October 5-8, 2010

Porto, Portugal

GENETICS of ADAPTATION IN RAINBOW TROUT: A MULTIDISCIPLINARY APPROACH.

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Introduction

Fish are poïkilotherm and live in tight physiological interactions with water, a highly fluctuating environment they have to face with. Variations can be either temporal, including global climatic changes, or spatial. Fish also experience multiple sources of stress (handling, sorting, diet changes, noise ...) which can affect welfare. Ability of fish to adapt to changing environments and stressors is a key trait for breeders, especially when they sell eggs or young fish all over the world. So far, this ability has not been introduced in any breeding program. Indeed, sensibility to environment is difficult to describe. Most often, either physiological traits, or behavioral traits are considered but each of them represent only a partial representation of the situation. In this study, we carried out a multidisciplinary approach to better understand the genetics of adaptation and prospect for possible selection strategies. Physiological traits (cortisol level after acute stress) and behavioral ones (spatial exploration and risk-taking behaviors) were used together with an innovative trait: phenotypic plasticity measured through the homogeneity of weight. We assume that body weight and its fluctuations is integrative of all life events and of the different components of reaction to environment. The genetic variability of the three kinds of traits was assessed and correlations between all traits were especially focused on. Besides this biological approach, an anthropological survey focused on human-animal relationship in fish farms to compare how breeders and scientists try to appreciate fish welfare and adaptation.

Material and methods

An original biological material was used: heterozygous isogenic lines of Rainbow trout, strictly reserved to research purposes. Isogenic lines are groups of fish where all individuals within the same line exhibit the same genotype, as if they were all the replicate of each other. In such lines, the genetic variance is null, and the phenotypic variability in weight performance among individuals is a measure of environmental variance, *i.e.* the sensibility to environment of the line genotype.

Ten isogenic lines were produced, by crossing all-homozygous females from one line to ten all-homozygous sires from ten different lines. To avoid bias due to maternal effects, females with similar mean weight of ova were chosen and all groups were fertilized the same day. Sires brought the genetic variability. Homozygoty of breeders was checked using four microsatellites.

The ten lines were reared at PEIMA (Brittany, France) INRA experimental fish farm. Each line was reared in triplicate during ten months (from January to November, until 250 g) under standard rearing conditions and was then submitted to a chronic stress (high density) during the next five months (350 g at end). Each month, 100 fish per replicate were randomly sampled and individually weighed. Three acute stress were applied to each replicate in June, November (2008) and March (2009, during chronic stress): fish were confined in 10cm depth-water during one hour, anesthetized with a lethal dose of phenoxy-ethanol and then a blood sample was taken from 10 fish per replicate to measure cortisol level. From each replicate, fish were transferred to Ifremer experimental facilities (L'Houmeau, France). Exploration and swimming activities were studied on 10 fish of seven lines before, during and 40 min after a stimulation (standardized fall of an object). A single fish was quickly moved from its maintenance tank to the experimental tank (400 l) 2 h before the experiment started. The experimental tank was divided into four zones, and the time spent, the distance traveled in each zone and the swimming complexity were quantified for each period from video recording.

Three mixed populations were also constituted with 50 fish of each line. A behavioral test was conducted on these three tanks at PEIMA facilities. A risk-taking score was evaluated *via* a preference choice between a safe zone (shaded, without food) and a risky zone (enlighten, potentially with food) by recording the number of fish passages per hour and the latency before the first passage through an opening in an opaque divider equipped with a PIT tag reader. The test lasted 23 hours and was repeated three times (including one during chronic stress).

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Phenotypic plasticity was characterized through either weight coefficient of variation (CV) within each line, or using heteroscedastic models to estimate environmental variability (Ve) for each line. Similar results were obtained, and only results with CV records will be presented. All data were analyzed with classical ANOVA. Finally Pearsons correlations between all parameters were calculated. A principal component analysis was also used.

For the anthropological analysis, qualitative interviews mainly about rearing practices, work conception and relationships with fish were conducted with 10 fish farmers.

Results and discussion

For all traits, a line effect was found significant showing existence of genetic variability. Pseudo heritability of weight CV estimated with a simple longitudinal model was 0.16. Differences between lines were significant at almost all dates but become small when fish were older than nine months (about 250 g). Finally, correlations between CV measured at distant dates were low. Thus CV of weight evolved according to life history and the mean ability of the genotype to manage with successive environments. Based on exploration and swimming activities, lines could be classified as either low reactive line or high reactive lines. Repeated measures such as cortisol level after acute stress and risk- taking evolved with time: differences between lines were significant at each date but line ranking was different. Again, life history probably had a large influence.

During the first rearing period, significant correlations were recorded between CV and physiological or behavioral traits: most heterogeneous lines (high CV) had higher cortisol levels after first acute stress and exhibited decrease of velocity and distance travelled after stimulation and higher number of passages between safe and risky zones. These correlations disappeared later on. This could be due to low differences of CV after nine months of survey or directly to fish age.

Fish farmers interviews show that, despite the difficulty to imagine if fish feel and in which way, fish farmers have a complex know-how to apprehend the positive or negative experience of their fish to take their welfare into account.

Conclusion

This study showed that different criteria of adaptation and welfare are actually connected during the first year of life. Correlations loosen later on, probably because it is easier for old fish to adapt to their environment. We suggest that a selection for robustness based on several criteria should be the most efficient. Finally, one should find the way to use fish farmers know how in selection decisions. However, important questions for selection choice are still raised, among others: which combination of traits should be targeted and when is it the most efficient/relevant to measure them?