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Keywords

Cannibalistic behaviour, early development, intra-specific predation, prey selection, teleost.

Abstract

Cannibalism has been described in 390 teleost fish species belonging to 104 families. In rearing conditions, intra-cohort cannibalism is one of the major bottlenecks during the early life stages of pikeperch (Sander lucioperca). This study aims to describe pre-cisely the occurrence and the onset of cannibalism of this species during the first two post-hatching months. In a first step, the cannibalistic behaviour was analysed through observations in three experiments. In each experiment, the number of cannibalistic cases was similar. From 14 to 48 days post-hatching (dph), more than 60 % of prey ingestions were realized by tail-first, but after 48 dph, the number of prey ingestions by head-first increased. In a second step, the behavioural sequence exhibited to attack and to capture a conspecific was analysed, taking into account the size ratio between the cannibal and its prey. The behavioural sequence to capture was always the same, similar to a predatory sequence and it seems that the cannibal chose its prey about 10 s before attacking. Moreover, prey choice by a cannibal was based more on the size ratio between the cannibal and its prey than the previous activity of the potential prey. These new results could lead to solutions to decrease cannibalism in pikeperch rearing.

1. Introduction

Few decades ago, cannibalism, which is defined as the act of killing and consuming whole or a major part of an individual belonging to the same species, irrespective of its stage of development, was most often considered as an aberrant behaviour appearing only under stressful or unusual conditions such as captivity (Baras, 2012; Hecht & Pienaar, 1993; Polis, 1981; Smith & Reay, 1991). Yet, it has now been described in a large number of species, from microorganisms to mammals, under natural conditions (Elgar & Crespi, 1992; Fox, 1975; Smith & Reay, 1991), and is considered as an adaptive response to environmental constraints. Besides, as cannibalism is a predatory be-haviour where the prey and the predator belong to the same species, it could be considered as an intra-specific predation (Polis, 1981) in involving a behavioural sequence for the capture of a conspecific similar to the behavioural sequence of a predator to capture its prey.

Cannibalism was described in 390 teleost fish species (only in captivity for 150 species) belonging to 104 families (Pereira, Agostinho, & Winemiller, 2017). In fish farms, as cohorts are generally not mixed, inter-cohort cannibalism is less or never observed (for more details see Baras, 1998), whereas intra-cohort cannibalism is commonly observed: for example, Atlantic cod Gadus morhua (Folkvord, 1997), dorada Brycon moorei (Baras, Maxi, Ndao, & Mélard, 2000; Baras, Ndao et al., 2000), Eurasian perch Perca fluviatilis (Baras, Kestemont, & Mélard, 2003), Northern pike Esox lucius (Bry, Basset, Rognon, & Bonamy, 1992) or pikeperch Sander lucioperca (Molnár et al., 2004). In teleost fish, intra-cohort cannibalism implying that both the cannibal and the prey have the same age has been classified into two categories: (a) sibling and (b) non-kin intra-cohort cannibalism (Smith & Reay, 1991). In fish, intra-cohort cannibalism was described mostly at the larval and juvenile stages (Baras, 1998, 2012). Two types of cannibalism have been described. Type I, also called precocious type, appears during larval stages and is totally independent of the size heterogeneity between fish. In this type, the prey is partially eaten and ingested tail-first (Cuff, 1980). Type II, which appears later in development, is directly linked to the size heterogeneity between individuals (Baras, 2012; Naumowicz, Pajdak, Terech-Majewska, & Szarek, 2017). It is characterized by a complete prey ingestion by the cannibal (Baras & Jobling, 2002), and this ingestion is performed either by head or by tail-first. Both types of cannibalism are observed under farm conditions.

In the last 20 years, many studies have focused on the impact of biotic, abiotic or feeding factors on cannibalism during the early life stages of both marine and freshwater species (for review see Naumowicz et al., 2017; Pereira et al., 2017) without considering the onset of this particular behavior. Despite the large literature on cannibalism, there is a lack of studies about the description of the cannibal's behaviour and the behavioural sequence leading to the capture of a conspecific. Among freshwater species, pikeperch has gained attention as a promising new species in intensive fish farming (Kestemont, Dabrowski, & Summerfelt, 2015; Nyina-wamwiza, Xu, Blanchard, & Kestemont, 2005). Until now, several bottlenecks have prevented the successful larval rearing due mainly to high mortality resulting from intra-cohort cannibalism (larvae and juveniles). The cannibalism rate can vary from 7.4 % to 53.0 % (Kestemont, Xueliang, Hamza, Maboudou, & Imorou Toko, 2007; Lappalainen, Olin, & Vinni, 2006; Mamcarz, Kucharczyk, Kujawa, & Skrzypczak, 1997; Molnár et al., 2004; Szczepkowski, Zakęś, Szczepkowska, & Piotrowska, 2011). In pikeperch, cannibalism appears two weeks after hatching (Kestemont et al., 2007) and fish larvae show a typical hunt behaviour performing an 'S-Shape' (Houde, 2001): at short distance of the prey, larva stops, folds as a spring and attacks (Turesson, Persson, & Brönmark, 2002). At the juvenile stage, the hunting behaviour called 'hide and chase' appears: the predator moves towards the prey, does not stop, but attacks it with a small and fast movement of the tail, which projects the predator forwards quickly (Sullivan & Atchinson, 1978).

In this study, we describe precisely the onset of cannibalism in pikeperch under farming conditions. First, we observed the cannibalistic behaviour during early development and under different combinations of biotic and abiotic factors during 3 years. Second, we described the behavioural sequences exhibited to attack and capture a conspecific, with an ethogram, taking into account the size ratio between the cannibal and its prey.

2. Material and Methods

All fish treatments and procedures used in this study were in accordance with the guidelines of the Council of European Communities (2010/63/UE) and the French Animal Care Guidelines (Animal approval No. APAFIS#1813-2015111618046759v2).

2.1. Fish housing and experimental conditions

Observations were conducted during three consecutive years (2015, 2016, 2017) in the frame of three successive rearing sessions with the main aim to test the interactions of different rearing factors on growth, survival and cannibalism (Table A1; Fontaine, Colchen, Gisbert, Pasquet, & Teletchea, 2016; Fontaine, Colchen, Pasquet, & Gisbert, 2017; Fontaine, Colchen, Pasquet, Teletchea, & Gisbert, 2016). For each year, larvae were obtained from a same local broodstock (native from Czech Republic) maintained at the SARL Asialor (57120, Pierrevillers, France) and transferred to the Experimental Platform in Aquaculture (URAFPA, Vandœuvre-lès-Nancy, France http://www.urafpa.fr/main.php) at the University of Lorraine. Observations were carried out in eight sub-squared 700-L indoor tanks (two rows of four tanks with green wall) of a recirculated aquaculture system equipped with mechanical and biological filters as well as a UV sterilization unit. For the first and second years of observations (2015 and 2016), fish were reared until 69 days post-hatching (dph) in eight tanks (density = 90 larvae/L and 43 larvae/L, respectively), and until 52 dph in seven tanks (50 or 100 larvae/L) for the third one (2017). Artificial lighting followed a 12 hr light/12 hr darkness cycle with light on from 08:00 to 20:00 with a 30-min simulation of dawn and dusk. Temperature was similar in all tanks and was incrementally increased by 1° C per day from 16° C at hatching up to 20 ± 0.3° C, and was maintained constant thereafter (Kestemont et al., 2007; Szkudlarek & Zakęś, 2007). The physicochemical properties of the water were monitored once or twice per week in each experiment (Table 1). Fish were fed commercial food (first with nauplii of Artemia: 550–600 µm, Sep-Art Artem ia cyst), then Larviva PROWEAN 100, 300, 500, 700 µm [BIOMAR®, France] and INICIOplus 0.8 mm [BIOMAR®, France]) according to the experimental modalities tested. Fish were fed seven times per day between 8:30 and 17:30 during the light period (a meal every 1.5 hr).

From the first day of exogenous feeding (i.e. 4 dph), 30 fish were randomly sampled in tanks during each weekly observation session. From these samplings, the average fish total length was calculated to determine the growth rate (%) per week and the size heterogeneity with the coefficient of variation of total length (%) in each tank. All fish were euthanized by over-anesthetizing in a MS 222 bath (240 mg/L) and measured from 4 to 25 dph with a binocular microscope linked to a computer with a camera (Sony CCD-Lw1235C) and Archimed software®. From 32 to 67 dph, larvae and juveniles were measured with a ruler. In our study, with our experimental conditions, larval phases were from 5 ± 1 dph (5.87 ± 0.77 mm, Ott, Löffler, Ahnelt., & Keckeis, 2012) to 32 ± 3 dph (21.78 ± 2.12 mm, Ott et al., 2012) and juveniles phases were from 40 ± 5 dph (33.38 ± 1.94 mm, Ott et al., 2012).

2.2. Description of the onset of cannibalism

2.2.1. Behavioural observations and measured variables

During the 3 years, observations lasting 5 min per tank were realized from 10 dph, on a daily basis (including week-ends) between 9:00 and 9:50 a.m. (the period of hunting activity in the field, Horký, Slavík, & Bartoš, 2008). During the observation sessions, the number of cases of cannibalism followed by a successful capture was recorded. These observations of 5 min allowed to obtain several parameters:

The first day of cannibalism for each tank (n = 23 tanks: eight in 2015 and 2016, seven in 2017),

The number of cases of cannibalism summed per week observed in each observation,

The type of cannibalism, by tail or head-first that is, when a fish was observed with a conspecific in its mouth and where head or tail of the conspecific was visible. Furthermore, a third type was considered when individuals were 7 weeks old or more because, after this age, fish were too fast to be caught with a net when they attacked a prey. An individual was considered a cannibal due to its large stomach and the presence of a conspecific in it, and was caught when possible. When digestion was not too advanced, the cannibal was euthanized and the prey was removed from the cannibal's stomach to be measured.

For the third year, the attempts of attacks were observed and counted. They were characterized 'by flank' when pikeperch attacked at the level of the pectoral fin and 'by tail' when pikeperch attacked at the level of the caudal fin.

When the cannibal and its prey were caught with a net, they were euthanized by over-anesthetizing in a MS 222 bath (240 mg/L) and measured (total length [cm]) to calculate the size ratio between the cannibal and its prey.

2.2.2. Data analysis

The growth rate between 4 and 52 dph was compared between the 3 years. After controlling normality and homogeneity of data, the growth rate between the three experiments was compared using a unifactorial ANOVA for data with a normal distribution (lm, R Core Team, 2016). Then, links between the first day of cannibalism in each tank, the growth rate and the coefficient of variation of the total length have been sought. As the first days of cannibalism were comprised between 14 and 36 dph (depending of the year), the growth rate and its coefficient of variation were calculated between 4 and 39 dph to highlight the links between growth and the onset of cannibalism. These links were statically tested with a Spearman correlation due to the lack of normality and homogeneity of variance of the data (Savicky, 2015). The percentages of the type of ingestion per week, cumulated on the 3 years, were compared with the chi-squared test. Finally, as data of size ratio fit with normality and homogeneity of variance, the size ratio between cannibals and prey was compared between the 3 years with a unifactorial ANOVA for data with a normal distribution (lm, R Core Team, 2016) followed by a Tukey's post hoc test.

Data normality and homogeneity of variance were tested on residuals with Shapiro–Wilk test (shapiro.test, R Core Team, 2016) and Levene test (leveneTest, Fox & Weisberg, 2016) respectively. All statistics were realized with R software (version 3.2.4) except for the chi-squared test, which was realized with StatView software (version 5.0). The level of significance used in all tests was $p < 0.05$.

2.3. Description and analysis of the cannibal behaviour

2.3.1. Behavioural observations and measured variables

During the second year (2016), from 14 to 69 dph, when a case of cannibalism was observed, the observer tried to catch the cannibal with a net during the 5 min of observation. Among the 192 cases of cannibalism observed, 35 cannibals were sampled; of these, a sub-sample of 25 cannibals (Mean ± SD = 34.6 ± 9.4 mm) between 28 and 52 dph old was used for further observations.

Each of these 25 cannibals was captured and isolated individually during 24 hr in a net (15 × 25 cm) immerged in an 80-L aquarium. After 24 hr, they were transferred in a smaller device (20 × 7 × 4 cm with 2 cm of water) divided into two zones by a divider. Test was performed in a dedicated room separate from rearing tanks but with the same rearing conditions (temperature, light). We placed the cannibal (called focal fish) in one zone, and three conspecifics of the same age but smaller (Mean ± SD = 24.2 ± 6.6 mm) in the other one (unfamiliar from the tested fish). After 30 min of acclimatization, the divider was removed and fish were left together for 20 min. Behaviours were video recorded with a camera (Sony HDR-CX550VE) placed on top of the device. The videos were analysed and all variables were measured with continuous focal observations (on the whole 20 min period). Behavioural variables were analysed with the Observer XT (Noldus, The Netherlands, version 10.0). Six variables were taken into account: the orientation towards a potential prey, the approach, the pursuit, the 'S-shape' behaviour in front of a conspecific but without an attack, the attack and the capture (for definitions see Table 2). Furthermore, distances before and after an attack, between a cannibal and its prey (thereafter called larva 1) or the two other larvae (larva 2 and 3) were measured at 60, 50, 40, 30, 20,10 s and at each second from 9 to 1 s before and after the attack. Only one attack per cannibal taken randomly through all the performed attacks by this cannibal during the 20 min of the test was analyzed. Furthermore, the swimming activity time of the three larvae was measured during 1 min before and after the attack. Two different types of swimming activities were considered: fast swimming (rapid displacement of the fish, with the tail flick no longer visible and fish moving more than its body length in less than 1 s) and slow swimming (a tail flick and the fish moved more than its body length during more than 1 s). Finally, all tested fish in the behavioural test (cannibal and conspecifics) were measured (total length) on video recording and the size ratio was calculated. The size ratio was compared between the cannibals that attacked and those that did not.

2.3.2. Data analysis

Swimming activity and size ratio were analyzed by a unifactorial ANOVA for data with a normal distribution (lm, R Core Team, 2016). The swimming activity before and after the attack for each conspecific was compared with a paired t test (t.test, R Core Team, 2016). To compare the size ratio between the cannibal and conspecific larvae, when an attack occurred or not, a t test was used.

The normality and the homogeneity of variance were tested on residuals with Shapiro–Wilk test (shapiro.test, R Core Team, 2016) and Levene test (leveneTest, Fox & Weisberg, 2016). All statistics were realized with R software (version 3.2.4). The level of significance used in all tests was $p < 0.05$.

3. Results

3.1. Description of the onset of cannibalism

Overall, 438 cases of cannibalism were observed in all tanks during the 141 days of observation for the 3 years combined. In the first year (2015), 142 cases of cannibalism were observed with the first one occurring at 21 dph. There was an increase in the number of cases of cannibalism between 21 and 41 dph, followed by a decrease between 41 and 48 dph, and a second increase till the end of the experiment (69 dph) (Figure 1). In the second year, 192 cases of cannibalism were observed starting 14 dph and a similar pattern for the first year was observed, and the increase after 48 dph was higher (Figure 1). For the third year, 104 cases of cannibalism starting at 17 dph were observed. There was an increase in the number of cases from 17 to 41 dph, followed by a decrease after 41 dph (Figure 1). The rearing was stopped at 52 dph due to external constraints.

The first day of cannibalism in each tank of the 3 years was significantly and positively correlated to the growth rate ($S = 357.2$; $\rho = 0.82$; $p < 0.0001$; Figure 2a) and to the coefficient of the total length variation ($S = 772.7$; $\rho = 0.62$; $p = 0.001$; Figure 2b). Between 4 and 52 dph, no significant difference was found for the growth rate between the 3 years ($F = 3.4$; $df = 2$; $p = 0.053$): 2015 = 16.2 ± 1.1 %; 2016 = 15.6 ± 0.5 %; 2017 = 15.1 ± 0.6 % (Figure 3). At the beginning of the three experiments, there was no difference between larval total length (for the 3 years: 5.5 ± 0.3 mm; Figure 3). At 53 dph, there was a significant difference between each year for the total length of the juveniles ($F = 6.6$; $df = 2$; $p = 0.005$; 2015 = 52.9 ± 4.1 mm; 2016 = 46 ± 5 mm; 2017 = 50.9 ± 5.4 mm) with 2015 and 2016 sig-nificantly different ($p = 0.003$). There was also significant difference between each year for the weight of the juveniles ($F = 7.2$; $df = 2$; $p = 0.002$; 2015 = 1,497.8 ± 708.8 mg; 2016 = 911.3 ± 272.9 mg; 2017 = 1,476.8 ± 354.6 mg) with 2015 and 2016 ($p = 0.01$) and 2016 and 2017 ($p = 0.004$) significantly different.

The type of prey ingestion (by tail or head first) differed between weeks when the 3 years were combined ($\chi^2 = 214.0$; $df = 7$; $p < 0.0001$). From 14 to 48 dph, more than 60 % of the ingestions were realized by the tail-first (Figure 4). After 48 dph, the number of prey ingested head-first increased (Figure 4) and the difference in ingestion by the tail became significant between the fifth (from 42 to 48 dph) and sixth weeks (from 49 to 52 dph) ($\chi^2 = 6.7$; $df = 1$; $p = 0.009$). Furthermore, in the first 2 years (2015, 2016) after 49 dph, individuals with a large stomach represented 46 % and 91 %, respectively, of cases of cannibalism.

In the third year (2017), 85 attacks were observed. Between 17 and 26 dph, 100 % of the attacks (n = 26) were realized close to the pectoral fin. From 27 to 52 dph, 64.4 % of the attacks (n = 38) were realized by the flank, also close to the pectoral fin, and 35.6 % of the attacks (n = 21) at the level of the caudal fin. Between 17 and 26 dph, 11 % of the attacks on the flank were successful and only 8 % between 27 and 52 dph.

The size ratio between the cannibals and their prey was significantly different between the 3 years ($F = 13.4$; $df = 2$; $p < 0.0001$; Figure 5). There was no difference between the first and the second years ($z = 0.7$; $p = 0.45$; Figure 5), but there was a significant difference between the first and third years ($z = –3.7$; $p < 0.0001$; Figure 5), and between the second and third years ($z = –4.01$; $p < 0.0001$; Figure 5).

3.2. Description and analysis of the cannibal behaviour

Among the 25 cannibals tested in the behavioural test, 7 attacked a conspecific and 3 of them were successful. Cannibals that attacked exhibited 83 orientations, 65 approaches, 3 attacks without an S-shape, 30 S-shaped attacks, 15 attacks after an S-shape, 3 pursuits and 3 captures in total (Figure 6). The other cannibals did not exhibit any agonistic behaviours towards their conspecifics. We can observe that 78 % of the orientations by the cannibal towards a conspecific were followed by an approach. After the approach, there was an attack without an S-shape in 5 % of the cases but 46 % of the larvae made an S-shape (Figure 6). When the larvae showed an S-shape, in 50 % of the cases, they attacked conspecifics or conspecifics moved away, which resulted in a pursuit in 10 % of the cases. Pursuits were followed by a new ‘S’ position in 100 per cent of the cases. Finally, after an attack with an S-shape, cannibals were successful in 20 % of the cases (Figure 6).

Cannibals seem to choose their prey at least 10 s before the attack (Figure 7). From 60 to 10 s before the attack, the distances between the cannibal and the three conspecifics were similar. However, from 10 s, the distance between the cannibal and the potential prey (larva 1, the one targeted) decreased significantly (Figure 7). After the attack, the distance between the attacked conspecific and the cannibal increases rapidly from 0 to 4.97 cm in 1 s (Figure 7). Four seconds after the attack, distances between the cannibal and conspecifics were similar as they were before the attack (Figure 7). Furthermore, there was no difference between the swimming activity’s time of the three conspecifics before or after the attack. Indeed, the larva that was attacked did not show a significant difference in swimming activity’s time from the two others during the minute before the attack (larva 1:31.8 ± 20.3 s; larva 2:22.1 ± 23.1 s; larva 3:32.6 ± 25.6 s; $F = 0.44$; $df = 2$; $p = 0.65$) and also after the attack (larva 1:27.2 ± 20.1 s; larva 2:23.8 ± 23.7 s; larva 3:18.3 ± 19.4 s; $F = 0.29$; $df = 2$; $p = 0.75$). However, after the attack, larva 1 used fast swimming to escape when the cannibal did not catch it during 9.83 ± 4.31 s (mean ± SD). Fast swimming was only used after an attack by conspecifics to escape from the cannibal. There is no significant difference between swimming activity’s time before the attack and after the attack for the three conspecifics (larva 1: $t = 1.15$; $df = 5$; $p = 0.3$; larva 2: $t = –0.17$; $df = 5$; $p = 0.9$; larva 3: $t = 1.99$; $df = 5$; $p = 0.09$). There is no significant difference between the size ratio of the cannibal and each conspecific ($t = 1.82$; $p = 0.09$). The prey/cannibal size ratio tended to be lower when there was an attack (0.63 ± 0.12) than when no attack occurred (0.75 ± 0.16).

4. Discussion

For the first time, to our knowledge, our study described and analyzed cannibalism focusing at an individual level. First, in our study, we counted the number of cannibals during the 5-min period per tank, while in previous studies, the number of cannibals resulted in the difference between the initial and final numbers of individuals in the aquaria (i.e., Baras, Ndao et al., 2000; Kestemont et al., 2007; Król, Flisiak, Urbanowicz, & Ulikowski, 2014). Hence, in these cases, all disappeared larvae were considered as prey of a cannibal. This method implied to work with a limited number of fishes per aquarium to be able to count precisely the initial and final numbers. To work with a low number of fish could not be representative of the cannibalism rate in farm or natural conditions due to the density dependence relationship (Molnár et al., 2004). Second, with this method to evaluate cannibalism, the authors had no precise count of cannibals; only one cannibal in the aquarium could be responsible for the death of several prey by bite or by ingestion. Even if the 5-min observation does not allow to demonstrate the total number of cannibals in tanks for the whole period, our method allowed to discuss cannibalism in farm conditions at an individual scale and not at a population scale as other studies.

In our study, the description of cannibalism began with an observation of the first day of cannibalism between 14 and 21 dph, which is earlier than the 22 dph, previously found (Kestemont et al., 2007). A large fish attacking and ingesting the smaller fish from 22 dph is representative of type II cannibalism (Kestemont et al., 2007). In our study, the first day of cannibalism was positively correlated to the growth rate and the coefficient of the total length variation. Consequently, cannibalism appears earlier in a population with a more homogeneous size and a low growth rate. These correlations could be explained by the onset of cannibalism of type I, called precocious type, which is independent of size heterogeneity and occurred in young and small larvae (Cuff, 1980). Conversely, in populations with large size heterogeneity and a strong growth rate, type I cannibalism was never or rarely observed, and consequently, in this population, a late beginning of cannibalism was observed, directly with type II (Baras et al., 2011).

Then, the onset of cannibalism description highlights two peaks of cannibalism during the three observation sessions. A first one between 35 and 41 dph, and a second one after 50 dph, whereas an increase in the cannibalism rate between 22 and 25 dph and then a decrease after 30 dph was previously demonstrated (Kestemont et al., 2007). Moreover, a peak of mortality in pikeperch mainly due to cannibalism between 22 and 34 dph was also described (Hamza, Mhetli, Khemis, Cahu, & Kestemont, 2008). Thus, it seems that there is a clear peak of cannibalism in pikeperch between the third and sixth weeks after hatching, even if the precise time differs between studies due to rearing conditions. This could be due to a high level of size heterogeneity between fish during this specific period of time (Baras & Jobling, 2002) and also due to developmental differences between the individuals. In Atlantic cod larvae, the peak of cannibalism was linked to the ontogenetic shift (morphological and physiological modifications) due to metamorphosis, allowing a better swimming activity and improving the attack behaviour (with vertebrae and fin rays' development) and ingestion process (with the stomach and pyloric caeca development) (Puvanendran, Laurel, & Brown, 2008). In pikeperch, between 17.71 ± 1.58 and 33.38 ± 1.94 mm, all fins are fully developed (Ott et al., 2012). In our observations, this larval size range corresponds approximately to an age of 32 to 42 dph coinciding with our first peak of cannibalism. Furthermore, an early study on pikeperch reported that cannibalism stopped at about 5 cm length (Hilge & Steffens, 1996) that corresponds in our study to an age of 50 dph or 7 weeks after hatching. However, in our study, it is not the case at least for the first and the second years of observation. Our study demonstrated that in the first year, cannibalism decreased effectively at 50 dph (46 ± 4.9 mm of total length) but increased again later on: we observed a second peak of cannibalism after 56 dph (8 weeks after hatching). At this age and size, pikeperch juveniles were definitively developed (Ott et al., 2012) that could explain this second peak of cannibalism and the appearance of a new method of capture with the shift in prey ingestion from the tail to the head-first.

Numerous attempts of attacks in young pikeperch larvae were mostly realized close to the pectoral fin, on the flank. These findings are in agreement with a previous study, which reported that in young larvae of walleye (Sander vitreus), between 4 and 10 dph, 92 % to 100 % of the attacks were done at the level of the pectoral fin and only 2 % of them were successful (Loadman, Moodie, & Mathias, 1986). In pikeperch, at this age, we did not observe attack attempts on the head directly, but we observed several cases of cannibalism by head. It seems that attacks by head are characteristic of older larvae (Loadman et al., 1986). We could hypothesize that larvae which realized an attack by head-first were more successful. However, an attack by head is more dangerous for the predator due to the presence of teeth of the prey and its abilities to defend itself causing injuries to the cannibal. Fish which attacked the prey by head take high risk to be bitten by the conspecific. Despite this danger, after 49 dph, there was an important shift to this new capture tactic from tail-first to head-first attack and ingestion. At this age, there is a high heterogeneity of size in the population of pikeperch which could lead to a decrease in risks for cannibals to be injured by a smaller conspecific. This shift has already been described in catfish Clarias gariepinus larvae (Hecht & Appelbaum, 1988). The authors characterized, from 8 to 45 mm, a tail-first caught and swallowed up to head, which was subsequently bitten off and discarded, as type I cannibalism and then type II cannibalism where the prey was swallowed head-first as a whole (Hecht & Appelbaum, 1988). We could hypothesize that, in pikeperch, this shift from tail-first to head-first ingestion is necessary due to the development of spiny rays on the prey, which are totally developed after 20 mm (Ott et al., 2012) and could result in suffocation and, consequently, prevent the tail-first ingestion by the cannibal. Further, the decrease in cannibalism between the sixth and eighth weeks could be explained by the necessity to learn an alternative strategy of ingestion without suffocation due to the development of spiny rays making the first strategy to swallow tail-first more difficult than a head-first ingestion.

The size ratio between the tested larva (cannibal) and the three conspecifics was smaller for the attacked pikeperch than those that were not attacked. We could hypothesize that there was an active selection of prey based on size. This hypothesis is reinforced by the fact that at least 10 s before the attack, cannibals had already chosen their prey (among the three proposed). After this period of time, the distance between the cannibal and its chosen prey decreased significantly. This selection seems to be based more on the size of the prey than on the swimming activity, as described for adult pikeperch (Lappalainen et al., 2006). After an attack, the attacked conspecific used fast swimming to increase the distance between the cannibal and it. In few seconds, the swimming activity and the distance between the cannibal and the conspecifics quickly returned to the same level before the attack (except for the conspecific caught by the cannibal).

In conclusion, first day of cannibalism in pikeperch larvae was directly linked to the growth rate and size homogeneity. Cannibalism appears earlier in a homogeneous population with a low growth rate. It seems that size sorting should not be done too soon. Furthermore, daily observations allowed demonstrating, for the first time, that there were two peaks of cannibalism (one between 35 and 41 dph and another one after 50 dph). In contrary to previous observations, in pikeperch, cannibalism does not stop after 50 dph. Finally, it seems that cannibals were able to choose their prey in terms of their size and they chose them 10 s before attacking.

Table 1. Mean ± SD values for the five water quality parameters measured for each experiment (n: number of measures)

	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Ammonium ion content (mg/L)	Nitrous nitrogen content (mg/L)
Experiment 1 (2015)	20.0 ± 0.3 (*n* = 9)	7.6 ± 0.4 (*n* = 9)	8.0 ± 0.2 (*n* = 9)	0.20 ± 0.1 (*n* = 19)	0.02 ± 0.02 (*n* = 15)
Experiment 2 (2016)	20.0 ± 0.3 (*n* = 12)	8.1 ± 0.5 (*n* = 12)	6.9 ± 0.8 (*n* = 19)	0.13 ± 0.1[a] (*n* = 10)	0.08 ± 0.06 (*n* = 14)
Experiment 3 (2017)	20.1 ± 0.14 (*n* = 19)	6.9 ± 0.8 (*n* = 19)	6.9 ± 0.7 (*n* = 42)	0.07 ± 0.1 (*n* = 20)	0.07 ± 0.05 (*n* = 20)

a The mean of ammonium ion content was calculated based on 10 values rather than 14 due to a peak of ammonia at the end of the experiment (Mean ± SD: 18.25 ± 12.02 mg/L).

Table 2. Description of the seven behavioural variables analysed in the test of cannibalism. Focal fish was defined as the cannibal tested in front of conspecifics

Behaviours	Definitions
Orientation	The focal fish turned its body head-first towards the conspecific and visually tracked it (Bell & Sih, 2007).
Approach	The movement of the focal fish towards the other fish with slow swimming (Colchen, Faux, Teletchea, & Pasquet, 2017).
S-shape	The focal fish make an 'S' position in front of a conspecific without the attack (rapid movement with open mouth). Fish does not propel itself.
Attack without an S-shape	The focal fish moves towards the prey and attacks it with a small and fast movement of the tail, which projects it forwards rapidly (Sullivan & Atchinson, 1978).
Attack after a an S-shape	A rapid movement of the focal fish towards other fish, with the mouth open (Colchen, Dias, Gisbert, Teletchea, & Pasquet, in prep). Attack is characterized by a 'S' position or a modification of the orientation of the tail of the focal fish before the rapid movement (Houde, 2001; Turesson et al., 2002).
Capture	The focal fish bites a conspecific (Colchen et al., in prep).
Pursuit	The focal fish follows a conspecific, which moves after the S-shape of focal fish.

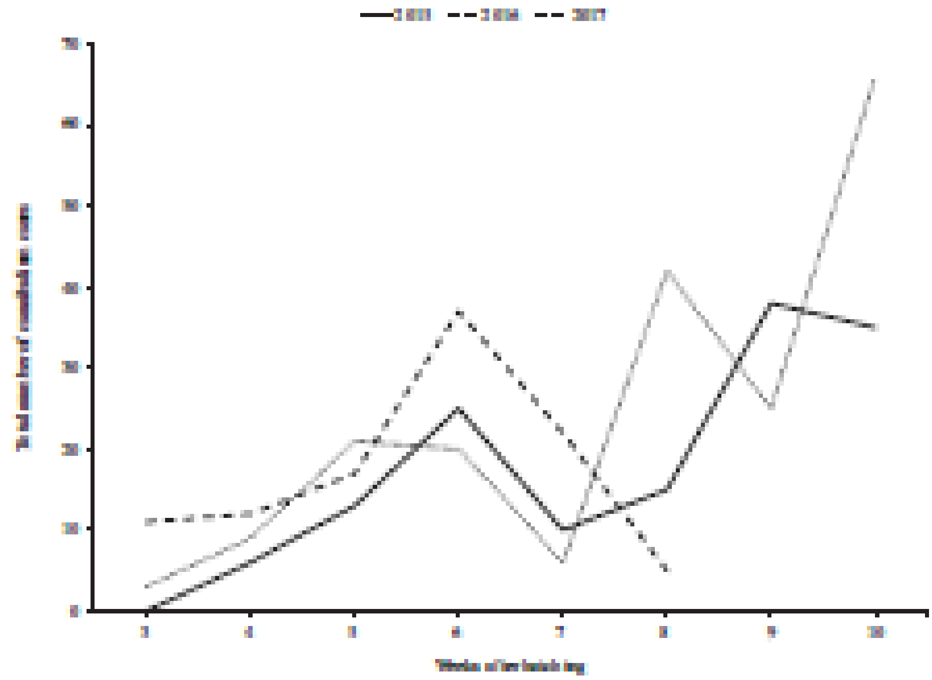

Figure 1. Total number of cannibalism cases observed during the 3 years of experimentation with pikeperch considering the age of fish (number of weeks after hatching), in which cannibalism was observed

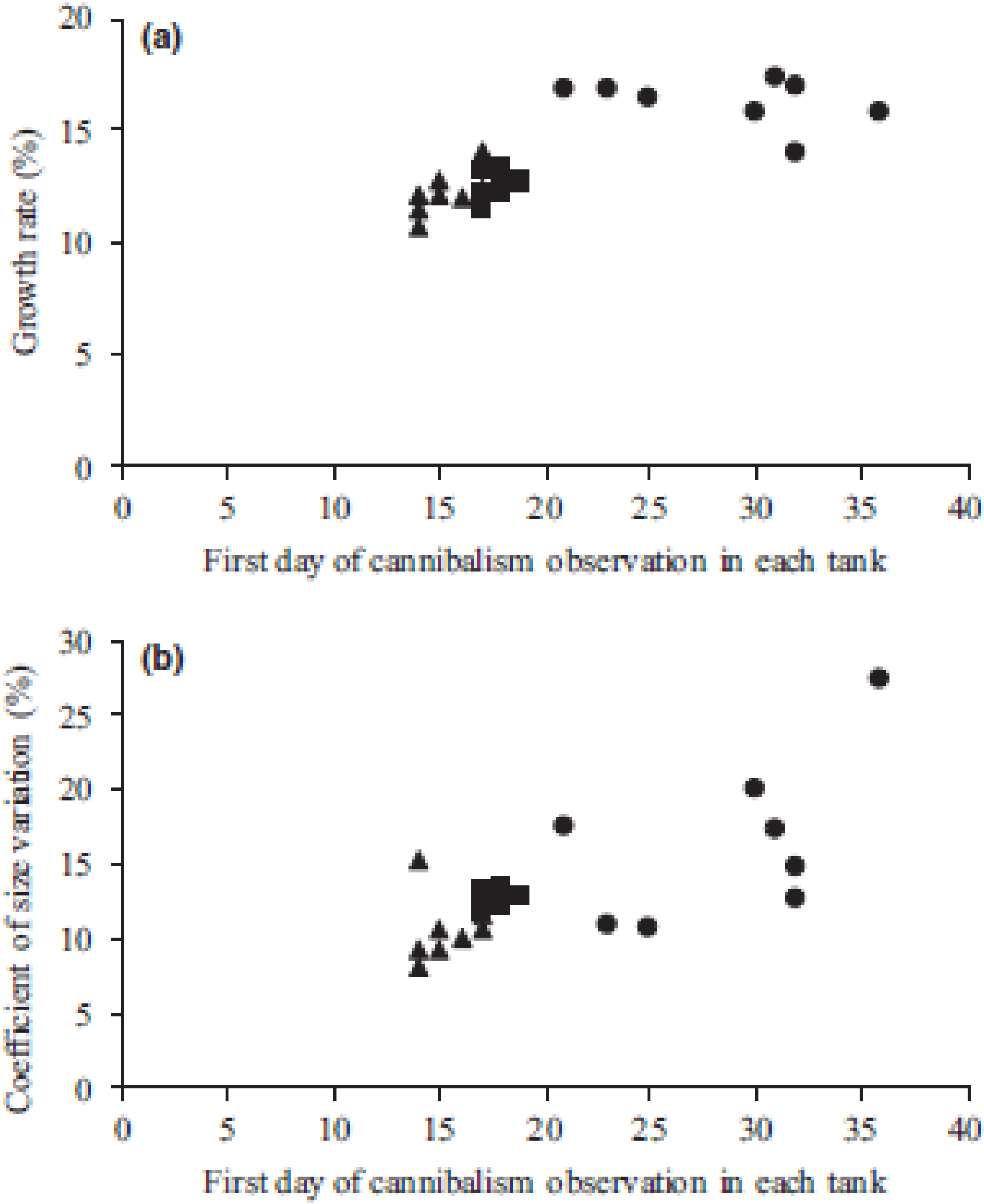

Figure 2. Spearman correlations between the first day of cannibalism for pikeperch in each tank (n = 23), and (a) the growth rate, and (b) the coefficient of variation of size. Circles represent the eight tanks of 2015, triangles those of 2016 and squares the seven tanks of 2017

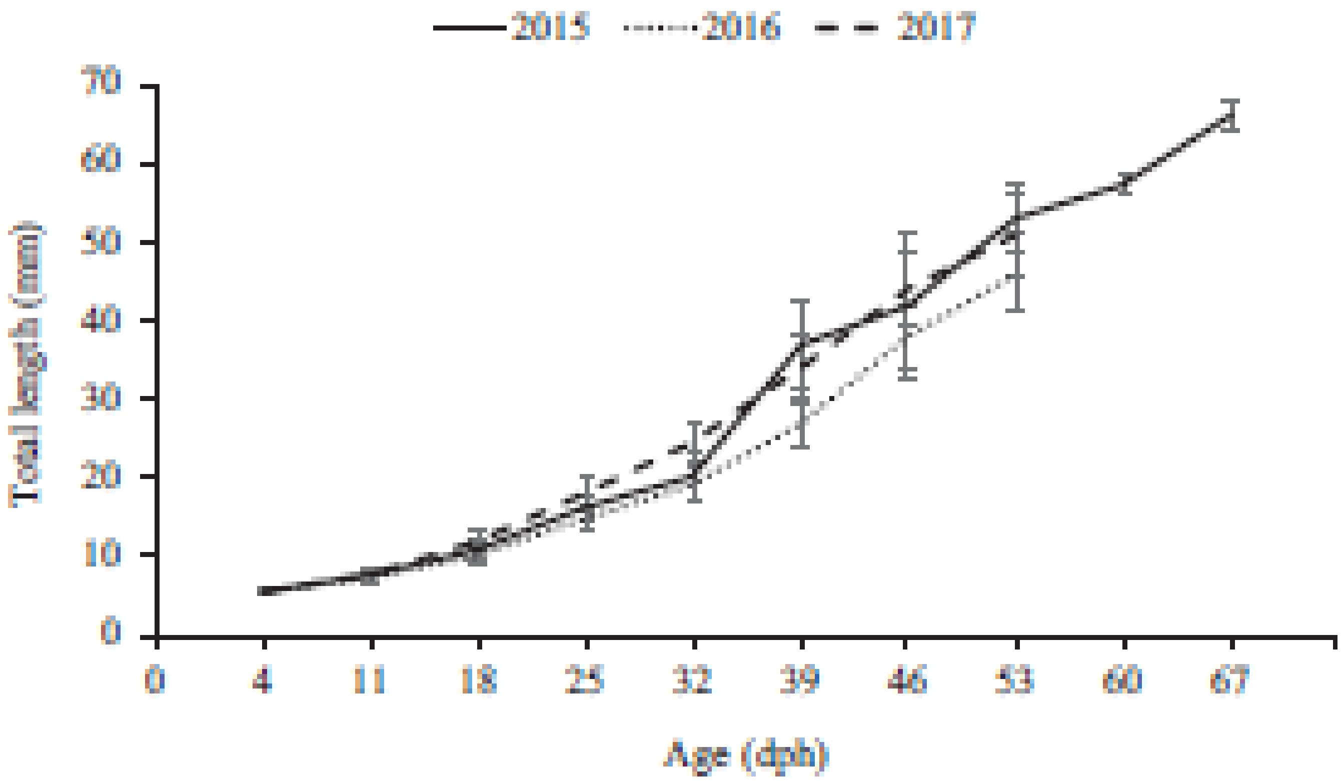

Figure 3. Growth curves (mean ± SE) of pikeperch calculated for the 3 years of experimentation considering the number of days post-hatching (age of larvae and juveniles)

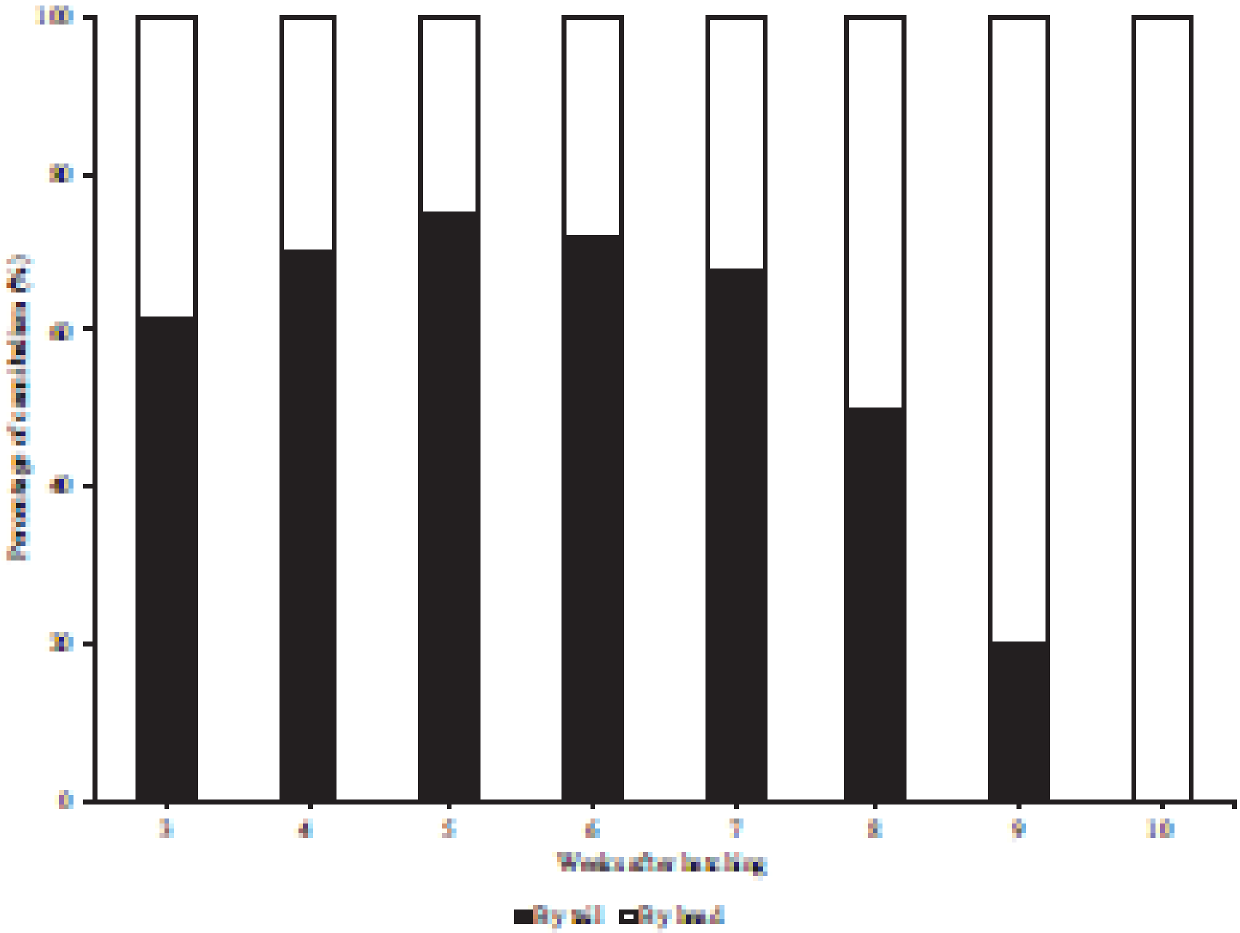

Figure 4. Cumulated percentage of cannibalism cases during the 3 years of experimentation for each week after hatching in function of the type of ingestion (type I or II)

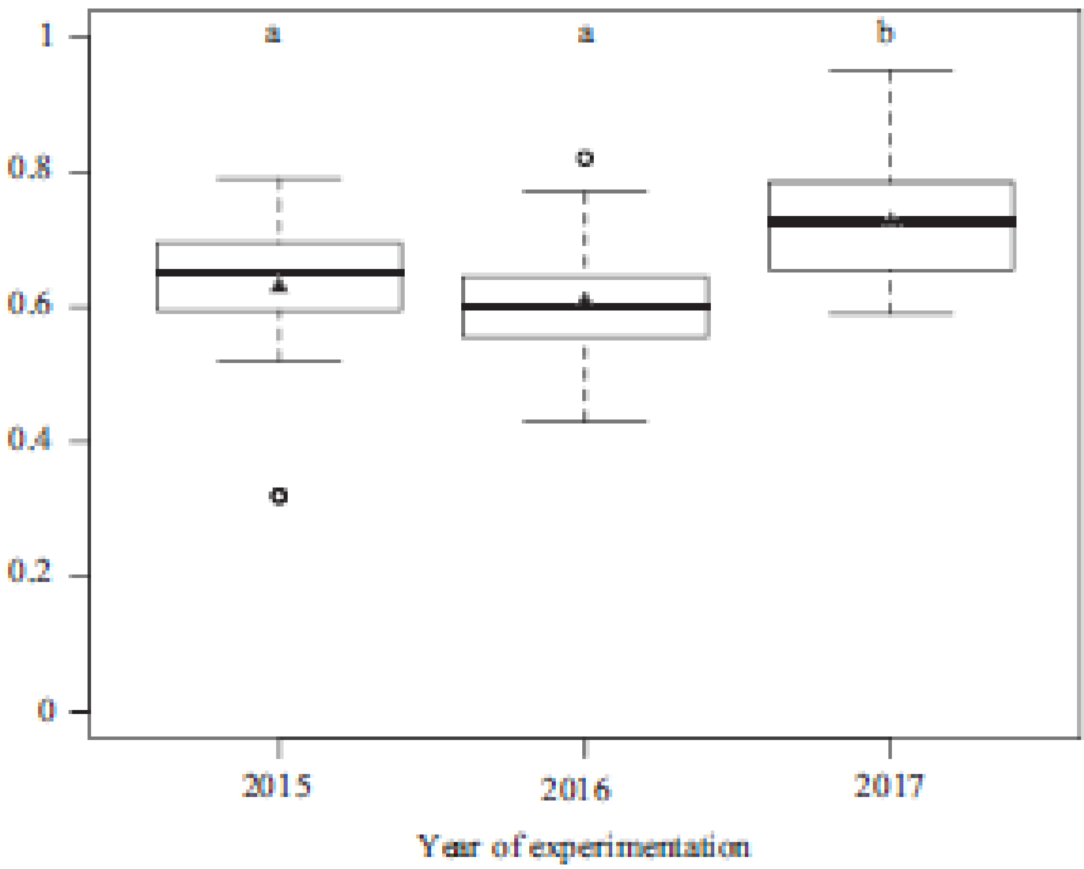

Figure 5. Size ratio between the cannibal and its prey considering the 3 years of experimentation with pikeperch. Size ratio was calculated with 31 (2015), 19 (2016) and 40 (2017) couples of cannibal/prey. The black line is the median, the black triangle is the mean, white dots are outsiders, and top and bottom lines are the first and third quartiles. Different letters indicate significant differences between years at $p < 0.05$ using Tukey's post hoc test

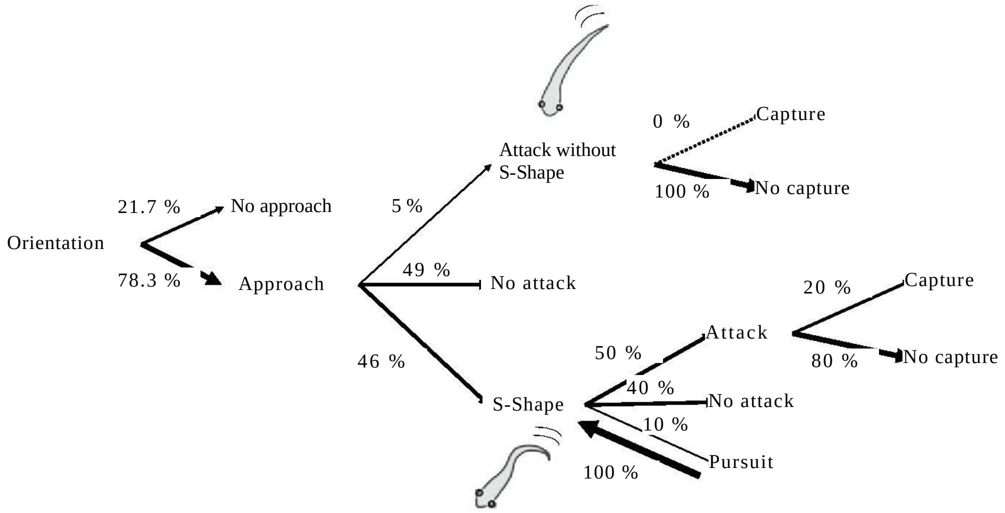

Figure 6. Ethogram of behavioural sequences of a cannibal to capture a conspecific among three larvae of the same age, but smaller proposed during the recorded behavioural test. See Table 2 for definitions

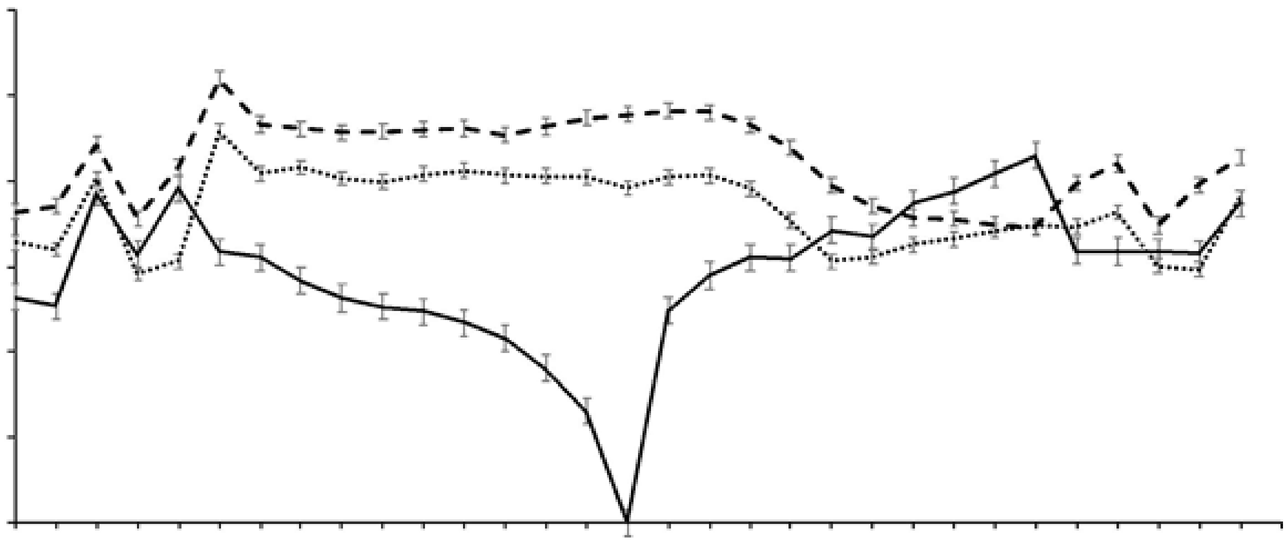

Figure 7. Mean ± SE (cm) of distances between cannibals and conspecifics before and after an attack (represented by 0 (s) on X-axis) on a recorded behavioural test. Larva called 'Larva 1' was the larva, which was attacked

5. References

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