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Intracohort cannibalism in European catfish (*Silurus glanis* L.) larvae under controlled rearing conditions at different stocking densities

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Abstract

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Under controlled conditions intracohort cannibalism is one of the main factors that affect the growth performance and survival of fish, especially during the early life stages of carnivorous species. The present study investigated the influence of different stocking densities on the frequency of occurrence of intracohort cannibalism, as an opportunity to study and mitigate the negative effects of this common behavior. The research was carried out at the Institute of Fisheries and Aquaculture, Plovdiv in two consecutive phases (Phase I and Phase II) with an overall period of 32 days. During Phase I three variants of stocking densities were evaluated: variant A - 10 ind.l^{-1}, variant B - 20 ind.l^{-1} and variant C - 40 ind.l^{-1}, whereas in Phase II the stocking densities were reduced (A-5 ind.l^{-1}, B-10 ind.l^{-1}, C-15 ind.l^{-1}, D-28 ind.l^{-1}). During Phase I of the experiment, only cannibalism type I was observed with a significant effect of the stocking density on the index of cannibalism (%) ($P<0.05$). At the beginning of Phase II cannibalism type II was observed, as its frequency increased with the increasing of the stocking density ($P<0.05$).

Keywords: European catfish; larvae; indexes of cannibalism; stocking densities

Introduction

Cannibalism is a predatory behavior and feeding strategy that involves killing and eating individuals of the same species. It is reported in both wild and farmed fish, particularly in aquaculture of predatory fish (Naumowicz et al., 2017). Cannibalisms is mainly observed in airbreathing catfish (*Clariidae*), pikes (*Esocidae*), percids (*Percidae*), characids (*Characidae*), latids (*Latidae*), gadids (*Gadidae*) and in more than 30 other families, including cyprinids (*Cyprinidae*) and salmonids (*Salmonidae*) (Smith & Reay, 1991; Hecht & Pienaar, 1993; Qin et al., 2004). The interactions between genotype and environment play a major role in the expression of cannibalism (Baras & Jobling, 2002; Yang et al., 2015). Under rearing conditions, the intensity of cannibalism is directly influenced by population and abiotic factors, along with genetic predispositions (Naumowicz et al., 2017).

Intracohort cannibalism is cannibalism among members of the same age cohort and it is common for fish farming conditions. It is divided into two types: type I (early type), which occurs in the larval phase and it is independent of a diversity in fish sizes, where the victim is not completely ingested or consumed; and type II (later form) associated with heterogeneous growth, when the victim is consumed whole (Hecht & Appelbaum,1988; Smith & Reay,1991; Baras & Jobling, 2002; Naumowicz et al., 2017). Intracohort cannibalism may also be sex-based,especially in species that show sexual growth dimorphism during early ontogeny (Naumowicz et al., 2017).

The impact of different stocking densities levels has been studied in many fish species. A proportional increase in cannibalistic behaviour resulting from rising stock density, following an increase in mortality, has been observed in pike (*Esox lucius*) (Kucharczyk et al.,1998), pikeperch (Szkudlarek & Zakes, 2007), European perch (Baras et al., 2003) and African sharptooth catfish (*Clarias gariepinus*) larvae (Solomon & Udoji,2011). For these species, it is recommended to initially rear larvae at higher stock densities and then to reduce the stock proportionally (Kestemont et al.,2003). An opposite relation is seen in such species as Atlantic cod (*Gadus morhua*) (Baskerville-Bridges & Kling, 2000), Vundu catfish (Imorou Toko et al.,2008) or European chub (*Leuciscus cephalus*) (Zarski et al., 2008), in which there is a reduction in cannibalistic behaviour at high stocking densities.

Król et al. (2014) state that under controlled conditions, intracohort cannibalism is one of the main factors that affects the growth and survival of fish, especially during the early life stages of carnivorous species. The authors established that cannibalism was the main cause of mortality and that losses to type II (complete) cannibalism were higher than those caused by type I (incomplete) cannibalism.

Kozłowski & Poczyczyński (1999) investigate the effect of light and stocking density on the results of rearing of European catfish. They established that cannibalism is more pronounced in illuminated tanks, reaching 53.7% compared to 46.7% in dark tanks, and the difference was statistically significant. This is explained by the fact that light is causing aggressive behavior in fish; they gather at the bottom, near the walls, and in tank corners. Fish reared in darkness are evenly dispersed in the entire water volume and do not crowd. In natural waters, under conditions of feeding competition, cannibalism among juveniles of the same year-class is considered a natural way of regulating population density, especially in predatory fish (Loadman et al., 1986; Hecht & Pienaar, 1991).

In fish, prey size is related to the mouth size, since prey is ingested without mincing (Dabrowski & Bardega, 1984). Thus, in fish such as catfish or carp, producing relatively small larvae of high growth rate (SGR up to 40% per day, or higher), individual body size differences very early, and bigger fish are able to prey on smaller ones after several days from the beginning of rearing. Cannibalism may be reduced by fish rearing in darkness, at stocking densities under 25 ind./dm^{-3} (Kozłowski & Poczyczyński, 1999).

Król et al. (2019) carried out an experiment to establish the effect of size sorting on growth, cannibalism and survival in European perch (*Perca fluviatilis* L.). The authors observed significant lower losses to type I cannibalism in small and large size groups than in the unsorted group ($P<0.05$). Losses to type II cannibalism ranged from 2.2 to 4.9%, but did not differ significantly between groups ($P>0.05$). At the end of the experiment, survival in small and large size groups exceed 92% and were significantly higher than in unsorted groups (75.6%) ($P<0.05$). The results from the experiment prove the importance of size sorting when rearing predatory fishes under controlled conditions.

Cannibalism represents a problem under intensive commercial production of different fish species and is impossible to manage without understanding the factors impacting its occurrence and degree of severity. Each case of cannibalism should be considered in the context of biology and specificity of a given species. In addition, a reduction in cannibalistic behaviour is not necessarily correlated with the best outcomes in production and, hence, the parameters should be carefully selected (Naumowicz et al., 2017).

Despite the fact that *S. glanis* is an economically valuable predatory fish in the aquaculture production, very few studies were done to investigate the effect of cannibalism in European catfish under controlled rearing conditions. The present study was designed to investigate intracohort cannibalism in *S. glanis* larvae reared at different stocking densities under controlled conditions.

Material and Methods

The present research was conducted at the Institute of Fisheries and Aquaculture, Plovdiv in April - May 2018. The experiment had two consecutive phases (Phase I and Phase II), each with duration of 16 days. The overall study period was 32 days.

Phase I

The studied individuals were European catfish larvae obtained from semi-artificial propagation with morphometric characteristics presented in Table 1. The total number of individuals used in the experiment was 6300. Three variants of experimental stocking densities, each with triplicate, were applied: 10 ind.l^{-1} (variant A), 20 ind.l^{-1} (variant B) and 40 ind.l^{-1} (variant C). At the beginning of the experiment, the assessment of the morphometric parameters total body length (TL) and body weight (BW) was performed by random selection and measurements of 50 individuals. Thus, the initial TL and BW of the larvae are the same. At the end of the experiment, 60 individuals were measured from each experimental stocking density. In Phase I and in Phase II the individuals were weighed with analytical balance „Kern AEJ" and measured with electronic gauge "Digital Caliper".Clove oil with concentration of 0.02 ml.l^{-1} was applied as anesthesia during the performance of the measurements in order to avoid stress and injures.

Phase II

The European catfish larvae from Phase I were used in the corresponding variant in Phase II. Therefore, the final body weight and final total length of each of the individuals from Phase I are considered as initial for the variants in Phase II. The total number of individuals used in the experiment was 5 860 with characteristics presented in Table 2.

Table 1. Technological characteristics of European catfish larvae at the start of Phase I

Parameter	Phase I		
	A	B	C
ISD[1] (ind.l^{-1})	10	20	40
INF[2]	900	1800	3600
IB[3] (mg)	16920	33840	67680
MIW[4] (mg/ind) MIW range, CV%	18.8±1.4 7.28	18.8±1.4 7.28	18.8±1.4 7.28
MITL[5] (mm/ind) MITL range, CV%	13.8±1.2 8.83	13.8±1.2 8.83	13.8±1.2 8.83

[1]ISD – initial stocking density; [2]INF – initial number of fish; [3]IB – initial biomass; [4]MIW – mean individual weight; [5]MITL – mean individual total length

Table 2. Technological characteristics of European catfish larvae at the start of Phase II

Parameter	Phase II			
	A	B	C	D
ISD[1] (ind.l^{-1})	5	10	15	28
INF[2]	600	1200	1800	2260
IB[3] (mg)	119580	219000	333900	386686
MIW[4] (mg/ind) MIW range, CV %	199.3±70.9 35.58	182.5±58.0 31.80	185.5±51.3 27.66	171.1±40.0 23.31
MITL[5] (mm/ind) MITL range, CV %	27.0±3.7 13.74	26.5±2.7 10.37	27.9±3.9 14.01	26.5±2.5 9.50

During Phase II the stocking density was reduced as follows: 5 ind.l^{-1} (variant A), 10 ind.l^{-1} (variant B), 15 ind.l^{-1} (variant C) and 28 ind.l^{-1}(variant D). At the end of the experiment 60 randomly selected larvae were weighed and measured.

Experimental conditions

The experimental unit was a flow-through production system consisting of tanks 60×40×40 cm in size and volume of 30 l. Water flow rate was 0.7 l.min^{-1} and complete water cycle took 43 min. The water for the experimental system was supplied from drill with average temperature of 12ºC, heaters in the collecting reservoir were installed to maintain admissible temperature. In each tank micro compressors for continuous air supply were installed. During the experiment water quality parameters were sustained within the optimal range for *Silurus glanis* species as follows: temperature 24.5±1.4ºC, dissolved oxygen 6.3±1.7 mg.L^{-1} and pH 8.2±1.3, UV lamp was used for the sterilization of the water. During the research, the tanks were siphoned twice a day to remove feces and uneaten feed; any injured and dead fish were countered and removed. The daily feeding rate was equal to 50% of the fish weight at the beginning of Phase I and at the 6th day of the study it was decreased to 30% till the end of Phase II. The larvae were fed with commercial dry pellet food “Ocean nutrition” with pellet size 0.5 mm and protein content 58%, during the whole duration of experiment.

Identification of cannibalism

Injured and dead larvae were counted daily, and were identified and classified using Hecht & Appelbaum (1988). The occurrences of cannibalism were classified as either type I, where the prey is bitten on the tail, head or abdomen (most often on the tail) and has visible gaping wound, or as type II, where the prey is swallowed whole. The injured ones, although not dead, were also considered as victims of cannibalism. Missing larvae were assumed to have been consumed (Baras et al., 2010).

The indices of cannibalism were calculated using the following formulas:

$$\text{Cannibalism (\%)} = [NcNj^{-1}]100 \quad (1)$$

$$\text{Survival rate (\%)} = [(Nj\text{-}Nc\text{-}Nnd)Nj^{-1}]100 \quad (2)$$

$$\text{Frequency of occurrence of type I or II cannibalism (\%)} = [NctNc^{-1}]100, \quad (3)$$

where: *Nj* was the initial number of larvae;

Nc – total number of fish cannibalized throughout the experiment;

Nnd – the total number of fish that died naturally;

Nct – the total number of cannibalized larvae which belong to the victims of type I or type II;

The coefficient of variation of total body length and body weight was established using the formula:

$$\text{CV (\%)} = \left(\frac{\text{Standard deviation}}{\text{Mean}}\right) \times 100 \quad (4)$$

Statistical analyses

The morphometric characteristics and the technological parameters are presented as mean values (± S.E.M).T-test (Two Samples for Means), at significant level of P<0.05, was used to compare the effect of the initial stocking density on cannibalism (%) during Phase I and on the frequency of occurrence of type I or II cannibalism (%) during Phase II. Correlation analysis (Pearson coefficient) was performed by applying scatter plot and linear regression in order to determine the correlation between stocking density and occurrence of cannibalism (%). The statistical analysis of the results was conducted with Data Analysis Tool (Microsoft Excel 2010).

Results and Discussion

During Phase I of the experiment, only cannibalism type I was observed (Figure 1). The survival rate (%) and the indices of cannibalism for each stocking density are presented in Table 3.

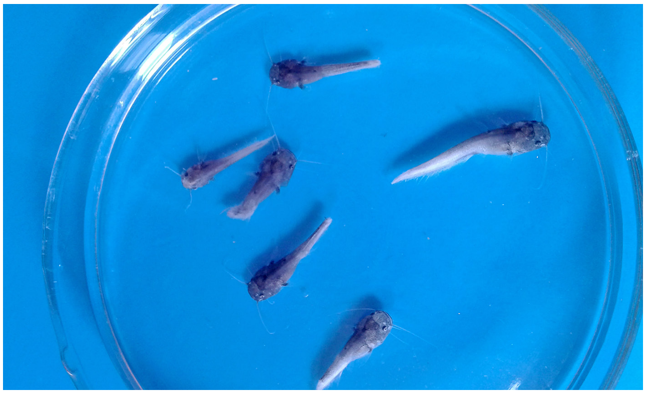

Figure 1. Cannibalism type I

Survival rate at the end of Phase I was high from 88.9% to 89.3%, the values are similar and the stocking density did not have a significant effect on it (P<0.05). As opposed to survival rate, the index of cannibalism increased with the increasing of the stocking density with significant difference (P<0.05). The total number of cannibalized fish (Nc) were the lowest in variant A and the highest in variant C with significant effect of the stocking density on this index (P≤0.01). The statistical analysis revealed insignificant influence of the stocking density on the total number of fish that died naturally (Nnd), although the higher stocking densities increased the value of the index (P<0.05).

Pearson coefficient established high correlation between cannibalism type I and the stocking density, with higher cannibalism values corresponding to higher stocking densities (Figure 2).

At the beginning of Phase II cannibalism type II was registered, with cannibalized fish being swallowed from the head region (Figure 3). Cannibalism type I was also established and it was expressed by biting in the tail region.

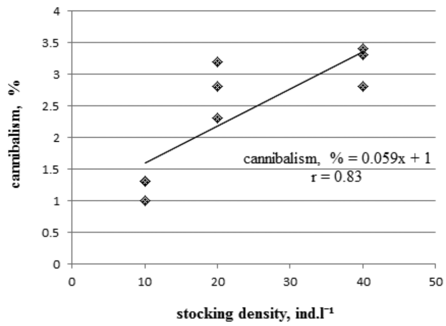

Figure 2. Correlation between cannibalism (%) and initial stocking density

Table 3. Classification of mortality rate, indices of cannibalism and morphometric assesments – Phase I

Parameter	Phase I			Level of significance
	A	B	C	
Nj (individuals)	300	600	1200	
TM (individuals)	33.3±18.2^{a}	60.7±40.8^{ab}	128.7±16.3^{b}	*
Nnd (individuals)	29.7±18.3	44.0±43.3	90.7±18.8	NS
Nc (individuals)	3.7±0.6^{a}	16.7±2.5^{b}	38.0±3.6^{c}	**
Cannibalism, %	1.2±0.^{2a}	2.8±0.4^{b}	3.2±0.3^{bc}	*
Survival rate, %	88.9±6.1	89.9±6.8	89.3±1.4	NS
MIW (mg/ind)	199.3±70.9	182.5±58.0	185.5±51.3	
MIW range, CV %	35.58	31.80	27.66	
MITL (mm/ind)	27.0±3.7	26.5±2.7	27.9±3.9	
MITL range, CV %	13.74	10.37	14.01	

Values connected by different superscripts are significantly different (P≤0.05)

***P≤0.001; **P≤0.01; *P≤0.05; NS – non significant

Nj – initial number of larvae; TM – total mortality; Nnd – total number of fish that died naturally; Nc – total number of cannibalized fish; MIW – mean individual weight; MITL – mean individual total length

Figure 3. Cannibalism type II

The survival rate was between 81.0% and 66.6%. The results show that at this stage of the rearing of European catfish larvae, the stocking density affects the survival rate. As the stocking density increased, the survival rate decreased ($P<0.05$). In contrast to survival rate, the index of cannibalism, based on the total number of cannibalized fish (Nc), increased with the increasing of the stocking density ($P<0.05$). When comparing the number of dead fish resulting from cannibalism type I and type II, it was found that in variant 1 and variant 2 the victims of cannibalism type I were more than those of cannibalism type II, whereas in variant 3 and variant 4 the victims of cannibalism type II exceeded in numbers those of cannibalism type I. The stocking density has a significant effect on the victims of cannibalism type I and type II ($P<0.05$). These results completely corresponded with the frequency of cannibalism type I and type II. At lower densities (variant 1 and 2), frequency of cannibalism type I was higher than the frequency of cannibalism type II. At higher densities (variant 3 and 4), the frequency of cannibalism type II exceeded the frequency of type I with statistically significant difference ($P<0.05$) (Table 4).

Strong correlation between the frequency of cannibalism type I and the rearing density was established ($r=0.82$), with negative regression equation. Contrary to type I cannibalism, the regression equation of cannibalism type II is positive, as increasing frequency of cannibalism type II corresponds to higher stocking densities. Pearson coefficient value indi-

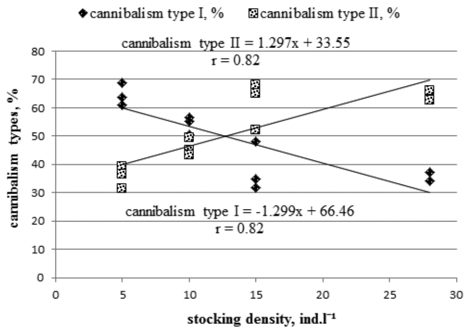

Figure 4. Correlation between cannibalism type I (%) and cannibalism type II (%) and initial stocking density

Table 4. Classification of mortality rate, indices of cannibalism and morphometric assessments – Phase II

Parameter	Phase II				Level of significance
	A	B	C	D	
Nj (ind.)	200	400	600	1130	
TM (ind.)	38±14.1^{a}	102.3±27.2^{b}	165.3±23.5^{c}	378.0±38.2	**
Nnd (ind.)	14.0±6.1^{a}	31.7±11.8^{b}	35.0±7.8^{ab}	75.0±12.7	*
Nc (ind.)	24.0±8.5^{a}	70.7±15.4^{b}	130.3±30.3^{bc}	303.0±25.5	*
$Nct_{type\ I}$ (ind.)	15.3±5.1^{a}	38.0±7.0^{b}	48.3±6.1^{bc}	107.5±2.1	*
$Nct_{type\ II}$ (ind.)	8.7±3.5^{a}	32.7±8.7^{b}	82.0±27.4^{c}	195.5±23.3	*
Cannibalism (%)	12.0±4.3^{a}	17.7±3.8^{b}	21.7±5.0^{ab}	26.8±2.3	*
Survival rate (%)	81.0±7.1^{a}	74.4±6.8^{b}	72.4±3.9^{ab}	66.5±3.4	*
Frequency, type I (%)	64.4±4.0^{a}	54.1±3.1^{b}	38.2±8.6^{c}	35.6±2.3	*
Frequency, type II (%)	35.6±4.0^{a}	45.9±3.1^{b}	61.8±8.6^{c}	64.4±2.3	*
MIW (mg/ind)	668.8±299.7	655.6±414.9	715.5±533.8	646.2±462.5	
MIW range, CV %	44.8	63.3	74.6	71.6	
MITL (mm/ind)	42.8±5.5	41.9±7.4	42.0±6.7	41.4±6.7	
MITL range, CV %	12.8	17.8	15.9	16.1	

Values connected by different superscripts are significantly different ($P\leq0.05$)
***$P\leq0.001$; **$P\leq0.01$; *$P\leq0.05$; NS – non significant
$Nct_{type\ I}$ – total number of cannibalized fish which belong to type I
Nct_{typeII} – total number of cannibalized fish which belong to type II

cates a strong connection between cannibalism type II and the stocking density.

Conclusion

The present study established that with the increasing stocking density the index of cannibalism also increases in both phases of the experiment. During Phase II, when both forms of cannibalism are present, at lower densities (5 ind.l^{-1} and 10 ind.l^{-1}) the frequency of cannibalism type I is higher than the frequency of cannibalism type II, while at higher stocking densities (15 ind.l^{-1} and 28 ind.l^{-1}) the mortality due to cannibalism type II exceeded that of cannibalism type I. It can be concluded that one of the biotic factors which influence the frequency of cannibalism is the stocking density. During the experiment it was observed that lower stocking densities reduce cannibalistic behaviour in European catfish larvae.

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