *Aquaculture* 25: 688-693.
- Hargrove L.L., Westerman P.W., Losordo T.M. 1996 – Nitrification in three-stage and single-stage floating bead biofilters in a laboratory scale recirculating aquaculture system – *Aquacult. Eng.* 15: 67-80.
- Kolman R. 1999 – Closed systems for the production of hatch and fry – *Wyd. IRS, Olsztyn*, 180 p. (in Polish).
- Kujawa R., Kucharczyk D., Mamcarz A. 1999 – A model system for keeping spawners of wild and domestic fish before artificial spawning – *Aquacult. Eng.* 20: 85-89.
- Kujawa R., Mamcarz A., Kucharczyk D., Skrzypczak A. 2000 – An experimental unit for rearing of larval freshwater fish – *Folia Univ. Agric. Stetin., Piscaria*, 205: 103-108.
- Oliva-Teles A., Pereira J.P., Gouveia A., Gomes E. 1998 – Utilisation of diets supplemented with microbial phytase by seabass (*Dicentrarchus labrax*) juveniles – *Aquat. Liv. Res.* 11: 255-259.
- Parimala V., Krishnani K.K., Gupta B.P., Ragunathan R., Pillai S.M., Ravichandran P. 2007 – Removal of ammonia and nitrite from coastal water using low-cost agricultural waste – *B. Environ. Contam. Tox.* 78: 288-293.
- Remen M., Imsland A.K., Steffanson S.O., Jonassen T.M., Foss A. 2008 – Interactive effects of ammonia and oxygen on growth and physiological status of juvenile Atlantic cod (*Gadus morhua*) – *Aquaculture* 274: 292-299.
- Ridha M.T., Cruz E.M. 2001 – Effect of biofilter media on water quality and biological performance of the Nile

- tilapia *Oreochromis niloticus* L. reared in a simple recirculating system – *Aquacult. Eng.* 24: 157-166.
- Rodehutsord M., Pfeffer E. 1995 – Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*) – *Water Sci. Technol.* 31: 143-147.
- Singh S., Ebeling J., Wheaton F. 1999 – Water quality trials in four recirculating aquacultural system configurations – *Aquacult. Eng.* 20: 75-84.
- Smutna M., Vorlova L., Svobodova Z. 2002 – Pathobiochemistry of ammonia in the internal environment of fish (Review) – *Acta Vet. Brno.* 71: 169-181.
- van Rijn J. 1996 – The potential for integrated biological treatment systems in recirculating fish culture. A review – *Aquaculture* 139: 181-201.
- van Rijn J., Tal Y., Shreier H.J. 2006 – Denitrification in recirculating systems, Theory and applications – *Aquacult. Eng.* 34: 364-376.
- Wolnicki J. 2005 – Intensive rearing of early stages of cyprinid fish under controlled conditions – *Arch. Pol. Fish.* 13: 5-87 (in Polish).
- Żarski D., Kucharczyk D., Targońska K., Chyła B., Dobrołowicz A. 2008 – Dynamics of changes in nitrogen and phosphorus compounds during intensive culture of ide *Leuciscus idus* (L.) in a recirculating system – *Arch. Pol. Fish.* 16: 459-467.

## Streszczenie

### Dynamika związków azotowych i fosforu w zamkniętym (cRAS) i półzamkniętym (scRAS) doświadczalnym systemie recyrkulacyjnym, podczas intensywnego podchowu narybku złotej rybki, *Carassius auratus auratus* (L.)

Celem pracy było porównanie dynamiki związków azotowych i fosforu w zamkniętym (cRAS) i półzamkniętym (scRAS) doświadczalnym systemie recyrkulacyjnym, podczas intensywnego podchowu narybku złotej rybki. Uzyskane wyniki zwracają uwagę na różną efektywność biologicznej nityfikacji w zależności od wielkości obciążenia systemu recyrkulacyjnego w związki azotowe, która zależała od wymiany wody. Ponadto stwierdzono wysoki stopień akumulacji azotu (22878.18 mg) oraz fosforu (1878.55 mg) w cRAS w porównaniu do scRAS (maksymalnie 3797.44 i 117.41 mg odpowiednio dla azotu i fosforu), co wskazuje na usuwanie do środowiska naturalnego dużej ilości związków biogennych na

skutek wymiany wody. Dane uzyskane w niniejszej pracy mogą być przydatne na etapie projektowania systemów recyrkulacyjnych do intensywnych produkcji akwakultury. Ponadto wskazują na konieczność prowadzenia co najmniej 10 dniowego okresu wstępnego, z zastosowaniem niskiego poziomu żywienia, dla cRAS przed planowanym podchowem. W przypadku scRAS podchów może natomiast być prowadzony już od czwartego dnia po dezynfekcji złoża biologicznego. Jednakże należy zwrócić szczególną uwagę na zachowanie odpowiedniej intensywności żywienia przez cały okres podchowu w scRAS z uwagi na jego niewielką zdolność nityfikacyjną.