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## BEHAVIOURAL ANALYSIS OF INTRA-COHORT CANNIBALISM IN YOUNG FISH

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### Introduction

Cannibalism is a predatory feeding strategy that involves killing and consuming the whole, or major part, of an individual belonging to the same species, irrespective of its stage of development (Baras, 2012). It occurs in 30 fish families and is reported in both wild and farmed conditions. In aquaculture, cannibalism is highly undesirable and is one of the major bottlenecks in larviculture. Several studies look for causes of cannibalism (for review, see Naumowicz et al., 2017) searching for rearing factors (environmental, nutritional, populational) able to stop cannibalism (Hecht and Pienaar, 1993; Baras, 2012). Manipulating some of these factors reduces cannibalism but never stops it. Consequently, the existence of cannibals could be explained by individual characteristics that rearing factors can not regulate. A new concept called personality, defined as behavioural individual differences stable over time and/or in varying environmental contexts, has emerged (Réale et al., 2007). Several personality traits have been recognized in animals in relation to diverse behavioural responses of individuals within the same population and the performances of these traits followed a continuum along the personality axis. Five traits have been identified: boldness-shyness, exploration-avoidance, activity, aggressiveness, and social tolerance (Sih et al., 2004; Réale et al., 2007). As a consequence of inter-individual variability, we can assume that cannibals may have a different personality compared to other individuals of the population. To confirm this hypothesis, pikeperch (*Sander lucioperca*) is a good model because it has a strong economic potential for inland aquaculture diversification in Europe (Wang et al., 2009), and there is a high mortality rate due to cannibalism during early life stages (up to 29% in that species, Kestemont et al., 2007).

### Materials and methods

Eggs were obtained from stripping two domesticated females reared in a French fish farm (Asialor, Pierrevillers 57, France) on January 22, 2016. After hatching

(27/01/2015), larvae were raised at URAFPA ([www.orafpa.fr](http://www.orafpa.fr), France) in a RAS (Recirculating Aquatic System). Fish were hand fed commercial food (first with nauplii of *Artemia* (550-600µm, Catvis, Hertogenbosch, The Netherlands) from 4 (days post-hatching: dph) to 18dph, followed by Larviva PROWEAN 100, 300, 500, 700µm (BIOMAR®, France) until 35dph and INICIOplus 0.8mm (BIOMAR®, France). We compared the behavioural responses of 42 fish taken randomly (unknown status) in the eight tanks to those of 25 cannibals of the same age (between 29 and 54dph) in two different tests of personality: a cross-maze test and a sociability test.

Swimming activity, exploration and boldness were evaluated using a cross-maze test. The cross-maze (25cm×7cm with 2.5cm of water) was divided into five zones, and placed on a translucent table with a light below. Fish were tested one by one. Each fish was placed in the acclimatization zone (7cm×5cm) separated from the maze by a vertical divider. After 30min of acclimatization, the divider was opened and fish behaviour was video recorded for 20min. If a fish did not go out of the acclimatization zone during the 20 minutes' period, a latency of 1200s was attributed. For exploration, three parameters were analysed: the individual latency to emerge from the acclimatization zone (in s), the total number of visited zones, and the swimming activity. They were analysed over the whole 20 minutes' period, except for swimming activity that was calculated over three periods of time: from the 1<sup>st</sup> to the 3<sup>rd</sup> minute, from the 9<sup>th</sup> to the 11<sup>th</sup> minute, and from the 17<sup>th</sup> to the 19<sup>th</sup> minute.

Relationships between fish were evaluated with a sociability test. A similar device as the cross-maze test was used for this test. Cross-maze was also divided into five zones with three zones occupied by small box separated from other zones by a transparent wall, to let the tested larvae to see congeners and small holes to let water circulated. In these small boxes, we put 0, 3, or 6 congeners. After an acclimatization period of 30min, larvae were tested for sociability during 20min. We measured four parameters: the latency to emerge from the acclimatization zone, and the time spent near 0, 3, and 6 congeners.

For all parameters, we tested the assumption of normality (Shapiro test) and of homogeneity of variances (Levene test). Consequently, data were compared with t-test for independent data. All statistical tests were performed with "R" software (version 3.0.3) and results are presented as mean, median, and outliers in box plots for all the parameters. Results were considered significant at  $P < 0.05$ .

## **Results and discussion**

In the exploration test, cannibals were not significantly more active than random fish (cannibals:  $171.7 \pm 110.3s$ ; unknown status:  $137.5 \pm 111.9s$ ;  $t=1.22$ ;  $df=65$ ;  $p=0.23$ ; Fig.1A). Cannibals did not emerge significantly faster than fish with

unknown status (cannibals:  $237.3 \pm 346.1$ s; unknown status:  $182.0 \pm 335.5$ s;  $t=0.64$ ;  $df=65$ ;  $p=0.52$ ). Cannibals did not explore significantly more zones than fish with unknown status (cannibals:  $68.7 \pm 74.8$ ; unknown status:  $52.1 \pm 65.4$ ;  $t=0.95$ ;  $df=65$ ;  $p=0.34$ ; Fig.1B).

In the sociability test, cannibals did not emerge significantly faster than fish with unknown status (cannibals:  $159 \pm 233.1$ s; unknown status:  $251.6 \pm 395.1$ s;  $t=-1.06$ ;  $df=65$ ;  $p=0.29$ ). Cannibals did not spend more time near zone without congener than fish with unknown status (cannibals:  $124.6 \pm 271.03$ s; unknown status:  $152.4 \pm 271.4$ s;  $t=-0.4$ ;  $df=65$ ;  $p=0.69$ ; Fig.1C). Cannibals did not spend more time near zone with three congeners than fish with unknown status (cannibals:  $126.4 \pm 134$ s; unknown status:  $130.8 \pm 177.02$ s;  $t=-0.11$ ;  $df=65$ ;  $p=0.91$ ; Fig.1D). Cannibals did not spend more time near zone with 6 congeners than fish with unknown status (cannibals:  $257.7 \pm 267.4$ s; unknown status:  $176.02 \pm 229.6$ s;  $t=1.32$ ;  $df=65$ ;  $p=0.2$ ; Fig.1E).

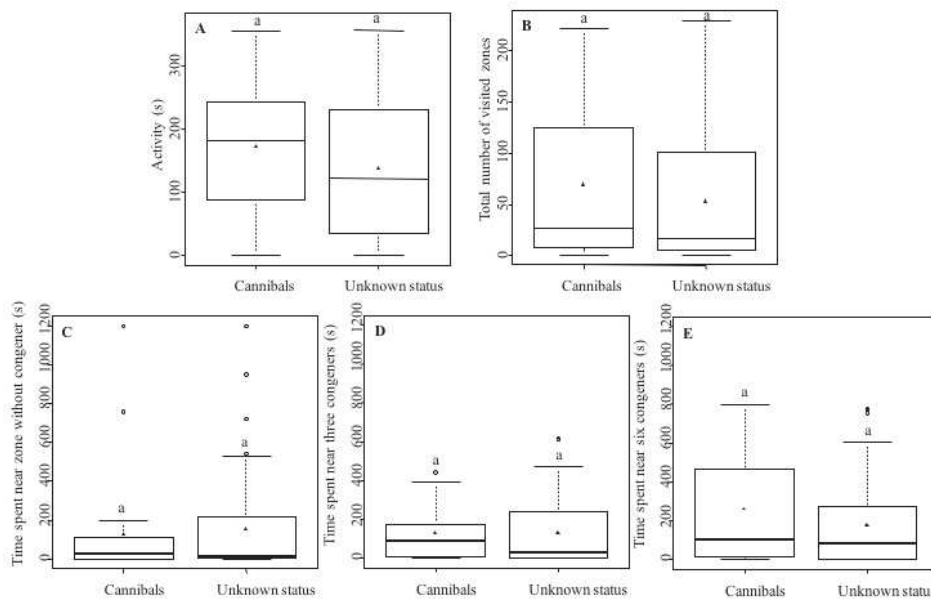


Fig. 1. Parameters used in both the exploration and the sociability tests: (A) activity (s), (B) total number of visited zones, (C), time spend near zone without congener (s), (D) time spend near three congeners (s), and (E) time spend near six congeners (s) in function of groups (cannibals and individuals with an unknown status). The black line is the median, the black triangle is the mean and the white dots are outliers.

To our knowledge, the present study is the first one that investigated the behavioural responses of cannibals compared to random sample of the population. We

showed that behavioural responses of cannibals did not differ from those of fish taken randomly within the same population. It seems that behavioural responses of personality did not allow us to characterize cannibals in young pikeperch juveniles.

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