

COMPARATIVE STUDY OF NITROGEN MANAGEMENT IN TWO DIFFERENT CYPRINID AQUACULTURE TECHNOLOGIES: INTEGRATED MULTI-TROPHIC AQUACULTURE VS POLYCULTURE

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Abstract

The aquaculture sector registers the fastest growth compared to other food production systems. Most of the Romanian aquaculture production is based on cyprinid species reared in ponds. This technology raises certain concerns related to environment sustainability, because aquaculture effluent contains high concentration of nitrogen, which can cause water pollution and eutrophication. Thus, the objective of this study is to determine if technologies based on integrated multi-trophic aquaculture (IMTA) design can optimize the sustainability of cyprinids pond-based production systems. A number of 4 fish species (common carp - CC; grass carp - GC; bighead carp - BC and silver carp - SC) were used in two rearing technologies (polyculture vs. IMTA). The experimental design includes 2 variants: PCP - polyculture pond (CC + GC + BC + SC) and CP-PP - IMTA partitioned pond (CC monoculture in CP part and CC + GC + BC + SC polyculture in PP part, where no feed was administrated). Total nitrogen (TN) was determined from water, sediments, reed and fish. A main conclusion of this study is that CP-PP variant registered a better TN utilization, fact leads to a reduced environmental impact of cyprinids pond aquaculture.

Key words: nitrogen balance, IMTA, environmental impact, cyprinids aquaculture, pond partition.

INTRODUCTION

According to FAO reports, the aquaculture sector registers the fastest growth, compared to other major food production sectors, which provides an important source of protein at a global level (Tenciu et al., 2020).

In aquaculture environments, nitrogen represents a primary concern as the component of waste products generated by rearing fish (Simionov et al., 2016). Expansion and intensification of land-based aquaculture farms can cause the release of a large quantity of wastewater (Simionov et al., 2020). The high nutrients load (such as nitrogen) in the aquaculture effluent can cause water pollution by inducing eutrophication (Herbeck et al., 2014). As well, the discharged nutrients can cause degradation of benthic and pelagic habitats.

Nitrogen is an important nutrient in aquatic ecosystems. It is found in water in many forms: molecular nitrogen, nitrogen oxides, ammonia, nitrates and nitrites (Popa et al., 2020). In the ecosystem, nitrogen enters the biogeochemical

cycle, determined by a complex network of interactions of factors in the aquatic ecosystem (Popa et al., 2020). Bacteria have an important role in nitrogen cycle in the aquatic ecosystem and the nitrogen transformations are reversible (Popa et al., 2020).

The present study aims to evaluate the nitrogen dynamics in two aquaculture pond production systems, by applying integrated multi-trophic aquaculture (IMTA) vs. polyculture technologies. Also, this research will evaluate the dynamics of other important nutrients and quality parameters of pond technological water.

MATERIALS AND METHODS

The experiment was conducted at a cyprinid fish farm, located at 24 km from Iasi city, Romania, for 83 days, from June to September. Water inlet and outlet in the farm is effectuated gravitationally.

Two earthen ponds (0.45 ha area and 1.5 m water depth) were used for the present research. The experimental design and sampling areas are represented in Figure 1.

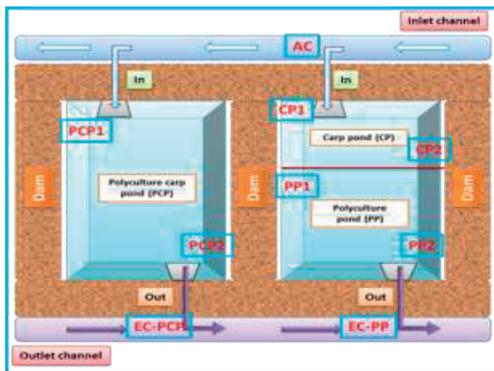


Figure 1. Experimental design and sampling stations (Metaxa et al., 2019)

(AC - inlet channel, EC-PCP - outlet channel from polyculture carp pond, EC-PP - outlet channel from polyculture pond, PCP1 - polyculture carp pond 1, PCP2 - polyculture carp pond 2, CP1 - carp pond 1, CP2 - carp pond 2, PP1 - polyculture pond 1, PP2 - polyculture pond 2) (Metaxa et al., 2019)

The first pond (PCP) was used for polyculture rearing of the common carp with grass carp, bighead carp and silver carp. The second pond (IMTA) was divided, by using a net (Figure 1), as it follows: first part with an area of 0.15 ha CP (carp pond) and the second part with an area of 0.30 ha PP (polyculture pond).

The following fish stocking formula was applied:

- PCP was populated with 2500 specimen of common carp (*Cyprinus carpio*), 40 specimen of silver carp (*Hypophthalmichthys molitrix*), 40 specimen of bighead carp (*Hypophthalmichthys nobilis*) and 100 specimen of grass carp (*Ctenopharyngodon idella*);

- CP was populated with 2000 specimen of common carp (*Cyprinus carpio*);

- PP was populated with 500 specimen of common carp (*Cyprinus carpio*), 40 specimen of silver carp (*Hypophthalmichthys molitrix*), 40 specimen of bighead carp (*Hypophthalmichthys nobilis*) and 100 specimen of grass carp (*Ctenopharyngodon idella*).

The administered fish feed had a crude protein content of 28% and was represented by a mix of cereals (wheat lees, dry maize dregs and sunflower grouts) in equal amounts. Feed was manually administered twice/day, only in PCP and CP, for five days/week, which makes a total of 59 days of feeding during the entire

experimental period. A total quantity of feed of 285.41 kg at CP-PP pond and 756.82 kg at PCP pond was administrated among the experimental period.

Table 1. Individual average length of specimen used in experiment (cm)

Pond	Common carp	Silver carp	Bighead carp	Grass carp
PCP	8.7±0.40	37.9±1.40	35.5±1.70	17.2±0.99
CP	8.5±0.90	-	-	-
PP	8.3±0.7	38.7±1.80	34.7±1.20	17.1±1.00

Table 2. Individual average weight of specimen used in experiment (g)

Pond	Common carp	Silver carp	Bighead carp	Grass carp
PCP	63.0±7.80	2006.3±213.8	1937.0±191.48	200.4±20.01
CP	61.2±11.60	-	-	-
PP	60.0±10.45	2044±289.8	1824.1±182.59	199.4 ± 20.0

Samples of water, sediments, fish and reed were collected from the ponds for analysis. Sampling was undertaken in different timeline stages during the experimental period, as it follows: initial stage (June 2016), intermediary stage (August 2016) and final stage (September 2016).

For the water samples, the following parameters were analysed: dissolved oxygen (DO), temperature (T°C), pH, nitrates (N-NO₃), nitrites (N-NO₂), ammonia nitrogen (N-NH₄), chemical oxygen demand (COD) and total suspended solids (TSS).

Table 3. Determination methods and the equipment used for the analysis of water samples

Analysed parameter	Used method	Used equipment
DO mg/L	Sensor method	HQ40d Portable pH, Dissolved Oxygen, Multi-Parameter (HACH)
T°C		
pH		
N-NO ₃ mg/L	Spectrophotometric method, Merk kits	Spectroquant photometer, Nova 400
N-NO ₂ mg/L		
N-NH ₄ mg/L		
COD mg/L		
TSS mg/L	Total Suspended Solids Procedure, Mass Balance (Dried at 103-105°C)	

For the samples of sediments, fish and reed the following parameters were analysed: total Kjeldahl nitrogen (TKN), nitrates (N-NO₃) and nitrites (N-NO₂).

The data obtained in the present experimental research was statistically analysed using descriptive statistics and ANOVA test. The programs used to carry out the aforementioned tests were Microsoft Excel 2010 and IBM SPSS Statistics 20.0. The results were presented as minimum, maximum and mean±standard deviation.

Table 4. Determination methods and the equipment used for the analysis of sediments, fish and reed samples

Analysed parameter	Used method	Used equipment
TKN (g % FW)	Macro-Kjeldahl and Acidimetric	Gerhardt Kjeldahl Nitrogen/Protein Equipment
N-NO ₃ (mg/kg FW)	Spectrophotometric method-Nitrite based on the diazotization-coupling reaction of sulfanilamide with N-(1-naphthyl) ethylenediaminedihydrochloride	SpecordAnalytik Jena 210
N-NO ₂ (mg/kg FW)	Spectrophotometric method- Nitrate is determined by reduction to nitrite with cadmium	SpecordAnalytik Jena 210

RESULTS AND DISCUSSIONS

A. WATER QUALITY

The concentration of DO in technological water registered a large variation interval (1.07-13.78 mg/L), during the experimental period.

A clear upward tendency in the final stage of the experimental period was observed in most of the investigated stations. In case of inlet and channels, the DO concentration registered lower values compared to the rest of the study stations, during the production cycle.

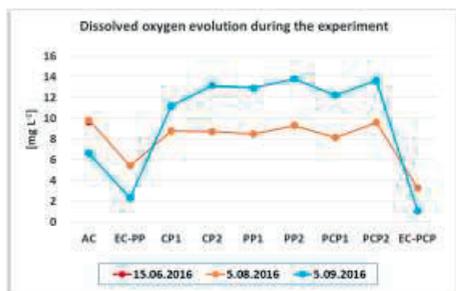


Figure 2. DO dynamics during the experimental period

The outlet channels registered significant lower water average DO concentrations (EC-PP:

3.87±1.54 mg/L and EC-PCP: 2.17±1.10 mg/L), compared with the inlet channel (AC: 8.21±1.60 mg/L). The highest DO average concentrations were recorded in the polyculture carp pond, at PCP2 (11.62±2.02 mg/L), followed by PP2 (11.53±2.25 mg/L) in IMTA pond (Figure 2).

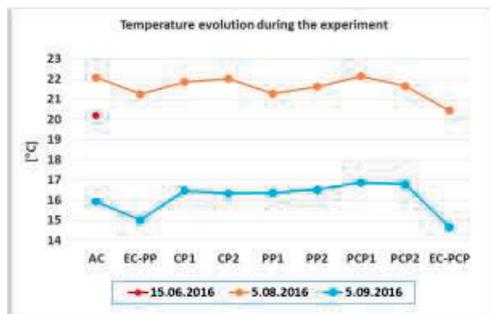


Figure 3. Temperature dynamics during the experimental period

The values registered in case of water temperature show a clear downward tendency during the experimental period from an average value of 21.60±0.50°C (beginning of August) to 16.10±0.72°C (beginning of September) (Figure 3). No significant differences were registered between the experimental sampling points.

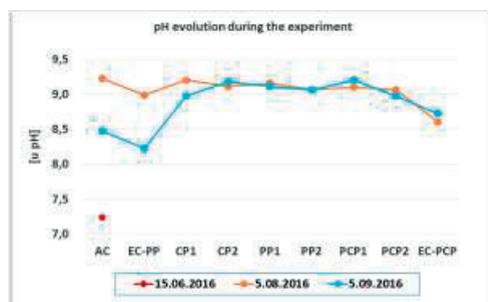


Figure 4. pH dynamics during the experimental period

The pH values registered in the technological water showed a constant trend during the entire production cycle period, in the ponds from both IMTA and polyculture systems, with an average value of 9.09±0.04 u pH, respectively 9.11±0.11u pH. The lowest pH values were registered at the outlet channels (EC-PCP: 8.67±0.06 u pH; EC-PP: 8.61±0.38 u pH), while the highest ones are recorded at PCP1

and CP2: 9.16 ± 0.05 u pH, respectively 9.15 ± 0.03 u pH (Figure 4).

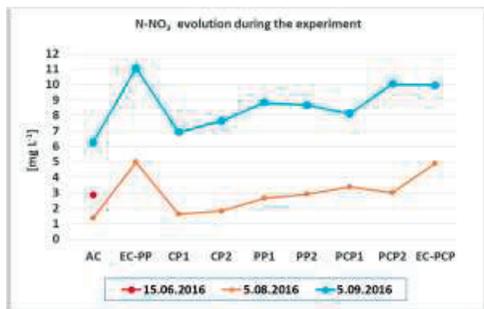


Figure 5. N-NO₃ dynamics during the experimental period

The concentration of N-NO₃ in the technological water registered an accumulation tendency, manifested especially at the end of the production cycle (Figure 5), from an average value of 2.96 ± 1.23 mg/L to 8.61 ± 1.47 mg/L. The outlet channels registered significant higher water average N-NO₃ concentrations (EC-PP: 7.03 ± 3.05 mg/L and EC-PCP: 7.43 ± 2.53 mg/L), compared with the inlet channel (AC: 3.81 ± 2.45 mg/L). Regarding the N-NO₃ average concentrations from both experimental production systems, the highest values were recorded in polyculture pond, at PCP2 (6.52 ± 3.52 mg/L), while the lowest are corresponding to CP1 (4.27 ± 2.65 mg/L), followed by CP2 (4.73 ± 2.91 mg/L).

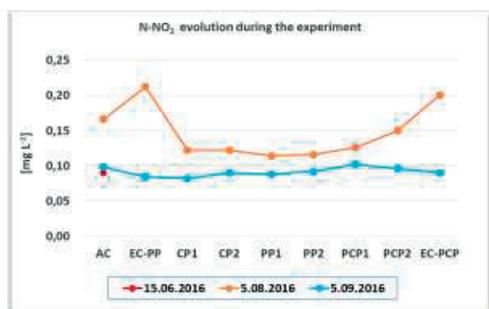


Figure 6. N-NO₂ dynamics during the experimental period

The concentration of N-NO₂ in the technological water had a relatively constant evolution in both experimental production systems, with highest concentrations registered at the end of the intermediary stage of the

experimental period (beginning of August). From the intermediary stage to the end of the experimental period, an increase of nitrogen oxidation rate is observed, fact confirmed by the upward tendency of N-NO₃ concentrations, correlated with the downward trend of N-NO₂ concentrations (Figure 6). The highest values of N-NO₂ concentrations were registered in the outlet channels, EC-PP and EC-PCP, with average values of 0.15 ± 0.06 mg/L, respectively 0.15 ± 0.05 mg/L (Figure 6).



Figure 7. N-NH₄ dynamics during the experimental period

The concentration of N-NH₄ in the technological water has a relatively constant evolution in both experimental production systems, with highest concentrations registered at the end of the experimental production cycle. Also, the N-NH₄ concentrations from the outlet channels sampling points are superior compared to the ones registered in the ponds production systems sampling points, except for EC-PP at the final stage of the experimental period. Therefore, the highest average values during the production cycle are recorded at EC-PCP (0.43 ± 0.14 mg/L) and EC-PP (0.24 ± 0.11 mg/L), while the lowest are corresponding to PP2 (0.16 ± 0.01 mg/L) and PP1 (0.16 ± 0.03 mg/L). Also, PCP2 values of N-NH₄ concentration are higher comparing with PP2 values (Figure 7). These results are in direct correlation with the feeding regime applied.

The concentration in COD has an upward tendency during the production cycle, from an average value for all sampling points of 164.4 ± 15.25 mg/L, to 167.89 ± 25.29 mg/L. The COD concentrations registered in PCP (176.77 ± 0.83 mg/L, respectively 192.4 ± 0.8 mg/L) are superior to the ones registered in CP (156.8 ± 6.8 mg/L, respectively 154.4 ± 2.2 mg/L)

and PP (155.6 ± 3.2 mg/L, respectively 162.94 ± 10.26 mg/L), fact manifested most probably due to the different feeding management applied (Figure 8).

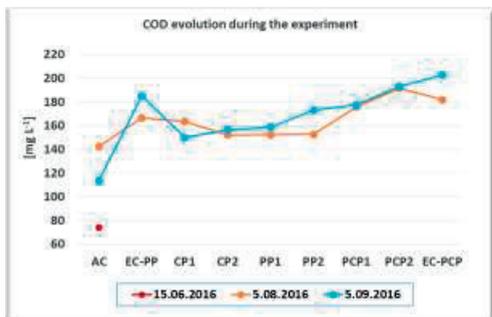


Figure 8. COD dynamics during the experimental period

The TSS dynamics presents an upward tendency during the production cycle, manifested especially at the end of the experimental period. Therefore, the lowest average value of 816.53 ± 362.89 mg/L is registered in case of all nine sampling points in the intermediary stage of the experimental period, while the maximum average value of 2254.13 ± 366.54 mg/L is registered in the final stage of the experimental period (Figure 9). Regarding the ponds sampling points, the IMTA pond registered lower (1390.7 ± 73.82 mg/L) values, compared to the polyculture pond (1756.75 ± 68 mg/L).

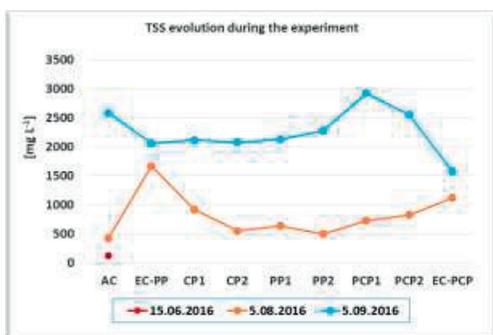


Figure 9. TSS dynamics during the experimental period

B. NITROGEN IN SEDIMENT SAMPLES

In the intermediary stage of the experiment, the TKN concentration in sediments had an average value of 0.50 ± 0.08 g % FW, which was lower compared to the average value in the

final stage of the experimental period, respectively 0.83 ± 0.09 g % FW (Figure 10). In the last experimental stage, the TKN concentration in the sediment's samples registered an upward tendency in case of all sampling stations.

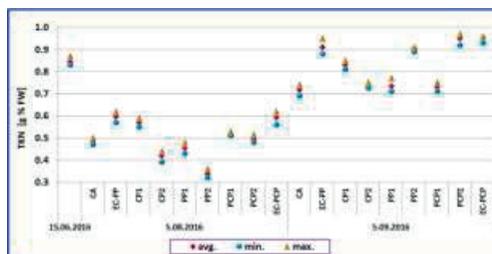


Figure 10. TKN dynamics in sediment samples during the experimental period

The IMTA pond registered significant ($p < 0.05$) higher TKN concentrations in sediments only on the inlet area (0.83 ± 0.02 g% at CP1, compared to 0.73 ± 0.02 g% at PCP1). However, comparing the TKN concentrations registered in sediments at the outlet of both ponds, it can be stated that IMTA pond registered significant ($p < 0.05$) lower values of TKN, compared to the polyculture pond (Figure 10). Also, by analysing the entire production cycle dynamics of TKN, it can be observed the long-term tendency of this nutrient to accumulate at the level of ponds sediments (Figure 10).

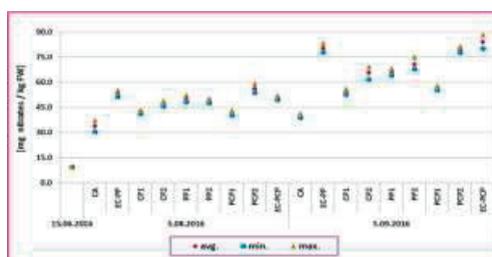


Figure 11. NO_3 dynamics in sediment samples during the experimental period

In the intermediary stage of the experimental period, lower values were registered for each of the sampling points in terms of nitrates concentrations in sediments, compared to the final stage (Figure 11), fact probably due to the change of fish feeding ratio, from 3% BW to 1.5% BW, which seems to stimulate the oxidation processes. The highest concentration

of nitrates in sediments is recorded at the outlet of polyculture pond (55.95±2.62 mg/kg FW at PCP2). The IMTA pond had registered significant ($p<0.05$) higher nitrite concentration in sediments on the middle area and also, on the outlet area (47.07±1.63 mg/kg FW at CP2 and 49.98±2.06 mg/kg FW at PP1, respectively 48.96±1.30 mg/kg FW at PP2). However, comparing the nitrates concentrations registered in sediments at the outlet of both ponds, it can be stated that IMTA pond registered significant ($p<0.05$) lower values of nitrates, compared to the polyculture pond (Figure 11). This demonstrates the capacity of IMTA pond to efficiently prevent the eutrophication at the level of pond and moreover, to become more sustainable by reducing the nitrogen outputs.

The final experimental stage registered an upward tendency of nitrates concentration in sediments, for all the sampling points. The IMTA pond had registered significant ($p<0.05$) lower nitrates concentration in sediments on the outlet area (70.64±3.68 mg/kg FW at PP2), compared to the polyculture pond 79.43±1.77 mg/kg FW at PCP2. This confirms the assumption described above. Also, by analysing the entire production cycle dynamics of nitrates, it can be observed the long-term tendency of this nitrogen compound to accumulate at the level of ponds sediments (Figure 11). Also, the highest nitrates accumulation rate was observed in case of both outlet channel sampling points (80.26±2.73 mg/kg FW at EC-PP, respectively 83.97±4.06 mg/kg FW at EC-PCP).

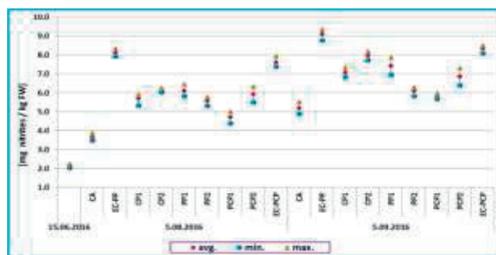


Figure 12. NO_2 dynamics in sediment samples during the experimental period

The highest concentration of nitrites in sediments was recorded in both outlet channel sampling points (8.14±0.21 mg/kg FW at EC-PP, respectively 7.62±0.29 mg/kg FW at EC-

PCP). The IMTA pond had registered significant ($p<0.05$) higher nitrite concentration in sediments on the inlet area (5.72±0.33 mg/kg FW at CP1, compared to 4.72±0.32 mg/kg FW at PCP1). However, when comparing the nitrites concentrations registered in sediments at the outlet of both ponds, it can be observed that IMTA pond registered significant ($p<0.05$) lower values of nitrites, compared to the polyculture pond (Figure 12). This demonstrates the capacity of IMTA pond to efficiently prevent the eutrophication at the level of pond and moreover, to become more sustainable by reducing the nitrogen outputs.

C. NITROGEN IN REED SAMPLES

The highest TKN average values were registered in case of the reed samples collected from the outlet channels (4.10±0.14 g% FW in EC-PP and 3.23±0.16 g% FW in EC-PCP), while the lowest values were registered in the IMTA pond, in PP1 (2.0±0.04 g% FW) and PP2 (1.93±0.04 g% FW) (Figure 13).

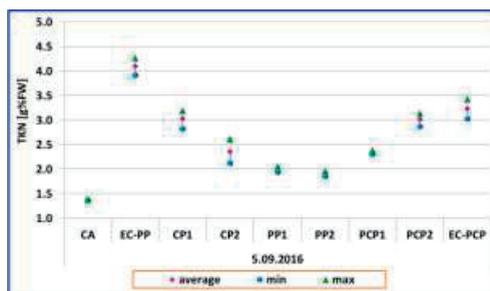


Figure 13. TKN dynamics in reed samples in the final stage of the experimental period



Figure 14. NO_3 dynamics in reed samples in the final stage of the experimental period

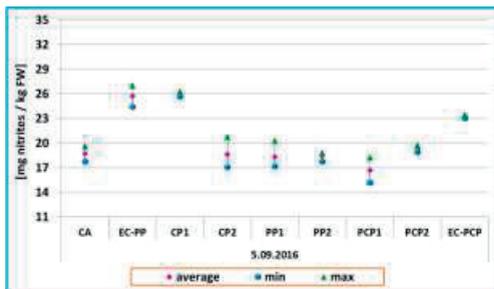


Figure 15. NO₂ dynamics in reed samples in the final stage of the experimental period

The highest NO₃ average values were registered in case of the reed samples collected from the outlet channels (875.62±12.8 g% FW in EC-PP and 880.07±29.5 g% FW in EC-PCP), while the lowest values were registered in the inlet channel, in CA (334.04±8.12 mg/kg FW) (Figure 14).

The highest NO₂ average values were registered in case of the reed samples collected from the outlet channels (25.72±2.25 g% FW in EC-PP and 23.54±1.60 g% FW in EC-PCP), while the lowest values were registered in the polyculture pond, in PCP1 (16.61±1.12 mg/kg FW) (Figure 15).

D. NITROGEN IN FISH SAMPLES

The highest values of TKN registered at the beginning of the experiment, before stocking, are recorded in case of grass carp (2.92 g%), followed by silver carp (2.89 g%) and bighead carp (2.85 g%), while the lowest concentration of TKN in meat is obtained at common carp (Figure 16). This may be due to the fact that common carp has an earlier growth stage, characterized by a lower individual biomass value, comparing with the rest of the experimental fish species.

At the intermediary stage of the experimental period, the CC specimen reared in the PCP polyculture pond registered the highest TKN concentration in meat (3.07±0.10 g%FW), followed by the IMTA pond specimen CP CC (2.21±0.07 g%) and PP CC (2.01±0.13 g%). Significant differences (p<0.05) were observed between IMTA ponds and the polyculture pond CC biomasses. Thus, the IMTA pond production system generates a lower accumulation of TKN in common carp meat, in

the intermediary stage of the production cycle, compared to the polyculture pond production system (Figure 17).

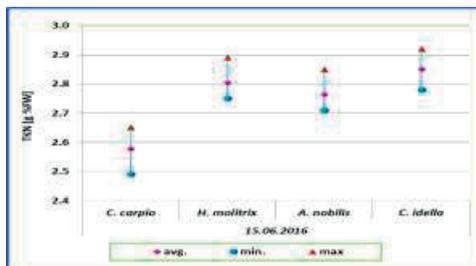


Figure 16. TKN dynamics in fish samples at the beginning of the experimental period

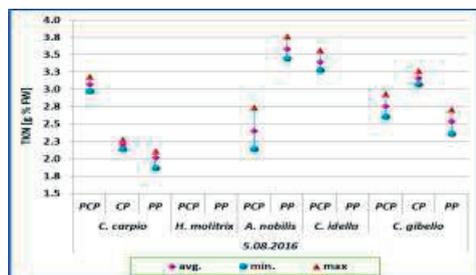


Figure 17. TKN dynamics in fish samples in the intermediary stage of the experimental period

At the intermediary stage of the experimental period, the CC specimen reared in the PCP polyculture pond registered the highest TKN concentration in meat (3.07±0.10 g%FW), followed by the IMTA pond specimen CP CC (2.21±0.07 g%) and PP CC (2.01±0.13 g%). Significant differences (p<0.05) were observed between IMTA ponds and the polyculture pond CC biomasses. Thus, the IMTA pond production system generates a lower accumulation of TKN in common carp meat, in the intermediary stage of the production cycle, compared to the polyculture pond production system (Figure 17).

The silver carp (SC) catches from both ponds, during the intermediary harvesting, were not enough in order to manage to characterize properly the TKN concentration in meat, therefore no results are available. The same situation is valid also for BC specimen from PP part of IMTA pond.

Regarding the BC meat concentration of TKN, significant (p<0.05) higher values can be observed at PP part of IMTA pond (3.59±0.17

g%), compared to PCP polyculture pond (2.40±0.31 g%) (Figure 17).

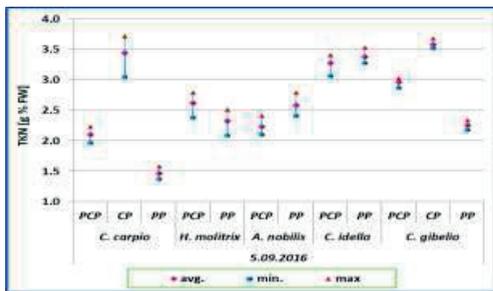


Figure 18. TKN dynamics in fish samples in the final stage of the experimental period

At the end of the experimental period, the CC specimen reared in CP part of IMTA pond registered the highest TKN concentration in meat (3.45±0.35 g%), followed by the PCP CC (2.10±0.13 g%) and PP CC (1.47±0.11 g%). Significant differences ($p < 0.05$) were observed between PP CC and PCP CC biomasses.

Thus, the IMTA pond part where feed was administrated generates the higher accumulation of TKN in common carp meat, in the last stage of the production cycle, compared to the polyculture pond production system (Figure 18). However, compared with the previous experimental stage (Figure 17), during this final stage (Figure 18) the TKN meat concentration of CC biomasses from all the experimental ponds registered an upward tendency (Figure 18).

The SC biomass registered a higher TKN concentration in meat ($p > 0.05$) in the polyculture pond (2.62±0.21 g%), compared to the PP part of IMTA pond (2.33±0.22 g%) (Figure 18).

The BC and GC biomass registered a higher TKN concentration in meat ($p > 0.05$) at PP part of IMTA pond (2.19±0.08 g%, respectively 3.38±0.13 g%), compared with to the polyculture pond (2.23±0.16 g%, respectively 3.28±0.19 g%) (Figure 18).

The TKN meat analysis of this prussian carp reveals the highest values in CP part of IMTA pond (3.58±0.09 g%), followed by the polyculture pond PrC (2.96±0.08 g%) and PP part of IMTA pond PrC (2.26±0.08 g%).

Therefore, it can be concluded that no significant differences ($p > 0.05$) were registered

between IMTA and polyculture pond systems in terms of meat TKN concentration of SC, BC and GC. However, the differences were significant ($p < 0.05$) when it comes to CC meat TKN concentration. Also, the invasive species PrC registered significant ($p < 0.05$) differences between PCP vs. CP vs. PP in terms of meat TKN concentration.

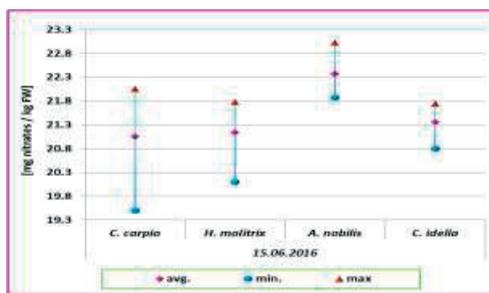


Figure 19. NO₃ dynamics in fish samples at the beginning of the experimental period

The highest values of nitrates concentration registered at the beginning of the experiment, before stocking, are recorded in case of the bighead carp (23.03 mg/kg FW), followed by the rest of three experimental species (22.06 mg/kg FW at CC, 21.78 mg/kg FW at SC and respectively, 21.74 mg/kg FW at GC) (Figure 19).

In the intermediary stage of the experimental period, the CC specimen from the polyculture pond registered the highest nitrate concentration in meat (25.62±2.88 mg/kg FW), followed by the IMTA pond specimen PP CC (14.75±1.59 mg/kg FW) and CP CC (12.52±1.21 mg/kg FW).

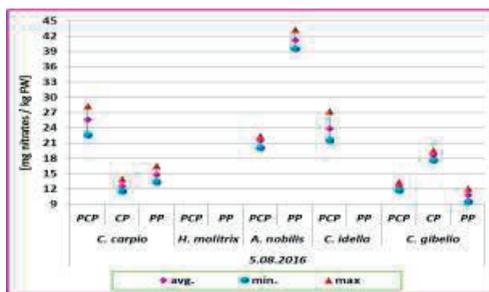


Figure 20. NO₃ dynamics in fish samples in the intermediary stage of the experimental period

Significant differences ($p < 0.05$) were observed between IMTA ponds and polyculture pond CC biomasses. Thus, the IMTA pond production system generates a lower accumulation of nitrates in common carp meat, in the intermediary stage of the production cycle (Figure 20), compared to the beginning stage of the experiment (Figure 20).

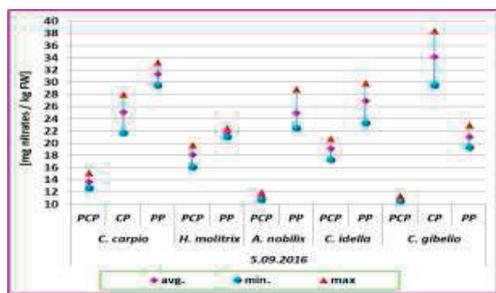


Figure 21. NO_3 dynamics in fish samples in the final stage of the experimental period

At the end of the experimental period, the CC specimen from PP part of IMTA pond registered the highest nitrates concentration in meat (31.32 ± 1.89 mg/kg FW), followed by the CP CC (25.09 ± 3.18 mg/kg FW) and PCP CC (13.69 ± 1.30 mg/kg FW). Significant differences ($p < 0.05$) were observed between all of the CC experimental variants.

Thus, the IMTA pond part where no feed was administrated generates the higher accumulation of nitrates in common carp meat, in the last stage of the production cycle, compared to the polyculture pond production system (Figure 21). Compared to the previous experimental stage (Figure 20), during this final stage (Figure 21) the nitrates meat concentration of CC biomasses from IMTA pond registered an upward tendency, while a downward tendency was recorded for CC at polyculture pond (Figure 21).

The SC biomass registered a higher nitrates concentration in meat ($p < 0.05$) at PP part of IMTA pond (21.73 ± 0.72 mg/kg FW), compared to the polyculture pond (18.16 ± 1.87 mg/kg FW) (Figure 21).

The BC and GC biomasses registered significant ($p < 0.05$) higher nitrates concentrations in meat at PP part of IMTA pond (24.97 ± 3.43 mg/kg FW for BC, respectively 26.94 ± 3.32 mg/kg FW for GC), compared to the polyculture pond (11.27 ± 0.61

mg/kg FW for BC, respectively 19.13 ± 1.73 mg/kg FW for GC) (Figure 21).

The nitrates meat analysis of the prussian carp reveals the highest values in CP part of IMTA pond (34.19 ± 4.50 mg/kg FW), followed by PP part of IMTA pond PrC (21.06 ± 1.81 mg/kg FW) and polyculture pond PrC (10.93 ± 0.40 mg/kg FW).

Therefore, it can be concluded that significant differences ($p < 0.05$) were recorded when it comes to all experimental fish species meat nitrates concentration from PP part of IMTA pond, compared to the polyculture pond. Also, the invasive species PrC registered significant ($p < 0.05$) differences between PCP vs. CP vs. PP in terms of meat nitrates concentration.

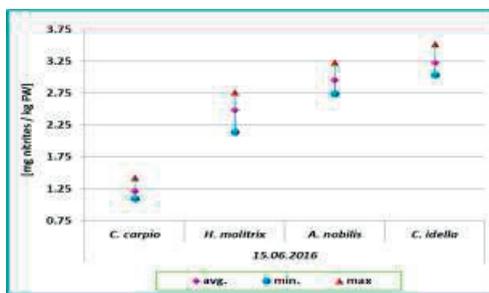


Figure 22. NO_2 dynamics in fish samples at the beginning of the experimental period

The highest values of nitrites concentration registered at the beginning of the experiment, before stocking, are recorded in case of grass carp (3.52 mg/kg FW), followed by bighead carp (3.23 mg/kg FW) and silver carp (2.76 mg/kg FW), while the lowest concentration of nitrites in meat is obtained at common carp (Figure 22). This may be due, same as it was mentioned in case of TKN concentration, to the fact that common carp has an earlier growth stage, characterized by a lower individual biomass value, compared to the rest of the experimental fish species.

In the intermediary stage of the experimental period, the CC specimen from the polyculture pond registered the highest nitrite concentration in meat (8.06 ± 0.48 mg/kg FW), followed by the IMTA pond specimen PP CC (4.09 ± 0.15 mg/kg FW) and CP CC (3.73 ± 0.13 mg/kg FW). Significant differences ($p < 0.05$) were observed between IMTA ponds and polyculture pond CC biomasses. Thus, the IMTA pond production

system generates a lower accumulation of nitrites in common carp meat, in the intermediary stage of the production cycle (Figure 23).

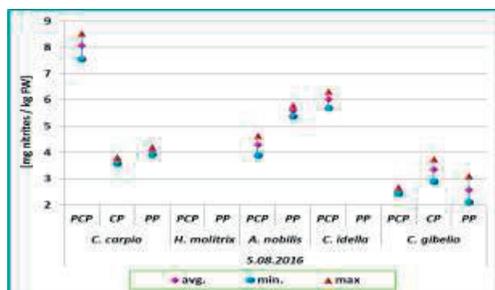


Figure 23. NO₂ dynamics in fish samples in the intermediary stage of the experimental period

The polyculture pond CC registered a notable upward tendency in terms of meat nitrite concentration in intermediary stage (Figure 23), compared to the beginning of the experiment.

The catches of silver carp (SC) specimen from both ponds, during the intermediary harvesting, were not enough in order to manage to characterize properly the nitrite concentration in meat, therefore no results are available. The same situation is valid also for BC specimen from PP part of IMTA pond.

Regarding the BC meat concentration of nitrites, significant ($p < 0.05$) higher values can be observed at PP part of IMTA pond (5.59 ± 0.21 mg/kg FW), compared to the PCP polyculture pond (4.29 ± 0.38 mg/kg FW) (Figure 23).

The grass carp (GC) specimen from PCP pond had an upward tendency in terms of nitrites concentration in meat (Figure 23), compared with the beginning of the experimental stage (Figure 22).

Since at this intermediary harvesting control prussian carp (PrC) specimen was harvested in both experimental ponds, the nitrites meat analysis of this species reveals the highest values in CP part of IMTA pond (3.35 ± 0.43 mg/kg FW), followed by PP part of IMTA pond PrC (2.56 ± 0.50 mg/kg FW).

Therefore, in case of the polyculture pond, after the intermediary stage of the production cycle, it can be concluded that CC and GC registered the highest meat nitrites concentrations, followed by BC. In the IMTA pond, BC

registered the highest nitrites concentration in meat, followed by the CC species, between which a small variation interval was registered (Figure 23).

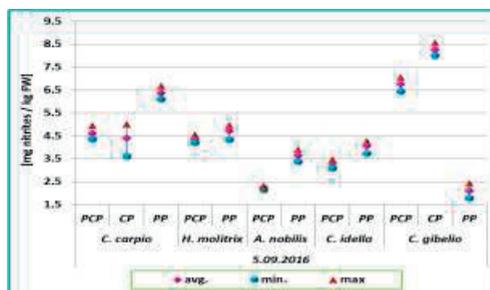


Figure 24. NO₂ dynamics in fish samples in the final stage of the experimental period

At the end of the experimental period, the CC specimen from PP part of IMTA pond registered the highest nitrites concentration in meat (6.36 ± 0.28 mg/kg FW), followed by the PCP CC (4.61 ± 0.60 mg/kg FW) and CP CC (4.38 ± 0.71 mg/kg FW). Significant differences ($p < 0.05$) were observed between PP CC and the rest of experimental CC variants.

Thus, the IMTA pond part where no feed was administrated generates the higher accumulation of nitrites in common carp meat, in the last stage of the production cycle, compared to the polyculture pond production system (Figure 24). Comparing with the previous experimental stage (Figure 23), during this final stage (Figure 24), the nitrites meat concentration of CC biomasses from IMTA pond registered an upward tendency, while a downward tendency was recorded for CC in the polyculture pond (Figure 24).

The SC biomass registered a higher nitrites concentration in meat ($p > 0.05$) at PP part of IMTA pond (4.70 ± 0.33 mg/kg FW), compared to the polyculture pond (4.35 ± 0.18 mg/kg FW) (Figure 24).

The BC and GC biomasses registered significant ($p < 0.05$) higher nitrites concentrations in meat at PP part of IMTA pond (3.63 ± 0.28 mg/kg FW for BC, respectively 4.04 ± 0.28 mg/kg FW for GC), compared to the polyculture pond (2.23 ± 0.12 mg/kg FW for BC, respectively 3.30 ± 0.19 mg/kg FW for GC) (Figure 24).

The nitrites meat analysis of this prussian carp reveals the highest values in CP part of IMTA

pond (8.26 ± 0.28 mg/kg FW), followed by the polyculture pond PrC (6.76 ± 0.31 mg/kg FW) and PP part of IMTA pond PrC (2.10 ± 0.32 mg/kg FW).

Therefore, it can be concluded that no significant differences ($p > 0.05$) were registered between IMTA and polyculture pond systems in terms of meat nitrites concentration of SC. However, the differences were significant ($p < 0.05$) when it comes to CC, BC and GC meat nitrites concentration from PP part of IMTA pond, compared to the polyculture pond. Also, the invasive species PrC registered significant ($p < 0.05$) differences between PCP vs. CP vs. PP in terms of meat nitrites concentration.

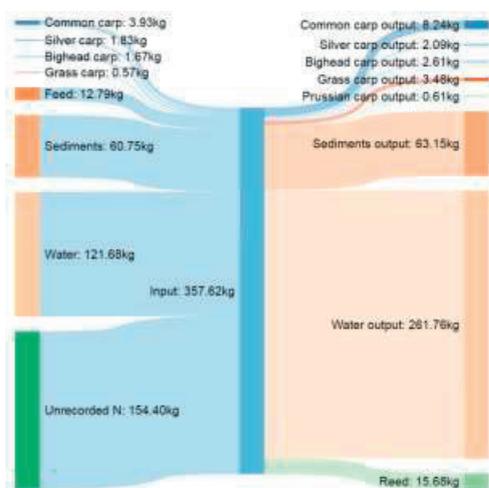


Figure 25. Sankey Diagram for TN in IMTA pond

The TN balance registered a percentage increase of TN recovery, at the end, compared to the beginning of the experimental period, as it follows: for PCP pond - 112.88% in case of fish biomass, 3.95% in case of sediments, 115.12% in case of water, for CP-PP pond - 108.43% in case of fish biomass, 10.06% in case of sediments, 41.78% in case of water. Also, considering the quantity of TN, registered at the end of the experiment: 4.76% - CP-PP, respectively 3.65% - PCP represents the recovered quantity of TN in fish biomass; 17.66% - CP-PP, respectively 14.94% - PCP recovered quantity of TN in sediments; 73.2% - CP-PP, respectively 72.2% - PCP recovered quantity of TN in water; 4.38% - CP-PP,

respectively 9.21% - PCP recovered quantity of TN in reed biomass.



Figure 26. Sankey Diagram for TN in PCP pond

CONCLUSIONS

It can be concluded that the CP-PP feeding management, together with the tested technical solution (pond dividing) generated a higher common carp nitrogen intake and also, better water conditioning performances. Thus, implementing the IMTA technical solution applied in CP-PP generates a reduced loading of nitrogen on the water environment.

Nitrogen compounds dynamics in cyprinids ponds can be significantly influenced by applying the IMTA technology described in this present study.

It is recommended that a long-term monitoring should be made in terms of nitrogen compounds concentration in fish meat, water, sediments and aquatic plants, in order to make a better evaluation of the IMTA technology. Also, future studies on this subject will imply also changes in the fish stocking structure.

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