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Is a cannibal different from its conspecifics? A behavioural, morphological, muscular and retinal structure study with pikeperch juveniles under farming conditions.

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Highlights

- Visual processing was linked to the onset of cannibalism in pikeperch.
- Two retinal layers (ganglion cell layer and inner nuclear layer) were thicker for cannibals.
- The non behavioural differences could explain asynchrony in the onset of cannibalism.
- Cannibalism would be driven by rearing condition-dependent individual development.

Abstract

Cannibalism is defined as the act of killing and consuming the whole, or major part, of an individual belonging to the same species, irrespective of its stage of development. Intra-cohort cannibalism in fish larval or juvenile stages, which is a major economic problem, has been widely studied in captive fish populations. In our study, we investigated the influence of animal personality (with cross-maze and conspecific choice tests) on intra-cohort cannibalism using pikeperch *Sander lucioperca* as a model species. Furthermore, we investigated the morphological (geometric morphological analysis) and anatomical (histological analysis of retinal and muscle tissue sections) differences between cannibal (C) fish (TL = 34.6 ± 9.4 mm, n = 25) and conspecific fish randomly sampled from rearing tanks, herein called 'potential non-cannibal fish' (PNC) (TL = 31.4 ± 10.5 mm, n = 42). We did not find any behavioural differences (swimming activity, exploration, conspecific choice) between cannibal and potential non-cannibal fish that could explain asynchrony in the onset of cannibalism. Moreover, we did not observe any morphological differences between the two groups (C and PNC fish). However, we did detect anatomical differences in two retinal layers (ganglion cell layer and inner nuclear layer) that were thicker for cannibals. These two layers are involved in the collection of information by photoreceptors and allow the shapes, colours and movements of objects to be detected in the water column. The onset of cannibalism therefore appears to be linked to environmental condition-dependent individual development, with some individuals exhibiting precocious anatomical, and probably physiological, development, rather than to individual personality.

Keywords: cannibalism; multi-trait approach; fish; freshwater species; *Sander lucioperca*

1. Introduction

Cannibalism could be considered as a predatory strategy that involves capturing, killing and eating a part or the whole of individuals of the same species (Polis, 1981; Elgar and Crespi, 1992). It has been recorded in more than 500 vertebrate species (Soulsby, 2013). Among them, about 200 species are fishes (Smith and Reay, 1991), for which cannibalism has been reported in the wild and under farming conditions. The most commonly farmed fish species are piscivorous predators: out of 26 fish species commonly found in rearing systems, 18 are classified as carnivorous and piscivorous and only 8 are considered as omnivorous and do not feed on other fish species). In the wild, cannibalism can be considered as 'a lifeboat mechanism' defined as the survival of a cannibalistic population when food for the adults is too scarce to support a non-cannibalistic population (van den Bosch et al., 1988). Under farming conditions, where yet there is usually no food limitation since fish are fed *ad libitum*, larviculture performance is also often affected by intra-cohort cannibalism. Intra-cohort cannibalism in fish larval or juvenile stages has been widely studied in captive fish populations since such a behaviour is a major economic problem in farmed piscivorous species (Naumowicz et al., 2017; Pereira et al., 2017).

In order to explain intra-cohort cannibalism, size heterogeneity has been largely documented. Indeed, it is known that the cannibal is larger in size than its prey, reflecting differences in development. Two types of cannibalism have been described during fish larval and early juvenile development stages: Type I, which occurs generally during the larval stage, does not imply any size difference between the cannibal and its prey, which is not entirely ingested (Baras, 2013); Type II, which occurs at a later stage, is characterized by the entire consumption of the prey and greater size heterogeneity between the cannibal and its prey (Baras and Jobling, 2002). However, even though size heterogeneity facilitates cannibalism, and many studies have attempted to identify environmental or population factors that could affect it (for review, see Pereira et al., 2017). To our knowledge, however, very few studies have used a multi-trait approach to identify the traits that could be involved in the onset of cannibalism (e.g. Baras and Jobling, 2002).

In fish, cannibalism implies performing as a piscivorous predator with particular characteristics. First, it is necessary for a piscivorous fish to have physical abilities to detect (i.e. visual cues), pursue and

capture (developing jaw and trunk musculature), and digest (digestive enzyme function) a prey (Sakakura and Tsukamoto, 1996; Cahu and Zambonino Infante, 2001). It is easier for a cannibal because its prey, belonging to the same species, has the same movement abilities. Finally, for fish, the mouth size is a limiting factor for ingesting a prey, particularly for Type II cannibalism (Hetch and Appelbaum, 1988; Sogard and Olla, 1994). The size of the mouth could be a consequence of an allometric growth of the mouthparts (Baras and Jobling, 2002). In Type II cannibalism, the mouth must be large enough to ingest the prey headfirst, in order to avoid the spiny dorsal fin and pectoral rays that may cause injury or even death of the cannibal (Qin et al., 2004). These morphological and anatomical characteristics have been largely studied by comparing cannibals and their prey (Baras and Jobling, 2002; Baras, 2012), and the former authors have concluded that the cannibals have a larger mouth gape, stronger musculature and better vision than their prey (Baras, 1998; Baras, 2012). However, the behavioural differences between cannibals and conspecifics have received less attention, which may be due to the difficulties in setting up these kinds of experimental designs.

Behavioural traits of personality allowed to reveal consistent behavioural differences over time and/or in different contexts between individuals of the same population (Koolhaas et al., 1999; Sih et al., 2004; Réale et al., 2007). This concept of personality makes the difference between bold individuals, which take risks, and are more aggressive, and shy individuals, which are less active and more sociable. Within a population, individuals may be classified between these two extremes of behavioural profiles along the bold-shy axis (Bell, 2007). Personality plays an important role in the onset of several behaviours during ontogeny, among which foraging performance in birds (Kurvers et al., 2009; Patrick and Weimerskirch, 2014), mammals (Mella et al., 2015) and fish (Cutts et al., 1998; 2001; Wilson and McLaughlin, 2007). In fish, foraging abilities are correlated to individual's level of activity in the Atlantic Salmon *Salmo salar L.* (Cutts et al., 1998), and the Arctic charr *Salvelinus alpinus L.* (Cutts et al., 2001). In this context, it seems that bold individuals were the best performing foragers (Conrad et al., 2011). In light of these findings, as personality could influence foraging performances, we can hypothesize that in a population, cannibals and non-cannibals lie at different points on the bold-shy continuum.

In our study, we investigated the influence of animal personality on intra-cohort cannibalism using

pikeperch *Sander lucioperca* as a fish model. This freshwater fish exhibits a high degree of cannibalism (<50%) under intensive rearing conditions (Molnár et al., 2004; Kestemont et al., 2007). However, it is necessary to understand what may differentiate a cannibal (C) from the other conspecifics (called 'potential non-cannibal fish' (PNC) in this study) to regulate this major bottleneck in pikeperch farming (Kestemont et al., 2007; 2015). Cannibalism mainly occurs in early development, when pikeperch larvae are between 14 and 17 days post-hatching (dph), and there is a first peak between 32 and 42 dph at 20°C (Colchen et al., 2019). In order to complete the comparison between cannibals and their conspecifics, we also investigated morphological (body shape by geometric morphometric analysis) and anatomical differences (retina and muscle development by histological analysis). Thereby, we wanted to determine whether there were behavioural, morphological and/or anatomical differences between cannibals and their conspecifics. Using behavioural, morphological and anatomical traits evolving during the ontogenetic development to compare cannibal fish with potential non-cannibal fish of pikeperch, we can hypothesize that cannibals should (i) be bolder, (ii) have more developed caudal musculature and a larger gape size, and (iii) have better visual abilities. These predictions mean that, if cannibalism depends on animal personality, some individuals will never be cannibals in this population.

2. Materials and methods

2.1. Rearing of the fish

The experiment was carried out at the Aquaculture Experimental Platform (AEP, registration number for animal experimentation C54-547-18) belonging to the URAFPA lab and located at the Faculty of Sciences and Technologies of the University of Lorraine (Nancy - France). Eggs came from two mature females (2.7 and 2.9 kg) previously injected with sGnRHa (25 and 50 $\mu g.kg^{-1}$, respectively; ovaRH, Syndel laboratories, Ltd) and fertilized by one male in a fish farm (SARL Asialor, Pierrevillers, Moselle, France). At their arrival at the AEP on 1st February 2016, just before hatching, the fertilized eggs were transferred into eight 700 L tanks where larvae hatched and developed until 52 dph. Artificial lighting (50 lx) followed a 12L/12D cycle with light on from 08:00 to 20:00 with 30 min simulation of dawn and dusk. The water was maintained at 16°C until hatching and then increased by 1°C per day until

reaching 20°C. Water parameters (mean ± standard deviation, SD) were measured once or twice a week: dissolved oxygen = 8.0 ± 0.5 $mg.L^{-1}$, pH = 6.9 ± 0.8, salinity = 0.2 ± 0.05 $g.L^{-1}$, ammonia (NH_4^+) = 5.3 ± 1.0 $mg.L^{-1}$ and nitrite (NO_2^-) = 0.08 ± 0.07 $mg.L^{-1}$. Fish were fed seven times per day between 8:30 and 17:30 during light period every one and a half hours. They were fed live prey and a commercial inert feed as follows: firstly, nauplii of *Artemia* (550-600 µm, Sep-Art *Artemia* cyst) from 4 to 16 dph, then Larviva PROWEAN 100, 300, 500, 700 µm (BIOMAR®, France) and INICIOplus 0.8 mm (BIOMAR®, France), following the protocol used by Schram and Philipsen (2003) for weaning.

2.2. Sampling of the fish

Our aim was to compare traits (anatomical, morphological and behavioural) between cannibal and potential non-cannibal fish, as we can be sure that at the sampling time fish were not cannibal, but we have no information about their attack activity on conspecifics before the sampling. In order to detect fish displaying cannibalism, 5 min observations were made at each tank every morning (after 9:40 a.m.) from 10 to 52 dph. When a case of cannibalism was observed, the observer attempted to capture the cannibal with a dip net. Out of 192 observed cases of cannibalism, 25 cannibals were sampled (total length (mean ± SD) = 34.6 ± 9.4 mm). To allow for comparison, each time we captured a cannibal, we also captured one or two fish randomly (potential non-cannibal fish) of similar size (total length (mean ± SD) = 31.4 ± 10.5 mm). For these potential non-cannibal fish (n = 42), we verified that they were not eating a conspecific or that there was no other fish in their digestive tube. All fish (potential non-cannibals and cannibals) were transferred into individual cages (15 x 12 x 11 cm) in a 52.5 L aquarium (50 x 35 x 30 cm) for 24 hours before their use in behavioural tests. The light cycle and the water temperature were the same as those in the 700 L tanks.

2.3. Behavioural tests

To establish the personality of pikeperch larvae and early juveniles, two behavioural tests were used: a cross-maze test to analyse swimming activity, exploration and boldness, and a choice test between

conspecifics or not to analyse the relationship behaviours.

The cross maze apparatus (16 x 5 cm with 2.5 cm of water) consisted of four arms, divided into five zones (**Fig. 1A**) and placed on a translucent table with a light below (50 lx). Fish were tested one by one. Each fish was placed in an acclimatization zone (7 x 5 cm) separated from the maze by a vertical divider (**Fig. 1A**). After a 30 min acclimatization period, the divider was removed and fish behaviour was video recorded for 20 min Several independent behavioural measures were analysed: i) the individual latency to emerge from the acclimatization zone (E_LS) (in seconds), if a fish did not emerge from the acclimatization zone (AZ) during the 20 min period, a latency period of 1,200 s was attributed. ii) the total number of visited zones (E_NVZ) and iii) swimming activity (E_SA) (in seconds). All variables were analysed over the entire 20 min period except E_SA, which was calculated over three periods of time: from the 1st to the 3rd minute, from the 9th to the 11th minute and from the 17th to the 19th minute (adapted from Pasquet et al., 2015).

The conspecific choice tests were realized in the same type of device. The apparatus consisted of four arms divided into five zones (**Fig. 1B**). Three arms of the cross-maze were separated from the central zone by transparent dividers perforated with small holes (less than 1 mm in diameter). These holes allowed constant water flow between the arms of the cross-maze. The three zones contained zero, three, and six pikeperch larvae of the same age and reared under the same conditions as the tested fish, and unknown from the tested fish. The main goal was to determine if a cannibal prefer stay near conspecifics or not and if the size of the group impact the choice. Each tested fish was placed in an acclimatization zone (7 x 5 cm) separated from the maze by a vertical divider (**Fig. 1B**). After 30 min of acclimatization, the divider between the acclimatization zone and the cross-maze was removed. The behaviour of the tested larvae was video recorded for 20 min. Five variables were taken into account during this period: the latency to emerge from the acclimatization zone (S_LS) (in seconds) and the time spent close (less than 1 cm from the divider) to the groups of zero (S_Z0), three (S_Z3) or six conspecifics (S_Z6), and aggressive behaviours (e.g. the attack attempts against the transparent divider). This last behaviour was never observed, consequently only the first four variables have been considered in the statistical analyses.

2.4. Geometric morphological analysis

After the behavioural tests, all larvae (cannibal and potential non-cannibal fish) were euthanatized with an overdose of tricaine methane-sulfonate (MS-222, Sigma; 240 $mg.L^{-1}$). The right side of each fish was photographed with a digital camera (Panasonic, DMC-FZ18). For each photographed fish, total length (TL) was measured and the coordinates of 15 morphological landmarks (LMs) were recorded (**Fig. 2**), using tpsDig 2.16 (Rohlf, 2008). The scale was calibrated for each photograph. The LMs were selected to provide a definition of the fish morphology in which the LMs are given as *x* and *y* coordinates. The distances and angles between specific LMs were determined from their coordinates. The LMs were digitized on the lateral side of each fish by the same observer (**Fig. 2**). Body shape was analysed using LM-based geometric morphometric methods (Rohlf, 1990; Bookstein, 1991). The LMs were superimposed to have a common centroid and rotated to minimize the distances between corresponding LMs. Once all the fish were aligned, the mean configuration of LMs was computed (consensus or reference shape). At that time, LM 11 and 12 were excluded because of the inaccuracy of their positions for each fish, and so were all spine-malformed fish (n = 29) and fish with one LM missing (n = 5). Finally, we compared the geometrical morphologies of 16 cannibal fish and 17 potential non-cannibal fish. Fish were projected to a tangent space by orthogonal projection where the distances between shapes were linear functions. This process then permitted the use of multivariate statistical methods to evaluate shape variation. Principal component analysis (PCA) was performed after computing the variance-covariance matrix of the procruster shape coordinates and projecting the data onto the corresponding eigenvectors. All geometric morphometric-related analyses were carried out with R (version 3.5.3) with 'shapes' (Dryden, 2018) and 'factoextra' (Kassambara and Mundt, 2017) packages. Thin-plate spline deformation grids of fish body shape to compare cannibal fish with potential non-cannibal fish were generated on MorphoJ® software (Klingenberg, 2011).

2.5. Histological data

After taking pictures of each fish for the morphological analysis, larvae were fixed in 10% buffered

formalin (Sigma-Aldrich, HT501128-4L). Twenty-five cannibals and 18 potential non-cannibal fish were used for this histological study, whose steps were all conducted at IRTA (Aquaculture Program, Sant Carles de la Rapita, Spain). Two types of fish tissue were analysed: the eyes, because vision is key to the development of predatory behaviour, and the muscles, which are essential for mobility to pursue and capture prey. To perform the various analyses, an eye and caudal muscles (vertical cut just behind the anus) were collected from each larva. All samples (eyes and muscles) were dehydrated with graded series of ethanol (from 50% to 100%) and embedded in paraffin with a Histolab ZX-60Myr automatic tissue processor (Especialidades Médicas MYR SL, Spain). Then, paraffin blocks were prepared in an AP280-2Myr station and cut into serial sagittal sections (3 µm thick) with a Microm HM automatic microtome (Leica RM2155 Microsystems Nussloch GmbH, Nussloch, Germany). Paraffin-embedded eyes and muscle sections were kept at 40°C overnight. Then, samples were deparaffined with graded series of xylene and stained by means of Hematoxylin (5 min) and Eosin (5 min). Stained sections were examined using an upright optical light microscope (Nikon Eclipse Ni-U) at 40x magnification (Nikon France, Champigny-sur-Marne, France).

For each cannibal (n = 19) and potential non-cannibal (n = 10) fish, the thickness of the seven retinal layers (Ganglion Cell Layer (GCL), Inner Plexiform Layer (IPL), Inner Nuclear Layer (INL), Outer Plexiform Layer (OPL), Outer Nuclear Layer (ONL), Photoreceptor Layer (P) and Pigmentary Epithelium (PE)) was measured using Nikon BR software (**Fig. 3**). Furthermore, the number of muscle fibres was counted on four defined zones for each cannibal (n = 16) and potential non-cannibal (n = 9) fish, and maximal and minimal diameters measured on 40 fibres for each fish (**Fig. 3**). The muscle fibres were classified as large (> 30 µm) or small (< 30 µm) depending on their diameters.

2.6. Statistical analysis

For the behavioural variables, we calculated the mean and standard deviation (SD) to assess the variability of pikeperch behavioural responses. For each variable of interest, in each group (cannibal and potential non-cannibal fish), inter-individual variability was assessed by calculating the coefficient of variation (CV, % = SD/mean x 100) as a normalized measure of dispersion. We checked the normality

of the data (Shapiro-Wilk test, R Core Team, 2017) and the homogeneity of the variances (Levene's test; Fox and Weisberg, 2016). For each group, we assessed the correlation between the values of each variable of interest between fish with Spearman correlations. Furthermore, comparisons of the same variables between cannibal and potential non-cannibal fish were carried out with a parametric Student's t-test for independent data. A multifactorial analysis (PCA) was conducted taking into account all the behavioural variables (FactoMineR; Husson et al., 2019) and all the individuals were projected on the graph of the PCA analysis. Data analyses were performed using R software (version 3.0.3) and the level of significance used in all tests was $P < 0.05$.

For the morphological analysis, all the coordinates of the landmarks obtained on cannibal and potential non-cannibal juveniles were analysed with a generalized procrustes analysis (GPA), with MorphoJ® software (Klingenberg, 2011). This procedure allowed us to eliminate all variations due to translation, rotation and scale effects. Then, the standardized coordinates obtained with this method were analysed with the Relative Warp Analysis (Rohlf, 1993), which is a principal component analysis (PCA).

For the histological parameters, as the data fitted the normality and the homogeneity of the variances, we used an ANCOVA, taking the TL of each individual as covariate. The analysis was performed with R software (version 3.5.3) and the level of significance used in all tests was $P < 0.05$.

2.7. Ethical note

During all procedures, we took care to minimize handling and stress as much as possible for the study animals. All fish treatments and procedures used in this study were in accordance with the guidelines of the Council of the European Union (2010/63/UE) and the French Animal Care Guidelines (Animal approval No. APAFIS#1813-2015111618046759v2).

3. Results

3.1. Behavioural analyses of each group: cannibal and potential non-cannibal fish.

3.1.1. Inter-individual variability

The analysis of coefficients of variation revealed considerable inter-individual variability in the variables measured in both tests carried out for cannibal and potential non-cannibal fish (**Table 1**), indicating that there was a high level of behavioural variability in both groups of fish.

3.1.2. Correlations between behavioural variables

Regarding cannibal fish, swimming activity was positively correlated to the total number of visited zones and the time spent near three conspecifics (S_Z3) (**Table 2**). Then, the time spent near six conspecifics was negatively correlated to the latency to emerge from the acclimatization zone (S_LS) in the conspecific choice test and positively correlated to the time spent near three conspecifics (S_Z3). Finally, the total number of visited zones was positively correlated to the time spent near three conspecifics (**Table 2**). For potential non-cannibal fish, swimming activity was positively correlated to the total number of visited zones, to the time spent near the zone without conspecifics (S_Z0), to the time spent near three conspecifics (S_Z3) and to the time spent near six conspecifics (S_Z6) (**Table 2**). Then, the total number of visited zones was positively correlated to the time spent near three and six conspecifics (**Table 2**). Finally, the latency to emerge from the acclimatization zone in the conspecific choice test was negatively correlated to the time spent near the zone without conspecifics (S_Z0), to the time spent near three conspecifics (S_Z3) and to the time spent near six conspecifics (S_Z6) (**Table 2**).

3.2. Comparison between cannibal and potential non-cannibal fish.

3.2.1. Behavioural test analysis

In the cross-maze test, the swimming activity (E_SA) of cannibal fish was similar to that of potential non-cannibal fish ($t = 1.22$; $df = 65$; $p = 0.23$; **Table 1**). The time to emerge from the acclimatization zone (E_LS) was similar between cannibal and potential non-cannibal fish ($t = 0.64$; $df = 65$; $p = 0.52$; **Table 1**). Cannibal fish visited statistically as many maze zones (E_NVZ) as potential non-cannibal fish did ($t = 0.95$; $df = 65$; $p = 0.34$; **Table 1**).

Regarding the conspecific choice test, cannibal fish emerged nearly as rapidly as potential non-cannibal fish from the acclimatization zone (S_LS) ($t = -1.06$; $df = 65$; $p = 0.29$; **Table 1**). Values of S_Z0 ($t = -$

0,4; df = 65 ; p = 0.69; **Table 1**), S_Z3 (t = -0.11; df = 65 ; p = 0.91; **Table 1**) and S_Z6 (t = 1.32; df = 65; p = 0.2; **Table 1**) were similar between cannibal and potential non-cannibal fish.

When all these variables were analysed using a PCA, the first two axes of the PCA represented 56.4% of the total variance (first axis = 39.0%, second axis = 17.4%; **Fig. 4**). The first axis contrasted the time to emerge from the acclimatization zone in both behavioural tests from swimming activity and conspecifics choice variables. This axis highlighted a bold-shy continuum with fish, which emerged rapidly from the acclimatization zone and were more active and attracted by conspecifics. The second axis was represented by the time spent near the zone without conspecific fish. Swimming activity (E_SA) was positively correlated to the total number of visited zones (E_NVZ) ($r = 0.81$; $p < 0.001$; **Fig. 4A**) and to the time spent near zones with conspecifics (S_Z3: $r = 0.44$; $p < 0.001$ and S_Z6: $r = 0.33$; $p < 0.01$; **Fig. 4A**). The time to emerge from the acclimatization zone (S_LS) in the conspecific choice test was negatively correlated to swimming activity (E_SA) ($r = - 0.32$; $p < 0.01$; **Fig. 4A**) and to the time spent near zones with conspecifics (S_Z3: $r = - 0.34$; $p < 0.01$ and S_Z6: $r = - 0.34$; $p < 0.01$; **Fig. 4A**). Projection of individuals on axes highlighted that cannibal and potential non-cannibal fish were equally distributed on both axes and consequently did not demonstrate a difference on behavioural traits (**Fig. 4B**).

3.2.2. Geometric morphological analysis

The PCA of aligned coordinates for the 13 selected landmarks yielded 10 principal components (PCs; **Fig. 5A**). The first two axes (PC1 and PC2) explained 38.1% and 22.8% of the body phenotypic variability, respectively, which accounted for 60.9% of the total variance. In contrast, the third axis (PC3) only accounted for 13.4% of the variance and, consequently, this axis and the subsequent ones were not included in further analyses. In addition, the morphospace (ellipses) from each group revealed no clear separation between cannibal and potential non-cannibal fish, with a total overlap between groups (**Fig. 5B**). A transformation grid for visualizing changes in body shape for cannibal and potential non-cannibal fish did not reflect changes in both relative shifts and body shape in pikeperch juveniles (**Fig. 5C; Supplementary materials**).

3.2.3. Histological analyses

Regarding the retina, the ganglion cell layer (GCL) and the inner nuclear layer (INL) were much thicker in cannibal fish than in potential non-cannibal fish (**Fig. 3; Table 3**). There were no marked differences in the thickness of all other layers between the two groups (**Table 3**), and fish size (TL) had no effects on all morphological parameters taken into account ($p > 0.05$). Considering trunk musculature, cannibal and potential non-cannibal fish exhibited similar diameters and numbers of muscle fibres. Both parameters were however found to be significantly affected by fish size (TL) ($p < 0.05$; **Fig. 3; Table 3**).

4. Discussion

Inter-individual variability, which is behaviourally, anatomically, morphologically and physiologically observable, characterizes the pikeperch population analysed in our study. Such variability is clearly visible under farming conditions leading for example to size heterogeneity, which may be regulated by control of environmental parameters. Under farming conditions, the existence of intra-cohort cannibalism has been shown repeatedly in various pikeperch populations (Ljubobratović et al., 2015; Steenfeldt, 2015; Król and Zakęś, 2016; Molnár et al., 2018). In our study, we investigated behavioural, morphological and anatomical potential differences between individuals of a population in order to explore such differences between a cannibal and its conspecifics and, consequently, the traits that could be involved in its differentiation from other individuals of the population.

It is well known that rearing factors such as population density, light intensity, feeding frequency, water turbidity or presence of alternative prey could affect the cannibalism rate (for review, see Naumowicz et al., 2017; Pereira et al., 2017), and result in individual behaviour changes (Coppens et al. 2010). These studies have demonstrated a range of individual responses directly influenced by environmental stimuli that could be associated with behavioural plasticity. Indeed, cannibals react to environmental stimuli by decreasing or increasing their cannibalism rates (Smith and Reay, 1991; Hecht and Piennar, 1993; Folkvord, 1997), but it seems that no biotic or abiotic factors can eradicate cannibalism (Baras and Jobling, 2002). Rather than focusing on individual behavioural plasticity to understand why an

individual becomes a cannibal at a given time of its development, our study looked at the differences in several traits between cannibals and other individuals of the population. Thus, it has provided results on several parameters (behaviour, morphology and anatomy) to highlight differences between cannibals and their conspecifics of a given population in order to evaluate whether a specific trait could differentiate them.

The study of personality allowed us to work on behavioural differences between individuals and look for differences between cannibal fish and their conspecifics (Torres et al., 2017; Meager et al., 2018). In our study, we demonstrated that there are no behavioural differences between cannibals and conspecifics. For several years, a number of behavioural differences observed under fish farming conditions have been explained by the personality paradigm, such as self-feeding triggering (Ferrari et al., 2014), aggressive behaviour (Martins et al., 2012), susceptibility to infection (Kittilsen et al., 2009a, b) or resuming feeding after transfer to a new tank (Vaz-Serrano et al., 2011). To our knowledge, no previous studies have compared the personality of cannibals with that of conspecifics, but several studies characterized personality first and then observed cannibalism rates in different groups composed of shy, bold or mixed individuals (Sih et al., 2004; McGhee and Travis, 2010; Réale et al., 2010; Colléter and Brown, 2011; Dahlbom et al., 2011; Mesquita et al., 2016). A recent study on catfish larvae *Lophiosilurus alexandri* showed that when sorting larvae by personality traits, there was a higher cannibalism rate in bold and mixed groups than in shy groups (Torres et al., 2017), thus suggesting a link between personality and cannibalism. However, our results were in disagreement with those of the above-mentioned authors, since we did not find any differences in personality traits associated with cannibalism. In our study, the non-difference between the two groups could be explained by the fact that some cannibals may have been mistakenly classified in the potential non-cannibal group because they did not display cannibalistic behaviour at the time of observation. We do not know if all potential non-cannibal fish had already been a cannibal or not. However, we were able to demonstrate a personality continuum in our pikeperch population with extreme personality as shown by a uniform repartition of fish personality scores on the first axis of the PCA. These results were in agreement with those previously reported in a study conducted on the same species by our research group (Colchen et al., 2017). Therefore, even if potential non-cannibal fish were not all non-cannibals, assuming that

cannibals have a different personality from the others, they should be found on either side of the point cloud when projecting all individuals on the PCA, meaning that cannibals have extreme personality (bold or shy). As a second argument, there were potentially few cannibals in the population with 30.9 ± 8.6 jumpers (the biggest fish in a population) per week (in 700 L tanks, with initial densities of 100 larvae.L^{-1}, unpublished data). It was therefore unlikely that we had captured a large number of cannibals when randomly collecting potential non-cannibal fish. So, we could hypothesize that, in pikeperch, personality is not a major characteristic for distinguishing cannibals from other individuals, but it could be associated with anatomical and/or morphological variables.

Cannibalism may not occur at the same time in all individuals because it is a piscivorous behaviour and, in the particular case of larvae, the shift from a planktivorous to a piscivorous diet requires morphological, anatomical and physiological modifications (Buijse and van Densen, 1992; Galarowicz and Whal, 2005; Hart and Ison, 1991; Kaji et al., 2002; Mittelbach and Persson, 1998). It has been shown that the onset of predation in pikeperch larvae is not synchronic for all individuals in a population and occurs from three to six weeks after hatching when larvae are reared at 20°C (Colchen et al., submitted). As cannibalism is an intra-specific predation phenomenon, its onset, like that of predation, was not synchronic between individuals and therefore could explain the non-behavioural differences between cannibal and potential non-cannibal fish (fish sampling between 10 and 52 dph). In pikeperch, cannibalism may have more to do with the onset of piscivory than with personality differences between individuals in a population.

Furthermore, our results demonstrated that there were no morphological differences between cannibals and other individuals in the population. In other species, morphological differences between cannibals and non-cannibals have already been shown such as in salamander (Pfennig and Collins, 1993). At the early juvenile stage, coloration was the only missing pattern for our pikeperch specimens to look like adults, they were therefore assumed to be already morphologically developed. Thus, we could hypothesize that at larval stages morphological differences (e.g. mouth development) may be a good visual guide for distinguishing a cannibal from another conspecific, but at juvenile stages visual differentiation is not possible because fish are fully morphologically developed. Moreover, there were no differences in trunk musculature development between cannibal and potential non-cannibal fish.

Under farming conditions, where high animal density is common practice, we can assume that capture conditions may not be complicated, so fish do not need to have developed musculature, they just have to catch smaller, deformed or sick fish. However, there appeared to be anatomical differences in the retina between cannibal and potential non-cannibal fish. Although there was the same number of layers for the two groups, two layers were thicker in cannibals: the ganglion cell layer and the inner nuclear layer. These two layers are involved in the collection of information by photoreceptors and allow shapes, colours and movements to be detected, which is useful for prey detection. These retina layers may therefore play an important role in prey capture. We can speculate that cannibals had better visual abilities with high detection accuracy. Fish have retinas that keep growing after embryogenesis (Lyall, 1957 ; Fernald, 1989), so the retinal layers increase in size continuously throughout development. The inner nuclear layer has the cell bodies of Müller cells (Mack et al., 1998), which are able to undergo a change and multiply in order to maintain glial functions and improve visual performance in growing fish (Mack et al., 1998). When larvae grow up, the density of Müller cells decreases but their total number increases compensating the thickening of the inner nuclear layer to keep visual performance at its maximum (Mack et al., 1998). It seems that in pikeperch larval cannibals this compensation was maximal allowing for better vision. Piscivory feeding habits (including cannibalism) require more refined detection and sensory-motor abilities compared to planktivory and microzooplankton feeding habits (Smith and Reay, 1991; Margulies, 1997). In pikeperch cannibals the ontogeny of the visual system appeared to be more advanced, which may have contributed significantly to a rapid improvement in their predatory abilities and the development of early piscivory, and thus to cannibalism. In order to complete the present study, it could also be interesting to look at differences in the development of the digestive systems of cannibal and potential non-cannibal fish to assess their abilities to digest fish prey.

5. Conclusion

Under the present experimental conditions there were no behavioural differences between cannibal and potential non-cannibal fish that could explain asynchrony in the onset of piscivory in pikeperch. Furthermore, no external morphological differences were found between the two groups. However, we did observe anatomical differences in the development of the eyes with two thicker retinal layers

(ganglion cell layer and inner nuclear layer) in cannibals, which are involved in the collection of information by photoreceptors and allow the shapes, colours and movements of objects to be detected in the water column. These findings have led us to conclude that cannibalism would be driven by rearing condition-dependent individual development, with some individuals exhibiting precocious anatomical and probably physiological developments, rather than by individual personality traits governed by genetic determinism (Stamps, 2007).

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Figure captions

Figure 1: Experimental set-up for behavioural tests. (A) Cross-maze test with an acclimatization zone (AZ) and four zones for exploration. (B) Conspecific choice test with an acclimatization zone (AZ), an entry zone (EZ), a central zone (CZ) and three zones with no (0), three (3) or six (6) conspecifics. The asterisk (*) represents a divider that was removed after the acclimatization period.

Figure 2: Landmarks collected on pikeperch larvae. 1. Tip of the premaxillary; 2. Insertion of the operculum on the profile; 3. Anterior insertion of anal fin; 4. Posterior insertion of anal fin; 5,7. Insertion of caudal fin; 6. Posterior extremity of the lateral line; 8. Posterior insertion of second dorsal fin; 9. Anterior insertion of second dorsal fin; 10. Posterior insertion of first dorsal fin; 11. Anterior insertion of first dorsal fin; 12. Insertion of the operculum on the profile; 13, 14, 15. Posterior extremity, anterior extremity and centre of eye.

Figure 3: Frontal histological sections of retina and muscles. (A) Retina: Lens (L), Ganglion Cell Layer (GCL), Inner Plexiform Layer (IPL), Inner Nuclear Layer (INL), Outer Plexiform Layer (OPL), Outer Nuclear Layer (ONL), Photoreceptor Layer (P) and Pigmentary Epithelium (PE). (B) Muscles. The black squares represent the focus and expansion carried out to count and measure muscle fibres. (C-D) Expansions of parts of muscles. The yellow asterisks (*) represent small fibres and the yellow arrows represent large fibres.

Figure 4: (A) PCA conducted with seven behavioural variables of the two tests (cross-maze and conspecific choice tests). For the cross-maze test: Swimming activity (E_A), Latency to emerge from the acclimatization zone (E_LS), Total number of visited zones (E_NVZ); for the conspecific choice test: Latency to emerge from the acclimatization zone (S_LS), Time spent near the zone without congeners (S_Z0), Time spent near three congeners (S_Z3), Time spent near six congeners (S_Z6). (B) Projection of individuals of each group: cannibal and potential non-cannibal fish as a function of PCA variables. The white dots represent cannibal fish and the black dots potential non-cannibal fish.

Figure 5: Results of the geometric morphological analysis of cannibal and potential non-cannibal pikeperch considering the set of 13 landmarks (LM). (A) Percentage variance explained by various principal components (PC) obtained by the principal component analysis (PCA). PC1 and PC2 explained 38.1% and 22.8% of the phenotypic variability, respectively. (B) Bivariate plot of the PCA scores obtained from cannibal (blue dots) and potential non-cannibal (red dots) fish on the morphospace depicted by PC1 and PC2; barycentre and 95% ellipses are shown for both fish groups. (C) Thin-plate spline deformation grid of fish body shape to compare cannibal with potential non-cannibal fish.

Figure 1. Colchen et al.

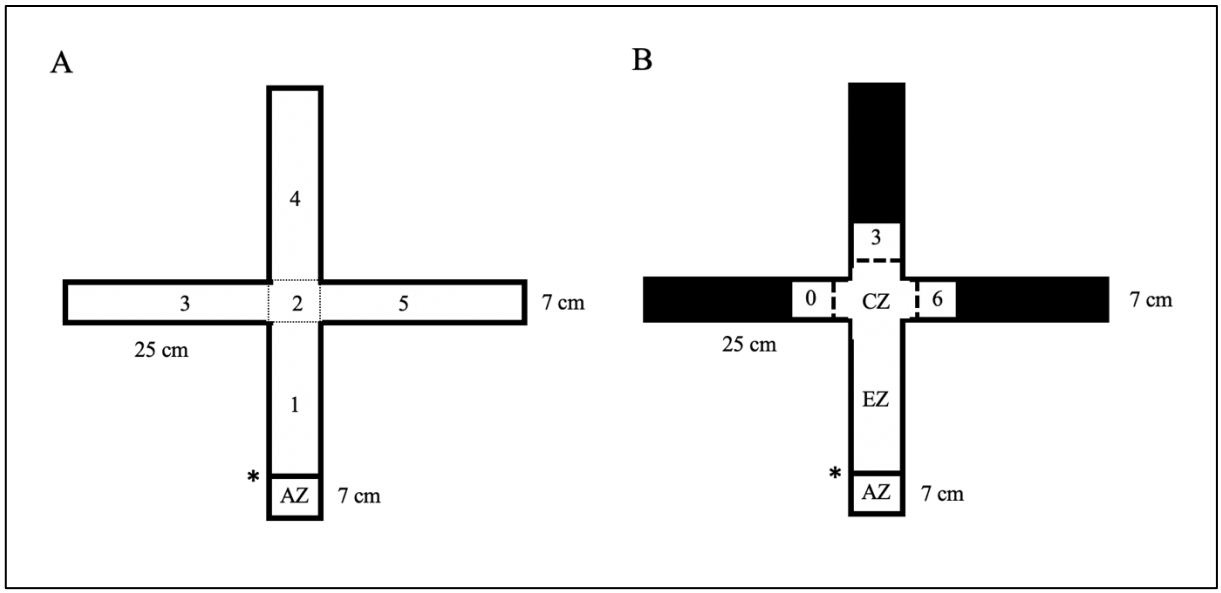

Figure 2. Colchen et al.

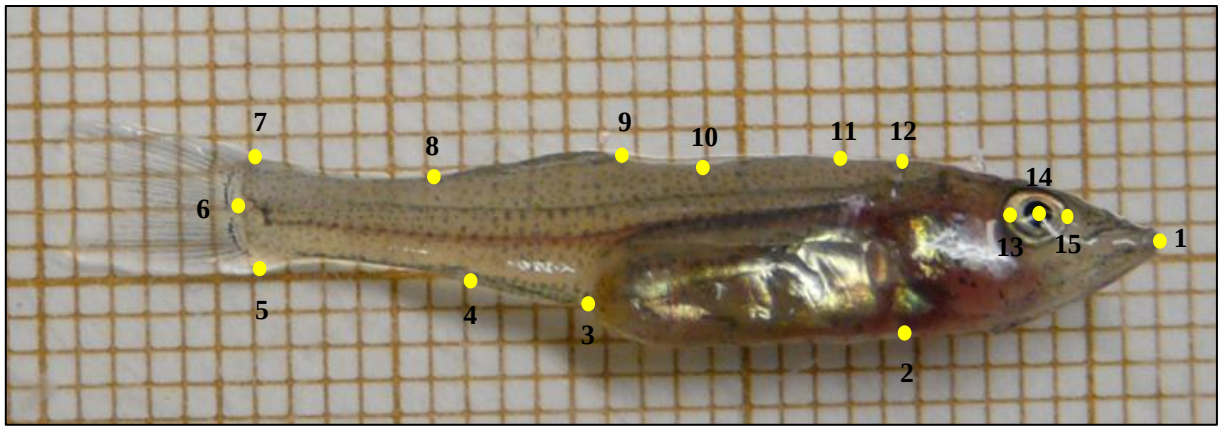

Figure 3. Colchen et al.

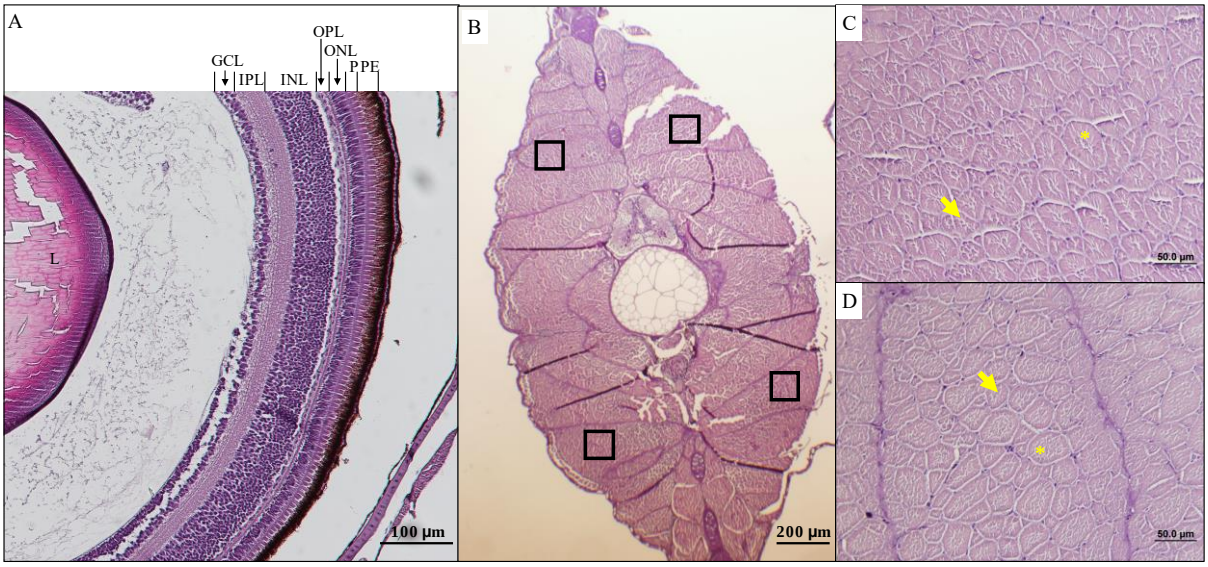

Figure 4. Colchen et al.

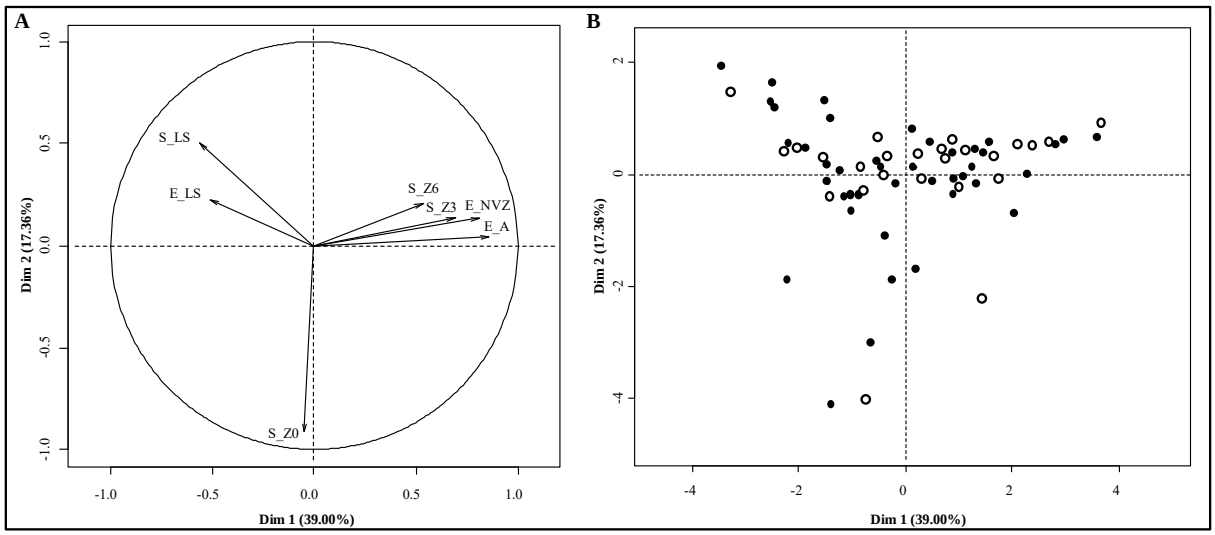

Figure 5. Colchen et al.

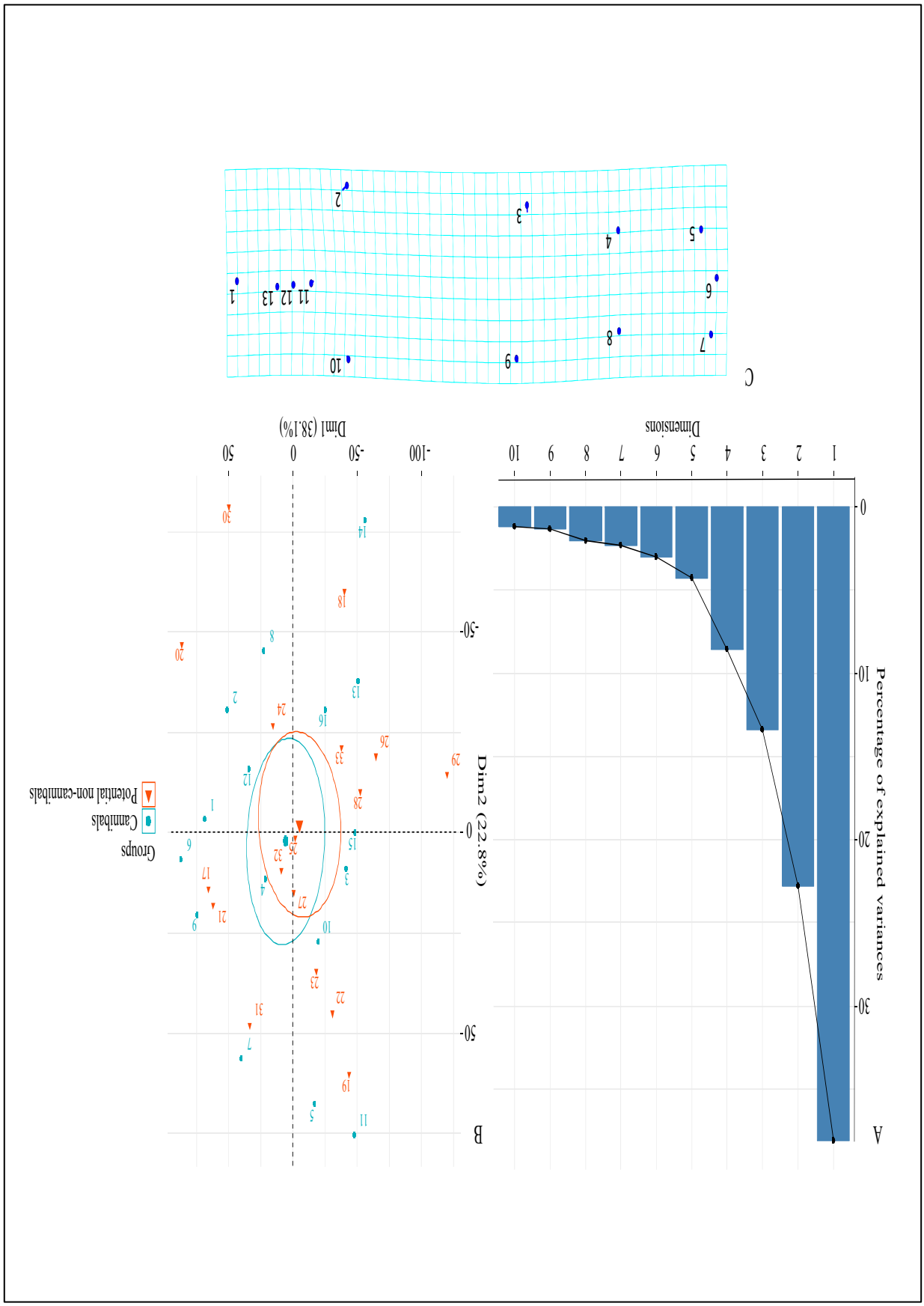

Table 1: Mean (± SD) of the variables of interest measured in cross-maze and conspecific choice tests for cannibal and control fish; inter-individual variation is represented by the coefficient of variation (CV).

Behavioural test	Variables	Cannibal fish		Potential non-cannibal fish	
		Mean ± SD	CV (%)	Mean ± SD	CV (%)
Cross-maze test	Swimming activity (E_A)	171.7 ± 110.3	64.2	137.5 ± 111.9	81.3
	Latency to emerge from the acclimatization zone (E_LS)	237.3 ± 346.1	145.9	182.0 ± 335.5	184.3
	Total number of visited zones (E_NVZ)	68.8 ± 74.9	108.9	52.1 ± 65.4	125.4
Conspecific choice test	Latency to emerge from the acclimatization zone (S_LS)	159.0 ± 233.1	146.6	251.6 ± 395.1	157.0
	Time spent near zone without congener (S_Z0)	124.6 ± 271.0	217.4	152.4 ± 271.4	178.1
	Time spent near three congeners (S_Z3)	126.4 ± 134.0	106.0	130.8 ± 177.0	135.3
	Time spent near six congeners (S_Z6)	257.7 ± 267.4	103.8	176.0 ± 229.6	130.4

Table 2: Correlations (Spearman correlations) between behavioural variables in both tests: cross-maze and conspecific choice tests for cannibal and potential non-cannibal fish. For exploration and boldness, three variables were analysed: the individual latency to emerge from the acclimatization zone (E_LS) (in seconds), the total number of visited zones (E_NVZ) and swimming activity (E_SA) (in seconds). For sociability test, four variables were analysed: the latency to emerge from the acclimatization zone (S_LS) (in seconds) and the time spent close (less than one centimetre of the divider) to the groups of 0 (S_Z0), three (S_Z3) or six conspecifics (S_Z6).

Variables	**E_A**	**E_LS**	**E_NVZ**	**S_LS**	**S_Z0**	**S_Z3**	**S_Z6**
	Cannibal fish						
E_A	/	S=3151.4; p=0.3; rho=-0.21	S=259.3; **p<0.0001**; rho=0.90	S=3339.3; p=0.1; rho=-0.28	S=1808.3; p=0.14; rho=0.30	S=1237.2; **p=0.007**; rho=0.52	S=1688.3; p=0.08; rho=0.35
E_LS	S=12058; p=0.88; rho=0.02	/	S=3337.5; p=0.16; rho=-0.28	S=2862.9; p=0.63; rho=-0.10	S=2656; p=0.92; rho=-0.02	S=2842.7; p=0.66; rho=-0.09	S=2500.9; p=0.85; rho=0.04
E_NVZ	S=2826.5; **p<0.0001**; rho=0.77	S=13181; p=0.66; rho=-0.07	/	S=3094.7; p=0.36; rho=-0.19	S=1874.3; p=0.18; rho=0.28	S=1359.6; **p=0.01**; rho=0.48	S=1816.1; p=0.14; rho=0.30
S_LS	S=13205; p=0.66; rho=-0.07	S=9915.2; p=0.21; rho=0.19	S=12388; p=0.98; rho=-0.004	/	S=3174.3; p=0.28; rho=-0.22	S=2984.6; p=0.48; rho=-0.15	S=3855.1; **p=0.01**; rho=-0.48
S_Z0	S=8626.2; **p=0.05**; rho=0.30	S=12744; p=0.84; rho=-0.03	S=9003.5; p=0.08; rho=0.27	S=17760; **p=0.004**; rho=-0.43	/	S=1959.5; p=0.23; rho=0.25	S=2439.7; p=0.77; rho=0.06
S_Z3	S=6702.6; **p=0.002**; rho=0.46	S=15232; p=0.13; rho=-0.23	S=6575.4; **p=0.002**; rho=0.47	S=16360; **p=0.03**; rho=-0.32	S=9539.1; p=0.15; rho=0.23	/	S=983.2; **p=0.0009**; rho=0.62
S_Z6	S=7090.2; **p=0.005**; rho=0.42	S=12789; p=0.81; rho=-0.04	S=7750.3; **p=0.01**; rho=0.37	S=16526; **p=0.02**; rho=-0.33	S=9303.6; p=0.12; rho=0.24	S=5294.6; p<0.0001; rho=0.57	/
	Potential non-cannibal fish						

Table 3: Means ± SD of different layers of retina (Ganglion Cell Layer (GCL), Inner Plexiform Layer (IPL), Inner Nuclear Layer (INL), Outer Plexiform Layer (OPL), Outer Nuclear Layer (ONL), Photoreceptors Layer (P) and Pigmentary Epithelium (PE)) and numbers and diameters of muscles measured on histological cuts for cannibal and control fish and statistical results of comparison between both groups.

	Variables	Cannibal fish (Mean ± SD)	Potential non-cannibal fish (Mean ± SD)	F	p
Retina	Thickness of GCL (µm)	35.69 ± 15.24	24.60 ± 6.51	3.90	**0.05**
	Thickness of IPL (µm)	48.61 ± 16.40	53.46 ± 20.27	0.49	0.49
	Thickness of INL (µm)	61.90 ± 23.05	45.44 ± 9.43	4.98	**0.03**
	Thickness of OPL (µm)	15.59 ± 10.91	17.54 ± 7.42	0.27	0.61
	Thickness of ONL (µm)	24.02 ± 11.73	23.21 ± 5.39	0.04	0.83
	Thickness of P (µm)	21.78 ± 13.96	21.49 ± 7.65	0.004	0.95
	Thickness of PE (µm)	36.84 ± 23.17	43.31 ± 15.44	0.72	0.40
Muscles	Number of large fibres	27.62 ± 7.19	25.58 ± 6.27	0.74	0.40
	Number of small fibres	22.28 ± 9.69	29.00 ± 10.78	2.64	0.12
	Maximal diameter of fibres (µm)	35.84 ± 6.31	34.95 ± 5.76	0.33	0.57
	Minimal diameter of fibres (µm)	24.46 ± 4.75	23.53 ± 5.82	0.76	0.39