Science of the Total Environment 714 (2020) 136759

Contents lists available at ScienceDirect

Science of the Total Environment

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

Screening anti-predator behaviour in fish larvae exposed to environmental pollutants

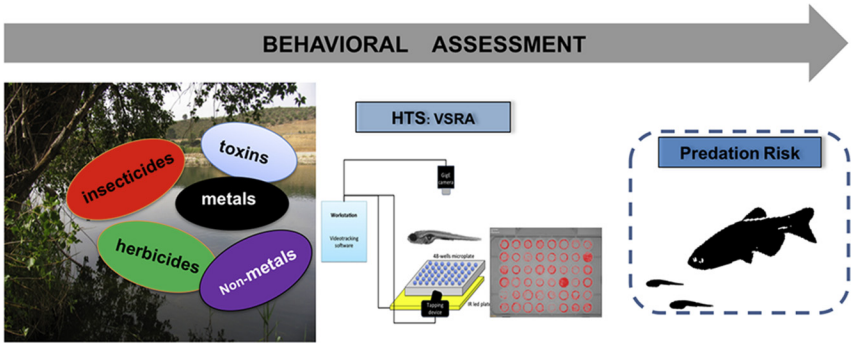
Melissa Faria [a,*], Xiaona Wu [a], Marlenne Luja-Mondragón [b], Eva Prats [c], Leobardo Manuel Gómez-Oliván [b], Benjamin Piña [a], Demetrio Raldúa [a]

[a] IDAEA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain
[b] Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón intersección Paseo Tollocan s/n. Col. Residencial Colón, 50120 Toluca, Estado de México, Mexico
[c] CID-CSIC, Jordi Girona 18, 08034 Barcelona, Spain

HIGHLIGHTS

- Behavioural screening of 30 neurotoxic environmental pollutants was conducted.
- Fish key antipredator responses, startle response and its habituation were assessed.
- The selected pollutants were tested at two environmental relevant concentrations.
- Both behavioural responses were significantly compromised by chemicals at ERCs.
- Respectively, 10 and 13 chemicals impaired the startle response and habituation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 November 2019
Received in revised form 13 January 2020
Accepted 15 January 2020
Available online 16 January 2020

Editor: Daqiang Yin

Keywords:
Neurotoxic pollutants
Zebrafish larvae
Behaviour
Startle response
Habituation

ABSTRACT

Predation is one of the main sources of mortality for fish larvae. During evolution, they have developed different anti-predator behaviours, as the vibrational-evoked startle response and its habituation, for promoting survival to predator's strikes. Whereas these two behaviours can be altered by the exposure to some neurotoxicants, it is currently unknown if the exposure to environmentally relevant concentration (ERC) of neurotoxic pollutants could impair them. In this study thirty neurotoxic environmental pollutants from nine chemical groups, including: herbicides; carbamate, organophosphate (OP), organochlorine (OC), neonicotinoid and pyrethroid insecticides; toxins; metal and non-metal elements, have been screened at two concentrations, including one environmental relevant concentration (ERC), for adverse effects on anti-predator behaviours by using the Vibrational Startle Response Assay on zebrafish larvae. Significant effects over anti-predator responses were equally observed in both exposure concentrations. Focusing on the ERC scenario, it was found that the startle response was the less affected behaviour, where ten pollutants from all chemical groups except for organochlorine, neonicotinoid and pyrethroids, altered this response. Interestingly, organic and inorganic pollutants showed opposite effects on this response: whereas all organic pollutants decreased the startle response, the three remaining inorganic pollutants increased it. On the other hand, more pollutants affected habituation of the startle response of the larvae, where thirteen of the pollutants from all groups, except for herbicides, altered this behaviour at ERC,

Abbreviations: AChE, acetylcholine esterase; AUC, area under the curve; ERC, environmental relevant concentration; dpf, days post fertilization; HCA, hierarchical clustering analysis; hpf, hours post fertilization; ISI, interstimulus interval; VSRA, vibration startle response assay; WSC, worst-case scenario concentration.

* Corresponding author.
E-mail address: mdfqam@cid.csic.es (M. Faria).

https://doi.org/10.1016/j.scitotenv.2020.136759

generally resulting in a faster habituation except for one OP and one marine toxin, which were able to delay this response. Ultimately, only one chemical from the OP, toxin, metal and non-metal element groups altered both the startle response and its habituation at both ERC and WSC. These results emphasize the environmental risk of the current levels of some neurotoxicants present in our aquatic ecosystems, as they are high enough to impair essential anti-predator behaviours in fish larvae.

1. Introduction

It has been estimated that up to 30% of all commercially used chemicals (~30,000 chemicals) may have neurotoxic potential (Legradi et al., 2018). Neuroactive chemicals, including neurotoxic pesticides, pharmaceuticals and illicit drugs, are the largest group of micropollutants present in European rivers, where nearly 30% of all detected chemicals were linked to neurotoxicity (Busch et al., 2016). Furthermore, neurotoxic actions of environmental contaminants on non-target species have been determined for several species, including fish (Carlson et al., 1998; de Melo Tarouco et al., 2017; Huang et al., 2014; Larsen et al., 2016; Sastre et al., 2018; Walker, 2003). It is suspected that such actions include changes in the behaviour of organisms (Hellou, 2011), for example, environmental pollutants such as trace metals and organic toxicants have been reported to increase fish susceptibility to predation (Scott and Sloman, 2004; Weis et al., 2001). Predator-prey interactions are important in structuring communities and can therefore function as important links between toxicant-induced effects on individuals and effects at higher levels of organization. In fish species, as part of an innate behavioural repertoire enabling larvae to escape from predator strikes, they have developed the acoustic/vibrational startle response characterized by an extremely fast C-bend followed by a bout of high-amplitude and low frequency fast swimming (Fero et al., 2011). The startle response is mediated by a relatively simple circuit. An abrupt acoustic/vibrational stimulus mechanically stimulates the hair cells from the inner ear and/or lateral line organ, and the signal is conducted by first-order sensitive neurons comprising the VIIIth nerve that synapse onto the Mauthner cells. The axons of the Mauthner cells extend along the trunk, contacting with the spinal motor neurons (Nicolson, 2006). However, in natural conditions larvae are exposed to many irrelevant stimuli, and unnecessary escape responses have a high energetic cost and increase the risk of predation (Killen and Brown, 2006). Larvae "learn" to ignore these irrelevant stimuli through a process known as "habituation", a primitive form of non-associative learning (Best et al., 2008). Thus, habituation of the startle response to irrelevant stimuli is also essential for larval survival. Exposure of fish larvae to some neurotoxic compounds in aquatic ecosystems has been reported to change the startle response evoked by vibrational stimuli and its habituation (Carlson et al., 1998; Faria et al., 2019b; Scott and Sloman, 2004). Such changes in larvae anti-predator behaviour may have dramatic effects over an individual's fitness and survival, which may lead to population declines and ultimately severe impacts on ecosystems (Weis et al., 2001). However, the available information on the potential effect of known environmental neurotoxic pollutants at relevant concentrations on the startle response and its habituation is currently scarce. In this regard, it is important to address if fish larvae survival can be compromised by exposure to such pollutants at realistic concentrations and also address the possibility to use automated and easily implemented methods with high-throughput potential to test a broad array of known and unknown neurotoxic pollutants present in the aquatic ecosystems. Recently we developed and validated the Vibrational Startle Response Assay (VSRA), an automated in vivo assay for identifying chemicals impairing the escape response and its habituation in zebrafish larvae (Faria et al., 2019a; Faria et al., 2019b). The assay is based on measuring the distance moved by the larva during the startle responses evoked by repetitive vibrational stimuli. The magnitude of the response to the first vibrational stimulus allows evaluating larvae escape response, whereas the decrease in the motor response resulting from repeated exposure to the same vibrational stimuli provides information on the habituation process of this response in the larvae.

In this manuscript we have used VSRA for screening the neurotoxicity of a panel of thirty neurotoxic pollutants, including herbicides, insecticides, metals, non-metals, and marine biotoxins, at two environmental relevant concentrations. The results of this study demonstrate that after only 24 h of water-exposure to environmentally relevant concentrations of some these compounds, fish larvae exhibited impaired startle response and/or its habituation, a deleterious effect in natural conditions.

2. Material and methods

2.1. Fish husbandry and larvae production

Adult wild-type zebrafish, with 3.8 ± 0.3 cm of body length, purchased from Piscicultura Superior SL, Parets del Vallès, Barcelona, were maintained in fish water [reverse-osmosis purified water containing 90 μg/mL of Instant Ocean (Aquarium Systems, Sarrebourg, France) and 0.58 mM $CaSO_4 \cdot 2H_2O$] at 28 ± 1 °C in the Research and Development Centre of the Spanish Research Council (CID-CSIC) facilities under standard conditions. Embryos were obtained by in-tank group breeding with a 5:3 female:male ratio per tank. Breeding tanks are homemade and include a solid external tank and an internal plastic net. Embryos deposited in the bottom of the tank were collected using a 3 mL plastic Pasteur pipette and maintained at a 1 individual/mL density in fish water at 28.5 °C on a 12 light:12 dark photoperiod. Larvae were not fed during the experimental period. All procedures were approved by the Institutional Animal Care and Use Committees at the CID-CSIC and conducted in accordance with the institutional guidelines under a license from the local government (agreement number 9027).

2.2. Chemicals and stock solutions

Thirty neurotoxic pollutants, from 5 chemical groups (herbicide, insecticide, metal elements, non-metal elements, and toxins) were selected (Table 1). The following compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA): acephate, acetamiprid, aldicarb, cadmium(II) sulfate (Cd), carbaryl, carbofuran, chlorpyriphos, clothianidin, diazinon, dichlorvos, endosulfan, esfenvalerate, fenitrothion, malathion, manganese(II) chloride (Mn), mercury(II) chloride (Hg), lead(II) nitrate (Pb), permethrin, selenium dioxide (Se), sodium fluoride (fluorine), thiamethoxam and β-cyfluthrin. Atrazine and deltamethrin were purchased from Riedel-de Haën (Germany). Dinotefuran and fenvalerate were purchased from Supelco™ Analytical (USA). Glyphosate was purchased from Santa Cruz Biotechnology (USA), whereas domoic acid, okadaic acid and saxitoxin were purchased from Cifga lab SA (Spain). CAS numbers of each chemical are provided in Table 1. Stock solutions of each compound were prepared on the day of the experiment. Solutions were either prepared directly in fish water or in dimethyl sulfoxide (DMSO), according to compound solubility. Experimental solutions were prepared in fish water from the stock solutions. DMSO was added to all conditions, including control, to a final concentration of 0.1%, which has been previously found not to affect larvae behaviour (Faria et al., 2019c).

Table 1
Selected environmental neurotoxic pollutants classified into groups and subgroups. Additionally, indicated are compounds CAS n° and reported concentrations, with respective references (ref.), in surface water bodies and the selected test concentrations.

Compound group	Compounds	Subs. groups	CAS n°	Reported concentration ranges (ref.)	Test concentrations
Herbicide	Glyphosate	OP	56-81-5	0.030–12 μg/L (Byer et al., 2008) 3–139 μg/L (Puértolas et al., 2010)	15 & 150 μg/L
	Atrazine	OC	1912-24-9	<0.01–62.5 μg/L (Pennington et al., 2001)	65 & 650 μg/L
Insecticide	Carbaryl	CB	63-25-2	4.6–6.3 μg/L (Hossain et al., 2015)	0.66 & 6.6 μg/L
	Carbofuran	CB	1563-66-2	1.9–5.6 μg/L (Vieira et al., 2016)	0.9 & 9 μg/L
	Aldicarb	CB	116-06-03	>2 μg/L (Pennington et al., 2001)	0.07 & 0.7 μg/L
	Fenitrothion	OP	122-14-5	<0.1–33.1 μg/L (Hossain et al., 2015)	1.7 & 17 μg/L
	Malathion	OP	121-75-5	7.93 ng/L (Ccanccapa et al., 2016) 50.4 ng/L (Sposito et al., 2018)	1.3 & 13 ng/L
	Dichlorvos	OP	62-73-7	1.4–1552 ng/L (Gao et al., 2009)	1.5 & 15 μg/L
	Chlorpyriphos	OP	2921-88-2	3.27–9.31 μg/L (Hossain et al., 2015) 0.5–729.5 ng/L (Delgado-Moreno et al., 2011)	0.22 & 2.2 μg/L
	Diazinon	OP	333-41-5	7.86 μg/L (Hossain et al., 2015) 0.5–172.8 ng/L (Delgado-Moreno et al., 2011)	0.2 & 2 μg/L
	Acephate	OP	30560-19-1	138 μg/L (Anderson et al., 2013) 1.0699 μg/L (Badach et al., 2007)	0.2 & 2 μg/L
	Endosulfan	OC	115-29-7	106.7 ng/L (Bonansea et al., 2013) 1.44 μg/L (Stehle et al., 2013) 215 ng/L (Zhang et al., 2003)	0.1 & 1 μg/L
	Permethrin	Pyrethroid	52645-53-1	5 ng/L (Jorgenson et al., 2013)	0.5 & 5 ng/L
	β-Cyfluthrin	Pyrethroid	68395-37-5	77-297 ng/L (Aznar et al., 2017)	0.02 & 0.2 ng/L
	Deltamethrin	Pyrethroid	52918-63-5	~ 60 ng/L (Feo et al., 2010)	60 & 600 ng/L
	Fenvalerate	Pyrethroid (synthetic)	51630-58-1	0.11 μg/L (Stehle et al., 2013) 17–21 ng/L (Ge et al., 2010) 0.2–6.2 μg/L (Liess et al., 1999)	50 & 500 ng/L
	Esfenvalerate	Pyrethroid (synthetic)	66230-04-4	941-1325 ng/L (Aznar et al., 2017)	25 & 250 ng/L
	Dinotefuran	Neonicotinoid	165252-70-0	220 ng/L (Yamamoto et al., 2012) 6.4–133 ng/L (Hladik and Kolpin, 2016)	0.13 & 1.3 μg/L
	Clothianidin	Neonicotinoid	210880-92-5	0.003–3.1 μg/L (Morrissey et al., 2015)	3 & 30 μg/L
	Acetamiprid	Neonicotinoid	135410-20-7	2.2 μg/L (Anderson et al., 2013) 39.5 ng/L (Hladik and Kolpin, 2016) 0.008–44.1 μg/L (Morrissey et al., 2015) <0.012–0.38 μg/L (Sánchez-Bayo and Hyne, 2014)	40 & 400 ng/L
	Thiamethoxam	Neonicotinoid	153719-23-4	3.2 μg/L (Anderson et al., 2013) 1.9–190.4 ng/L (Hladik and Kolpin, 2016) 0.001–225 μg/L (Morrissey et al., 2015) 0.014–0.2 μg/L (Sánchez-Bayo and Hyne, 2014)	0.19 & 1.9 μg/L
Toxins	Domoic acid	Neurotoxin	14277-97-5	12 μg/L (Wang et al., 2007)	12 & 120 μg/L
	Okadaic acid	Toxin	78111-17-8	3.6 ng/L (Torgersen et al., 2008)	3.6 & 36 ng/L
	Saxitoxin	Neurotoxin	35523-89-8	110 μg/Kg (Wood et al., 2006) ~ 25 μg/L (Hoeger et al., 2004) 600 μg/L (Grachev et al., 2018)	100 & 1000 μg/L
Metal element	Lead ($PbNO_3$)	Metal	10099-74-8	0.3–97 μg/L (Belzuncea et al., 2004) 0.35–4.5 μg/L (Ramos et al., 1999) 0.4–350 μg/L (Bervoets et al., 1994)	7 & 35 μg/L
	Mercury ($HgCl_2$)	Metal	7487-94-7	<0.3–3 μg/L (Belzuncea et al., 2004) 2.09–1502 μg/L (Ramos et al., 1999) 2.8–5.7 μg/L (Fernandez et al., 1992)	5 & 50 μg/L
	Cadmium ($CdSO_4$)	Metal	15244-35-6	<0.2–60 μg/L (Belzuncea et al., 2004) 0.11–2.06 μg/L (Ramos et al., 1999) 0.2–19.2 μg/L (Bervoets et al., 1994)	8 & 80 μg/L
	Manganese ($MnCl_2 \cdot 4H_2O$)	Metal	13,446–34-9	0.2–3510 μg/L (Belzuncea et al., 2004) 10.8–24.3 mg/L (Bouza-Deaño et al., 2008)	0.25 & 2.5 mg/L
Non-metal element	Selenium (Selenium oxide)	Metalloid	7446-08-4	0.2 μg/L (Lemly, 2004)	0.16 & 1.6 μg/L
	Fluoride (NaF)	Halogen	7681-49-4	0.66–10 mg/L (Genxu and Guodong, 2001)	5 & 50 mg/L

OP – organophosphates; OC – organochlorines; CB - carbamates.

2.3. Experimental procedure

Two concentrations of each environmental neurotoxic pollutant were selected (Table 1). The lowest concentration used was an environmentally relevant concentration (ERC) previously reported in scientific bibliography. The highest concentration used corresponded to a value 10-fold higher than ERC, and this concentration was included as the worst-case scenario concentration (WSC). Then, selected concentrations were screened for systemic toxicity (death and gross morphology impairment) and swimming impairment. Although none of the compounds exhibited systemic toxicity and/or swimming impairment at ERC, larvae exposed to Pb at WSC showed some lethality (21%). As a result, WSC used for lead was only 5 fold higher than ERC, where toxicity was reduced to control levels, ~2%.

For pyrethroids, highly lipophilic compounds (Wheelock et al., 2005), the potential absorption to well walls was prevented by pre-incubating the plates for 6 h with test medium, and then the solution was replaced with a new one following the addition of zebrafish larvae.

Exposures were conducted in 48-well microplates with 1 larva per well and 1 mL of medium. After 24 h of exposure (from 7 to 8 dpf),

larvae were directly tested in the VSRA without further manipulation. Exposure period was selected considering the multiple modes of action implicated in screening many chemicals at different concentrations. Whereas shorter exposure periods could suggest a reduced effect of certain chemicals, longer exposure periods compromised the high-throughput potential of the method and may imply the need to feed larvae, hence introducing a variability factor. All the exposures were performed at 28.5 °C (POL-EKO APARATURA Climatic chamber KK350, Poland) with 12L:12D photoperiod. For each compound, startle response and its habituation were determined in 2–3 independent experiments, with 48 larvae for experimental group in each experiment. Thus, 96–144 larvae were analysed for each experimental condition, and the total number of larvae used during the screening was about 16,200. This high number of larvae results from the high inter-individual variability of the behaviours in 8 dpf larvae.

2.4. Vibrational startle response assay (VSRA)

Vibrational startle response assay was performed as described in Faria et al. (2019b). Video tracking and the escape response were analysed using the EthoVision XT 9 software (Noldus, Wageningen, The Netherlands). Trials were performed at 28 °C with near infrared light. Tapping stimulus was selected at the highest intensity (intensity level: 8) and 50 sequences of the vibrational stimuli were delivered at an interstimulus interval (ISI) rate of 1 s. Before delivering the first stimulus, larvae were left in the observation chamber for 15 min to acclimate. Videos were recorded at 30 frames per second and the VSR was analysed for each individual larva by measuring the distance moved (cm) over the 1 s period after stimulus.

2.5. Data analysis

Data were analysed with IBM SPSS 19.0 (Statistical Package 2010, Chicago, IL). Data are presented as the mean ± SEM of all subjects from 2 to 3 independent experiments, unless stated otherwise. Behavioural responses are represented as: "*Startle Response or Startle*": measured as the total distance moved (cm) in response to the first stimulus and as "*Habituation*": represented as area under the curve (AUC) of plots of distance moved relative to the response to the first stimulus according to Faria et al. (2019c) (Fig. S1). Single plots of exposed larvae responses vs control are provided in the Supplementary material (Fig. S2). One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests were used to compare behavioural responses of each condition with that of control. *P* value was set to 0.05 for statistical differences.

Heatmaps and hierarchical clustering were performed using the function heatmap2, from the gplots package in R (https://CRAN.R-project.org/package=gplots).

3. Results and discussion

3.1. Effects on startle response

The startle response of the larvae evoked by the first vibrational stimulus was significantly altered by 60% of the environmental pollutants tested (18/30) (Table 2). This was confirmed by ANOVA test that detected statistical differences between group means of all chemical families (Table 3). Multiple comparison Dunnett's test discriminated ten of the pollutants which were able to alter larvae startle at ERC, with seven of them (glyphosate, carbaryl, atrazine, fenitrothion, acephate, domoic acid, and saxitoxin) decreasing and three (Hg, Pb and fluorine) increasing this behaviour. Moreover, eight compounds (malathion, diazinon, endosulfan, clothianidin, acetamiprid, fenvalerate, permethrin and deltamethrin) altered the startle response of larvae only at WSC. Interestingly, all these compounds were insecticides, and all but the permethrin increased the startle. The chemicals exhibiting the highest increase in startle response were endosulfan (WSC), deltamethrin (WSC) and acetamiprid (WSC), with a distance moved in response to the first stimulus respectively 19%, 18% and 14% higher than the control larvae. Only two compounds, atrazine and fluorine, altered startle at the two selected concentrations. Finally, the chemicals exhibiting the highest decrease in the startle response were saxitoxin (ERC), atrazine (WSC) and acephate (ERC), with a distance moved 32%, 21% and 21% lower than the control larvae.

A limited number of studies has addressed the neurotoxic effect of some environmental pollutants on the startle response of fish early life stage (Faucher et al., 2006; Painter et al., 2009; Rice et al., 2011; Sloman and McNeil, 2012; Stanley et al., 2009). In the seminal manuscript, Carlson et al., 1998, reported the neurological effects on startle response in juvenile medaka exposed for 24 h to sublethal concentrations of some organic chemicals, including chlorpyrifos, carbaryl, endosulfan and fenvalerate (Carlson et al., 1998). In that study chlorpyrifos and carbaryl altered the startle circuit at two different levels, and the ratio between the number of startle responses evoked to the number of stimuli was affected by most of the tested chemicals. However, caution must be taken when comparing results from the present study with Carlson's work (Carlson et al., 1998), as there are important differences in the fish species (*Danio rerio* vs *Oryzias latipes*), developmental stage (larvae vs juveniles), type of stimulus used for evoking the startle response (vibration vs touch) and concentrations of the chemicals used in both studies. Effects of metals on the startle response have also been addressed. For example, a significant decrease in the number of startle responses was reported in juvenile European sea bass after 4 h exposure to 5 μg/L cadmium (Faucher et al., 2006). On the other hand, Rice et al., 2011, found that exposure of zebrafish embryos from 2 to 24 h to 2 or 6 μg/L Pb^{2+} did not change the first startle response of 7 dpf larvae (Rice et al., 2011); Weber, 2006, observed that the startle response of zebrafish larvae exposed to Hg^{2+} during early development (0–24 hpf) was unaffected at 25–50 μg/L and impaired at 75 μg/L (Weber, 2006). Finally, in a recent study using VSRA, an increase in startle responses in zebrafish larvae exposed to 8.4–16.7 μg/L of chlorpyrifos-oxon, and a decrease in startle responses in zebrafish larvae exposed to high concentrations (12.8 mg/L) of the neonicotinoid pesticide imidacloprid were reported (Faria et al., 2019b). Thus, results presented in this manuscript on the effect of neurotoxic compounds on the startle response of zebrafish larvae are consistent with other reports in different fish species and different experimental conditions, indicating that this anti-predator behaviour is an important target for neurotoxic compounds in aquatic environments. Moreover, these results emphasize the convenience to include the startle response as a relevant endpoint for the assessment of neurotoxicity of the chemicals.

3.2. Effects on habituation

The habituation of the larvae to the startle response evoked by repetitive vibrational stimuli was significantly altered by seventeen compounds, 57% of the environmental pollutants tested, after only 24 h water exposure (Table 2). Differences between group means of all chemical families was confirmed by ANOVA, where *P* values from each group was ≤0.001 (Table 3). Furthermore, multiple comparison Dunnett's test discriminated that thirteen of the chemicals altered habituation at ERC, with eleven of them (carbofuran, diazinon, endosulfan, dinotefuran, acetamiprid, fenvalerate, Cd, Hg, Mn, Se and fluorine) resulting in a faster habituation than the control larvae (AUC exposed < AUC control), and the remaining two (acephate and domoic acid) resulting in delayed habituation (AUC exposed > AUC control). Moreover, four compounds (glyphosate, dichlorvos, chlorpyrifos, and deltamethrin) altered habituation of larvae only at the highest tested concentration. Interestingly, all these compounds are agrochemicals and the common effect observed is a faster habituation than the control larvae. Eight compounds (carbofuran, diazinon, endosulfan, dinotefuran, acetamiprid, fenvalerate, Se and fluorine) altered

Table 2
Effect of 24 h water-exposure to 30 environmental pollutants on the startle response and habituation to vibrational stimuli in 8 days post-fertilization zebrafish larvae. For each chemical two concentrations were selected, an environmental relevant concentration (ERC) and a worst-case scenario concentration (WSC) concentration. *P* value resulting from Dunnett's test is provided, with statistical differences set to $P < 0.05$.

			Startle				*Habituation*			
			Mean	±	SEM	*P* value	Mean	±	SEM	*P* value
Control			0.750	±	0.01		597.69	±	12.7	
Herbicide	Glyphosate	15 μg/L	0.648	±	0.01	0.000	592.26	±	24.0	0.975
		150 μg/L	0.756	±	0.02	0.947	452.82	±	22.7	0.000
	Atrazine	65 μg/L	0.682	±	0.02	0.007	555.94	±	31.1	0.359
		650 μg/L	0.594	±	0.02	0.000	542.39	±	24.3	0.179
Insecticide	Carbaryl	0.66 μg/L	0.643	±	0.02	0.000	634.25	±	46.2	0.640
		6.6 μg/L	0.733	±	0.02	0.735	579.96	±	30.5	0.894
	Aldicarb	0.07 μg/L	0.723	±	0.03	0.501	627.58	±	37.6	0.733
		0.7 μg/L	0.717	±	0.02	0.368	598.80	±	38.6	1.000
	Carbofuran	0.9 μg/L	0.791	±	0.03	0.314	504.05	±	30.5	0.021
		9 μg/L	0.804	±	0.03	0.146	472.55	±	29.9	0.001
	Fenitrothion	17 μg/L	0.639	±	0.02	0.000	656.72	±	40.0	0.259
		170 μg/L	0.755	±	0.03	0.979	534.55	±	28.9	0.204
	Dichlorvos	0.15 μg/L	0.725	±	0.03	0.600	580.03	±	31.1	0.821
		1.5 μg/L	0.769	±	0.02	0.739	487.78	±	23.9	0.003
	Malathion	1.3 μg/L	0.739	±	0.03	0.875	575.30	±	29.9	0.705
		13 μg/L	0.822	±	0.02	0.012	551.18	±	21.5	0.251
	Diazinon	0.2 μg/L	0.779	±	0.02	0.335	532.16	±	24.3	0.033
		2 μg/L	0.804	±	0.02	0.032	443.80	±	19.0	0.000
	Chlorpyrifos	0.22 μg/L	0.741	±	0.02	0.929	551.74	±	49.0	0.502
		2.2 μg/L	0.771	±	0.03	0.696	492.87	±	27.1	0.038
	Acephate	0.2 μg/L	0.596	±	0.03	0.000	746.10	±	37.7	0.001
		2 μg/L	0.725	±	0.03	0.642	595.78	±	29.8	0.998
	Endosulfan	0.1 μg/L	0.695	±	0.03	0.262	472.10	±	30.0	0.000
		1 μg/L	0.896	±	0.04	0.000	403.49	±	23.0	0.000
	Clothianidin	3 μg/L	0.788	±	0.03	0.280	609.09	±	38.4	0.934
		30 μg/L	0.812	±	0.01	0.041	552.25	±	21.9	0.369
	Dinotefuran	0.13 μg/L	0.832	±	0.05	0.136	423.46	±	27.2	0.000
		1.3 μg/L	0.741	±	0.04	0.971	425.76	±	30.1	0.000
	Thiamethoxam	0.19 μg/L	0.795	±	0.04	0.448	525.84	±	28.0	0.117
		1.9 μg/L	0.722	±	0.03	0.703	535.09	±	35.5	0.176
	Acetamiprid	40 ng/L	0.817	±	0.02	0.119	516.18	±	25.9	0.035
		400 ng/L	0.854	±	0.04	0.008	500.20	±	30.5	0.010
	Esfenvalerate	2.5 μg/L	0.785	±	0.02	0.348	627.30	±	33.0	0.680
		25 μg/L	0.768	±	0.02	0.749	567.18	±	34.0	0.665
	Fenvalerate	50 ng/L	0.756	±	0.02	0.928	504.37	±	20.4	0.000
		500 ng/L	0.794	±	0.01	0.037	491.11	±	18.3	0.000
	β-Cyfluthrin	0.02 ng/L	0.734	±	0.02	0.729	604.08	±	28.1	0.972
		0.2 ng/L	0.760	±	0.02	0.872	636.84	±	25.7	0.372
	Permethrin	0.5 μg/L	0.771	±	0.02	0.682	634.05	±	33.4	0.604
		5 μg/L	0.672	±	0.02	0.004	641.69	±	33.0	0.477
	Deltamethrin	60 ng/L	0.801	±	0.03	0.284	615.40	±	24.9	0.859
		600 ng/L	0.885	±	0.04	0.001	496.67	±	30.8	0.005
Toxin	Domoic acid	12 μg/L	0.625	±	0.02	0.000	779.79	±	43.7	0.000
		120 μg/L	0.795	±	0.02	0.151	567.34	±	23.3	0.689
	Okadaic acid	3.6 ng/L	0.738	±	0.02	0.871	626.06	±	27.0	0.647
		36 ng/L	0.752	±	0.02	0.995	626.35	±	32.5	0.642
	Saxitoxin	100 μg/L	0.508	±	0.03	0.000	674.77	±	45.9	0.249
		1000 μg/L	0.804	±	0.03	0.235	618.38	±	43.9	0.890
Metal element	Cadmium	8 μg/L	0.789	±	0.02	0.387	499.48	±	21.9	0.032
		80 μg/L	0.751	±	0.03	0.999	634.47	±	44.1	0.589
	Mercury	5 μg/L	0.819	±	0.02	0.033	477.73	±	29.6	0.003
		50 μg/L	0.780	±	0.03	0.491	561.25	±	33.1	0.524
	Lead	7 μg/L	0.831	±	0.02	0.002	576.06	±	33.1	0.825
		35 μg/L	0.777	±	0.02	0.420	580.68	±	19.2	0.735
	Manganese	0.25 mg/L	0.735	±	0.02	0.814	470.04	±	25.5	0.001
		2.5 mg/L	0.786	±	0.03	0.334	572.51	±	33.5	0.707
Non-metal element	Selenium	0.16 μg/L	0.761	±	0.03	0.922	420.63	±	26.0	0.000
		1.6 μg/L	0.758	±	0.03	0.956	433.20	±	23.0	0.000
	Fluorine	5 mg/L	0.820	±	0.02	0.004	489.99	±	24.3	0.000
		50 mg/L	0.821	±	0.02	0.004	506.56	±	21.5	0.003

habituation at the two selected concentrations. Finally, exposure to endosulfan (WSC), Se (ERC) and dinotefuran (ERC) resulted in the fastest habituation, with AUCs 32%, 29% and 29% lower than the control values, whereas acephate and domoic acid, both at ERC, were the most potent chemicals in delaying habituation, with AUCs 25% and 30% higher than the control larvae.

Information about the neurotoxic effects of environmental pollutants on habituation of the startle response in fish is still scarce. When zebrafish embryos were exposed from 2 to 24 h to 2–6 μg/L Pb^{2+} and then the habituation to the startle response was tested at 7 dpf, a faster habituation than control larvae was reported (Rice et al., 2011). Faster habituation to the startle response evoked by vibrational stimuli has been also reported (Weber, 2006) in zebrafish larvae exposed during early development (0–24 hpf) to 50–75 μg/L Hg^{2+}. Another study found that whereas the 24 h exposure of 7 dpf zebrafish larvae to 1.7 μg/L chlorpyrifos-oxon resulted in a delayed habituation, exposure

Table 3
One way ANOVA results of the assessment of effects of each environmental contaminant group, over the variance of zebrafish larvae behavioural responses. Degrees of freedom (*df*), F-value and *P* value are given. Statistical differences were set to $P < 0.05$.

	df	F	*P*
Startle			
Herbicides	4239	15.894	<0.001
Insecticides	37,1844	5.892	<0.001
Toxins	6323	18.602	<0.001
Metal elements	8524	3.866	<0.001
Non-metal elements	4238	2.785	0.027
Habituation			
Herbicides	4235	6.14	<0.001
Insecticides	37,1838	5.544	<0.001
Toxins	6331	4.066	0.001
Metal elements	8511	3.948	<0.001
Non-metal elements	4239	10.297	<0.001

to 6.4 mg/L imidacloprid resulted in a faster habituation (Faria et al., 2019b). There are several studies addressing habituation of the startle response in adult zebrafish after developmental exposure to environmental contaminants. Both Eddins et al., 2010 and Sledge et al., 2011 found a delay in habituation of the vibrational startle response in adult zebrafish after exposure to 100 μg/L chlorpyrifos during early developmental stages (0–5 dpf) (Eddins et al., 2010; Sledge et al., 2011). The reported studies demonstrate different outcomes of the startle habituation, similar to what was observed in this study. However, interpretation of possible consequences is complex, as the same response can have opposite meanings. For example, a faster habituation may reflect higher alertness, but may also reflect memory dementia (Best et al., 2008). Understanding the pathophysiological mechanisms behind the habituation impairment induced by one chemical implies deciphering of toxicity pathways at molecular level. Nevertheless, if habituation of the startle response has been refined during evolution to improve the survival of fish larvae, any change in this behaviour induced by the exposure to chemicals might compromise larvae survival.

3.3. Effect on both startle response and habituation

Nine of the thirty chemicals screened altered both the startle response and its habituation (Table 2). Acephate, domoic acid and Hg were able to modify both behaviours only at ERC, decreasing the startle response and delaying habituation (AUC > control). The agrochemicals diazinon, endosulfan, acetamiprid, fenvalerate and deltamethrin, altered these two behaviours only at WSC. The effect of this group of chemicals was, however, an increase in the startle and a faster habituation (AUC < control). Fluorine was able to alter the two behaviours at selected concentrations, increasing the startle response and promoting a faster habituation. Only four chemicals (aldicarb, esfenvalerate, β-cyfluthrin, and okadaic acid) didn't have any effect on startle or habituation.

In order to identify chemicals with a similar behavioural profile, a hierarchical clustering analysis (HCA) of the startle response and habituation of larvae after exposure to the twenty-six chemicals that significantly alter any of the behavioural parameters evaluated was performed (Fig. 1). Heatmap representations of the changes in behavioural responses related to the control larvae showed one cluster of five chemicals, the marine toxins saxitoxin and domoic acid and the three AChE inhibitors acephate, fenitrothion and carbaryl, with a common pattern: decreasing startle and delaying habituation at ERC. Another clear cluster includes those chemicals increasing startle and decreasing the habituation time after exposure at WSC. In this cluster there are nine chemicals, including two pyrethroids (deltamethrin and fenvalerate), three organophosphates (diazinon, dichlorvos and chlorpyrifos), one neonicotinoid (acetamiprid), one carbamate (carbofuran) and one non-metal element (Fluorine). Although the compounds included in each group share a similar mode of action, Fig. 1 shows that they are usually included in different clusters. This result is probably related to the fact that in this screening the concentrations selected for each chemical were based on the reported ERC, so in each group there are chemicals with different potency and different concentrations.

Whereas previous studies have also found neurotoxic effects for some of the chemicals screened in this study, including chlorpyrifos, carbaryl, endosulfan, Cd, Pb or Hg, it is not possible to directly compare these studies with the one presented here, since there are differences in fish species, developmental stage, type of stimulus used, inter-stimuli interval and exposure conditions.

Finally, the performance of the VSRA for screening of neurotoxic chemicals present in the aquatic environment is noteworthy as it simultaneously provides impact on two key anti-predator behavioural responses. The results of the screening reported in this manuscript strongly suggest that the current environmental levels of neurotoxicants in many aquatic ecosystems are high enough to impair a key antipredatory behaviour in fish larvae. First, the impaired startle response due to exposure to contaminates has been reported to have a direct impact on fish survival following predator strikes, which could have dramatic effects on recruitment (Bhattacharyya et al., 2019; Mesa et al., 1994; Scott and Sloman, 2004; Weis and Candelmo, 2012). Second, whereas animals have an innate ability to recognize their predators and are less likely to habituate to predator forms (Magurran and Girling, 1986), altered habituation of the startle response is still a major problem for larvae, since unnecessary escape responses supposes a high cost of energy resulting in the exhaustion of organisms or it may attract the attention of both visual and mechanoreceptive predators (Batabyal et al., 2017; Killen and Brown, 2006). Therefore any deficiency in either direction of habituation is very likely to compromise larvae survival.

4. Conclusions

The present study describes the screening potential of a simple behavioural assay to identify neurotoxicants present in our aquatic ecosystems. The assay, which is able to evaluate two behavioural endpoints simultaneously, was found to be sensitive enough to detect changes in the anti-predator behaviour at realistic concentrations of known neurotoxic pollutants. Screening of the 30 selected neurotoxic environmental pollutants found that 19 of them were able to affect zebrafish larvae anti-predator behaviour at environmental relevant concentrations. In general, habituation was more affected than the startle response and most of the chemicals resulted in a faster habituation and a decrease in the startle response. Curiously, certain response patterns were observed, at ERC the startle response was decreased and increased only by organic and inorganic pollutants, respectively while on the other hand, also at ERC, only the herbicides were unable to impaired larvae habituation. These results suggest prospect of an environmental risk of the current levels of some of these pollutants, as they are high enough to impair essential anti-predator behaviours in fish larvae, as well as, the importance of evaluating more than one behavioural endpoint during screening studies. Altered predator/prey interactions involve organisms from two trophic levels and can cause changes in populations of predators, prey or both, and thus affect the community. However, due to the large amount of pollutants detected in the environment and the lack of knowledge of their neurotoxic potential, much more screening research remains to be done.

5. Suggestions

Despite the VSRA method has been proven to be sensitive to low concentrations of environmental neurotoxic pollutants, experimental layout may be adjusted, especially considering the time of exposure, to detect specific contaminants that have a rapid effect or that require a longer time of exposure to impair behaviour. For example, our

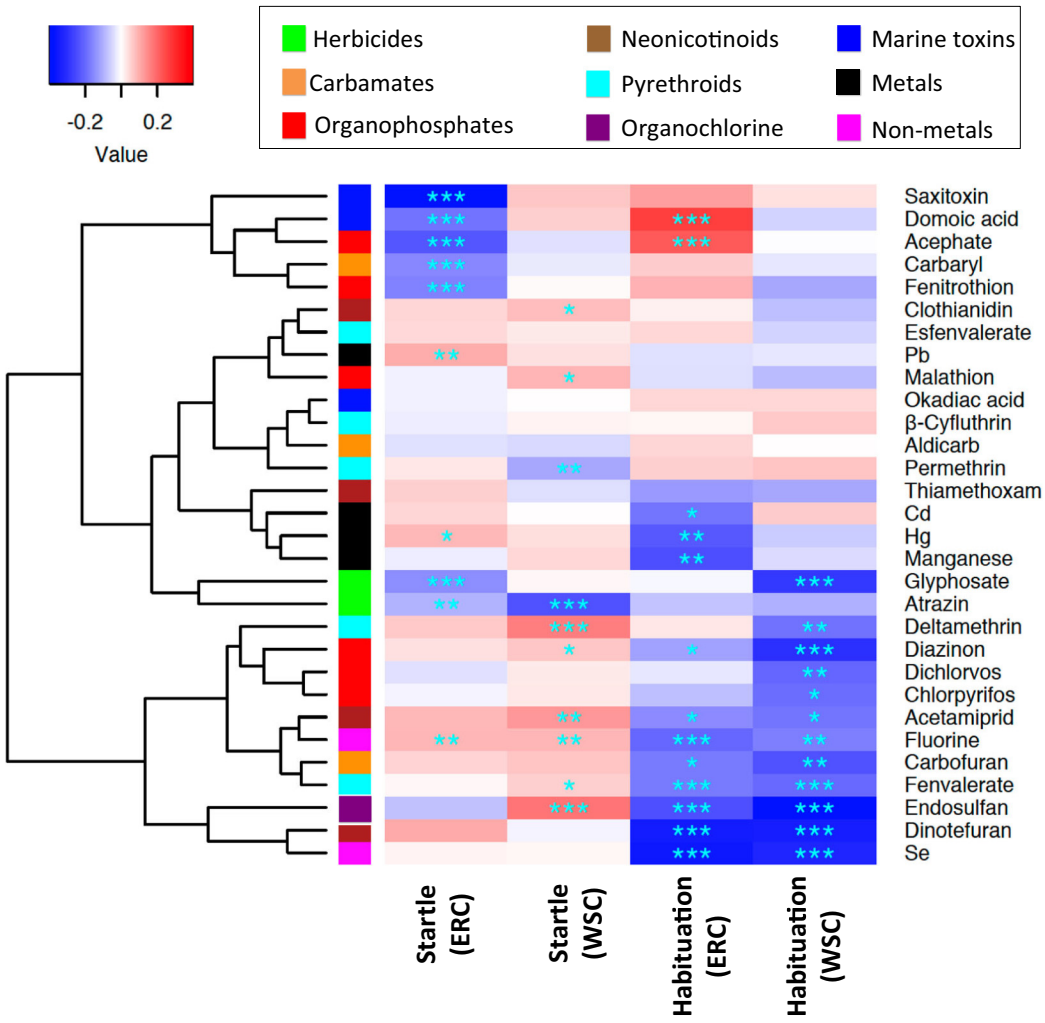


Fig. 1. Graphic representation of the effects on startle response and habituation for 30 compounds at environmental relevant (ERC) and worst-scenario (WSC) concentrations. Exposures with similar behavioural response patterns were grouped by hierarchical clustering; values are expressed and color-coded as relative to control levels. White, blue, and red sectors represent control, below control and above control values, respectively (see the color scale on top). The left column side bar indicates the type of compound for each exposure: herbicides (green), carbamates (orange), organophosphates (red), organochlorine (violet), neonicotinoid (brown), pyrethroid (cyan), marine toxins (dark blue), metals (black) and inorganic, non-metal toxins (magenta, see the legend on top). Blue stars indicate statistically significant differences between exposed and control animals for each compound and dose (Dunnett's test, *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

research group has also conducted a study of neurotoxic effects over zebrafish larvae of an industrial contaminant, acrylamide (Prats et al., 2017) at relevant concentrations, which required a 72 h exposure window to induce neurotoxicity. In this sense, the assay can allow adjustments to be made to better adapt to the targeted chemical group.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Spanish Government (CTM2017-83242-R; D.R.). M.F acknowledges financial support from the Beatriu de Pinós programme (grant N°: 2016 BP 00233) provided by the Secretariat of Universities and Research Department of the Ministry for Business and Knowledge, Catalonia Government.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.136759.

References

Anderson, T.A., Salice, C.J., Erickson, R.A., McMurry, S.T., Cox, S.B., Smith, L.M., 2013. Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains. Chemosphere 92, 84–90.
Aznar, R., Sánchez-Brunete, C., Albero, B., Moreno-Ramón, H., Tadeo, J.L., 2017. Pyrethroids levels in paddy field water under Mediterranean conditions: measurements and distribution modelling. Paddy Water Environ. 15, 307–316.
Badach, H., Nazimek, T., Kaminska, I.A., 2007. Pesticide content in drinking water samples collected from orchard areas in central Poland. Ann. Agric. Environ. Med. 14.
Batabyal, A., Balakrishna, S., Thaker, M., 2017. A multivariate approach to understanding shifts in escape strategies of urban lizards. Behav. Ecol. Sociobiol. 71, 83.
Belzuncea, M.J., Solauna, O., Valenciaa, V., Péreza, V., 2004. Contaminants in estuarine and coastal waters. Forest 1, 850.93.
Bervoets, L., Panis, L.I., Verheyen, R., 1994. Trace metal levels in water, sediments and Chironomus gr. thumni, from different water courses in Flanders (Belgium). Chemosphere 29, 1591–1601.
Best, J.D., Berghmans, S., Hunt, J.J., Clarke, S.C., Fleming, A., Goldsmith, P., et al., 2008. Non-associative learning in larval zebrafish. Neuropsychopharmacology 33, 1206.

Bhattacharyya, K., McLean, D.L., MacIver, M.A., 2019. Intersection of motor volumes predicts the outcome of predator-prey interactions. bioRxiv 626549 >626549.
Bonansea, R.I., Amé, M.V., Wunderlin, D.A., 2013. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River basin (Argentina). Chemosphere 90, 1860–1869.
Bouza-Deaño, R., Ternero-Rodriguez, M., Fernández-Espinosa, A., 2008. Trend study and assessment of surface water quality in the Ebro River (Spain). J. Hydrol. 361, 227–239.
Busch, W., Schmidt, S., Kühne, R., Schulze, T., Krauss, M., Altenburger, R., 2016. Micropollutants in European rivers: a mode of action survey to support the development of effect-based tools for water monitoring. Environ. Toxicol. Chem. 35, 1887–1899.
Byer, J.D., Struger, J., Klawunn, P., Todd, A., Sverko, E., 2008. Low cost monitoring of glyphosate in surface waters using the ELISA method: an evaluation. Environ. Sci. Technol. 42, 6052–6057.
Carlson, R.W., Bradbury, S.P., Drummond, R.A., Hammermeister, D.E., 1998. Neurological effects on startle response and escape from predation by medaka exposed to organic chemicals. Aquat. Toxicol. 43, 51–68.
Ccanccapa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., Barceló, D., 2016. Pesticides in the Ebro River basin: occurrence and risk assessment. Environ. Pollut. 211, 414–424.
de Melo Tarouco, F., de Godoi, F.G.A., Velasques, R.R., da Silveira Guerreiro, A., Geihs, M.A., da Rosa, C.E., 2017. Effects of the herbicide Roundup on the polychaeta Laeonereis acuta: cholinesterases and oxidative stress. Ecotoxicol. Environ. Saf. 135, 259–266.
Delgado-Moreno, L., Lin, K., Veiga-Nascimento, R., Gan, J., 2011. Occurrence and toxicity of three classes of insecticides in water and sediment in two Southern California coastal watersheds. J. Agric. Food Chem. 59, 9448–9456.
Eddins, D., Cerutti, D., Williams, P., Linney, E., Levin, E.D., 2010. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. Neurotoxicol. Teratol. 32, 99–108.
Faria, M., Bedrossiantz, J., Prats, E., Garcia, X.R., Gómez-Canela, C., Piña, B., et al., 2019a. Deciphering the mode of action of pollutants impairing the fish larvae escape response with the vibrational startle response assay. Sci. Total Environ. 672, 121–128.
Faria, M., Prats, E., Novoa-Luna, K.A., Bedrossiantz, J., Gómez-Canela, C., Gómez-Oliván, L.M., et al., 2019b. Development of a vibrational startle response assay for screening environmental pollutants and drugs impairing predator avoidance. Sci. Total Environ. 650, 87–96.
Faria, M., Valls, A., Prats, E., Bedrossiantz, J., Orozco, M., Porta, J.M., et al., 2019c. Further characterization of the zebrafish model of acrylamide acute neurotoxicity: gait abnormalities and oxidative stress. Sci. Rep. 9, 7075.
Faucher, K., Fichet, D., Miramand, P., Lagardère, J.P., 2006. Impact of acute cadmium exposure on the trunk lateral line neuromasts and consequences on the "C-start" response behaviour of the sea bass (Dicentrarchus labrax L.; Teleostei, Moronidae). Aquat. Toxicol. 76, 278–294.
Feo, M.L., Ginebreda, A., Eljarrat, E., Barceló, D., 2010. Presence of pyrethroid pesticides in water and sediments of Ebro River Delta. J. Hydrol. 393, 156–162.
Fernandez, M.A., Hernandez, L.M., Gonzalez, M.J., Tabera, M.C., 1992. Organochlorinated compounds and selected metals in waters and soils from Doñana National Park (Spain). Water Air Soil Pollut. 65, 293–305.
Fero, K., Yokogawa, T., Burgess, H.A., 2011. The behavioral repertoire of larval zebrafish. Zebrafish Models in Neurobehavioral Research. Springer, pp. 249–291.
Gao, J., Liu, L., Liu, X., Zhou, H., Lu, J., Huang, S., et al., 2009. The occurrence and spatial distribution of organophosphorous pesticides in Chinese surface water. Bull. Environ. Contam. Toxicol. 82, 223–229.
Ge, J., Cong, J., Sun, Y., Li, G., Zhou, Z., Qian, C., et al., 2010. Determination of endocrine disrupting chemicals in surface water and industrial wastewater from Beijing, China. Bull. Environ. Contam. Toxicol. 84, 401–405.
Genxu, W., Guodong, C., 2001. Fluoride distribution in water and the governing factors of environment in arid north-west China. J. Arid Environ. 49, 601–614.
Grachev, M., Zubkov, I., Tikhonova, I., Ivacheva, M., Kuzmin, A., Sukhanova, E., et al., 2018. Extensive contamination of water with saxitoxin near the dam of the Irkutsk hydropower station reservoir (East Siberia, Russia). Toxins 10, 402.
Hellou, J., 2011. Behavioural ecotoxicology, an "early warning" signal to assess environmental quality. Environ. Sci. Pollut. Res. 18, 1–11.
Hladik, M.L., Kolpin, D.W., 2016. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. Environ. Chem. 13, 12–20.
Hoeger, S.J., Shaw, G., Hitzfeld, B.C., Dietrich, D.R., 2004. Occurrence and elimination of cyanobacterial toxins in two Australian drinking water treatment plants. Toxicon 43, 639–649.
Hossain, M.S., Chowdhury, M.A.Z., Pramanik, M.K., Rahman, M.A., Fakhruddin, A.N.M., Alam, M.K., 2015. Determination of selected pesticides in water samples adjacent to agricultural fields and removal of organophosphorus insecticide chlorpyrifos using soil bacterial isolates. Appl Water Sci 5, 171–179.
Huang, Y., Zhang, J., Han, X., Huang, T., 2014. The use of zebrafish (Danio rerio) behavioral responses in identifying sublethal exposures to deltamethrin. Int. J. Environ. Res. Public Health 11, 3650–3660.
Jorgenson, B., Fleishman, E., Macneale, K.H., Schlenk, D., Scholz, N.L., Spromberg, J.A., et al., 2013. Predicted transport of pyrethroid insecticides from an urban landscape to surface water. Environ. Toxicol. Chem. 32, 2469–2477.
Killen, S.S., Brown, J.A., 2006. Energetic cost of reduced foraging under predation threat in newly hatched ocean pout. Mar. Ecol. Prog. Ser. 321, 255–266.
Larsen, K.E., Lifschitz, A.L., Lanusse, C.E., Virkel, G.L., 2016. The herbicide glyphosate is a weak inhibitor of acetylcholinesterase in rats. Environ. Toxicol. Pharmacol. 45, 41–44.
Legradi, J., Di Paolo, C., Kraak, M., Van Der Geest, H., Schymanski, E., Williams, A., et al., 2018. An ecotoxicological view on neurotoxicity assessment. Environ. Sci. Eur. 30, 46.
Lemly, A.D., 2004. Aquatic selenium pollution is a global environmental safety issue. Ecotoxicol. Environ. Saf. 59, 44–56.
Liess, M., Schulz, R., Liess, M.H.D., Rother, B., Kreuzig, R., 1999. Determination of insecticide contamination in agricultural headwater streams. Water Res. 33, 239–247.
Magurran, A.E., Girling, S.L., 1986. Predator model recognition and response habituation in shoaling minnows. Anim. Behav. 34, 510–518.
Mesa, M.G., Poe, T.P., Gadomski, D.M., Petersen, J., 1994. Are all prey created equal? A review and synthesis of differential predation on prey in substandard condition. J. Fish Biol. 45, 81–96.
Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., et al., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ. Int. 74, 291–303.
Nicolson, T., 2006. Methods for exploring the auditory/vestibular system in zebrafish. Using Zebrafish to Study Neuroscience, p. 1.
Painter, M.M., Buerkley, M.A., Julius, M.L., Vajda, A.M., Norris, D.O., Barber, L.B., et al., 2009. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (Pimephales promelas). Environ. Toxicol. Chem. 28, 2677–2684.
Pennington, P.L., Daugomah, J.W., Colbert, A.C., Fulton, M.H., Key, P.B., Thompson, B.C., et al., 2001. Analysis of pesticides runoff from mid-Texas estuaries and risk assessment implications for marine phytoplankton. J. Environ. Sci. Health B 36, 1–14.
Prats, E., Gómez-Canela, C., Ben-Lulu, S., Ziv, T., Padrós, F., Tornero, D., et al., 2017. Modelling acrylamide acute neurotoxicity in zebrafish larvae. Sci. Rep. 7, 13952.
Puértolas, L., Damásio, J., Barata, C., Soares, A.M., Prat, N., 2010. Evaluation of side-effects of glyphosate mediated control of giant reed (Arundo donax) on the structure and function of a nearby Mediterranean river ecosystem. Environ. Res. 110, 556–564.
Ramos, L., Fernández, M., González, M., Hernández, L., 1999. Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. Bull. Environ. Contam. Toxicol. 63, 305–311.
Rice, C., Ghorai, J.K., Zalewski, K., Weber, D.N., 2011. Developmental lead exposure causes startle response deficits in zebrafish. Aquat. Toxicol. 105, 600–608.
Sánchez-Bayo, F., Hyne, R.V., 2014. Detection and analysis of neonicotinoids in river waters – development of a passive sampler for three commonly used insecticides. Chemosphere 99, 143–151.
Sastre, S., Torija, C.F., Pertusa, I.A., Beltrán, E.M., Pablos, M.V., González-Doncel, M., 2018. Stage-dependent effects of chlorpyrifos on medaka (Oryzias latipes) swimming behavior using a miniaturized swim flume. Aquat. Toxicol. 200, 37–49.
Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392.
Sledge, D., Yen, J., Morton, T., Dishaw, L., Petro, A., Donerly, S., et al., 2011. Critical duration of exposure for developmental chlorpyrifos-induced neurobehavioral toxicity. Neurotoxicol. Teratol. 33, 742–751.
Sloman, K.A., McNeil, P.L., 2012. Using physiology and behaviour to understand the responses of fish early life stages to toxicants. J. Fish Biol. 81, 2175–2198.
Sposito, J.C.V., Montagner, C.C., Casado, M., Navarro-Martín, L., Jut Solórzano, J.C., Piña, B., et al., 2018. Emerging contaminants in Brazilian rivers: occurrence and effects on gene expression in zebrafish (Danio rerio) embryos. Chemosphere 209, 696–704.
Stanley, K.A., Curtis, L.R., Massey Simonich, S.L., Tanguay, R.L., 2009. Endosulfan I and endosulfan sulfate disrupts zebrafish embryonic development. Aquat. Toxicol. 95, 355–361.
Stehle, S., Knäbel, A., Schulz, R., 2013. Probabilistic risk assessment of insecticide concentrations in agricultural surface waters: a critical appraisal. Environ. Monit. Assess. 185, 6295–6310.
Torgersen, T., Miles, C.O., Rundberget, T., Wilkins, A.L., 2008. New esters of okadaic acid in seawater and blue mussels (Mytilus edulis). J. Agric. Food Chem. 56, 9628–9635.
Vieira, D.C., Noldin, J.A., Deschamps, F.C., Resgalla, C., 2016. Ecological risk analysis of pesticides used on irrigated rice crops in southern Brazil. Chemosphere 162, 48–54.
Walker, C., 2003. Neurotoxic pesticides and behavioural effects upon birds. Ecotoxicology 12, 307–316.
Wang, Z., King, K.L., Ramsdell, J.S., Doucette, G.J., 2007. Determination of domoic acid in seawater and phytoplankton by liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1163, 169–176.
Weber, D., 2006. Dose-dependent effects of developmental mercury exposure on C-start escape responses of larval zebrafish Danio rerio. J. Fish Biol. 69, 75–94.
Weis, J.S., Candelmo, A., 2012. Pollutants and fish predator/prey behavior: a review of laboratory and field approaches. Curr. Zool. 58, 9–20.
Weis, J.S., Smith, G., Zhou, T., Santiago-Bass, C., Weis, P., 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences: killifish from a contaminated site are slow to capture prey and escape predators; altered neurotransmitters and thyroid may be responsible for this behavior, which may produce population changes in the fish and their major prey, the grass shrimp. AIBS Bull. 51, 209–217.
Wheelock, C.E., Miller, J.L., Miller, M.J., Phillips, B.M., Gee, S.J., Tjeerdema, R.S., et al., 2005. Influence of container adsorption upon observed pyrethroid toxicity to Ceriodaphnia dubia and Hyalella azteca. Aquat. Toxicol. 74, 47–52.
Wood, S.A., Holland, P.T., Stirling, D.J., Briggs, L.R., Sprosen, J., Ruck, J.G., et al., 2006. Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. N. Z. J. Mar. Freshw. Res. 40, 585–597.
Yamamoto, A., Terao, T., Hisatomi, H., Kawasaki, H., Arakawa, R., 2012. Evaluation of river pollution of neonicotinoids in Osaka City (Japan) by LC/MS with dopant-assisted photoionisation. J. Environ. Monit. 14, 2189–2194.
Zhang, Z.L., Hong, H.S., Zhou, J.L., Huang, J., Yu, G., 2003. Fate and assessment of persistent organic pollutants in water and sediment from Minjiang River estuary, Southeast China. Chemosphere 52, 1423–1430.