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Gammarids Species in Tunisia, what Indicator Interest Cans it Prove on Fresh Waters Bio-monitoring?

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Abstract

The objective of this study is to test for the first time, the ecotoxicological interest of gammarids in Tunisia. We proceed in two steps: first, we test, in laboratory conditions, the influence of Methomyl on feeding rate (FR) and acetylcholinesterase (AChE) activity of different gammarids populations. Then we examine, *in situ*, the sensitivity of gammarids to contaminated stations.

Exposure to Methomyl had 20 µg/l significant effect on both FR and AChE in all exposed animals. Multivariate test prove that despite the variability in sensitivity, gammarids show high similarity level (80%).

For in field exposure, we observed 1) a total mortality of *Echinogammarus simoni* in oued Kasseb downstream station 2) In Bouhertma station, values of the two biomarkers were significantly less than that of animals deployed at control station, Oued Beja ($p<0.05$). Based on previous results, we are led to confirm the crucial role of gammarids in Tunisia freshwater biomonitoring.

Keywords: Methomyl; Fields' exposure; Feeding rate; AChE activity; Bioindicator; Tunisia

Introduction

In Tunisia, it is increasingly asked to ecotoxicologists to develop tools allowing to determine the intensity and duration of contamination events and to assess associated ecological risks, through the prediction of potential effects of contaminant exposure in freshwater. One approach to meet this social demand for bio monitoring methods is the development of biomarkers. This approach considers that the best method to detect the biological impact of contaminant exposure is to investigate the effects of contaminants on organism level responses. Indeed, compared to traditional methods focusing on physical and chemical properties of soils or waters, biomarkers are assumed to focus on the effects of the bioavailable (i.e. transmitted to living organisms) fraction of environmental chemicals and to integrate the putative interactive effects of complex mixtures of chemicals in the Ecological Risk Assessment (ERA). Theoretically, a "biomarker" can be defined from any observably and/or measurable functional response to exposure to one or several contaminants that can be characterized at the sub individual level of biological organization (molecular, biochemical, cellular, physiological, behavioral) [1]. Importantly, the response is assumed to indicate a departure from healthy status that cannot be detected from an intact organism [1]. The concept of biomarker is thus based on the causal relationship between the contamination of environments by any chemical inducing a stress (pesticides, polycyclic aromatic hydrocarbons (PAHs), metals, etc.) and biological changes induced by the contaminated environment.

The application of biomarkers for ERA purposes relies on more technical issues. Therefore, biomarkers should be used on sentinel species, i.e. on wild organisms sampled in natural populations from the field rather than on laboratory specimens [2]. Working on sentinel species implies that biomarkers may be developed on varying species corresponding to the taxonomic diversity of the ecosystem of interest. Considering the ERA of soil pollution in aquatic ecosystems, it is well admitted that, because they represent important ecological functions of freshwater ecosystems, species from the macrofauna should be considered as potential indicators of water quality.

The use of gammarids freshwater biomonitoring is relevant [3], because their sensitivity to pollutants and other disturbances, had offered to them to be widely used in experimental toxicity tests [3]. Many published studies exist on toxicity of a wide range of chemicals and natural water samples toward gammarids such as pesticides, metals, and surfactants [4-11].

In gammarids, toxicant-induced, reductions in feeding rate can result in reduced growth, size, fecundity, and survival of individuals [12-14], thereby affecting the stream community structure [15]. Irreversible effects of a toxicant on a behavioral mechanism or expression are also observed in the behavioral response of an organism, after the toxicokinetic and toxicodynamic processes have started (e.g., acetylcholinesterase (AChE) inhibition exerted by neurotoxins [16].

The inhibition of feeding rate was one of the first and interest observed responses to large variety of environmental contaminants [17-19]. Thus,

1. It can be correlated with ecosystem processes [20,21].
2. It has an ecological concern because it can be related to alteration in life-history traits [22-25] and
3. Its interpretation can be linked with the modulation of molecular biomarkers of specific modes of action [24,26]. The inhibition of cholinesterase (ChE) activity has been used as a specific biomarker for exposure to Organophosphore sand

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Carbamate pesticides [27,28], heavy metals [29], surfactants [30,31], hydrocarbons [32,33] and pharmaceuticals [34].

Applying gammarids, as biondicators of freshwater quality, has been extensively developed during recent decades in different countries, since many research provide its interest role environmental assessment [19,35-38]. However, the appropriate use of Tunisian gammarid species in biomonitoring freshwater required testing its bioindicator role, since no study has confirmed its ecotoxicological interest. For this, we have chosen two biomarkers jugged ideals reliable, robust, and easy applied and only modulated by contaminants. In this context, we have proposed feeding rate and AChE activity [27,19].

The present study aims to illustrate the importance gammarids as an indicator of water quality in Tunisia. We proceed in two steps: first, we test, in laboratory conditions, the influence of Methomyl on feeding rate and acetylcholinesterase activity of different gammarids populations. Then we examine, *in situ*, the sensitivity of gammarids to contaminated stations

Materials and Methods

Sampling and maintenance of gammarids

Gammarids were collected using a net (by kick sampling) from the northern rivers of Tunisia (Table 1). Stations have good water quality according to CRDA data records (Administration of Water) and a high density of gammarids was found. Different size classes were separated by sieving. Immediately after sampling, specimens were stored in plastic bottles containing stream water and quickly transferred to the laboratory. Gammarids were kept during an acclimatization period of at least 10 days in 30 L tanks continuously supplied with drilled groundwater adjusted to the sampling site conductivity (i.e., 600 $\mu S.cm^{-1}$). The tanks were under constant aeration. An 8/16-h light/dark photoperiod was maintained and the temperature was kept at 16 ± 1°C. The organisms were fed with leaves of *Quercus canariensis*. The leaves were conditioned for at least 6 ± 1 days in water. Thanks to Dr. Chritophe Piscart, seven populations of *Echinogammarus simoni* and two populations of *Gammarus nsp* were identified.

Locality	Sampling stations	GPS coordinates	Species
Hamem Saiala	Torech	36°40.96.8 N 009°09. 677 E	*E. simoni* (si1)
Bousalem	Kasseb	36°, 38.431N 009°, 00.303E	*E. simoni* (si2)
Nefza	Ain Zouraa		*E. simoni* (si 3)
Abaissia	Ain Changoula	36°48.004N 009°, 08.305E	*E. simoni* (si 4)
	Saidia	36°45.137 N 009°, 08.991 E	*E. simoni* (si 5)
Nefza	Ain Ghrab		*E. simoni* (si 6)
Balta Bouaoen	Rbaania	36°, 46.232N 008°, 55.292E	*E. simoni* (si 7)
Joumine	Ziatine	-	*E. macrophtalmi* (nsp1)
Tborba	-	-	*E. macrophtalmi* (nsp2)

Table 1: Detailed information of sampling stations.

Laboratory exposure

Choice of contaminant: Methomyl (MT) [IUPAC: S-methyl N (methylcarbamoyloxy) thioacetimidate] was tested in our experiments. The compound was widely used as carbamate insecticide because of its high insecticidal activity with rapid reversibility and its relative low persistence when compared with other insecticidal classes [39]. This insecticide has been thoroughly studied in terms of its efficiency in controlling target pests [40,41]. However, considering the non-ecologically selective profile of methomyl [41], its toxicity to aquatic non-target organisms and the further consequences of the aquatic ecosystem need scrutiny. According to Xuereb et al. [38], this pesticide inhibits the feeding rate and AChE activity in gammarids.

Methomyl exposure: The methomyl (MT) dose of 20 µg/l was chosen as a suitable concentration for our experiments. It conduced to low mortality (between 0 and 13%). MT stock solutions were prepared in ultrapure water. The contaminated media were obtained by adding 2 mL of MT stock solution to 2 L of uncontaminated drilled ground water (i.e., 600 $\mu S.cm^{-1}$; temperature previously kept to 16 ± 1°C). Water controls without toxicant were included as well as a solvent control. Four replicates of 20 male gammarids ranging in weight from 10 to 15 mg were exposed in 500 mL glass beakers maintained at 16 ± 1°C in a thermoregulated water bath. A piece of polyamide net (mesh size: 500 µm; length × width: 6 cm × 5 cm) was added to the vessel to provide a resting surface, thus minimizing cannibalism and the confrontations between organisms. To assess the feeding rate (FR), ten alder leaf discs (20 mm in diameter, without major veins) were supplied in each beaker (i.e., 5 per glass beakers). Ninety-six hour methomyl semi-static exposure tests were conducted at a temperature of 16 ± 1°C and under a photoperiod of 8/16 h light/dark. The media were renewed every 24 h, and at the same time the living organisms were counted and the dead ones were removed. Water quality parameters (pH, conductivity, temperature and dissolved oxygen) were recorded before and after the renewal of the test solutions.

***In situ* deployments:** *In situ*, exposures were adapted according to the method described by [42]. We deployed four replicates of 20 adult male gammarids (*G.simoni*) with homogenous body size in stations presented in Table 2 (near here. Organisms were placed in polypropylene cylinders (diameter 5 cm, length 10 cm) capped at their ends with pieces of net (mesh size: 1 mm). 20 alder leaf discs of *Quercus canariensis* (20 mm in diameter, without major veins) were supplied in each container. Two containers with only leaf material were deployed at each station as a control. After 7 days of exposure, the gammarids were counted (for survival rate assessment) and kept for the AChE activity measurement. The alder leaf discs were collected for FR computation.

Locality	River	GPS Coordinates	Type of pollution	Effect in invertebrates	T°C in exposure day
Beja	Oued	36°77'87.45''N	(DRRB)	-	19
	Beja	9°.15'10.40 E	-	-	-
Bousalem	Oued	36°37'42.00'' N	Industrielle	Decrese in diversity and equitability of meiofauna	20
	Kasseb	9°00'18.48'' E	Abidi Bejaoui	-	-
Bousalem	Oued	6°36'15'' N	Agricole	-	18
	Bouhertma	5°6'18.4''E	-	community	-

Table 2: Detailed informations of *in situ* deployments.

Biomarkers measurements

Feeding rate (FR): To assess the feeding rate (FR), the leaf discs were numerically scanned using an Epson perfection 3490 PHOTO® scanner at the beginning of the experiment (t_0) and every 24 h when the media were renewed. The surfaces of the ten discs were then measured daily using SigmaScan® Pro v5.0 Imaging Software (Systat Software). The FR expressed as a consumed surface per gammarid per day was (mm^2/day/organism).

Acethylcholinesterase activity: Concerning the measurements of the AChE activity, pools of five organisms were randomly sampled in each beaker of polypropylene cylinder to obtain five replicates for each population (n=5).

Immediately after sampling, the organisms were weighted, frozen in liquid nitrogen and stored at -80°C until the measure of enzyme activity. Pools of whole bodies gammarid species were homogenised in 1:10 (W: V) ice-cold phosphate buffer (100 mM; pH 7.8)+0.1% Triton X-100 with an Ultra-Turrax ®T25 basic at 24,000 rpm for 35 s. The homogenate was centrifuged at 9000 × g at 4° for 15 min then clear supernatant was collected and kept at 4°C to be used as an enzyme source. Enzyme activity was determined in triplicate for each sample according to the colorimetric method initially developed by [43] then adapted by Xuereb et al. [38]. Briefly, 990 µL of phosphate buffer (0.1 M, pH 7.8), 60 µL of the chromogenic agent DTNB (0.0076 M) and 60 µL of supernatant were added to the bath. The measurement of enzyme activity was initiated by adding 30 µL of acetylthiocholine iodide solution (0.076 M). The absorbance measurement was recorded at 405 nm every 60 s for 7 min using JENWAY 6300**.** The absorption kinetics were calculated in a linear range and then converted to Nano moles per minute according to the molar extinction coefficient of DTNB (ε=1.36 × 104 $Lmol^{-1}cm^{-1}$) [38].

Data Analysis

Statistical analyses were performed using Statistica 9 software (StatSoft, USA) and expressed as a mean ± standard deviation. The differences in variability of biomarkers' populations and species and the effect of methomyl *in situ* exposure on the FR and AChE activity were examined using the ANOVA test. Non parametric tests were used when data did not fulfill homogeneity requirements. Multivariate analysis was performed on both AChE activity and FR for every population. A similarity matrix based on Bray-Curtis coefficient was classified by hierarchical agglomerative clustering using Unweighted Pair Group Mean Arithmetic (UPGMA) and multi-dimensional scaling. The examination of relative 180 similarities of populations through relative ordination distance was done by means of non-metric multidimensional scaling ordination (MDS). The Analysis of Similarity Sample Statistic Global (ANOSIM tests) were carried out to determine if there were significant differences between gammarid species considering both markers at the same time. SIMPER (Similarity Percentage) led to assess the biomarker that contributed to the average of similarity between gammarid species. Calculations were performed with PRIMER 6.0. The level of significance was established at P<0.05 for statistical tests.

Results

For basic values of feeding rate and AChE, no significant differences were observed between the populations of *E.simoni* and the two populations of *Gammarus nsp* (p-value ≥ 0.05). At inter-specific level, FRs and AChE activity were homogeneous and no significative difference was found (p-value ≥ 0.05) (Figures 1a, 1b and 1c).

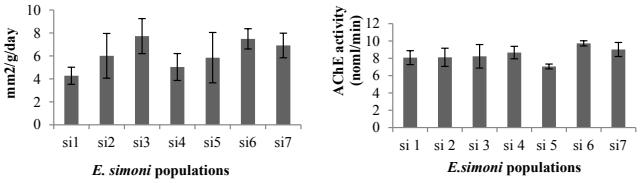

Figure 1a: Basic levels of AChE activity (nmol/min) and feeding rate (mm^2/G/j) in populations of *E. simoni* (si) and *G.* new species (nsp) respectively.

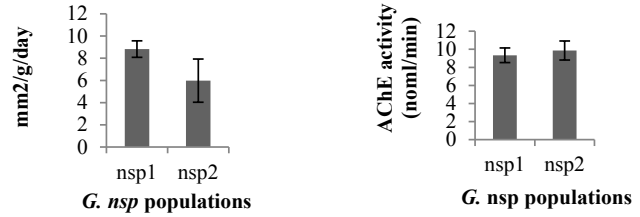

Figure 1b: Basic levels of AChE activity (nmol/min) and feeding rate (mm^2/G/j) in populations of and *G.* new species (*nsp*) respectively.

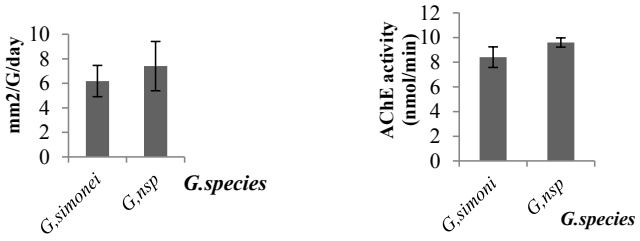

Figure 1c: Basic levels AChE activity (nmol/min) and feeding rate (mm^2/G/j) in the two species.

After 20 $\mu g.L^{-1}$ of methomyl exposure, there is no significant mortality in exposed gammarids (23%). However, this compound inhibits significantly (p-value ≤ 0.05) the feeding rate (FI between 50%-98%) and the AChE activity (RI between 20% and 60%). An intra-specific difference (p-value<0.005) was found in FI and RI. Based on inter-specific levels, our results showed a significant difference in value of AChE activity; however no significant variability was registered in FI (p value ≥ 0.5) between *E.simoni* and *Gammarus nsp* (Figures 2a, 2b and 2c).

MDS, Simper and ANOSIM test

MDS results and ANOSIM test (Figure 3 and Table 3) indicate that, for basic and sensitive values of the two biomarkers, all gammarid populations were arranged on one group with high percentage of similarity (80%) and no significant variability was registered between them (ANOSIM R=0.28 for basic value and 0.374 for sensitive value; p-value ≥ 0.05). The SIMPER results showed that both AChE activity and feeding rate (basic and sensitive values) contribute to average of similarity.

In situ exposure

In oued Kasseb downstream station, we observed a total mortality of *E.simoni*. However, in Bouhertma, level of mortality was less than 20%. In this station, values of the two biomarkers were significantly less than that of animals deployed at Oued Beja (p<0.05) (Figure 4).

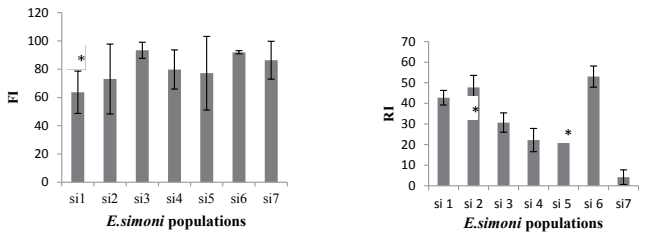

Figure 2a: Feeding inhibition (FI) and AChE inhibition(RI) in populations of *E.simoni* (si) respectively, after methomyl exposure (20µg/L).

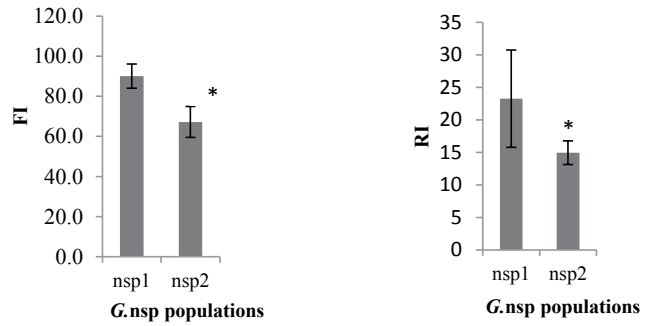

Figure 2b: Feeding inhibition (FI) and AChE inhibition(RI) in populations of *G.* new species (nsp) respectively after methomyl exposure (20µg/L).

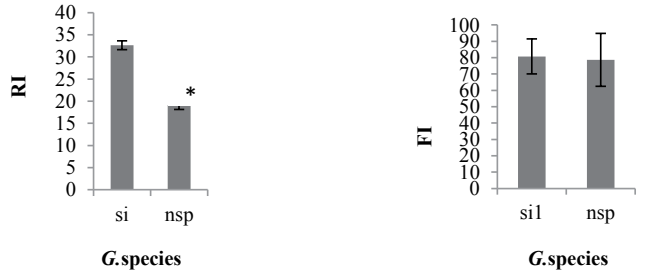

Figure 2c: FI and RI in the two species. Significant differences against the controls (0.lg/l) are indicated by asterisks (one way-ANOVA and HSD Tukey post hoc; (n=20; *p<0.005).

Discussion

Basic value of AChE activity and feeding rate

Our results showed that the feeding levels were similar throughout all tested populations. These observations suggest that Tunisian gammarids will feed whenever food is available regardless their origin. However, knowing that environmental conditions tend to vary over space and time in nature, these later are known to favor different species potentially leading to different behavior. Nevertheless, we suggest that the gammarid tested populations were more closely related species to still share more similar niches under a different

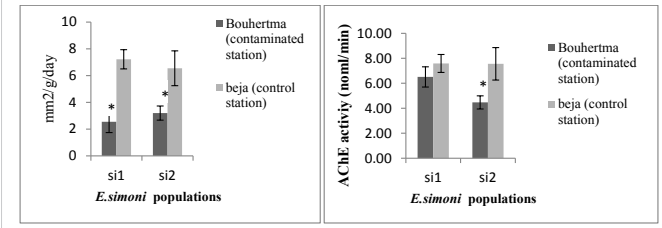

Figure 4: AChE activity rate in *E. simoni* populations (si1 and si2) exposed in reference station (Beja upstream) and in polluted station (Bouhertma downstream). Significant differences against the controls (0.lg/l) are indicated by asterisks (one way-ANOVA and HSD Tukey post hoc; (n=20; *p<0.005).

Groups	ANOSIM: Analysis of Similarities sample stastic Global R: 0.177 Significance level of sample stastic:18.8%		SIMPER : Similarity Percentages-species contributions		
	R statistic	Significance level %	Average similarity	Markers contribuate	% contribution to average similarity value
Basic values of the two tested biomarkers	0.374	0.1	84%	RI (AChE)	53.79
				activity	53.79
				FI	53.79
Sensitives values of the two tested biomarkers	0.287	0.2	91.1	AChE	58.61
				activity	41.39
				Feeding rate	-

Table 3: Pairwaise similarity in Gammarid species (*E. simoni* and *G. nsp*) and percentage of biomarkers contribution.

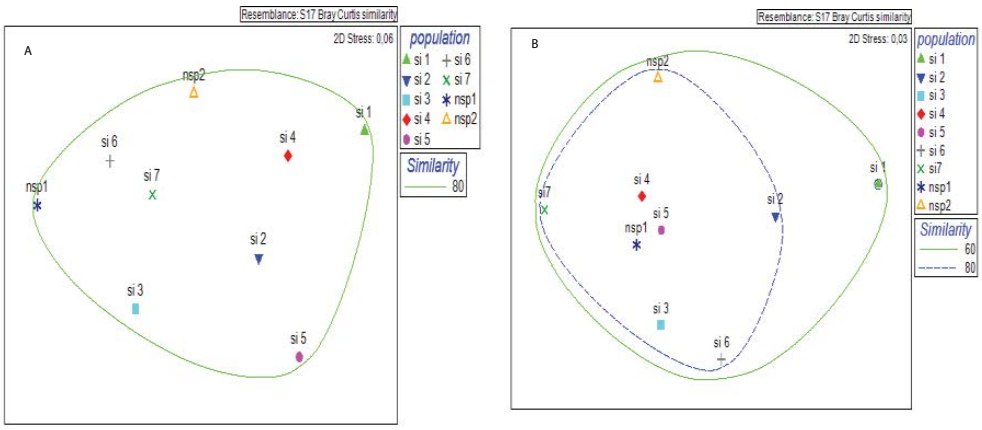

Figure 3: Arrangement of the gammarid populations according to the MDS (non- parametric Multi-Dimensional Scaling) method realized by considering the transformed basic (a) and sensitive (b) values (square root) of feeding rate (FR) and AChE activity of each population (si: *simoni*; nsp: new species).

environment, which may lead to found behavior similarity as it has been shown in our study.

Our results showed that AChE activity, varied between 7.5 and 9.75 nmol/min in all tested populations. These values were near those of 25 with different *G. fossarum* populations (between 7.4 and 9.5). Our results showed also absence of intra and interspecific variability of AChE values which could be probably explained by genetic stability. This state was also noted by [27] in *G. fossarum*. However, some other authors observed significance interspecific difference in mean activity of AChE in *Acartia* and *Siriella Genus* [44]. According to Toumi et al. [45], there is also intraspecific significance variability in AChE activity in the cladoceran *Daphnia magna*. This state may be explained by difference in expression of AChE value. Thus, in his study of AChE activity normalization, [27] affirm that when expression of AChE activities are normalized against the protein content in sample extracts and expressed in nanomoles of substrate hydrolyzed or DO units min^{-1} mg $protein^{-1}$, it may cause a variation in AChE activities. This state is due to the natural variation of structural protein contents, related to physiological changes (such as reproductive status) and constitutes a source of variability leading probably to an under- or over-estimation of the basal level of AChE activity. However, when AChE activity was expressed in nmol min^{-1} (our case), this variation is lower and generally no significative.

Sensitives values of AChE activity and feeding rate after methomyl exposure

Despite its different origins, organisms showed similar stress responses to methomyl (20 µg/L) exposure. The current study is in good agreement with previous research concerning the effects of methomyl in diverse species of gammarids such as *G. fossarum* and *G. pulex* [28,46,47]. Concerning classical mechanistic action of methomyl on AChE activity, [48] affirm that carbamic acid esters of methomyl attach to the serine hydroxyl group of the reactive site of AChE. When unbound acetylcholine accumulates at the cholinergic nerve endings, there is continual stimulation of electrical activity.

The significative variability in inhibition of the two biomarkers (at intra and interspecific level) could probably assign to variability of organism response after methomyl exposure. It's clear that pollution create disturbance in behavior and that each population response according to its physiology and capacity to defend itself. Thus, difference on metabolic pathways predominantly employed in detoxification and excretion capabilities has been described by [46]. The genetic differences may, also, lead to a difference in inhibition rate [49]. Therefore, deviations among cryptic lineages regarding physiological and behavioral characteristics are conceivable as well [50].

Following the trend observed in inhibition of AChE activity, methomyl was chronically more toxic to the *Gammarus nsp* populations than to *E. simoni*. Considering that methomyl is a contact insecticide [51], the main intake route of the toxicant in gammarids will be mostly through body surface rather than via filtration of toxicant-bound food particles. Accordingly, a difference surface-to-volume ratio may explain the difference of sensitivity (RI) to methomyl of gammarids. Further research will be done to see difference with presence or no surface-to-volume ratio difference between the two species found if populations are genetically distinct certainly in mechanism of detoxification and therefore their tolerance to the toxicant can be constrained by the genotype.

Multivariate analysis proved that intra and interspecific variability of basic or sensitive values of two biomarkers (AChE activity, feeding rate) does not exclude the approach of using model gammarid populations for biomonitoring water quality.

In situ exposure

The biological monitoring of the environment or "biomonitoring" has the objective of integrating these various aspects, particularly by using sentinel species as model. Thus, in situ exposure takes into account the influence of the multiple parameters present in the environment that intervene under natural conditions to affect toxicity: abiotic ecological factors (such as temperature, salinity, conditions of oxygenation), interspecific variability and the interactions between species, the heterogeneity of populations in their interactions with pollutants, or between pollutants an abiotic factors of the environment [52]. For this, we proposed, for the first time an in situ exposure with gammarids.

The two used biomarkers have been proposed as ecologically relevant *in situ* indicator of water quality [19,21,26]. Values of AChE activity and feeding rate observed in reference station (Oued Beja upstream) was similar to those observed in our laboratory study, in controls. This state, led us to purpose this station as reference. However, several authors affirm that biomarkers values, in reference station, should not usually be defined as baseline values, such biotic and non-toxic environmental influences could lead to the misinterpretation of individual markers in water chemical quality assessment, during *in situ* or post exposure assays, with caged organisms such us temperature, conductivity, pH, season). However, for gammarids, [19] affirm that feeding rate was only modulated by season and temperature. In addition, AChE activity was not influenced by any abiotic factors [26]. Finally, our *in situ* exposure was done on the same season and same periods that let us purpose this station as reference.

Total mortality of *E. simoni* populations in Kasseb downstream, should be related to the low oxygen level, caused by high Biologic Oxygen Demand. Thus, [53] affirmed that effluent of dairy caused high organic matter pollution. The low percentage of mortality in Bouhetma downstream, does not means good water quality. Thus, feeding rate and Ache activity decrease significantly. These observations should be a result of an exposure of varied pollution such as pesticide, heavy metals, as affirmed by several authors [18,19,24,54-58].

Conclusion

Despite the interest of gammarids in biomonitoring in Tunisia, the use of this aquatic invertebrate in toxicology is still absent. In this context, we proposed to test in laboratory and in the fields the response of gammarids to methomyl and pollution respectively. Results show that despite the variability in sensitivity, in all exposed animals AChE activity and feeding rate decrease significantly. This state proves that tunisian gammarids are robust, responsive, and relevant for Tunisian fresh water biomonitoring. Clearly, there is a need for further studies in this area, to include both laboratory and field studies from clean and reference sites, we suggest the following priority research areas like a) Field monitoring is required to assess the quality of the majority of Tunisian rivers, b) Studies are required to develop and adapt other biomarkers in gammarids assessing water quality and c) Assessments into the impacts of known industrial pollutants in Tunisia on Gammarids.

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References

1. Van Gestel CA, Van Brummelen TC (1996) Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. Ecotoxicol 5: 217-225.
2. Beeby A (2001) What do sentinels stand for? Environ Pollut 112: 285-298.
3. Kunz PY, Kienle C, Gerhardt A (2010) *Gammarus* spp. in aquatic ecotoxicology and water quality assessment: toward integrated multilevel tests. Rev Environ Cont Toxicol 205: 1-76.
4. McCahon CP, Pascoe D (1988) Use of *Gammarus pulex* (L.) in safety evaluation tests: culture and selection of a sensitive life stage. Ecotoxicol Environ Saf 15: 245-252.
5. Macek KJ, Buxton KS, Derr SK, Dean JW, Sauter S (1976) Chronic toxicity of lindane to selected aquatic invertebrates and fishes. US Environmental Protection Agency. Ecological Research Series, EPA-60013-76-047, Washington DC.
6. Williams DD, Moore KA (1985) The role of semiochemicals in benthic community relationships of the lotic amphipod Gammarus pseudolimnaeus: A laboratory analysis. Oikos 44: 280-286.
7. Mian S, Mulla S (1992) Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems. J Agric Entomol 9: 73-98.
8. Pantani C, Pannunzio G, De Cristofaro M, Novelli AA, Salvatori M (1997) Comparative acute toxicity of some pesticides, metals, and surfactants to *Gammarus italicus* Geodm. and *Echinogammarus tibaltii* Pink. and Stock (Crustacea: Amphipoda). Bull Environ Contam Toxicol 59: 963-967.
9. Wogram J, Liess M (2001) Rank ordering of macroinvertebrate species sensitivity to toxic compounds by comparison with that of *Daphnia magna*. Bull Environ Contam Toxicol 67: 360-367.
10. Van Wijngaarden RPA, Cuppen JGM, Arts GHP, Crum SJH, Van den Hoorn MW, et al. (2004) Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: a microcosm study. Environ Toxicol Chem 23: 1479-1498.
11. Bloor MC, Banks CJ, Krivtsov V (2005) Acute and sub-lethal toxicity tests to monitor the impact of leachate on an aquatic environment. Environ Int 31: 269-273.
12. Anderson NH, Cummins KW (1979) Influences of diet on the life histories of aquatic insects. J Fish Res Board Can 36: 335-342.
13. Maltby L, Naylor C (1990) Preliminary observations on the ecological relevance of the gammarus scope for growth' assay: effect of zinc on reproduction. Funct Ecol 4: 393-397.
14. Geffard O, Xuereb B, Chaumot A, Geffard A, Biagianti S, et al. (2010) Ovarian cycle and embryonic development in *Gammarus fossarum*: application for reproductive toxicity assessment. Environ Toxicol Chem 29: 2249-2259.
15. Sutcliffe DW, Hildrew AG (1989) Invertebrate communities in acid streams. In: Morris R, Taylor EW, Brown DJA, Brown JA (eds) In acid toxicity and aquatic animals. Society for experimental biology, Seminar Series 34, Cambridge: Cambridge University Press, pp: 13-29.
16. Gerhardt A (1995) Monitoring behavioral responses to metals in *Gammarus pulex* (L.) (Crustacea) with impedance conversion. Environ Sci Poll Res 2: 15-23.
17. Macedo-Sousa JA, Pestana JL, Gerhardt A, Nogueira AJA, Soares AM (2007) Behavioural and feeding responses of Echinogammarus meridionalis (Crustacea, Amphipoda) to acid mine drainage. Chemosph 67: 1663-1670.
18. Mouneyrac C, Perrein-Ettajani H, Amiard-Triquet C (2010) Influence of anthropogenic stress on fitness and behaviour of a key-species of estuarine ecosystems, the ragworm Nereis diversicolor. Environ Pollut 158: 121-128.
19. Coulaud R, Geffard O, Xuereb B, Lacaze E, Queau H, et al. (2011) In situ feeding assay with Gammarus fossarum (Crustacea): modelling the influence of confounding factors to improve water quality biomonitoring. Water Res 45: 6417-6429.
20. Forrow DM, Maltby L (2000) Toward a mechanistic understanding of contaminant-induced changes in detritus processing in streams: direct and indirect effects on detritivore feeding. Environ Toxicol Chem 19: 2100-2106.
21. Maltby L, Clayton SA, Wood RM, McLoughlin N (2002) Evaluation of the *Gammarus pulex* in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. Environ Toxicol Chem 21: 361-368.
22. Maltby L (1999) Studying stress: the importance of organism level responses. Ecol Appl 9: 431-440.
23. Baird DJ, Brown SS, Lagadic L, Liess M, Maltby L, et al. (2007) In situ-based effects measures: determining the ecological relevance of measured responses. Integra Environ Assess Manage 3: 259-267.
24. Barata C, Damasio J, Lopez MA, Kuster M, De Alda ML, et al. (2007) Combined use of biomarkers and in situ bioassays in *Daphnia magna* to monitor environmental hazards of pesticides in the field. Environ Toxicol Chem 26: 370-379.
25. Coulaud R, Geffard O, Vigneron A, Quéau H, François A, et al. (2015) Linking feeding inhibition with reproductive impairment in *Gammarus* confirms the ecological relevance of feeding assays in environmental monitoring. Environ Toxicol Chem 34: 1031-1038.
26. Xuereb B (2009) Development of biomarkers of neurotoxicity and endocrine disruption in the freshwater amphipod *Gammarus fossarum*. pp: 348.
27. Xuereb B, Chaumot A, Mons R, Garric J, Geffard O (2009) Acetylcholinesterase activity in *Gammarus fossarum* (Crustacea Amphipoda). Intrinsic variability, reference levels, and a liable tool for field surveys. Aquat Toxicol 93: 225-233.
28. Xuereb B, Noury P, Felten V, Garric J, Geffard O (2007) Cholinesterase activity in *Gammarus pulex* (Crustacea Amphipoda): characterization and effects of chlorpyrifos. Toxicol 236: 178-189.
29. Jebali J, Banni M, Guerbej H, Almeida E, Bannaoui A et al. (2006) Effects of malathion and cadmium on acetylcholinesterase activity and metallothionein levels in the fish Seriola dumerilli. Fish Physiol Biochem 32: 93-98.
30. Guilhermino L, Lacerda MN, Nogueira AJA, Soares AMVM (2000) In vitro and in vivo inhibition of *Daphnia magna* acetylcholinesterase by surfactant agents: possible implications for contamination biomonitoring. Sci Total Environ 247: 137-141.
31. Jifa W, Zhiming Y, Xiuxian S, You W, Xihua C (2005) Comparative researches on effects of sodium dodecylbenzene sulfonate and sodium dodecyl sulfate upon Lateolabrax japonicus biomarker system. Environ Toxicol Pharmacol 20: 465-470.
32. Kang JJ, Fang HW (1997) Polycyclic aromatic hydrocarbons inhibit the activity of acetylcholinesterase purified from electric eel. Biochem Biophys Res Commun 238: 367-371.
33. Oropesa AL, Perez-Lopez M, Hernandez D, Garcia JP, Fidalgo LE (2007) Acetylcholinesterase activity in seabirds affected by the Prestige oil spill on the Galician coast (NW Spain). Sci Total Environ 372: 532-538.
34. Nunes B, Carvalho F, Guilhermino L (2006) Effects of widely used pharmaceuticals and a detergent on oxidative stress biomarkers of the crustacean Artemia parthenogenetica. Chemosph 62: 581-594.
35. Gerhardt A, Carlsson A, Ressemann C, Stich KP (1998) Newonline biomonitoring system for *Gammarus pulex* (L.) (Crustacea): in situ test below a copper effluent in south Sweden. Environ Sci Technol 32: 150-156.
36. Schulz R, Liess M (1999) A field study of the effects of agriculturally derived insecticide input on stream macroinvertebrate dynamics. Aquat Toxicol 46: 155-176.
37. Dedourge-Geffard O, Palais F, Biagianti-Risbourg S, Geffard O, Geffard A (2009) Effects of metals on feeding rate and digestive enzymes in *Gammarus fossarum*: an in situ experiment. Chemosph 11: 1569-1576.
38. Xuereb B, Lefevre E, Garric J, Geffard O (2009) Acetylcholinesterase activity in *Gammarus fossarum* (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration. Aquatic Toxicol 94: 114-122.
39. Hutson DH, Roberts TR (1985) Progress in pesticide biochemistry and toxicology. In: John Wiley & Sons (eds), Chichester, UK, pp: 368.
40. Reitz SR, Kund GS, Carson WG, Phillips PA, Trumble JT (1999) Economics of reducing insecticide use on celery through low-input pest management strategies. Agric Ecosyst Environ 73: 185-97.
41. Ehler LE (2004) An evaluation of some natural enemies of *Spodoptera exigua* on sugarbeet in Northern California. Biocontrol 49: 121-35.
42. Maltby L, Crane M (1994b) Responses of *Gammarus pulex* (Amphipoda, crustacea) to metalliferous effluents: identification of toxic components and the importance of interpopulation variation. Environ Pollut 84: 45-52.
43. Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid

colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88-95.

44. Minutoli R, Fossi MC, Guglielmo L (2002) Evaluation of acetylcholinesterase activity in several zooplanktonic crustaceans. Mar Environ Res 54: 799-804.

45. Toumi H, Boumaiza M, Immel F, Sohm B, Felten V, et al. (2014) Effect of deltamethrin (pyrethroid insecticide) on two strains of *Daphnia magna* (Crustacea, Cladocera): a proteomic investigation. Aquat Toxicol 148: 40-47.

46. Kuhn K, Streit B (1994) Detecting sublethal effects of organophosphates by meusuring acetylcholinesterase activity in *Gammarus*. Bull Environ Contam Toxicol 53: 398-404.

47. McLoughlin N, Yin D, Maltby L, Wood RM, Yu H (2000) Evaluation of sensitivity and specificity of two crustacean biochemical biomarkers. Environ Toxicol Chem 19: 2085-2092.

48. Costa LG (2008) Toxic effects of pesticides. In: Casarett & Doull's Toxicology. (7thedn) the basic science of poisons. (7thedn), New York: McGraw-Hill.

49. Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22: 148-155.

50. Feckler A, Thielsch A, Schwenk K, Schulz R, Bundschuh M (2012) Differences in the sensitivity among cryptic lineages of the *Gammarus fossarum* complex. Sci Total Environ 439: 158-164.

51. Clive T (2001) Methomyl. In: Tomlin CDS (eds) The pesticide manual. Surrey: British Crop Protection Council, pp: 60-621.

52. Smolders E, Buekers J, Oliver I, McLaughlin MJ (2004) Soil properties affecting toxicity of zinc to soil microbial properties in laboratory-spiked and field-contaminated soils. Environ Toxicol Chem 23: 2633-2640.

53. Abidi S, Bejaoui M (2011) Influence of pollution on water quality and the meriowave of oued kasseb (Northern Tunisia). Bull Soc zool Fr 136: 145-157.

54. Naylor C, Maltby L, Calow P (1989) Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia 188: 517-523.

55. Maltby L, Naylor C, Calow P (1990a) Effect of stress on a freshwater benthic detritivore: Scope for growth in *Gammarus pulex*. Ecotoxicol Environ Saf 19: 285-291.

56. Maltby L, Naylor C, Calow P (1990b) Field deployment of a scope for growth assay involving Gammarus pulex, a freshwater benthic invertebrate. Ecotoxicol Environ Saf 19: 292-300.

57. Crane M, Maltby L (1991) The lethal and sublethal responses of *Gammarus pulex* to stress: sensitivity and sources of variation in an in situ bioassay. Environ Toxicol Chem 10: 1331-1339.

58. Matthiessen P, Sheahan D, Harrison R, Kirby M, Rycroft R, et al. (1995) Use of a *Gammarus pulex* bioassay to measure the effects of transient carbofuran runoff from farmland. Ecotoxicol Environ Saf 30: 111-119.