Consideration of inter-population variability to improve ecological relevance of biomarkers

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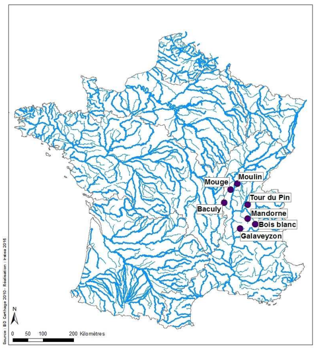
Introduction

Energetic metabolism markers appear to be a good predictive tool to detect physiological disturbances related to population dynamic. Indeed animal health depends on the availability of metabolic energy necessary to ensure maintenance, growth and reproduction [1]. Among biological processes to access energy, digestive capacities appear sensitive to contamination stress [2]. In recent studies, based on *Gammarus fossarum* males from one reference population and an active approach (caging), reference values (RV) for digestive enzymes, taking into account their natural variability, have been proposed [3]. In the same vein, we previously described link between digestive enzyme changes and reproductive impairments in females [4]. So digestive capacities appear as ecologically endpoints to provide information on the consequences likely to occur at a higher biological level (population). However this approach has been developed on only one population, consequently it does not take into account of population diversity. In this way, it appears necessary to assess the between-populations variability of these enzyme activities and its consequence on the reference values and its sensitivity to a stress.

- Step 1, the basal level of digestive enzyme activities was compared between 7 reference populations.
- Step 2, the sensitivity difference between the 7 populations was assessed after a trophic stress.



Materials and Methods

Gammarus fossarum males from 7 reference sites were collected at the same period (october, 30th, 2014). Population 1 is the population previously used for assessing the reference values (June 2010). Organisms were acclimated 2 weeks in lab and fed *ad libitum* with alder leaves [3]. Then, adult males (2-2.5 mm) were fed 21 days with alder leaves either *ad libitum* (7 /7) or only one day per week, corresponding to 75% starvation diet (1/7). At the end of the experiment, 6 pools of 3 organisms were collected to measure the activities of amylase, cellulase and trypsin [3].

Results and Discussion

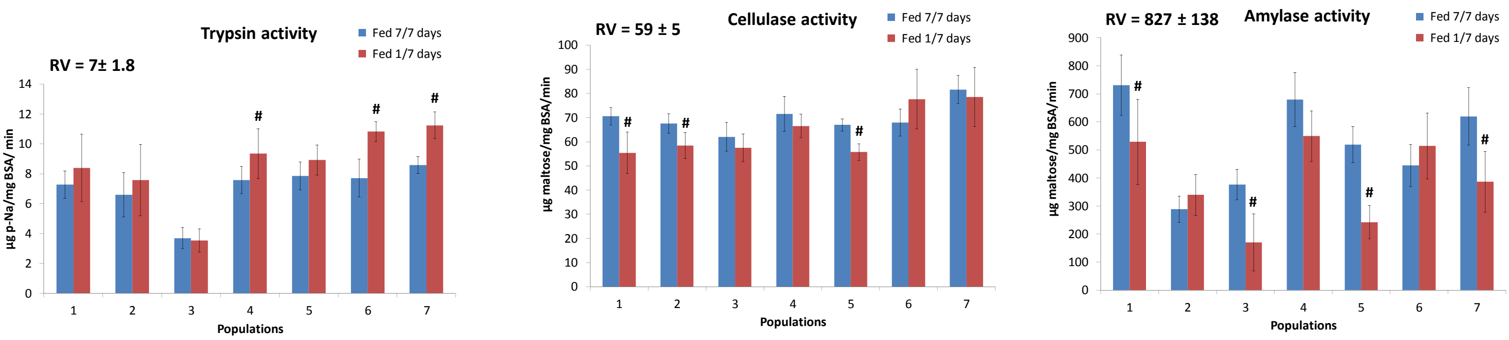


Figure 1. Digestive enzyme activities in *Gammarus fossarum* males exposed to two levels of diet stress (fed 7 days per week; fed 1 day per week) for 21 days (mean ± SD, n = 6). The hash (#) symbol indicates a significant difference between the starved organisms and the fed control organisms (Mann-Whitney test: $p<0.05$). RV = Reference value [3].

Basal level of digestive enzyme activities:

- Digestive enzyme activities in the population 1 are similar to the previous reference values (RV) except to cellulase, which shows significantly higher activity than RV (June 2010 vs October 2014, $p<0.05$). This result suggests temporal changes for cellulase activity.
- For trypsin and cellulase enzymes, few inter-populations differences (excepted trypsin in pop 3) are observed concerning the basal activity level, with a coefficient of variation (CV) of 9% for trypsin and cellulase. This result underlines a relatively steady enzyme activity among the different populations.
- Inversely for amylase, a strong variability is observed between the 7 populations studied (CV 32%). Even after 2 weeks of acclimatization, populations fed with same food, show clear differences. However, among environmental (temperature, conductivity) and endogen (weight or reproductive status) parameters considered, no one appears as an explanatory factor (not shown).

Inter - populations sensitivity in link with starvation stress:

- Concerning trypsin activity, the trophic stress have either no effect or increase the protease activity. These results are consistent with a previous study [5].
- At the opposite, the activity of the two carbohydrases decreases for some populations submitted to the starvation stress. However, strong variability is observed according to the population used and is not consistent between the two enzymes. This result is equally consistent with a previous study [5] and with contaminant stress.
- As previously discussed [5], the difference pattern (protease vs carbohydrases) between enzymes, under food starvation stress, could be linked to the use of a only one food source (alder leaves) limiting the diversity of nutrients. In the same way, the differences observed between populations should be linked to others environmental parameters and probably to an adaptation of populations to their habitat (physicochemical parameters, sources, diversity and amount of food). In the case of amylase, between population differences were also observed when gammarids were exposed to chemical stress, methomyl (80µg/L, not shown).

Conclusion:

To improve the ecological relevance of digestive biomarkers, it appears necessary to continue to increase our knowledge and the characterization of the explanatory factors which could be implied in the digestive enzyme activities changes. In this way, according to potential temporal changes (cellulase) and/or strong effect of habitat (amylase) on the basal level and sensitivity of digestive enzymes, it appears difficult to propose only one reference value for all *G. fossarum* populations.

References
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