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## Acute toxicity of two pyrethroid insecticides for five non-indigenous crayfish species in Europe

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**Abstract:** Pyrethroid insecticides are highly toxic to many aquatic organisms. The aim of this study was to evaluate the toxicity of the commercial products Cyperkill 25 EC (active compound 250 g/l cypermethrin) and Decis Mega (active compound 50 g/l deltamethrin) for European non-indigenous marbled crayfish *Procambarus virginalis*, red swamp crayfish *Procambarus clarkii*, signal crayfish *Pacifastacus leniusculus*, spiny-cheek crayfish *Orconectes limosus* and yabby *Cherax destructor*. These data will provide a baseline for potential programmes to eradicate alien crayfish from Europe (EU Regulation No. 1143/2014; Commission Implementing Regulation No. 2016/1141) and are also relevant globally. The 96hLC<sub>50</sub> values of Cyperkill 25 EC were 0.09, 0.17, 0.18, 0.19 and 0.30 µg/l for spiny-cheek crayfish, red swamp crayfish, marbled crayfish, signal crayfish and yabby, respectively. In the same order, the 96hLC<sub>50</sub> values of Decis Mega were 0.76, 0.16, 0.21, 0.03 and 0.27 µg/l. The toxicity of the insecticides was similar and species-specific, possibly reflecting the size difference of the tested animals. This study shows that cypermethrin and deltamethrin are highly toxic to the tested crayfish species at low concentrations. This high sensitivity, along with the low accumulation in the food chain and short-term persistence in the aquatic environment, suggests that they are suitable biocides for eradicating crayfish in the wild. Stagnant, closed water bodies with newly emerging invaders are ideal sites for possible application, although local conditions must be considered.

**Keywords:** biological invasion; insecticide; cypermethrin; deltamethrin; eradication; invasive species

Pesticide use has increased over the past 50 years, leading to a risk of environmental contamination, including that of freshwaters, and to considerable negative impact on non-target organisms (Tilman et al. 2011). Pyrethroids are synthetic insecticides with enhanced photostability and are based on the structure of naturally insecticidal pyrethrins extracted from *Chrysanthemum* (Elliott et al. 1974). They are commonly used for control or elimination of pests in agriculture and households and of ectoparasites

in veterinary and human medicine (Palmquist et al. 2012). Pyrethroids represent a major class of highly effective insecticides with relatively low toxicity to birds and mammals, including humans. They show lower persistence in the environment compared to carbamates, organophosphates and organochlorine pesticides but are toxic to organisms such as aquatic crustaceans and fish (Breckenridge et al. 2009).

Cypermethrin and deltamethrin are commonly used second-generation pyrethroids (Saha and

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Kaviraj 2008; Moid et al. 2012) that cause disruption of the nervous system via prolongation of the depolarization phase of sodium channels in neural axons and affect gamma-aminobutyric acid (GABA) receptors in nerve fibres, leading to hyperactivity, paralysis and death (Werner and Moran 2008).

Cypermethrin [(R,S)- $\alpha$ -cyano-3-phenoxybenzyl (1R,S)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] is used for control of ectoparasites infecting domestic animals, as an insecticide in agriculture (Breckenridge et al. 2009) and in aquaculture to control sea lice in salmonids (Treasurer and Wadsworth 2004). During the 1990s, the commercial preparation Betamax vet (Hart et al. 1997) was found to be highly toxic to aquatic crustaceans and was employed for eradication of signal crayfish *Pacifastacus leniusculus* populations in field conditions in Norway (Sandodden and Johnsen 2010). Cypermethrin is highly toxic to many aquatic organisms (Morolli et al. 2006; Velisek et al. 2006; Werner and Moran 2008; Velisek et al. 2009; Velisek et al. 2011) and is frequently detected in surface waters in concentrations ranging from 0.1 to 194  $\mu\text{g/l}$  (WHO 1989; Marino and Ronco 2005). The maximal environmental concentration detected in Czech rivers was 0.05  $\mu\text{g/l}$  (CHMI 2018).

Deltamethrin [(S)- $\alpha$ -cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] is commonly used in agriculture and in veterinary practice as a parasiticide (Wolansky and Harrill 2008). The commercial aquaculture formulation AlphaMax is used to treat salmonids in sea-cage culture during infestations with sea lice parasites (Terech-Majewska et al. 2016). Deltamethrin is classified as highly toxic to non-target aquatic organisms based on acute toxicity (96hLC<sub>50</sub>) tests (Mian and Mulla 1992; Velisek et al. 2011; Wu et al. 2012). The maximal environmental concentration detected in Czech rivers was 0.03  $\mu\text{g/l}$  (CHMI 2018).

The EU Parliament and Council has identified invasive non-indigenous species as being of high concern to European biodiversity (Regulation (EU) No. 1143/2014; Commission Implementing Regulation (EU) 2016/1141). Twenty-three of the 37 listed species are animals, of which five are freshwater crayfish, confirming the invasive potential of at least some members of this group, as is apparent in Europe and regions of North America (Lodge et al. 2000; Kouba et al. 2014). In certain circumstances, use of biocides is one of only a few

feasible methods of eradicating unwanted crayfish (Gherardi et al. 2011; Stebbing et al. 2014). Taking into account the considerable lack of information regarding the acute toxicity of pyrethroid-based insecticides for freshwater crayfish in general, we evaluated the toxicity of commercial cypermethrin and deltamethrin products for five non-indigenous crayfish species established in Europe, of which four are species of particular concern.

## MATERIAL AND METHODS

**Chemicals and animals.** The pyrethroid insecticides Cyperkill 25 EC (Agriphar S.A., Belgium) and Decis Mega (Bayer AG, Germany) were used. Decis Mega contains the active substance deltamethrin (50 g/l) and other additional substances (solvent naphtha, naphthalene, 2-ethylhexan-1-ol; 2,6-di-tert-butyl-4-methylphenol, cyclohexanone). Cyperkill 25 EC contains the active substance cypermethrin (250 g/l) and other additional substances (solvent naphtha and other nonspecific solvent).

Five crayfish species non-indigenous to Europe were used in the study (Table 1). Signal crayfish and spiny-cheek crayfish were obtained from sites of their natural occurrence in the Czech Republic. The remaining species originated from our laboratory cultures.

**Water quality.** Water contained ANC<sub>4.5</sub> (acid neutralization capacity) 1.10 mmol/l, COD<sub>Mn</sub> (chemical oxygen demand) 1.1 mg/l, total ammonia 0.03 mg/l, NO<sub>2</sub><sup>-</sup> 0.02 mg/l and NO<sub>3</sub><sup>-</sup> 5.92 mg/l,

Table 1. Crayfish species and size range of specimens

Crayfish species	Insecticide	Total length (mm)	Weight (mg)
Marbled c. ( <i>Procambarus virginalis</i> )		6.6–10.0	10.0–20.4
Red swamp c. ( <i>Procambarus clarkii</i> )		16.4–22.8	1100–4300
Signal c. ( <i>Pacifastacus leniusculus</i> )		10.1–12.8	30.1–50.1
Spiny-cheek c. ( <i>Orconectes limosus</i> )	Cyp	15.0–25.1	1300–2000
	Delt	59.8–76.8	5860–12 100
Yabby ( <i>Cherax destructor</i> )		9.0–11.1	18.1–26.4

Cyp and Delt indicate size of crayfish used in the trial with cypermethrin- and deltamethrin-based insecticides, respectively

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and the sum of  $\text{Ca}^{2+} + \text{Mg}^{2+}$  was 8.91 mg/l. The temperature was 20.0–21.1°C, oxygen saturation was > 80%, and pH was 7.8–8.2. These values were monitored twice daily.

Aquaria were gently aerated throughout the tests to ensure that the concentration of the tested substance did not drop below 80% of the nominal concentration within 12 h. To ensure agreement between nominal and actual pyrethroid concentrations, water was monitored using a gas chromatography/electron capture detector (Kocourek and Hajslova 1989). In this technique, the sample is extracted with methylene chloride (DCM) using a separatory funnel. The DCM extract is dried with sodium sulfate, evaporated and solvent exchanged into petroleum ether. The extract is concentrated with a Micro Snyder (micro K-D) apparatus to approximately 1 ml and adjusted to 2 ml with iso-octane. The extracts are analysed by gas chromatography using conditions which permit the separation and measurement of the target analytes in the extracts by GC/ECD. In the water samples, the limit of quantification (LOQ) of cypermethrin and deltamethrin was 0.002 µg/l. The concentration of cypermethrin or deltamethrin in the dechlorinated tap water, in control crayfish during the trial, and in crayfish prior to exposure, was below the LOQ. The concentrations of cypermethrin and deltamethrin in water over the course of 12 h as an average of the four sampling dates compared to the nominal value for all test groups did not drop below 80% of the nominal concentration.

**Experimental design.** Crayfish were inspected for apparent healthiness and for evidence of impending moulting. Acute toxicity tests were performed in accordance with OECD guidelines for testing of chemicals No. 203 (OECD 1992). The acute toxicity of insecticides was assessed by determination of the median 24 h, 48 h, 72 h and 96 h  $\text{LC}_0$ ,  $\text{LC}_{50}$  and  $\text{LC}_{100}$  for evaluated species. The test solutions were changed every 12 h (static renewal). Ten experiments were conducted in glass aquaria containing 10 l of test solution or a water-only control. Each exposure was tested in triplicate. Crayfish (total = 1188) were held individually in the aquaria in transparent plastic boxes to prevent aggression and cannibalism. This arrangement is commonly used for growth (Kozak et al. 2009) and toxicity tests (Velisek et al. 2013) in crayfish. The concentrations covered a dosage range resulting in 0–100% mortality according to preliminary tests.

Experimental concentrations of both pesticides ranged from 0.01 µg/l to 15 µg/l. Dead crayfish were removed and mortality recorded daily. Crayfish were not fed during the trials.

Crayfish behaviour was observed after change of test solution. Animals were considered dead if they failed to respond to prodding to the abdomen.

**Analysis of results.** Data were used to determine the  $\text{LC}_{50}$  of the crayfish within 95% confidence limits. Maximum likelihood linear regression with probit analysis was performed using EKO-TOX software, version 5.2 (INGEO Liberec, Czech Republic).

## RESULTS

### Crayfish behaviour

At concentrations of Cyperkill 25 EC of 1, 5, 10 and 15 µg/l in red swamp c. and 0.5, 1, 5 and 10 µg/l in other species) and of Decis Mega of 5, 10 and 15 µg/l in spiny-cheek c., 0.5, 1, 5 and 10 µg/l in yabby, 0.1, 0.5, 1, 5, 10 and 15 µg/l in signal c. and 0.5, 1, 5, 10 and 15 µg/l in other species, hyperactivity and rapid movement were observed almost immediately, sometimes combined with escape attempts. Sluggish movement, lethargy, loss of balance and immobility soon followed, with crayfish lying supine and showing only slight movement of antennae and walking with their legs. The time to onset of symptoms decreased with increasing concentrations of the tested substances. The highest concentrations (10 and 15 µg/l) were associated with shedding of claws in marbled and signal crayfish. Crayfish in control groups showed typical behaviour, usually remaining in a corner of the compartment or walking around the walls.

### Acute toxicity

No mortality or sublethal effects were observed in the controls in any of the acute toxicity tests. The estimated  $\text{LC}_0$ ,  $\text{LC}_{50}$  and  $\text{LC}_{100}$  values following 24, 48, 72 and 96-h exposure for Cyperkill 25 EC and Decis Mega are summarised in Tables 2 and 3, respectively. The 96-h  $\text{LC}_{50}$  values were 0.09, 0.17, 0.18, 0.19 and 0.30 µg/l Cyperkill 25 EC (corresponding to 0.0225, 0.0425, 0.045, 0.0475 and 0.075 µg/l of cypermethrin, respectively) for

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Table 2. Acute toxicity of Cyperkill 25 EC for selected crayfish species after 24, 48, 72 and 96 h exposure. Concentration is expressed as µg/l

Crayfish species	24 h			48 h			72 h			96 h		
	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>
Spiny-cheek c.	0.01	0.46	32.26	0.01	0.41	23.52	0.01	0.28	6.93	0.01	0.09	0.48
Red swamp c.	0.04	1.17	30.50	0.04	0.67	9.15	0.01	0.40	8.26	0.01	0.17	1.95
Marbled c.	0.06	1.34	28.39	0.05	0.76	13.48	0.01	0.34	8.55	0.005	0.18	6.78
Signal c.	0.03	0.79	10.48	0.01	0.24	5.10	0.01	0.21	3.59	0.01	0.19	3.13
Yabby	0.10	18.3	38.10	0.05	4.77	24.13	0.02	0.34	6.24	0.02	0.30	5.61

spiny-cheek crayfish, red swamp crayfish, marbled crayfish, signal crayfish and yabby, respectively. In the same order, the 96-h LC<sub>50</sub> values of Decis Mega were 0.76, 0.16, 0.21, 0.03 and 0.27 µg/l (corresponding to 0.038, 0.008, 0.0105, 0.0135 and 0.0015 µg/l of deltamethrin).

## DISCUSSION

Freshwater crustaceans are generally more sensitive to pyrethroids than are fish (Smith and Stratton 1986; Haya et al. 2005). We observed a high sensitivity of the tested crayfish species to the pyrethroid insecticides Cyperkill 25 EC and Decis Mega using OECD data as the criterion. Both Cyperkill 25 EC and Decis Mega showed high toxicity in the crayfish species tested. With the exception of signal crayfish, within-species toxicity of the insecticides was similar, reflecting their similar modes of action. Interspecific variability in acute toxicity was in part related to the different sizes of the tested crayfish, as apparent in the spiny-cheek crayfish (Tables 2 and 3). Our results differ substantially from those of other studies, possibly due to the composition of the evaluated insecticides. Differing sensitivity of animal taxa can be explained by the original development of pyrethroids to target in-

sects, hence the higher sensitivity of crayfish and closely related groups. Pyrethroids were developed to replace older classes of pesticides including organochlorine, organophosphate and carbamate insecticides, which are considered particularly toxic (Breckenridge et al. 2009).

Although the red swamp crayfish used in our study were juveniles, rates of toxicity of cypermethrin for adults of this species have been reported to be substantially higher (Table 4; Morolli et al. 2006). This may be attributed, at least in part, to the effects of additional compounds present in the tested insecticide preparation (Percitox 25) administered at higher doses than in the present study, since the concentration of cypermethrin used (2.5%) was only 25% of that used in our trials. Pyrethroids are usually introduced into the environment in the form of a commercial preparation. This should be considered when evaluating research results, since a mixture of the active and ancillary substances may affect the toxicity of the active substance or can potentially be dangerous to the aquatic organisms. No other data of acute cypermethrin toxicity specifically for crayfish are available. The toxicity of cypermethrin for some other freshwater invertebrates and fish is summarised in Table 4. Based on this information, the most sensitive organisms are Malacostraca, specifically freshwater shrimp

Table 3. Acute toxicity of Decis Mega for selected crayfish species after 24, 48, 72 and 96 h exposure. Concentration is expressed as µg/l

Crayfish species	24 h			48 h			72 h			96 h		
	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>	LC <sub>0</sub>	LC <sub>50</sub>	LC <sub>100</sub>
Spiny-cheek c.	3.35	9.48	31.63	1.27	6.35	16.82	0.40	2.24	12.52	0.07	0.76	8.55
Red swamp c.	0.12	0.90	6.49	0.04	0.24	1.45	0.03	0.21	1.37	0.03	0.16	1.29
Marbled c.	0.04	6.06	138.51	0.01	3.70	72.13	0.01	0.57	57.04	0.01	0.21	7.51
Signal c.	0.01	0.07	41.34	0.01	0.05	11.27	0.01	0.04	6.86	0.01	0.03	1.22
Yabby	0.13	3.57	95.50	0.10	1.88	35.48	0.01	0.49	19.26	0.01	0.27	11.33

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Table 4. Acute toxicity of cypermethrin-containing products for selected freshwater organisms. LC/EC values are recalculated to cypermethrin as the active compound

Taxonomic group	Species	Time (h)	LC <sub>50</sub> /EC <sub>50</sub> (µg/l)	References
Oligochaeta, Tubificida	<i>Branchiura sowerbyi</i> adults	96	71.12	Saha and Kaviraj (2008)
Branchiopoda, Diplostraca	<i>Daphnia magna</i> adults	72	0.2	Mian and Mulla (1992)
Maxillopoda, Calanoida	<i>Aglaodiaptomus forbesi</i> adults	96	0.03	Saha and Kaviraj (2008)
Malacostraca, Isopoda	<i>Asellus aquaticus</i> adults	72	0.008	Werner and Moran (2008)
Malacostraca, Decapoda	grass shrimp ( <i>Palaemon paludosus</i> ) adults/juveniles	96	0.021/0.019	DeLorenzo et al. (2014)
	freshwater shrimp ( <i>Palaemon argentinus</i> ) juveniles	96	0.002	Collins and Cappello (2006)
	red swamp crayfish ( <i>Procambarus clarkii</i> ) adults	24/48	0.16/0.10	Morolli et al. (2006)
Insecta, Hemiptera	<i>Ranatra filiformis</i> adults	96	0.07	Saha and Kaviraj (2008)
Insecta, Ephemeroptera	<i>Cloeon dipterum</i> adults	96	0.03	Werner and Moran (2008)
Osteichthyes	common carp ( <i>Cyprinus carpio</i> ) juveniles	96	2.91	Velisek et al. (2011)
	rainbow trout ( <i>Oncorhynchus mykiss</i> ) fry	96	3.14	Velisek et al. (2011)
	rohu ( <i>Labeo rohita</i> ) juveniles	96	4.0	Marigoudar et al. (2009)
	Nile mouthbreeder ( <i>Oreochromis niloticus</i> ) juveniles	96	2.0	Stephenson et al. (1984)
	green snakehead ( <i>Channa punctate</i> ) juveniles	96	0.4	Kumar et al. (2007)

(*Palaemon argentinus*) followed by Isopoda (*Asellus aquaticus*), Maxillopoda, Insecta, Branchiopoda, Osteichthyes and Oligochaeta.

The acute toxicity of Decis Mega expressed as the 96-h LC<sub>50</sub> ranged from 0.03 µg/l to 0.76 µg/l, corresponding to 0.0015 and 0.038 µg/l deltamethrin, respectively. Signal crayfish and yabby showed the highest and lowest sensitivities to Decis Mega, respectively. Similar 48-h LC<sub>50</sub> values (DeLorenzo et al. 2014) and slightly lower ones (Golow and Godzi 1994) than ours have been reported for adult red swamp crayfish. The toxicity of deltamethrin for selected freshwater invertebrates and fish is summarised in Table 5. Based on available information, the most sensitive organisms are Insecta (except *Cordulia aenea*) and Malacostraca, specifically larvae of *Cloeon dipterum* and *Palaemon paludosus*, respectively. They are followed by Branchiopoda, Malacostraca and Osteichthyes.

Pyrethroids are adsorbed into suspended organic fractions and bottom sediments of natural waters. This greatly reduces the exposure of organisms in the water column and lowers bioavailability and potential toxic effects. The probability of bioaccumulation of these relatively non-persistent substances in organisms or in the food chain is low

(Haya 1989). Pyrethroids persist from hours to days in the water column. The persistence of deltamethrin in the water column is 8–48 h (Erstfeld 1999). In pond sediment, deltamethrin has been detected 306 days after application (Muir et al. 1985). The reported half-life of most pyrethroids adsorbed to sediment particulates ranges from 150 to 200 days (Amweg et al. 2005). The most persistent pyrethroid in sediment is bifenthrin, with a half-life ranging from 428 to 483 days under aerobic conditions with low persistence in anaerobic sediment (Li et al. 2017). Cypermethrin rapidly degrades in soil and sediment, with hydrolysis and photolysis playing major roles in the degradation. This and the related high lipoaffinity (reported log K<sub>ow</sub> values 3.76–5.54) indicate a strong tendency to sorb to sediment and accumulate in aquatic biota (Muir et al. 1985). Pyrethroids in stream water were most frequently associated with suspended solids and particulates, with only 0.4% to 1.0% of added pyrethroids present in the freely dissolved phase. In runoff, the freely dissolved phase accounted for 10% to 27% of the total pyrethroid mass. Following pyrethroid addition to stream water, more than 97% of the total mass added was sorbed to suspended solids and particulates (Liu et al. 2004).

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Table 5. Acute toxicity of deltamethrin-containing products for selected freshwater organisms. LC/EC values are recalculated to deltamethrin as the active compound

Taxonomic group	Species	Time (h)	LC <sub>50</sub> /EC <sub>50</sub> (µg/l)	References
Branchiopoda, Diplostraca	<i>Daphnia magna</i> adults	96	0.0293	Beketov (2004)
Malacostraca, Amphipoda	<i>Gammarus pulex</i> juveniles	96	0.068	Adam et al. (2010)
	<i>Gammarus fossarum</i> juveniles	96	0.033	Adam et al. (2010)
Malacostraca, Decapoda	grass shrimp ( <i>Palaemon paludosus</i> ) adults/juveniles	96	0.006/0.005	DeLorenzo (2014)
	red swamp crayfish ( <i>Procambarus clarkii</i> ) adults	24/48	0.22/0.18	Morolli et al. (2006)
	red swamp crayfish ( <i>Procambarus clarkii</i> ) adults	96	0.056	Wu et al. (2012)
Insecta, Ephemeroptera	<i>Cloeon dipterum</i> larvae	96	0.005	Beketov (2004)
	<i>Caenis miliaria</i> larvae	96	0.0091	Beketov (2004)
Insecta, Odonata	emerald damselfly ( <i>Lestes sponsa</i> ) larvae	96	0.0145	Beketov (2004)
	downy emerald ( <i>Cordulia aenea</i> ) larvae	96	0.79	Beketov (2004)
Osteichthyes	common carp ( <i>Cyprinus carpio</i> ) juveniles	96	0.5	Velisek et al. (2011)
	rainbow trout ( <i>Oncorhynchus mykiss</i> ) fry	96	3.25	Velisek et al. (2011)
	guppy ( <i>Poecilia reticulata</i> ) adults	96	5.14	Viran et al. (2003)
	Nile mouthbreeder ( <i>Oreochromis niloticus</i> ) juveniles	96	0.36	Golow and Godzi (1994)

Concentrations of cypermethrin and deltamethrin in the interstitial (pore) water of sandy sediment were five to six times as high as concentrations measured in the overlying water. However, the differences between pyrethroid concentrations in overlying and pore water in silt or clay sediments were considerably lower, a 1.3 to 1.5-fold difference (Muir et al. 1985). Consequently, benthic and epibenthic invertebrates in systems with clay or silt sediments are likely to experience similar pyrethroid exposures. However, exposure of benthic organisms in sandy substrates may not necessarily be greater despite the higher total concentration of pyrethroids as a result of chemical adsorption to the larger particles; the mass adhered to sand is likely not as bioavailable to sediment-dwelling invertebrates (You et al. 2008). Pyrethroids are also relatively inexpensive compared to other insecticides, since doses are usually low (Li et al. 2017). These characteristics make pyrethroid potential biocides for eradication of invasive crayfish, but further research on the presence of active compounds and their metabolites in the environment is needed to assess possible adverse effects.

Freshwater crayfish represent a group of particularly successful biological invaders in Europe and regions of North America (Lodge et al. 2000; Kouba

et al. 2014). Use of biocides is one of the few feasible means for their eradication (Gherardi et al. 2011; Stebbing et al. 2014). The present study demonstrates that cypermethrin- and deltamethrin-based insecticides are highly toxic for the studied species. Lethal concentrations values represent a baseline for possible eradication programs in line with attempts to counteract biological invasions on the European continent, as well as globally (Regulation (EU) No. 1143/2014; Commission Implementing Regulation (EU) 2016/1141). Stagnant, closed water bodies with newly emerging crayfish invasion are possible sites of application (Patoka et al. 2016), although local conditions should be always carefully considered. Application of biocides to established and widely distributed alien crayfish populations is unfeasible, especially in running waters. In such cases, prevention of further spread should be the focus (Holdich et al. 2009; Booy et al. 2017).

Acute toxicity testing in the laboratory is usually conducted under controlled conditions with pure water, glass vessels, etc. Compared to natural situations, the sorption of active compounds on available surfaces is lower, requiring application of higher doses, ideally derived from LC<sub>100</sub> values. The spiny-cheek crayfish and marbled crayfish are facultative and obligatory parthenogenetic

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species, respectively (Buric et al. 2011), and total elimination is essential to prevent population renewal. Pyrethroids are less toxic to fish than to crayfish (Tables 4 and 5), but applied concentrations will presumably exceed their tolerance levels. Whenever possible, they should be removed from the site prior to biocide application. Eradication should be accompanied by exposure to captive crayfish to indirectly confirm efficacy of biocides applied at the site (Peay et al. 2006; Sandoden and Johnsen 2010). The burrowing behaviour of crayfish (Kouba et al. 2016) may negatively impact the success of eradication, as an effective concentration of the active compound may not reach the extremity of the burrows in which crayfish are hidden (Cecchinelli et al. 2012). Crayfish are also able, under extreme conditions (e.g. decreased oxygen levels), to leave water bodies, so biocide should also be applied to banks to prevent the escape of crayfish from the treated water (Peay et al. 2006; Gherardi et al. 2011).

In conclusion, a high sensitivity of all tested crayfish species was confirmed. Due to crayfish sensitivity, low accumulation of active compounds in the food chain and short-term persistence in the aquatic phase, pyrethroids are suggested to be suitable biocides for eradication of crayfish. In particular, stagnant, closed water bodies with newly emerging invaders are the prime places for possible applications, while local conditions should be always carefully considered. Further research is needed into the presence and effects in sediment of other commercial pesticide components and their metabolites.

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