Ecological Indicators 93 (2018) 351–357

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

An integrated biomarker response study explains more than the sum of the parts: Oxidative stress in the fish *Australoheros facetus* exposed to imidacloprid

Fernando G. Iturburu[a], Lidwina Bertrand[b], Julieta R. Mendieta[c], María V. Amé[b], Mirta L. Menone[a,*]

[a] *Laboratorio de Ecotoxicología, Instituto de Investigaciones Marinas y Costeras (IIMYC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de Mar del Plata, Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina*

[b] *Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI), Facultad de Ciencias Químicas, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de Córdoba, Haya de la Torre esq., Medina Allende, 5000 Córdoba, Argentina*

[c] *Instituto de Investigaciones Biológicas, Facultad de Ciencias Exactas y Naturales, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de Mar del Plata, Funes 3250, 7600 Mar del Plata, Argentina*

ARTICLE INFO

Keywords:
Neonicotinoid
Integrated biomarker response index
Antioxidant enzymes
Oxidative damage
Fishes

ABSTRACT

Integrated Biomarkers Response (IBR) index have been developed as a practical and robust tool to assess the susceptibility to pollutants using multiple biomarker responses. Neonicotinoid insecticides are nowadays one of the most sold pesticides worldwide. Nevertheless, imidacloprid (IMI) sub-lethal effects such as oxidative stress (OS) on fishes are scarcely studied. Hence, the aims of this work were: (1) to evaluate exposure- and damage biomarkers related to OS in the freshwater fish *Australoheros facetus* exposed to IMI and (2) to apply the IBR index to achieve a comprehensive understanding of OS in the fish. The results of the present study showed that all the biomarkers presented different responses in the three monitored tissues: liver, brain and gills. Results for an initial battery of 19 biomarkers were obtained and for the IBR index only those with significant differences have been considered. The biomarkers that had the most important weight on the IBR index were SOD activity in brain and gills, H_2O_2 concentration in liver, and carbonyl groups concentration in gills in fishes exposed to 100 and 1000 $\mu g\,L^{-1}$ IMI. This index allowed affirming that a short term exposure to environmentally relevant concentrations of IMI ($\geq 10\,\mu g\,L^{-1}$) produces OS in *A. facetus*. However, a more deep understanding of some biomarkers response is necessary to improve the index and for finally apply it in field studies.

1. Introduction

The identification of ecological risks due to exposure of aquatic organisms to environmental pollutants is a crucial point for environmental managers. Nevertheless, the complexity of direct and indirect interactions among different ecosystem components in wild populations makes assessment of the impacts of environmental pollutants on aquatic species challenging (Santos et al., 2016). In this context, systematic assessment methods of potential risk of pollutants (i.e., pesticides) could serve as valuable tools in decision making and policy formulation (Kookana et al., 2005).

In the last 30 years the literature showed the use of different parameters able to explain effects of pollutants on different organisms (i.e., fish) at community or individual levels. To synthesize this information, different authors have developed a wide variety of indices and metrics used in biological monitoring (Revenga et al., 2005). In the case of fish communities, the index of biotic integrity (IBI) was developed in the 1980 decade (Karr, 1981). It is an ecological approach that incorporates multiple attributes of a fish community into a composite index predictive of water quality (Eaton and Lydy, 2000), successfully used in several studies (i.e., Scott and Hall, 1997). Later, Oberdorff et al. (2002) developed a modification called fish based index (FBI), a biological indicator which integrate environmental factors acting on fish assemblage structure in natural conditions able to distinguish effects of human-induced disturbances from natural variation.

Beyond these community indices, morphometric indices are used frequently to estimate fish general health (growth, nutritional state and energy content) under the assumption that morphometric changes track to physiological changes (Sutton et al., 2000). These kind of indices, like body condition indices or condition factors, are common indices in

* Corresponding author at: Funes 3350, 7600 Mar del Plata, Buenos Aires, Argentina.
E-mail address: mirta.menone@gmail.com (M.L. Menone).

https://doi.org/10.1016/j.ecolind.2018.05.019
Received 9 December 2017; Received in revised form 7 March 2018; Accepted 7 May 2018

Table 1
Oxidative stress biomarkers response in different tissues of *Australoheros facetus* exposed to imidacloprid. Catalase, glutathione S- transferases and glutathione reductase activities are expressed as nkat mg^{-1} protein. SOD activity expressed as U mg^{-1} protein. H_2O_2 and TBARS concentration are expressed as nmol mg^{-1} fresh weight tissue, and carbonyl groups as µmol mg^{-1} protein. Data are expressed as the average ± standard deviation (SD). Different letters show significant differences among treatments ($p < 0.05$).

Biomarker	[IMI] (µg L^{-1})	Tissue						Tissue Difference
		Liver		Gills		Brain		
CAT	0	2575 ± 471	a	427.49 ± 68.09	a			YES
	1	2767 ± 640	a	488.42 ± 197.51	a			
	10	2628 ± 547	a	362.07 ± 101.88	a			
	100	2602 ± 936	a	372.76 ± 42.07	a			
	1000	2723 ± 804	a	250.77 ± 26.31	a			
SOD	0	4204 ± 384	a	3034 ± 307	a	4156 ± 470	a	YES
	1	4199 ± 710	a	2769 ± 349	a	4049 ± 610	a	
	10	4129 ± 191	a	1161 ± 45	b	3702 ± 304	ab	
	100	3870 ± 458	a	1077 ± 151	b	3492 ± 379	b	
	1000	3296 ± 381	b	739 ± 60	c	2968 ± 264	c	
GST	0	14.75 ± 3.87	a	5.98 ± 1.13	a	2.10 ± 0.63	a	YES
	1	12.99 ± 1.61	a	6.83 ± 1.70	a	2.10 ± 0.49	a	
	10	13.22 ± 2.50	a	5.59 ± 0.94	a	2.27 ± 0.48	a	
	100	13.43 ± 4.34	a	6.72 ± 1.47	a	1.91 ± 0.66	a	
	1000	14.24 ± 3.03	a	7.15 ± 2.41	a	1.57 ± 0.67	a	
GR	0	0.37 ± 0.05	a	2.56 ± 0.63	a			YES
	1	0.34 ± 0.21	a	2.78 ± 0.51	a			
	10	0.22 ± 0.04	a	2.41 ± 0.37	a			
	100	0.30 ± 0.09	a	2.97 ± 0.22	a			
	1000	0.38 ± 0.10	a	2.46 ± 0.42	a			
H_2O_2	0	1.15 ± 0.05	a	0.72 ± 0.01	a	0.83 ± 0.03	a	YES
	1	1.01 ± 0.06	b	0.72 ± 0.02	a	0.79 ± 0.01	b	
	10	1.00 ± 0.02	b	0.72 ± 0.01	a	0.81 ± 0.02	ab	
	100	2.39 ± 0.07	c	0.73 ± 0.01	a	0.81 ± 0.02	b	
	1000	3.42 ± 0.12	d	0.73 ± 0.01	a	0.80 ± 0.01	b	
TBARS	0	0.05 ± 0.01	a	0.06 ± 0.02	a	0.06 ± 0.00	a	YES
	1	0.05 ± 0.01	a	0.07 ± 0.02	a	0.06 ± 0.01	a	
	10	0.05 ± 0.02	a	0.07 ± 0.02	a	0.07 ± 0.02	a	
	100	0.07 ± 0.03	a	0.10 ± 0.02	a	0.05 ± 0.02	a	
	1000	0.06 ± 0.02	a	0.07 ± 0.02	a	0.10 ± 0.01	a	
CG	0	0.014 ± 0.002	a	0.015 ± 0.004	a	0.014 ± 0.005	a	YES
	1	0.012 ± 0.005	a	0.011 ± 0.004	a	0.016 ± 0.004	a	
	10	0.013 ± 0.005	a	0.014 ± 0.004	a	0.015 ± 0.006	a	
	100	0.009 ± 0.002	b	0.011 ± 0.004	a	0.016 ± 0.004	a	
	1000	0.012 ± 0.002	a	0.009 ± 0.003	b	0.014 ± 0.005	a	

pollution effect studies (i.e., Brodeur et al., 2017). More particularly, histopathological indices (Maggioni et al., 2012) and fish somatic indices that relate the weight of determined tissues (i.e. liver, spleen, gonads) with the total fish weight are able to show pollutants effects on fish (i.e., Guyón et al., 2016; Ballesteros et al., 2017). On the other hand, fish biomarkers are useful tools in several steps of the risk assessment process: effect, exposure and hazard assessment, risk characterization or classification, and monitoring the environmental quality of aquatic ecosystems (van der Ooost et al., 2003). However, data provided by this biomarker approach is difficult to interpret without an integrated overview that globally assesses the potential influence of the pollutant under study (Bertrand et al., 2016a). Hence, stress indices also have been developed from this type of parameters. A prominent example of them is the Integrated Biomarkers Response (IBR) index, which constitutes a practical and robust tool to assess the susceptibility to pollutants using multiple biomarker responses (Beliaeff and Burgeot, 2002; Serafim et al., 2012). Several studies used this index with field data (Damiens et al., 2007; Cravo et al., 2012; Pain-Devin et al., 2014), although it was also utilized as a promising tool to integrate and interpret responses measured in organisms exposed in laboratory experiments (Quintaneiro et al., 2015; Bertrand et al., 2016a).

Neonicotinoid insecticides are nowadays one of the most sold pesticides worldwide. These insecticides are applied as seed coating, leaf spray and soil drenches when used in crops (Bonmatin et al., 2015) and they act on the central nervous system of insects, interfering with neural transmission (Gibbons et al., 2015). The neonicotinoid imidacloprid (IMI) was first registered in France in 1991 (Sur and Stork, 2003) and after its patent expiration in 2006 products based on IMI have extended its application to a broader scale of use (Elbert et al., 2008). Its high water solubility (610 mg L^{-1} at 20 °C) and hydrophilicity (log Kow = 0.57; IUPAC PPDB, 2017), make possible IMI movement through plant tissues by the sap; protecting crops from roots to shoots (Fossen, 2006). In addition, these physical- chemical characteristics increase the chances of environmental contamination via surface- runoff or drainage into areas adjacent to the crops (Botías et al., 2016). Moreover, when it is applied as a seed coating, more than 80% of the active ingredient enter to the soil and soil water, and could leach into aquatic ecosystems (Goulson, 2014). The concentration of IMI in freshwater ecosystems has been well recorded in different regions of the world. The range of concentrations goes from ng L^{-1} (Masiá et al., 2013) to a reported maximum of 320 µg L^{-1} (van Dijk et al., 2013).

Toxic acute effects of IMI on aquatic organisms often happen on aquatic insects or other invertebrates, or at least with concentrations with several orders of magnitude less than in vertebrates (Morrissey et al., 2015). Acute toxicity of IMI on fishes has been established in the order of LC_{50} 200 mg L^{-1} for model species (Tišler et al., 2009; Fossen, 2006). Nevertheless, IMI sublethal effects such as oxidative stress (OS) on fishes are scarcely studied. Traditionally, biomarkers of exposure (antioxidant enzymes) as well as biomarkers of damage (oxidation products) are evaluated for studying OS without an integration tool for these responses.

Hence, the aims of this study were: (1) to evaluate exposure- and damage- biomarkers related to OS in the Southamerican fish *Australoheros facetus* exposed to IMI and (2) to apply the IBR index to