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1 **Variation in Variance means more than Mean Variations: What does variability tell us about**
2 **population health status?**

3

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12

1 **Abstract**

2 In environmental science, the variability of biological responses in natural or laboratory populations
3 is a well known and documented phenomenon. However, while an extensive literature aims to
4 explain and understand the origin of variability, few try to use it as a demonstration of the
5 population's response facing a stress. We propose here a theoretical framework that explores
6 various patterns of variability both within and among populations, and seeks methods useful in
7 bioevaluation methodologies. We also introduce the concept of "ecotoxicological niche" to
8 characterize the ability of a population to endure contamination.

9

10 **Keywords**

11 Biomarkers, Variability, Niche, Ecotoxicology, Risk assessment, Population acclimatation

12

13 **Highlights**

- 14
- 15 • Variance in biomarkers response along environmental gradients shouldn't be neglected
 - 16 • Variability reflects acclimatation abilities and could be a precocious response to stress
 - 17 • Ecotoxicological niche allow to interpret ecological significance of toxicity biomarkers

17

18

1 **Preamble**

2 What is one of the most annoying things that an ecotoxicologist is confronted with? A high variability
3 of responses between replicates, and perhaps most annoying, a variable variability across conditions.
4 Indeed, the within-site/condition variability of the measured biological responses is a strong
5 limitation on detecting the central tendency between site/condition differences, whatever the
6 statistical test used. As a consequence, the variance is generally only considered as a parameter
7 limiting the access to parametric statistical analysis, *i.e.* the (h)allowed student and ANOVAs tests.

8

9 **How is variability considered in ecotoxicology literature?**

10 Our scientific literature is strongly dominated by the tyranny of the Golden Mean introduced by
11 Bennett (1987), that restricts our vision of the dataset to a unique endpoint, and prevents us from
12 fully exploiting the richness of this dataset. Variance is therefore considered at best as illustrative
13 information, at worst as a noise, that prevents us from discerning/depicting the true biological
14 effects, *i.e.* variations of mean along environmental gradients, still considered to be the most useful
15 way to understand the studied phenomenon.

16 In a recent synthesis, Artigas et al. (2012) proposed a framework for ecotoxicology that pointed out
17 some new and some recurrent questions, including relevance of ecotoxicological tools. A key part of
18 this relevance is to go beyond the question of biological endpoints by defining some quantitative
19 parameters that we should focus on: the mean is of course a pertinent measure, but the variance is
20 another, as well as differences of basal levels across populations and their range of variation.

21 Indeed, variability is a key parameter in population genetics (Browne et al. 2002), that relies on the
22 partitioning of phenotypic variance between its genetic and environmental component , as well as in
23 physiology (Bennett 1987). A rebirth of interest for the variance is also observed in risk analysis (Maul

1 2014) and in ecology, after having been neglected for decades (Violle et al. 2012). In ecotoxicology
2 also, a quite abundant literature deals with the variability of biological responses. However, it mainly
3 tries to understand the mechanisms underlying the observed variability, proposes solutions to limit
4 this variability and deals with the statistical issues and methods to cope with the variability, but few
5 have explored the information contained in those variability patterns as a new method to better
6 understand the effect of a contaminant on the studied system (Calow 1996). By focusing on the
7 mean only, we neglect an important part of the information in our interpretation and probably
8 misunderstand the true effect of anthropogenic stressors on populations. Indeed, the mean is often
9 interpreted as a parameter that reflects the highest density of individuals in a distribution, while it's
10 only its gravity center. In some case (bimodal distribution, asymmetric distribution with extreme
11 values), it could be a real problem. However, data are often summarized by the mean and the
12 standard deviation, that gave some indications on the data distribution.

13 If we consider now a classic case of normal distribution of the biological response, the mean +/-
14 standard deviation interval encompasses roughly only 68% of the population. As pointed out by
15 Depledge (1990), we thus neglect 32% of the population. These 32%, far from being atypical
16 individuals, constitute a significant proportion of the population and need to be included in our
17 reflection about effect of the pollution. Indeed, those individuals could be particularly resistant to
18 some stresses (Depledge 1990) and survive high pollution, being the basis for a future population
19 which reconstitutes after an episodic stress or that has adapted in the case of a chronic stress.

20 Thus, since the eighties, regularly, some people have been advocating for a better consideration of
21 variance in data analysis, and desired that it become a topic in ecotoxicology (Depledge 1990;
22 Depledge and Fossi 1994; Handy and Depledge 1999; Keppler and Ringwood 2001; Williams 2008;
23 Corton et al. 2012; Smith et al. 2012). However, we have not yet observed a shift of paradigm from
24 mean to mean/variance studies, and therefore propose here an additional step in this direction.

1 So, how can we explain inter and intra-population variations of the biological responses, and what
2 are the general patterns we could define across contamination levels? Once these issues are
3 understood, the usefulness of this information can be assessed more accurately. The way variability
4 will be considered will affect environmental risk assessment strategies

5

6 **Observed patterns of variability**

7 First, we should define at which level we consider variability. The objects usually compared to each
8 other are populations. However, the term population can define different objects depending on the
9 context in which it is used. Calow (1996) deals with the responses of populations of several species
10 facing a stress, and debates their varying tolerance to pollutants. However, this is not the type of
11 variance that is most often considered in ecotoxicology, and we focus on variations within and
12 among populations of one species only. Within-population studies are generally laboratory
13 experiments where a field population is exposed to a gradient of pollutant, while between-
14 population studies are generally field experiments where several sites across an environmental
15 gradient are considered.

16 While such studies mostly focus on the mean, the importance of variance among individuals has
17 been described in several studies. The major observed patterns are detailed below. However,
18 explanations for these patterns are rarely proposed by the authors.

19 Depledge and Lundebye (1996) observed high inter-individual variations in the heart rate of crabs in
20 contaminated sites, and suggested that it was linked to a high response of sensitive individuals while
21 resistant ones exhibited low responses. Bard (2000) also noticed a higher inter-individual variability
22 of P-glycoprotein expression in a contaminated site, but only link this to variable abilities of
23 individuals to respond to P-gp inducers. Odum et al. (1979) presents the increase of variability with
24 increasing perturbation as a proof of a decrease of system stability, leading to a decrease of its global

1 performance. In the meantime, Luoma (1996) observed a narrowing of the variance of mussel
2 growth rates on polluted sites, which he explained by a selection of some typical response by the
3 contaminants. He stated that "high variance in a response may be typical at low pollutant
4 concentration". Apart from these two opposite patterns, some studies showed either less clear
5 patterns (Kepler and Ringwood 2001), or both patterns. For example, Baird and Barata (1999)
6 showed that variance within population can either increase or decrease between sublethal and lethal
7 contamination levels, depending on the contaminant tested. It thus became excessively low or high
8 compared to the basal variability observed without contamination (Cairns Jr. 1992).

9

10 **How can we explain differences of variability levels? What are the mechanisms are involved?**

11 While variability is not used *per se* to understand the effect of contamination on natural systems, a
12 quite abundant literature aims to explain why responses are variable. Mechanisms that structure
13 variability within populations are acting at four different levels.

14 The first two mechanisms have an intrinsic origin. Differences in the genetic heritage of individuals
15 enable populations sufficiently large to avoid genetic homogenization, through drift or inbreeding for
16 example. A conceptual framework for these interactions between genes and environment in the
17 specific context of contaminated ecosystems has been proposed by Morgan et al. (2007) or Steinberg
18 et al. (2008). It has been illustrated by Baird & Barata (1999) for *Daphnia* clonal populations, and by
19 Feckler et al. (2012) for natural populations of *Gammarus fossarum*. However, even in clonal
20 populations without genetic variability, physiological status (varying according to age, reproduction
21 or feeding status for example) may influence all the biological responses. Many works have focused
22 on this physiological status, seeking for sensitive stage (Depledge 1994; Hyne and Maher 2003;
23 Karimi and Folt 2006; Corton et al. 2012).

1 The latter two mechanisms involve the interactions of the population with its biocenosis and its
2 biotope, and are of extrinsic origin. They are generally considered through the angle of confounders
3 that could mask the effect of contaminant and lead to false evaluation of population and ecosystem
4 health. They could be abiotic factors of natural (temperature, salinity...) or anthropogenic origin
5 (contamination, but also pH, organic matter...) or biotic factors such species interaction, food
6 availability, parasitism (Beketov and Liess 2008; Nikinmaa and Tjeerdema 2010; Beketov et al. 2011;
7 Minguéz et al. 2012; Knillmann et al. 2013).

8 All those mechanisms generally act simultaneously, and the identification of the origin of the
9 variation is not easy. However, whatever the origin of variation, it contains information which is of no
10 profit.

11 **What are the expected patterns of variability along environmental gradients, and what is their**
12 **significance?**

13 We focus only on adverse effects of contaminants on biological systems, thus hormesis (Costantini et
14 al. 2010) is not implicitly considered here. However, whether beneficial effects of low doses are
15 homogenous within a population, or if resistant individuals are less affected by low doses than
16 sensitive individuals should also be considered. The different patterns explored below exclude
17 extrinsic "non mechanistic" sources of variation (measurement errors, experimental unit differences
18 - block effects, system stochasticity - see box 1 for the experimental design associated with variance
19 study). However, we can leave out that an initial source of variation lies on the field sampling
20 procedure of the population. This factor cannot be controlled easily, while it remains possible to
21 evaluate the sample size needed to stabilize the sample variance, for example by increasing sample
22 size until no change in variance was observed. To precisely estimate inter-individual variability and to
23 compare variability across samples (samples being either from the same population or from different

1 populations), the blame pseudoreplication frequently observed in ecotoxicology became the most
2 powerful experimental design, limiting the source of variation to the sole individual effect.

3 Finally, the patterns presented thereafter only considered sublethal biological response that
4 increased following exposure to contaminants. However, the theoretical framework we develop here
5 is also applicable to biological responses that decrease following exposure to contaminants. These
6 biological responses could be either a detoxication system, a defense or exposure biomarker, express
7 the modification of physiological performance, an increase or a decrease in growth rate or in
8 fecundity.....

9 *Intra-population variability along environmental/pollution gradients*

10 During laboratory experiments or active biomonitoring, one population is used in several
11 conditions. Two dimensions should be considered. For a given exposure condition, the intra-
12 population variation mainly reflects genetic variability. When all the exposure conditions are
13 considered, variance patterns show how this genetic variability allows populations to cope with
14 contamination. We first focus on sublethal levels of exposure, *i.e.* the stage where the contaminant
15 begin to have an impact on the organism without leading to the death of any individual. Obviously,
16 this stage is strongly dependant of the exposure duration, with differences between short and long
17 term lethality thresholds. However, a general pattern can be drawn, and the main difference
18 between these two exposure conditions is in the scale of the horizontal axis rather than in the
19 general aspect of the response. Thus, three patterns are possible: a stable variance, a decreasing or
20 an increasing variance. They are presented in box 2. Only the principal trends are detailed there, and
21 obviously intermediate and mix situations could be observed, with for example a canalization of the
22 response at sublethal levels, followed by an increase in variance at lethal levels, when
23 regulation/defense systems are overcome.

1 Once the lethality threshold is exceeded, that is, when the first deaths occur (indicated by CL_{10} in box
2 2), we should observe either no modification of the variance or a decrease, following the death of
3 individuals exhibiting the most extreme values. It could be accompanied by a shift of the mean,
4 according to the relationships between individual response and survival. It could be the driver of
5 stabilizing selection (box 2c), cases (d) and (e) are directional selection. They all led, over some
6 generations, to a type (a) response, but with probable differences in basal and maximum levels.

7 These variation patterns could help us to understand the results of active biomonitoring, with a
8 population sampled in a reference station and transplanted into a contaminated one. Thus, with this
9 theoretical framework, we will develop three situations of mean variation (Fig. 1).

10 Case 1 is the simplest one: when facing contamination, all individuals die. Thus the diagnosis is easy,
11 the environment is toxic! Case 2 is the favorite of scientists and editors: a clear and indubitable
12 increase in mean is observed. Beyond the mean, the variance could also change, with the patterns
13 detailed in box 2.

14 Finally, case 3 is classically considered as a "no effect" response. However, while it's true for the
15 population 1a, the two others presented variation in variance, if not in mean. We must not neglect
16 this effect that could be the early warning signal that everybody is looking for. Indeed, the shift in
17 variance can occur before the shift in mean, though numerous case studies are needed to correctly
18 interpret such results.

19

20 *Inter-population variability along environmental/pollution gradients*

21 Passive biomonitoring cases are the most difficult to interpret, because they are the outcome
22 of a gene x environment interaction. By comparing several populations to a reference system, several
23 patterns could be observed. The values observed are not the responses to a change of environmental

1 condition, but the chronic, basal level of the studied parameter in the local environmental condition.
2 Thus, the objective is to understand whether the differences observed could be relevant indicators of
3 population health (Fig. 2).

4 The simplest result is the stability of both mean and variance that could be easily interpreted as the
5 absence of environmental stress. If no difference in mean is observed, while a difference in variance
6 exists (Fig 2., A vs B vs C), we can postulate that the chronic levels of contamination are similar
7 among the two studied sites. However, the phenotypic plasticity revealed by the response variability
8 tells about the genetic variability and the adaptative abilities of the population. It has been shown
9 that genetic variability is a key factor for population health, thus populations at risk are those with
10 the lowest variability.

11 The most complicated cases are those where several populations exhibit different mean levels of
12 their responses (A-B-C vs D-E-F). Indeed, without information on the temporal variation, we cannot
13 distinguish short term induction in response to a temporary stress or long term adaptation or
14 acclimatation to a chronic stress. In such situations, even the study of the variance is of no help.
15 Either temporal trends of the biological response or results of laboratory stress on stress
16 experiments would be helpful to understand these complex interactions. This leads us to question, in
17 an interpopulation perspective, the meaning of the basal level (in mean and variance), and how
18 useful it is to predict and interpret the response pattern. Such approaches are poorly developed.
19 They reveal within and between population phenotypic variability, that could be the outcome of
20 stochastic phenomenon (genetic drift or founder effect), but also the results of local adaptation
21 through selection by the contaminant pressure, or a simple acclimatation to local conditions. More
22 studies are necessary, either in the field or laboratory, to explore and understand the mechanisms
23 and the consequences of modification of variance patterns.

24

1 **Towards the ecotoxicological niche: an ecological perspective to ecotoxicology**

2 Information necessary to understand and exploit the different patterns of response within and
3 between populations relies on the natural variability, *i.e.* the range of values of the studied biological
4 response in a range of healthy ecosystems and for healthy populations, to correctly define the
5 baseline level (Xuereb et al. 2009; Coulaud et al. 2011; Lacaze et al. 2011; Jubeaux et al. 2012).

6 We can then define the ecotoxicological niche as the ability of species to cope with contamination
7 and to maintain basal physiological function when facing anthropogenic stress. This ecotoxicological
8 niche is thus a particular case of the traditional ecological niche, where the environmental gradient is
9 replaced by a chemical compound. Using this well known conceptual framework, we can adapt the
10 Shelford law (1931) and define a well-being zone (Figure 3), corresponding to the realized niche of a
11 population, a stress zone, corresponding to the potential niche, and a lethal zone, where acclimation
12 abilities are overwhelmed.

13 The main dimensions of this niche should be the classical ecotoxicological parameters related to
14 genetic and cellular damages, physiological and behavioral modifications, defense or exposure
15 system activation, but also more integrative parameters directly related to fitness (reproduction,
16 survival and growth). For each of these dimensions, the realized niche is defined as the inter-
17 individual variability (95% confidence interval, for instance) observed *in situ*. To get a complete
18 overview of this realized niche, those parameters should at least be measured at several time scales,
19 depending on the life cycle of the species. The theoretical niche is not conceptually difficult to define,
20 as it should be drawn from the observation of several populations of the considered species, in
21 various environmental conditions (of pollution, geochemistry, hydromorphology...). Thus, it is just a
22 compilation of many realized niches. The most difficult parameter to evaluate is the potential niche,
23 which is the extreme deviation from the baseline that allows the population to maintain itself in the
24 ecosystem. This raises two problems: how to make sure that we measured the extreme deviation,

1 and how to ensure the sustainability of the population? It needs to combine an experimental and a
2 modeling approach. Experiments performed in realistic conditions (mesocosms), on long term to
3 allow acclimation phenomena, will be used to measure biological response levels. In the meantime,
4 population dynamics models based on the Dynamic Energy Budget in Toxicology (DEBtox) need to be
5 built to assess population sustainability (Billoir et al. 2007). The combination of those two
6 information will define the potential ecotoxicological niche defined above.

7 Once the three niche scales described, the population-level information (realized and potential) can
8 be compared to the species level information (theoretical niche), this comparison solving the
9 problem of interpopulation comparison, giving a general framework to interpret every response
10 observed. Indeed, if populations get different realized niches, but similar potential niches, we can
11 suppose that they are living in contrasted environments according to contamination. In the
12 meantime, if those differences in realized niche are small compared to the theoretical niche of the
13 species, we can suppose that the level of stress is far from the maximal level that they are able to
14 undergo. Finally, the potential niche breadth informs us on the sensitivity of each population to
15 stress. A next step should be to link ecotoxicological niche breadth to biological and ecological traits
16 such as feeding habits and food, maximum size, substrate preferences, trophic status (Poisot et al.
17 2011; Colas et al. 2014).

18

19 **Consequences for Environmental Risk Assessment (ERA) approaches**

20 The main objective of ERA approaches is to define the health status of a population. A frequent
21 confusion/debate is about the scale at which we should interpret biological responses measured on
22 an individual. We think that a misuse/misunderstanding is to consider that those parameters should
23 be interpreted as proxy of the individual health status. Well, they obviously reflect the individual
24 health status, but those individuals are sampled from a population, and thus also reflect the

1 population health status. Thus, individual-based measurements are population relevant indicators,
2 and as a consequence, variance between individuals should be interpreted as a populational
3 response.

4 *Sacrifice complexity (and realism) on the altar of reproducibility*

5 Biomarker approaches are often criticized for their lack of ecological relevance (McCarty et al 2002).
6 Indeed, to get fine information about the ecosystem health, a common approach is to focus on the
7 most sensitive species and on the most sensitive stages. However, finding such species looks like an
8 unattainable goal (Cairns Jr. 1992) and takes us away from a pertinent ecological risk assessment
9 (Baird and Barata 1999). In the same way, considering the spatio-temporal variability of physiological
10 status, the most sensitive stage at one time (which has been selected for ecotoxicology tests) could
11 be more resistant at another time (*i.e.* females should be more sensitive to lipophilic contaminants
12 before gestation than during).

13 Moreover, with the aim of controlling variability, ecotoxicology tests are based on a very specific
14 category of natural population. However, doing that, these tests are not suitable to build metric
15 representatives of the whole population. The response variability evidences phenotypic variants
16 within a population. For example, decreasing genetic diversity by selecting some clonal population of
17 *Daphnia* leads to an increase in uncertainty when performing field extrapolation (Barata et al. 2002).
18 Thus, with a limited, biased variability, we do not get a good idea of the adaptative possibilities
19 within the population, nor of the resilience/resistance abilities of the population. Therefore, rather
20 than minimize variability, it would be better to model the relative influence of intrinsic and extrinsic
21 factors on the studied biological response to better understand the system (Handy et al. 2003).

22 Finally, to demonstrate the effects of pollution and to predict population health, variance must
23 become a component of the evaluation scheme, as it can reflect system resistance and resilience

1 against perturbation (the assimilative capacity hypothesis (Cairns Jr. 1977, 1999; Death 2010; Steudel
2 et al. 2012)).

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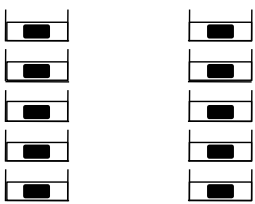
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- 1 Box 1: experimental design (for recent review and analysis of pseudoreplication, see(Bastos et al.;
- 2 Lazic 2010; Drummond and Vowler 2012; Nikinmaa M. et al. 2012).

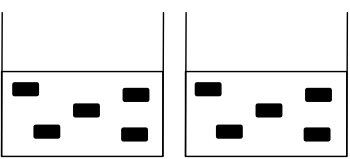
If you focus on mean, the good practice is :



5 « independant » experimental units per condition
 1 measure per unit
 = 5 replicates

Condition A Condition B

BUT if you focus on variance, the good practice is :



1 experimental units per condition
 5 measure per unit
 = 5 pseudoreplicates

Condition A Condition B

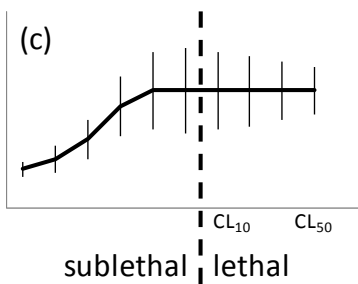
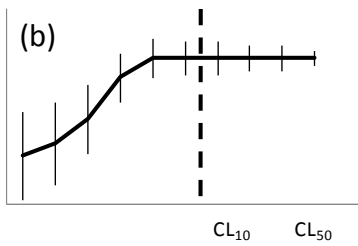
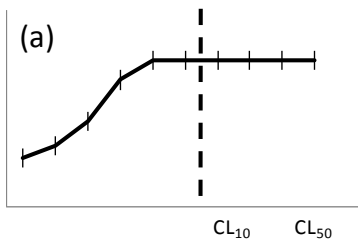
Replicated samples take into account two sources of variability : experimental units differences and inter-individual differences. Pseudo-replicated samples only consider the variability within population. The best way is to mix the two methods, that allows to work on the mean and on the variance.

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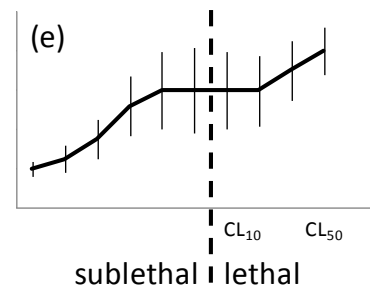
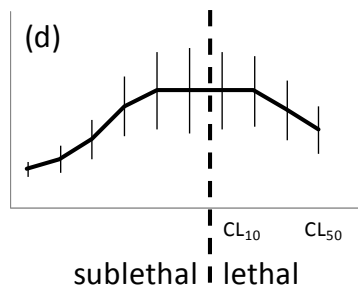
2 Box 2 :Intra-population variance scenarios



The case considered is an increase of the measured parameter in response to contamination. Beside the mean, several trends in the variance could be observed.

Sublethal exposure. The variance could be constant across conditions (a), revealing a low phenotypic variability, probably associated to a low genetic variability. A decrease in variance (b), often associated to a high variability of basal levels, could be interpreted as a canalization of the response to the most efficient one. Finally, the variance could increase (c). This last case could be linked to a continuous variation of the response in the interval observed, or to the constitution of several distinct groups of individuals (low, intermediate and high values for instance). Thus this high variability at concentrations near the lethality threshold is linked to individuals that exhibit extreme values, reflecting either low resistance abilities or a risk for the biological responses to be overcome (i.e. once the highest possible value is attained, health impairments are expected). Those two extreme situations can lead to death if the stress is maintained.

Lethal exposure. Over the lethality threshold, a decrease in variance indicates that all individuals displaying extreme values are dead. If the mean decreases (d), we can suppose that individuals exhibiting the highest responses are dead and that high induction is a signal of a soon overcoming of regulation/defense system. The last case (e) is an increase of the mean, indicating that low values of the response are linked to insufficient induction and an inability of individuals to protect themselves.

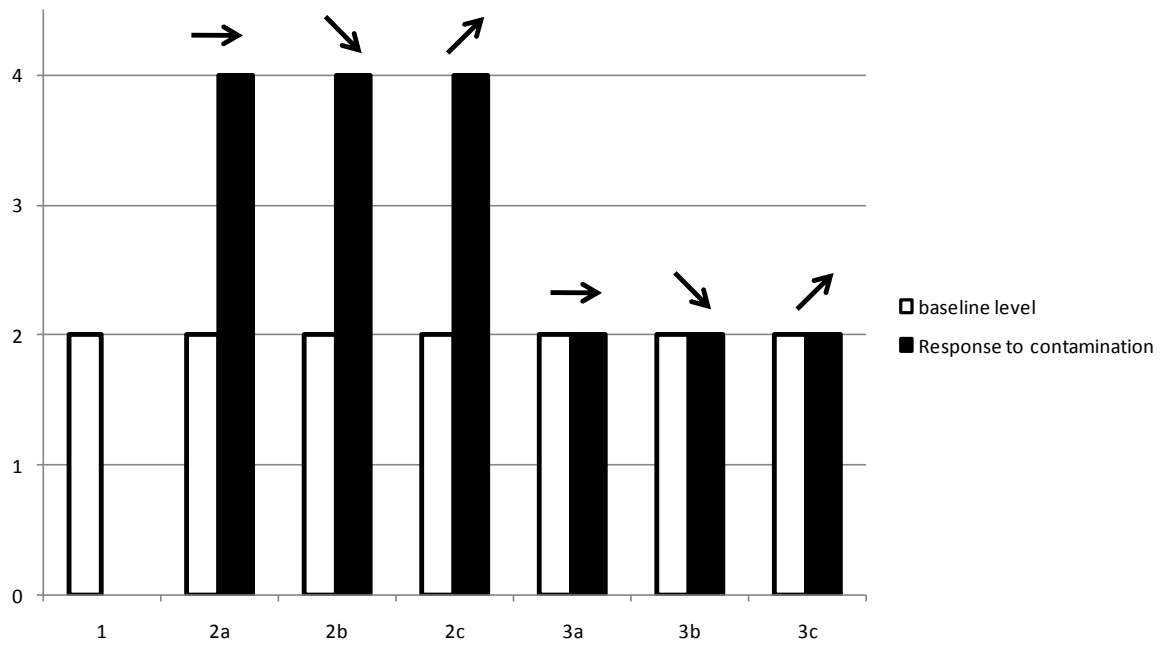


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2 Figure 1



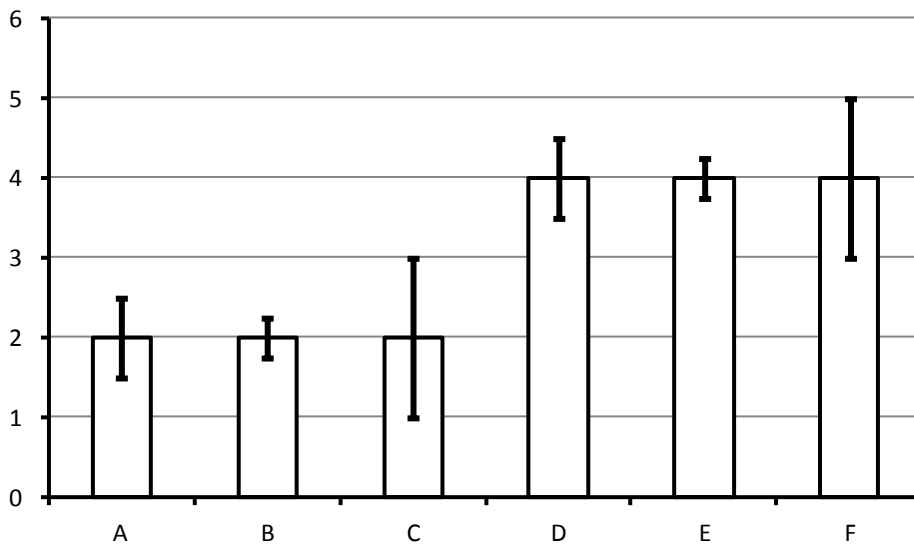
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4 Figure 1: Main mean/variance response to contamination in an active biomonitoring study. Arrows
5 indicates the direction of variance changes.

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2 Figure 2.

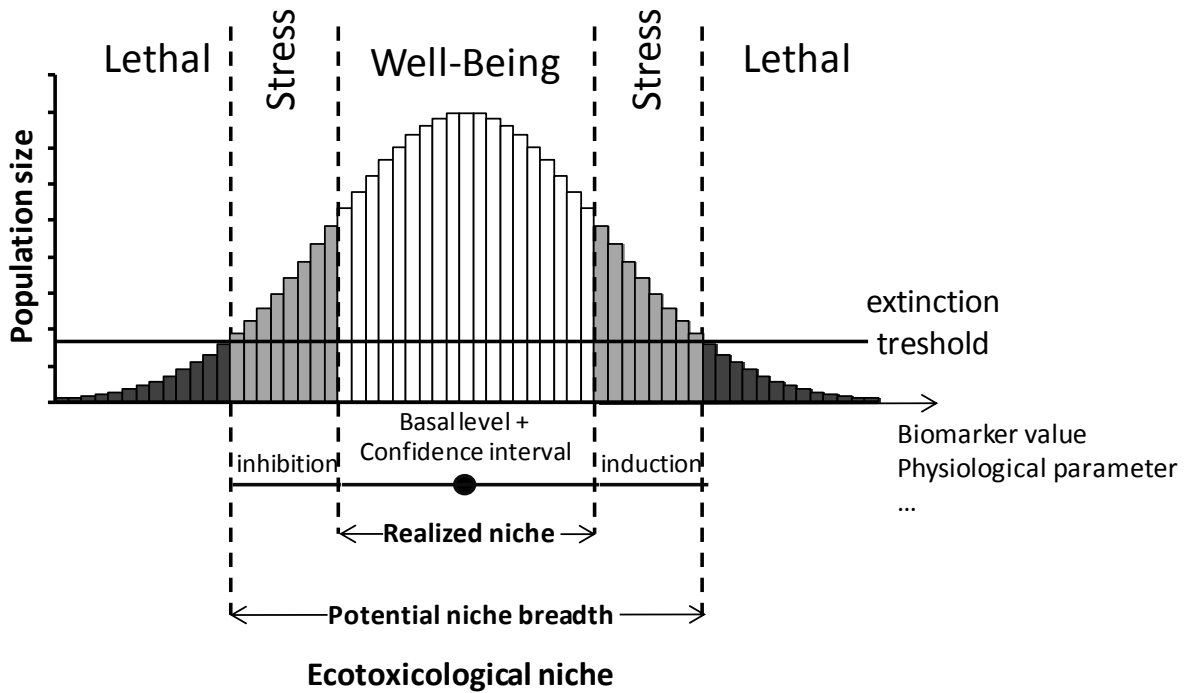


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4 Figure 2: Main mean/variance response to contamination in a passive biomonitoring study. The
5 observed biological response is induced when organisms are exposed to a toxicant. A is the reference
6 population. By comparing other population to the reference one, we can observe either a stability of
7 mean with differences in variance (B and C) or differences in means without (D) or with (E, F)
8 differences in variance.

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3 Figure 3: The ecotoxicological niche. The optimal health status for a given population is attained for
 4 its realized niche, *i.e.* when the natural, chronic conditions are encountered, contamination included.
 5 This niche thus encompasses acclimation and adaptation phenomenon. The presence of a
 6 supplementary contamination represents an important deviation from these standard conditions,
 7 leading to an increase or a decrease of the biological response, depending on the parameter
 8 considered. A first level still allows the population to maintain itself, with lower abundances: it's the
 9 potential niche. Then this potential niche is overcome, the environment became too far from the
 10 adaptative abilities of the population, which lead to local extinction.

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