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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction to reproductive and behavioural issues of crayfish

1.2. **Kubec, J.**, Kouba, A., Buřič, M., 2019. Communication, behaviour and decision making of crayfish: a review. Zool. Anzeig. 278, 28–37.

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1.1. Introduction to reproductive and behavioural issues of crayfish

Animals exhibit behavioural patterns characterized by responses to information received from internal and external stimuli. The ultimate response or decision is based on physical, chemical, or biological factors and their analysis by the nervous system, and may be influenced by previous experiences, stressors, and limitations. This general introduction summarizes behavioural patterns connected with decision making in crayfish, and includes similarities to vertebrates and other invertebrates that may be applied to freshwater crayfish. Concurrently, purpose of this review is introduce behavioural patterns from outside and inside of crustaceans taxa, in which might crayfish have potential exhibit them. Knowledge of patterns influencing behavioural responses and decisions in organisms is necessary for understanding basic biological relationships and for extraction of information applicable to crayfish. Information on sensory and nervous systems, communication, social behaviour, reproductive behaviour, and decision making within the context of species success and adaptations to changing environments will contribute to a model design for future studies of reproductive and social behaviour and behavioural ecology of crayfish. The study of crayfish behavioural patterns can extend knowledge of habitat and reproductive requirements of native crayfish as well as investigate success and impacts of non-native species in environment.

This general introduction provides an overview of studies investigating the behaviour of freshwater organisms, including social and reproductive interactions, decision making, and channels for recognition or processing of environmental stimuli. For ethological studies in aquatic ecosystems, crustaceans, and especially crayfish, represent suitable model organisms. Crayfish are advantageous over other invertebrates given their high level of social interaction in both the field and the laboratory. They exhibit unique eco- ethological behaviour in variety of feeding habits, activity cycles, habitat selection, and resource utilization at the level of species, sex, and age. Similar to vertebrates, crayfish demonstrate unique behaviours throughout their life. Crayfish are widely accepted as sensitive and rapid bioindicators of water quality changes. They are considered keystone species in freshwater ecosystems and are important ecosystem engineers. Subsequently, pollution impacts on native crayfish stock can result in ecosystem instability.

Effects of environmental pollutants on the behavioural patterns of crayfish

In recent decades, rapid economic development, and increasing population and urbanization have caused an enlargement of common synthetic pollutants entering water systems, including plastics, detergents, pharmaceuticals, pesticides, and petrochemicals (Faggio et al., 2016; Sehonova et al., 2018). The potential risks industrial products or their metabolites have created, frequent incidents of pollution and deterioration of water quality with impacts on the aquatic ecosystem (Buřič et al., 2018; Velíšek et al., 2018).

Given the inefficiency of wastewater treatment plants at eliminating a wide spectrum of pharmaceuticals consumed by modern society, compounds and their metabolites with residual activities, persist in surface waters (Cunha et al., 2017). Concentrations detected in effluent may cause measurable toxic effects in ecotoxicity assays (Kellner et al., 2018). Nevertheless, psychoactive pharmaceuticals are designed to be bioactive and therefore have considerable pharmacological effects on non-target aquatic organisms that share receptor and enzyme homology with humans. The most common effects of pharmaceuticals are survival rates, reproductive malformations and behavioural changes (Sehonova et al., 2018). Alterations in natural behaviour, such as shoal cohesion, activity, light or dark zone preferance provide evidence of environmental effects of pharmaceutical pollutants in vertebrates (Gebauer et al., 2011; Fong and Hoy, 2012; Kellner et al., 2015). The non-lethal effects of psychoactive drugs are displayed by disruption of prey/predator relationships or alternation in social interactions, defence strategies, reproduction and migration of aquatic organisms (Boxall et al., 2012; Fong and Ford, 2014, Bláha et al., 2019).

Herbicides are another type of aquatic environment contaminant based on chloroacetanilide compounds with an annual consumption over 200 tons to control grasses and some broad-level weeds in a wide range of crops (Zemolin et al., 2014). Most of these herbicides are soluble in water with a half-life greater than several months in freshwaters (Senseman, 2007). Irrespective, herbicides such as metolachlor, s-metolachlor or metazachlor are currently detectable in European surface waters in ranges from 0.01 to 5.30 µg L^{-1} (CHMI, 2018). Even environmentally relevant concentrations of chloroacetanilide herbicides may pose a risk to aquatic organisms (Maronic et al., 2018). Studies assessing long-term exposure to low concentrations of pesticides have been associated with motion alterations, changes in activity and heart rate, dysfunction of foraging and social behaviour, as well as abnormalities in response to alarm cues (Moore et al., 1991; Zulandt Schneider et al., 2001; Wolf and Moore, 2002; Kuklina et al., 2018; Velíšek et al., 2018; Velíšek et al., 2019).

The current study was devoted to the comprehensive investigation of the behavioural patterns of crayfish and pursuing the following objectives:

1. To study the whole processes of behavioural patterns underlying reproductive and environmental issues.
2. To investigate the mechanisms of decision making in crayfish in specified conditions.
3. To describe the effects of pollution on crayfish population, health condition and their future prosperity in the aquatic environment.

REFERENCES

Bláha, M., Grabicova, K., Shaliutina, O., Kubec, J., Randák, T., Zlabek, V., Buřič, M., Veselý, L., 2019. Foraging behaviour of top predators mediated by pollution of psychoactive pharmaceuticals and effects on ecosystem stability. Sci Total Environ, 662, 655–661.

Boxall, A.B., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ. Health Perspect. 120, 1221.

Buřič, M., Grabicová, K., Kubec, J., Kouba, A., Kuklina, I., Kozák, P., Grabic, R., Randák, T., 2018. Environmentally relevant concentrations of tramadol and citalopram alter behaviour of an aquatic invertebrate. Aquatic Toxicology, 200, 226–232.

CHMI (Czech Hydrometeorological Institute), 2018. On-lineWater Duality Database. http://hydro.chmi.cz/. (Accessed 11 June 2018).

Cunha, D.L., Araujo, F.G., Marques, M., 2017. Psychoactive drugs: occurrence in aquatic environment, analytical methods, and ecotoxicity: a review. Environ Sci Pollut Res 24, 24076–24091.

Faggio, C., Pagano, M., Alampi, R., Vazzana, I., Felice, M.R., 2016. Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternium-15 in *Mytilus galloprovincialis*. Aquat. Toxicol. 180, 258–265.

Fong, P.P., Hoy, C.M., 2012. Antidepressants (venlafaxine and citalopram) cause foot detachment from the substrate in freshwater snails at environmentally relevant concentrations. Mar Freshw Behav Physiol 45, 145–153.

Fong, P.P., Ford, A.T., 2014. The biological effects of antidepressants on the molluscs and crustaceans: a review. Aquat. Toxicol. 151, 4–13.

Gebauer, D.L., Pagnussat, N., Piato, Â.L., Schaefer, I.C., Bonan, C.D., Lara, D.R., 2011. Effects of anxiolytics in zebrafish: similarities and differences between benzodiazepines, buspirone and ethanol. Pharmacol Biochem Behav 99, 480–486.

Kellner, M., Porseryd, T., Porsch-HällströmI, I., Hansen, S.H., Olsén, K.H., 2015. Environmentally relevant concentrations of citalopram partially inhibit feeding in the three-spine stickleback (*Gasterosteus aculeatus*). Aquat Toxicol 158, 165–170.

Kellner, M., Porseryd, T., Porsch-Hällström, I., Borg, B., Roufidou, C., Olsén, K.H., 2018. Developmental exposure to the SSRI citalopram causes long-lasting behavioural effects in the three-spined stickleback (*Gasterosteus aculeatus*). Ecotoxicology 27, 12–22.

Kuklina, I., Ložek, F., Císař, P., Kouba, A., Kozák, P., 2018. Crayfish can distinguish between natural and chemical stimuli as assessed by cardiac and locomotor reactions. Environ Sci Pollut Res 25(9), 8396–8403.

Maronic, D.S., Camagajevac, I.S., Horvatic, J., Pfeiffer, T.Z., Stevic, F., Zarkovic, N., Waeg, G., Jaganjac, M., 2018. S-metolachlor promotes oxidative stress in green microalga *Parachlorella kessleri* - a potential environmental and health risk for higher organisms. Sci. Total Environ. 637-638, 41–49.

Moore, P.A., Scholz, N., Atema, J., 1991. Chemical orientation of lobsters, *Homarus americanus*, in turbulent odor plumes. J. Chem. Ecol. 17, 1293–1307.

Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., Faggio, C., 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Sci. Total Environ. 631–632, 789–794.

Senseman, S.A. (Ed.), 2007. Herbicide Handbook, 9 ed. Weed Science Society of America, Lawrence (458 pp).

Velíšek, J., Stará, A., Zusková, E., Kubec, J., Buřič, M., Kouba, A., 2018. Chronic toxicity of metolachlor OA on growth, ontogenetic development, antioxidant biomarkers and histopathology of early life stages of marbled crayfish. Sci. Total Environ. 643, 1456–1463.

Velíšek, J., Stará, A., Zusková, E., Kubec, J., Buřič, M., Kouba, A., 2019. Effects of s-metolachlor on early life stages of marbled crayfish. Pesticide biochemistry and physiology, 153, 87–94.

Wolf, M.C., Moore, P.A., 2002. The effects of the herbicide metolachlor on the perception of chemical stimuli by *Orconectes rusticus*. J. North Am. Benthol. Soc. 21, 457–467.

Zemolin, C.R., Avila, L.A., Cassol, G.V., Massey, J.H., Camargo, E.R., 2014. Environmental fate of S-metolachlor: a review. Planta Daninha 32, 655–664.

Zulandt Schneider, R.A., Huber, R., Moore, P.A., 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour 138, 137–153.

CHAPTER 1.2.

COMMUNICATION, BEHAVIOUR AND DECISION MAKING OF CRAYFISH: A REVIEW

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ABSTRACT

Animals exhibit behavioural patterns characterized by responses to information received from internal and external stimuli. The ultimate response or decision is based on physical, chemical, or biological factors and their analysis by the nervous system, and may be influenced by previous experiences, stressors, and limitations. This review summarizes behavioural patterns connected with decision making in crayfish, and includes similarities to vertebrates and other invertebrates that may be applied to freshwater crayfish. Concurrently, purpose of this review is introduce behavioural patterns from outside and inside of crustaceans taxa, in which might crayfish have potential exhibit them. Knowledge of patterns influencing behavioural responses and decisions in organisms is necessary for understanding basic biological relationships and for extraction of information applicable to crayfish. Information on sensory and nervous systems, communication, social behaviour, reproductive behaviour, and decision making within the context of species success and adaptations to changing environments will contribute to a model design for future studies of reproductive and social behaviour and behavioural ecology of crayfish. The study of crayfish behavioural patterns can extend knowledge of habitat and reproductive requirements of native crayfish as well as investigate success and impacts of non-native species in environment.

1. Introduction

From the perspective of ethology, studying communication and signal reception is important for understanding behavioural patterns of organisms. Behaviours are dependent on multiple factors, including but not limited to biological and chemical stressors (Moller & Milinski 1998). Communication signals play significant roles in animal societies during important events such as reproduction (sex and maturity recognition, mate choice) and in intra- and inter-specific competition. Animals employ a wide range of signals to share or send information regarding their identity, status, mood, position, and experience of the environment (Bradbury & Vehrencamp 1998). Organisms are able to make decisions and act on preferences for mating, feeding, homing, and survival based on interaction of signals and sensory organs, previously known information, and learning abilities (Atema 1988; Bierbower et al. 2013; Veselovský 2005). Fig. 1.

In this review, we provide an overview of current knowledge about communication processes and their effect on behaviour and decision making in crayfish. Within this topic, it is necessary to integrate the disciplines of neurophysiology, physiology, ecology, behaviour, and even psychology such as personality. Similar integration of disciplines is used in investigations of animals ranging from mammals (Veselovský 2005) to small arachnids (Chapin & Hebets 2016) and worms (Capowiez & Bérard 2006) which have been the subject of studies, with no limitations on behaviours observed.

Crustaceans are widely used as model animals for behavioural studies, largely due to their global distribution (Briffa 2013; Huxley 1880). They are ecologically important mostly benthic macroinvertebrates often regarded as keystone species (Crustacea, Decapoda, Astacida) as well as ecosystem engineers and bioindicators (Fureder & Reynolds 2003; Kouba et al. 2016). Freshwater crayfish, with their high level of social interaction in both the field and the laboratory, offer advantages over many other invertebrate groups (Gherardi 2002). Crayfish are physiologically, ecologically, and behaviourally remarkable animals occurring in a wide range of habitats including lentic and lotic waters such as springs, lakes, ponds, creeks, rivers, alpine and subtropical streams.

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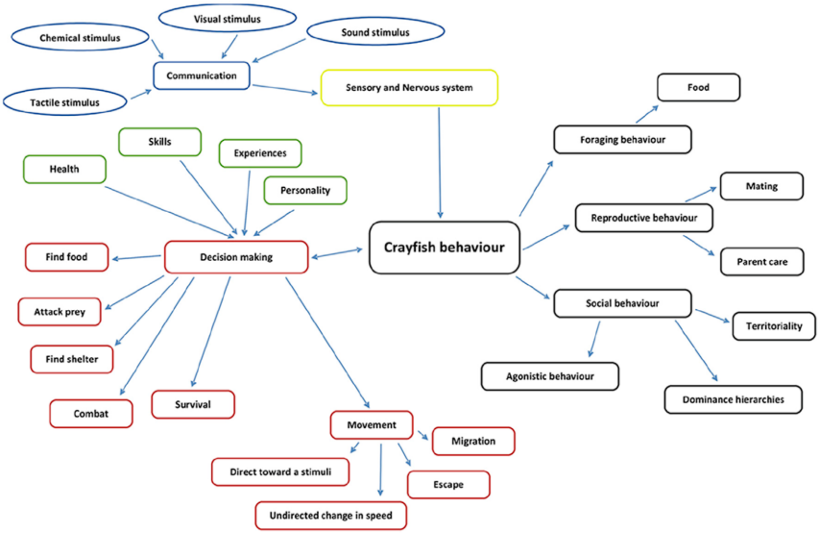

Fig. 1. Illustration of flow map of behaviour in crayfish.

They exhibit diverse life histories, including a broad spectrum of behavioural patterns and show widespread natural distribution patterns that have been further extended via introductions and subsequent spread into new areas (Souty-Grosset et al. 2006). Even their adaptive capacity as long-term survival of Astacidea into brackish water (Holdich et al. 1997) or tolerance of warm-water crayfish (Karplus et al. 1998; Veselý et al. 2015) to cold water in winter period plays role in their success in new areas. Some are significantly invasive, causing damage to freshwater habitats (Crandall & Buhay 2008; Holdich et al. 2009). They are able to dig burrows and can be found in subterranean and semi-terrestrial environments (Holdich 2002; McMahon et al. 2005; Taylor 2002). Crayfish exhibit their highest activity in darkness (McMahon et al. 2005) and possess highly developed sensory organs for movement, orientation, food searching, and communication (Basil & Sandeman 2000). All these features make the crayfish as a suitable model species for ethological studies with broad applicability across research disciplines.

2. Sensory and nervous system

Animals are closely connected to their physical and chemical environment, with which they maintain continuous interaction via their senses. They have adapted sensory organs for obtaining specific information characteristic of the environmental conditions under which they live (Atema 1988). Animals operate in different environments, and their sensory organs and behaviours have unique constraints and opportunities to acquire and filter stimuli from the variety of signals typical of their habitat (Atema 1988; Hu et al. 2003; Palacios et al. 1996).

In crustaceans, as in many other animals, the sensory system comprises interoceptors and exteroceptors that transmit information from the internal and external world to the central nervous system. Interoceptors relay information about the position and motion of the body, while chemo- and baro-receptors monitor the chemical state of body fluids and blood pressure. Exteroceptors include sense organs such as compound eyes, chemoreceptors, and mechanoreceptors that detect stimuli arriving at the surface of the body from a distance: light, scent, sound, and hydrodynamic stimuli (Anger 2001; Brusca & Brusca 2003; Vogt 2002).

The crayfish central nervous system comprises the cerebral ganglion (brain) formed by the fusion of anterior ganglia, together with a ventral nerve cord located below the alimentary tract (Holdich & Reeve 1988; Sandeman et al. 1992). Five paired nerves and two unpaired nerves originate in the crayfish cerebral ganglion. The paired nerves include the oculomotor, antennular, antennary, optic, and tegumentary nerves. The unpaired nerves link the brain with the neural network of the suboesophageal ganglion (Sandeman & Sandeman 2003; Tautz & Muller-Tautz 1983; Vogt 2002). This ganglion splits into nerve roots on each side that proceed to cephalothoracic limbs and continue caudally to connect with muscles of the thorax and the thoracic and abdominal ganglia (Brusca & Brusca 2003; Vogt 2002).

Both groups of nerves serve as conduits for axons of both sensory and motor neurons. The axons terminate in synapses, sites of transmission of information between neurons and motor cells, sensory receptors, and the brain (Anger 2001; Atema 1988; Brusca & Brusca 2003).

3. Bio–communication

Appropriate social, sexual, and defence behaviours depend on mutual understanding. Communication provides location and harmonic coexistence of social and reproductive partners, as well as warning against an enemy (Gherardi 2002; Veselovský 2005). It is a process of information exchange that includes participants or participant groups, a sender and a receiver. The sender creates a signal that contains codes transmitted by specific channels through the environment to the receiver. The selection of the proper channel, whether optic, acoustic, tactile, or olfactory (Table 1), is related to sender ability and the sensory facilities of the intended receiver (Bradbury & Vehrencamp 1998; Hopkins 1988). The receiver‘s sense organs intercept and decode the signal and send the information to the nervous system where it is processed (Vogt 2002). The receiver uses the information in the decision-making process and selects an appropriate response (Dawkins & Krebs 1978). In bio-communication, the transmitted signal generally prepares sender and receiver for new circumstances, which might be manipulation of one or the other or a challenge to mutual cooperation (Schaller 1978). However, reception of signals may vary both among species and among conspecifics. The communicating participants may encounter eavesdroppers such as parasites, predators, potential prey, or rivals (Brandley et al. 2013). A sender may err in the assessment of a new situation and imperfectly encode the signal. However, in bio-communication both sender and receiver should benefit from the information exchange (Bradbury & Vehrencamp 1998).

3.1. Vision and visual communication

Crayfish possess a complex visual system including well-developed compound eyes, one on each eyestalk that can be moved by oculomotor muscles (Vogt 2002). The compound eye contains multiple lenses, each associated with a receptor or a cluster typically made up of eight photoreceptors. The typical compound eye contains one hundred lens systems and receptor group units, called ommatidia (Land 2005; Porter et al. 2010) that constitutes part of a composite image that is assembled and transmitted to the brain (Bradbury & Vehrencamp 1998). In the majority of decapod crustaceans (crayfish, lobster, Caridean and penaeid shrimp, and the anomuran galatheids) the ommatidia on the eye surface are square, and the structures beneath are not rigid cylinders, but pyramids of soft jelly (Land 2004; Land 2005).

Advantages of vision are accurate determination of course, rapid dissemination, and detection of movement (Alcock 2001; Veselovský 2005). The transmission of visual signals from sender to receiver over long distances is influenced by terrain barriers, availability and quality of ambient light, and light transmission conditions such as turbidity (Bradbury & Vehrencamp 1998). Visual communication involves cues such as colour or patterning, bioluminescence (Haddock et al. 2010), size and shape of morphological structures (Palmer 2009), and resources (shelters), often in connection with elaborate courtship behaviour (Díaz & Thiel 2004). For example, ground-nesting birds can distinguish a flying predator according to body shape (Manning & Dawkins 2012). Honeybees use visual communication through dancing movements (Dornhaus & Chittka 2004; Wenner 2002). In the absence of light, the frequency of visually-mediated behaviours decreases (Bruski & Dunham 1987).

Exploitation of visual signals has been observed in male *Ocypode* crabs. Pyramids of sand placed in front of burrows are typical for this genus, and males use these readily visible signs to mark their territory and to attract females (Christy et al. 2002; Linsenmair 1965). Many animals possess ornamentation or weapons that may also function as visual signals (Hughes 1996), as in fiddler crab (genus *Uca*) males, which wave their larger claw in species-specific undulations during courtship (Zeil & Zanker 1997). Colour ornaments feature in mate choice of cichlid fish (Baldauf et al. 2010) and in crabs that display prominent colour patterns on the carapace and claw (Cummings et al. 2008) serve for recognition and mates attraction. A similar behaviour is seen in the snapping shrimp (Alpheidae) (Schein 1977). Equally in crayfish, vision plays a role during the initial stages of agonistic behaviour (Bruski & Dunham 1987), facial recognition of previous opponents (Van der Velden et al. 2008), and in selection of a reproductive partner (Aquiloni & Gherardi 2008c; Snedden 1990).

Table 1
Types of signals and general characteristics. Adapted from Alcock (2001).

Properties	Signal			
	visual	acoustic	tactile	chemical
Range	limited	long	short	long
Transmission	rapid	rapid	rapid	slow
Limited by barrier	yes	no	yes	no
Localization	accurate	variable	accurate	variable
Energy expenditure	low	high	low	low

3.2. Water–borne acoustic communication

In crayfish, receptors receive acoustic signals or vibrations using the hair pit organ on the chelae, well-suited to measure water velocity (Vogt 2002); statocysts; hair-like setae cells on the body surface; and chordotonal organs associated with antenna joints, legs, and other body appendages. These mechanoreceptors consist of one or more hairs that are associated with sensory cells. Mechanical disturbances from an acoustic signal result in stimulation of the sensory cells (Popper et al. 2001). These sensory cells are often organized tonotopically, *i.e.* different parts are stimulated by different frequencies (Rosenthal & Ryan 2000). Acoustic stimuli can also be sensed by mechanoreceptors that monitor the deformation of the antennal flagella, as well as by the statocysts. The antennae have external and internal joint receptors that react to weak water vibrations, gravity, and possibly to angular acceleration (Vogt 2002).

Similar to light signals, sound signals can be highly degraded when travelling over long distances. High frequencies attenuate and become scattered by obstacles to a greater extent than do low frequencies in all types of habitat including aquatic (Manning & Dawkins 2012). The transmission of a long-distance acoustic signal involves challenges such as attenuation, pattern loss, and ambient noise (Bradbury & Vehrencamp 1998).

Three successive steps in sound production are essential for acoustic communication in an aquatic environment: The production of vibrations carrying specifically coded information, modification of the acoustic vibrations to match biological function such as mechanoreceptors, and finally the coupling of the modified vibrations to the medium in which the sound is propagated (Bradbury & Vehrencamp 1998). In comparison to terrestrial animals, aquatic organisms have an advantage in coupling of sound to medium, as sound waves travel about 5 times faster in water than in air (Hopkins 1988). Acoustic organs of aquatic animals have an impedance sufficiently similar to water that the sounds they produce are transmitted over long distances without the aid of resonant structures (Bradbury & Vehrencamp 1998). Production of acoustic signals by some fish is used predominantly as a tool for attracting females and for mate choice. This pattern has been reported in male plainfin midshipman *Porichtys notatus*, which creates sound using the swimbladder (Brantley & Bass 1994); in gobiid fish *Zosterisessor ophiocephalus* males, which emit sound via head movements and enlargement of the branchial cavity (Malavasi et al. 2003); and in several cichlid species (Longrie et al. 2013). Some cyprinids and silurids also emit acoustic signals using muscular contraction and expansion of the swimbladder (Bradbury & Vehrencamp 1998; Radford et al. 2014). Evidence of acoustic signals is reported, albeit rarely, in crustaceans, such as in snapping shrimp, which strike parts of their claws to make cracking noises

(Versluis et al. 2000). The purpose of snapping shrimp behaviour seems to be to disturb the communication of other animals rather than to communicate (Lohse et al. 2001), whereas semi-terrestrial crabs produce and use sound for communication for social interaction and probably to attract mates (Popper et al. 2001). The acoustic signals of the spiny lobster *Panulirus interruptus* can be used as a startle or aposematic cue to predators (Staaterman et al. 2010).

Sensitivity to hydrodynamic stimuli has been described in the red swamp crayfish *Procambarus clarkii* (Monteclaro et al., 2011) and spiny-cheek crayfish *Orconectes limosus* (Tautz, 1987), but the use of acoustic signals for communication or defence has not been observed.

3.3. *Tactile communication*

Touch plays a prominent role in signalling and discrimination in invertebrates, to distinguish objects, for movement, and orientation, as well as for demonstration of social relationships and to calm aggressive behaviour (Alcock 2001; Bradbury & Vehrencamp 1998). Arthropods obtain information of the near surroundings through the tactile response (Wiese 1993). The major intermediaries for tactile sensory tasks are antennules and antennae. These organs possess both chemosensors and mechanosensors, but antennules primarily have an olfactory function, while the antennae respond chiefly to mechanosensory stimuli (Vogt 2002; Wiese 1993). In crayfish, each antenna consists of basal segments and a long segmented flagellum. In some species, the antennae are as long as the body and extremely flexible. These mechanoreceptive organs assist crayfish in determining the position of objects as well as their own position, posture, and movement by using information from receptors on the flagellum (Vogt 2002; Zeil et al. 1985). Stimulation of sensilla on the flagellum assists in sensing a stimulus (Masters et al. 1982) and this provides information about an object and the distance to it (Zeil et al. 1985). Despite this, evidence of the use of antennae during exploration of new environments is limited. There is no available information about the extent that crayfish explore or navigate in confined spaces or whether mechanosensors are actively involved. On the other hand, in natural aquatic environments, crayfish inhabit areas that contain crevices, stones, and wood debris that restrict or impede movement and force decisions about travel over or around obstacles primarily based on information gained from mechanoreceptors (McMahon et al. 2005).

3.4. *Olfactory receptors and chemical communication*

The chemical signals, pheromones, belong to the oldest communication systems, and are species-specific. Pheromones can have different functions, such as sexual, alarm, and territory-marking, depending on molecular weight (Alcock 2001; Bradbury & Vehrencamp 1998; Breithaupt & Thiel 2011). Some animals, especially aquatic, despite other available senses, primarily use olfactory cues in inter- and intra-specific interactions to decide what they should eat, run from, fight with, or mate with (Breithaupt & Thiel 2011; Hay 2011).

In an aquatic environment, chemical signals influence feeding, habitat, and mating choices and stabilize dominance in hierarchies (Hay 2011). Hence, chemical communication is well developed and widely used by aquatic organisms (Wyatt 2011). Chemical communication is widely exploited in mate attraction, such as reported in blind livebearer *Poecilia mexicana* females, which secrete a substance increasing their sexual attractiveness to males (Tobler et al. 2008). Goldfish and other teleost fish release reproductive pheromones by both females and males (Stacey et al. 1994; Van Den Hurk & Resink 1992). These pheromones not only attract mates, but mediate increasing milt production and enhance fertility in males (Barki et al. 2011). African jewelfish *Hemichromis bimaculatus* use the olfactory system in parental care. Females are able to distinguish their own offspring even at different ages of juveniles (Immelmann et al. 1996). A similar finding of recognition of independent juveniles in crayfish would be interesting. Olfaction is also used in fish including the Atlantic salmon *Salmo salar* (Johnstone et al., 2012; Stabell, 1984) and the European eel *Anguilla anguilla* (Churcher et al,. 2015) for the long-distance migration to their home locations for reproduction (Ueda & Shoji 2002).

Crustaceans, essentially occurring in all environments including aquatic as well as terrestrial habitats, face numerous challenges in communicating with conspecifics (Thiel & Breithaupt 2011). All crustaceans possess a carapace, which impedes diffusion of chemical substances into the environment (Hallberg & Skog 2011). In the aquatic environment, most pheromones are contained in the urine, which is released through the nephropores (Breithaupt & Thiel 2011). Chemoreceptors have evolved for interception of chemical signals in crustaceans (Vogt 2002).

In crayfish, the olfactory system comprises receptor neurons on the antennules, paired olfactory and accessory lobes, and various cell clusters in the brain (Vogt 2002). The chief olfactory detection system is represented by chemoreceptors, the chemosensilla, and the aesthetascs. The chemosensilla are innervated structures of small, often hair-like cuticular organs with sensory cells (See Breithaupt et al. 2016). The sensilla are mainly concentrated on the oral appendages and tips of the pereopods (Breithaupt 2011; Hallberg & Skog 2011). At these sites, chemoreceptors receive signals from chemical sources that are in direct contact with the animal, similar to taste in vertebrates (Hallberg & Skog 2011). The aesthetascs are generally located in the outer flagella on the antennules and are composed of cuticular hairs. Aesthetascs serve to mediate reception of chemical signals such as prey scent or sex pheromones released by conspecifics from a long distance too (Breithaupt 2011; Hallberg & Skog 2011; Vogt 2002). In addition, the major chelae contain necessary chemosensory structures for recognition of female scent and can be an alternative site of reception (Belanger & Moore 2006).

In crustaceans, chemical signals are one of the most significant messengers. They can effectively transmit coded information about specific properties such as sex, sexual receptivity, species identity, health status, motivation to fight, dominance, individual identity, and moult stage and communicate information about important functions throughout life stages (Thiel & Breithaupt 2011). Pheromones are powerful elements in agonistic behaviour and hierarchy establishment (Atema & Steinbach 2007). Lobsters can recognize individuals that they have previously encountered by chemical scent and behave appropriately given their relative dominance (Hay 2011). The hermit crab *Pagurus longicarpus* can distinguish individual conspecifics and their status by chemical scent (Gherardi et al. 2005). Memory of crustaceans in similar cases has been demonstrated to last from one to two weeks (Karavanich & Atema 1998). Chemical scents and signals are used by crustaceans in foraging, especially the food specialists such as the turtle-weed crab *Caphyra rotundifrons* (Hay, 2011).

A major role for pheromones is in mediating reproductive behaviour. The first evidence of a female sex pheromone in a decapod crustacean was demonstrated in the black-spotted swimming crab *Portunus sanguinolentus* (Ryan, 1966). Some male crustaceans, including the green crab *Carcinus maenas* and the American lobster *Homarus americanus*, often guard females through moulting and subsequent egg laying. Mating is restricted to a short time, signalled by sex pheromones. Intermoult females can be rejected by males, since they do not secrete the pheromone indicative of the correct moult stage (Atema & Engstrom 1971;

Hardege et al. 2002). Sex pheromones have also been detected in rock shrimp *Rhynchocinectes typus* (Díaz & Thiel, 2004) and signal crayfish *Pacifastacus leniusculus* (Stebbing et al., 2003).

The use of chemical signals in crayfish is similar to other crustaceans, but in reproduction is not related to the female moulting period (Reynolds & Holdich 2002; Stebbing et al. 2003). Mating in crayfish occurs during the intermoult stage and is dependent on prior agonistic interactions between the sexes (Reynolds & Holdich 2002). Females release chemicals in urine during aggressive acts. Males recognize female receptivity via these urinary components and initiate courtship (Berry & Breithaupt 2010). In dominance competition, importance of chemicals and olfactory receptors of the combatants was observed in red swamp crayfish (Schneider et al. 1999), rusty crayfish *Orconectes rusticus* (Bergman et al., 2003), and narrow-clawed crayfish *Astacus leptodactylus* (Breithaupt, 2011).

4. Social behaviour

In ethology, social behaviour is defined as interactions between two and more individuals, typically within a group of conspecifics (Krebs & Davies 1997; Manning & Dawkins 2012). Examples include coordination of activities between males and females in mating and caring for offspring and the integration of members of group-living species (Bradbury & Vehrencamp 1998; Krebs & Davies 1997). Female crayfish care for eggs while they are attached to the pleopods (Gherardi 2002) using shaking movements, and juveniles are cleaned and fanned to ensure that they receive sufficient oxygen (Vogt 2013). Crayfish males may prey upon juveniles (Mathews 2011). Social behaviour in parental care is demonstrated by recognition and care of conspecific offspring (Beermann et al. 2015).

In addition to providing active assistance and support, social behaviour may be aggressive (Manning & Dawkins 2012). Efforts of an individual to manipulate another member of society to obtain some advantage is an important aspect of social behaviour (Krebs & Davies 1997; Manning & Dawkins 2012). Aggressive behaviour is characteristic of all life stages of crayfish. It is typical of inter- and intra-specific relationships and appears during conflicts for resources such as food and shelter, preferred habitat, and a reproductive partner (Gherardi 2002; Krebs & Davies 1997). Agonistic behaviour is primarily demonstrated by increased aggressiveness between participants of conflict. The use of chemical or visual communication signals to intimidate an opponent can be a substitute for combat and avoid higher costs of physical aggression (Breithaupt & Thiel 2011) (Fig. 2). When pre-fight signals fail, opponents commence the characteristic fight for resources or a mate. This may lead to energy depletion, time lost from foraging or mating, and injury or death of one or both combatants. Opponents are also more prone to predation during conflicts (Jakobsson et al. 1995; Payne 1998). A functioning social hierarchy within the animal group can forestall overt fighting (Gherardi 2002; Manning & Dawkins 2012; Neumann et al. 2011) and ensure the safety of individuals. Generally, hierarchy is based on dominance from the strongest to the weakest, with the dominance rank determined by experience and fitness (Gherardi & Daniels 2003; Manning & Dawkins 2012; Neumann et al. 2011). Studies show dominance hierarchies to be based on strength, size, age category, sex, and ability to manipulate others, as well as ability to recognize individual (Aquiloni et al. 2008; Fero & Moore 2014; Seebacher & Wilson 2007). Even, balance in hierarchy is necessary in social behaviour, high winner effects with low loser effects result in an overall increasing of aggressiveness and lead to decrease of stability of social group (Hock & Huber 2009).

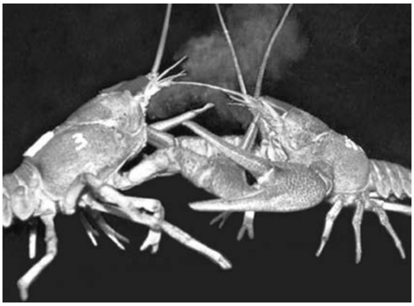

Fig. 2. Chemical communication visualized using Fluorescein dye during aggressive interaction of two males narrow-clawed crayfish (Breithaupt 2011).

Opponents can tolerate only a certain level of direct physical damage in fights. In some cases, fighting may not occur, due to lack of time and physical costs such as loss of body parts, and vulnerability to predation or death. Hence, a potential combatant may decide whether to initiate, persist in, or retreat from combat (Payne 1998).

Agonistic inter- and intraspecific interactions occur when a stranger invades to a crayfish's territory (Bruski & Dunham 1987). In the wild, crayfish are solitary and territorial animals, with rare cases of eusociality (Vogt 2013). Territoriality comes with a hierarchy in which a dominant animal has greater access to food and mates (Delgado-Morales et al. 2004; Fero & Moore 2014). Goessmann et al. (2000) observed that dominant animals and individuals occupying dominant positions became increasingly likely to escalate to higher fight intensity and are less likely to retreat, in the noble crayfish *Astacus astacus*. Manifestation of the agonistic behaviour in crayfish includes a combination of postural displays using the chelipeds, antennae whipping, or walking legs and physical contact in the form of grappling (Kravitz & Huber 2003). Individuals may also use chelipeds to inflict injuries on the opponent (Briffa 2013). Crayfish are able to not only recognize conspecifics, but also the position and characteristics of chelae and the length and thickness of antennae may decide (Delgado-Morales et al. 2004). Red swamp crayfish show the ability to recognize the dominance status of conspecifics (Schneider et al. 1999). Agonistic behaviour is more affected by distribution and availability of shelters and the distance to shelter than is past social experience and current social status (Chibucos et al. 2015). Competition for important resources indirectly increases vulnerability to predation and cannibalism (Figler et al. 1999). Agonistic behaviour and fighting occurs in females as well as males. Female on female competition occurs in establishing dominance and in competition for males (Jennions & Petrie 1997), but female–female combats are not fatalistic and frequent as in males.

Social interactions are not restricted to relationships between conspecifics, but also occur in encounters between native species and invaders. Invasive crayfish species are generally characterized by higher aggressiveness, which enables them to dominate native species. In European region, both signal crayfish and spiny-cheek crayfish are more capable of establishing clear dominance via their greater tendency to continue engagement in high-intensity combat than native species which are losing their habitats (Hudina & Hock 2012). Impact of invasive crayfish interactions is not European problem, it is global. Klocker & Strayer (2004) show in a study of interactions among native and non-native species that elsewhere dominant species spiny-cheek crayfish is oppressed by an even more aggressive rusty crayfish in native habitat.

5. Reproductive behaviour

Reproductive behaviour of animals is a significant manifestation of their life (Alcock 2001; Gherardi 2002; Krebs & Davies 1997) with the exception potential behavioural patterns of species with asexual reproduction such as the marbled crayfish *Procambarus fallax f. virginalis* (Scholtz et al., 2003), where this is still an unexplored field. Most animals are distinguished by sexual differentiation reflecting preferences and requirements of potential mates (Manning & Dawkins 2012). Reproductive behaviour is preceded by courtship, creating of pairs and mechanisms of synchronization and consolidation of relationships between male and female (Alcock 2001; Bradbury & Vehrencamp 1998). In crayfish, reproduction is often tied to factors such as temperature, photoperiod, and season (Gherardi 2002).

In sexual selection, individuals attempt to increase the chances of successful reproduction and the fitness of offspring by choosing the best available mate on the basis of visual (size, coloration), chemical (pheromones, major histocompatibility complex), and behavioural (courtship behaviour, agonistic interactions, dominance status) cues (Alcock 2001; Bakker et al. 1999; Chenoweth & Blows 2006; Partridge 1980; Roberts & Gosling 2003). The right partner most often is of the same population or conhabitant (Lande 1981; Roberts & Gosling 2003; Veselovský 2005). Factors involving sexual history, dominance status, size of body parts, investment in offspring, and fitness provide a decisive advantage in partner finding (Jennions & Petrie 1997). During the selection of a reproductive partner, individuals often engage in agonistic interactions with competitors (Alcock 2001; Hunt et al. 2009). Male competition for a female has been observed in crayfish. This competition is not always decisive, since the female may choose to receive or refuse either male, regardless of the outcome of the battle (Gherardi 2002; Mellan et al. 2014; Villanelli & Gherardi 1998). Males have been observed to remove spermatophores deposited by other males by eating them and subsequently replacing them with their own (Snedden 1990).

An important aspect of successful reproduction is recognition by both partners. Males can distinguish at a distance between receptive and non-receptive females based on pheromone signalling (Breithaupt & Thiel 2011; Gherardi 2002). In crayfish, both sexes are polygamous and mate with multiple partners for higher paternity success, obtaining benefits of greater investment in offspring (Bretman et al. 2009; Reynolds & Holdich 2002) such as larger and stronger juveniles from a larger and dominant partner.

Maternal behaviour is another aspect of social and reproductive behaviour. In crayfish, maternal care is important to egg incubation as well as early developmental stages of offspring (Gherardi 2002; Vogt 2013), with females engaging in extended care to protect juveniles from predation and cannibalism (Mathews 2011). Extent of maternal care in crayfish differs with species, from 1 to 2 weeks in the noble crayfish (Skurdal & Taugbøl 2002) to 5 months in *Paranephrops zealandicus* (Whitmore & Huryn/ 1999). In maternal care, chemical communication is employed for protection of juveniles from stresses (Little 1975; Tricarico 2015; Vogt 2013; Vogt & Tolley 2004), especially the brood pheromone, which attracts juveniles back to the female (Little 1975). This pheromone, possibly species-specific, has not been chemically isolated, but its existence has been described based on behavioural changes in mother and offspring (Aquiloni & Gherardi 2008b; Little 1976; Mathews 2011). Vogt (2013) reports return of juveniles to the mother's pleopods as a response to optical maternal signals. Brood protection is also associated with an increase in territoriality against non-maternal individuals and use of shelters by ovigerous and offspring-carrying females (Figler et al. 2001). Female red swamp crayfish and the Neotropical burrowing crayfish *Parastacus pilimanus* accept and protect offspring of other females without aggressive behaviour (Aquiloni & Gherardi 2008b; Dalosto et al. 2012). Mathews (2011) described a female spiny-cheek crayfish that collected unfamiliar juveniles, heavily overloading her pleopods, although genetic analysis of wild living *Orconectes placidus* has shown that females most often carry their own offspring (Walker et al. 2002). The preservation of own offspring remains paramount, as shown by behaviour after juveniles become independent, when females are likely to cannibalize offspring of other females (Mathews 2011; Tricarico 2015).

6. Decision making

The study of decision making is the core of behavioural ecology (Dill 1987). All living organisms must make decisions throughout life regarding feeding, courtship, sleep, escape, competition. Final decisions should maximize, as much as possible, chances for survival and reproductive success (Krebs & Davies 1997; Manning & Dawkins 2012; Tricarico 2015). In other words, they should represent the optimal strategy at a given time. Hypothetically, optimal behaviour can be compared with actual behaviour and decision making of the animal, to shed light on the evolution of behaviours based on experience, conditions, fitness, motivation, and individual personality (Krebs & Davies 1997; Manning & Dawkins 2012; Mcfarland 1977). Decision making processes in animals are complex and sophisticated, since, for any behaviour directed and coordinated by the brain, nerve impulses, muscle movements, and hormone levels must coordinate in specific ways (Krebs & Davies 1997; Manning & Dawkins 2012).

Basic models of optimal behaviour consider relatively simple problems, such as prey size choice, when the sole objective is maximizing net energy intake rate (Dill 1987; Krebs & Davies 1997). Lima (1998) reviewed decision making on diet selection and preference for certain food types when the animal is at risk of predation. In such situations, crayfish preference for food or shelter may be altered on the basis of environmental variations such as the relative risk of predation compared to the relative reward of resource (Fero & Moore 2014; Jurcak & Moore 2014; Lima 1998). On the other hand, food choices of native and non-native species crayfish may differ. The white clawed crayfish *Austropotamobius pallipes* tends to be selective, while invasive species, represented by signal crayfish, are primarily non-selective and choose larger prey than do natives (Haddaway et al. 2012). Buřič et al. (2016) described an unexpected behavioural pattern in signal crayfish and yabby *Cherax destructor*: both species buried their own exuviae after moulting. An animal behaviour may reflect the expression of evidence of decision making for preference of self-protection or creation of mineral storage (Buřič et al. 2016).

In recent years, animal behaviour is seen to reflect expression of individual personality. This phenomenon in non-human animals aids in understanding of many aspects of their ecology (Gherardi et al. 2012). Personality traits, which are typical for higher animals, are found in crayfish (Tricarico 2015). Pintor et al. (2008) observed positive correlations among activity, aggressiveness, and boldness in populations of signal crayfish. More aggressive behaviour of this species compared to non-aggressive and shy *Pacifastacus fortis* might explain the success of signal crayfish (*P. leniusculus*) invasion. Vainikka et al. (2011) suggested, based on observation of decreased resource holding potential, that boldness can be an individual personality trait. Two test groups of noble crayfish (naïve), upon exposure to predator scent showed differences in behaviour. Vainikka et al. (2011) found individuals with higher levels of boldness repeatedly left shelters during periods of varying risk of predation.

Another manifestation of decision making is choice of reproductive partner. Mate choice can be defined as a response to a pattern of sensory and behavioural properties exhibited by potential mates, grounded in benefits derived from choosing certain mates over others. This leads to higher likelihood of mating with the more attractive members of the opposite sex (Halliday 1983; Jennions & Petrie 1997). Many factors that can be seen as meeting criteria of mate quality play a role in mate choice including potential to mate, degree of investment in offspring, and resource holding potential reflected in the ability to defend resources or shelters from intruders (Bateson 1983; Mellan et al. 2014; Rahman et al. 2004; Tricarico 2015). Preference for conspecifics does not necessarily lead to mating with them. Mate choice may be based on mate quality or sperm competition (Galipaud et al. 2015), and leads to hybridization among the noble crayfish and the narrow-clawed crayfish (Jelić et al. 2013; Maguire et al. 2013).

Penn (2002) reviewed the preference observed in vertebrates for mating with individuals carrying dissimilar genes of the major histocompatibility complex (MHC), which may increase heterozygosity of offspring. The observation of mate choice according to the MHC in the female house mouse, *Mus musculus domesticus* confirms the selection of partners according to ability to produce offspring with greater survival (Ilmonen et al. 2009; Penn 2002). In addition to its role in the immune system, the MHC is responsible for scent secretion. Potential mates with dissimilar MHC loci, which produce a different scent than mates with similar loci, are more attractive for female house mice (Penn 2002; Roberts & Gosling 2003; Veselovský 2005). Evidence for MHC-dependent mating preferences has also been found in humans (Havlicek & Roberts 2009; Ober et al. 1997; Wedekind et al. 1995), the Swedish sand lizard *Lacerta agilis* (Olsson et al., 2003), and the Atlantic salmon (Evans et al. 2012; Landry et al. 2001). Most invertebrates do not possess an MHC system (Dishaw & Litman 2009), but similar polymorphic immune genes with a locus involved in histocompatibility have been described in the tunicate *Botryllus schlosseri* (De Tomaso et al., 2005) and the cnidarian *Hydractinia* (Rosa et al., 2010). Considering the capability for chemical communication in crustaceans, including crayfish, they may possess a similar mechanism enabling recognition of related individuals. Unfortunately, any kind of MHC system is still only speculative in crustacean taxa.

In crustaceans, the evidence for preferences in mate choice is similar to results of studies describing mate selection throughout taxa. The preference for larger sized mates has been reported in amphipods (Beermann et al. 2015; Sutherland et al. 2007), the big-clawed snapping shrimp *Alpheus heterochaelis* (Rahman et al., 2004), hermit crab *Pagurus middendorffii* (Wada et al., 2011), and in the spotted spiny lobster *Panulirus guttatus* (Robertson & Butler, 2013). Similar preference was shown in several species of crayfish, including the white-clawed crayfish *A. pallipes* species complex (Galeotti et al. 2012; Gherardi et al. 2006; Villanelli & Gherardi 1998) and the red swamp crayfish (Aquiloni & Gherardi 2008a, c), in which at least one of sexes spent significantly more time in proximity of larger individuals than smaller. Larger body size is positively correlated with fecundity in crayfish females (Aquiloni & Gherardi 2008c; Rahman et al. 2004). Similarly, females, which produce highly costly gametes in a limited reproductive season, prefer larger males which translates to higher dominance status (Aquiloni et al. 2008). It is positively correlate with size of males (Snedden 1990) which produce of larger quantities of sperm to increase chances of fertilization. Female red swamp crayfish are able to discriminate, and prefer, large-sized as opposed to large-clawed males (Aquiloni & Gherardi 2008c). The hypothesis that larger body size leads to higher investment in offspring is supported by Aquiloni & Gherardi (2008a) who confirmed that females copulating with large males produced larger and heavier juveniles. Larger males also exhibit a significantly higher probability of winning competition for acquiring or defending shelters (Figler et al. 2005; Figler et al. 1999).

Sub-preferences have been observed in mate choice. Aquiloni et al. (2008) and Aquiloni & Gherardi (2008c) described higher preference for victors in male–male competition in red swamp crayfish and signal crayfish. However dominance preference among size-matched males was not shown in naïve females, which had not eavesdropped on male–male competition (Aquiloni & Gherardi 2008c; Berry 2008). After the effect of body size, mate choice may be positively affected by shelter ownership, according to a study of big-claw snapping shrimp, with shelter competition success or occupancy and defence influencing pair formation. When presented with small size-matched males, females paired more frequently with those that occupied a shelter (Rahman et al. 2004).

In some cases, individuals may benefit from information gained from prior reproductive experience to make a mate choice. Previously unmated (virgin) spiny-cheek crayfish of both sexes were chosen more often for mating than mated ones by experienced mates. But virgin animals were choosier than those who had mated in mate choice (Mellan et al. 2014). A similar scenario was described in red swamp crayfish males, which were shown to preferentially discriminate and select virgin females rather than mated (Aquiloni & Gherardi 2008c). Hence, individuals seek unmated mates for highest reproductive success.

The speed of decision making as well as the final choice can be significantly affected by stress factors and predation pressure (Lima 1998). Crowley et al. (1991) reviewed studies of terrestrial and aquatic invertebrates such as the snapping shrimp *Alpheus armatus* (Knowlton, 1980), the katydid *Metaballus* sp. (Gwynne, 1985), and the túngara frog *Physalaemus pustulosus* (Ryan, 1985), all of which showed reduced discrimination in mate selection when under predation risk. We suggest that reduced discrimination may be useful in crayfish females, which are generally selective (Aquiloni et al. 2008).

Infection with pathogens and reduced fitness can also play a significant role in decision making. Beltran-Bech & Richard (2014) reviewed the impact of infection on mate choice with possible application to all animals, including crayfish. Parasites from eukaryotes to bacteria and viruses can reduce fitness and decrease attractiveness or lead to errors in decision making.

Mate choice may not be solely dependent on physical factors. Chemical signals, such as pheromones, can influence decision making by providing chemical cues to signal receptivity, specific phases in the reproductive phase, or mated/unmated status (Johansson & Jones 2007). Stebbing et al. (2003) demonstrated the influence of sex pheromones on male signal crayfish. In the presence of female scent transmitted through air-stones, males initiated seizure, mounting, and deposition of spermatophores on the surface of the air-stone.

7. Conclusions

This review provides an overview of social interactions and decision making, including channels for recognition and processing of environmental stimuli, with the primary focus on freshwater crayfish. We reviewed relationships between the environment and the organism and the basic biology of signal processing. These aspects are important, as every decision and interaction results in consequences for survival and reproduction, *i.e.* at some level of species success.

For ethological studies in aquatic ecosystems, crustaceans, especially crayfish represent very suitable model organism.

Crayfish show advantages over other invertebrates due to their high level of social interaction in both the field and laboratory. They exhibit unique eco-ethological behaviour in diversity of feeding habits, activity cycles, habitat selection, and resource utilization at the level of species, sex, and age (Gherardi 2002). Similar to vertebrates, crayfish demonstrate unique behaviours throughout life. They possess ability to recognize, not only conspecifics, but individuals such as mates and offspring as well.

The study of agonistic behaviour is useful for understanding hierarchy in social communities and resource partitioning, and consequently for creating diverse habitats to reduce conflict and possible losses. Knowledge of individual animal personality can be applied to commercial production and animal welfare (Gherardi et al. 2012).

Chemical stimuli have comparatively high impacts on behaviour of crayfish in mating, kin recognition, and social status. There is opportunity for future research to identify the compounds that influence specific behaviours.

The results of recent studies could be applied in conservation research, captive breeding, and recovery programs as well as to aquaculture. Native crayfish are usually studied to extend knowledge of habitat requirements and mating behaviour (Tricarico 2015), while the invasive crayfish species are investigated for their reproductive behaviour, plasticity, and for developing methods for their control (Gherardi et al. 2011). The study of behaviour in crayfish is currently an open research area in both the field and laboratory.

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References

Alcock, J., 2001. Animal Behavior: an Evolutionary Approach, seventh ed. Sinauer Publishers, Sunderland, Mass.

Anger, K., 2001. The Biology of Decapod Crustacean Larvae. A.A. Balkema Publishers, Lisse.

Aquiloni, L., Gherardi, F., 2008a. Evidence of female cryptic choice in crayfish. Biol. Lett. 4, 163–165.

Aquiloni, L., Gherardi, F., 2008b. Extended mother-offspring relationships in crayfish: the return behaviour of juvenile *Procambarus clarkii*. Ethology 114, 946–954.

Aquiloni, L., Gherardi, F., 2008c. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J. Zool. 274, 171–179.

Aquiloni, L., Buřič, M., Gherardi, F., 2008. Crayfish females eavesdrop on fighting males before choosing the dominant mate. Curr. Biol. 18, R462–R463.

Atema, J., 1988. Sensory Biology of Aquatic Animals. Springer-Verlag, New York.

Atema, J., Engstrom, D.G., 1971. Sex pheromone in lobster, *Homarus americanus*. Nature 232, 261–263.

Atema, J., Steinbach, M., 2007. Chemical communication and social behavior of the lobster *Homarus americanus* and other decapod Crustacea. In: Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms, pp. 115–144.

Bakker, T.C.M., Kunzler, R., Mazzi, D., 1999. Sexual selection – condition-related mate choice in sticklebacks. Nature 401, 234-234.

Baldauf, S.A., Bakker, T.C.M., Herder, F., Kullmann, H., Thunken, T., 2010. Male mate choice scales female ornament allometry in a cichlid fish. BMC Evol. Biol. 10, 301.

Barki, A., Jones, C., Karplus, I., 2011. Chemical communication and aquaculture of decapod crustaceans: needs, problems, and possible solutions. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 485–506.

Basil, J., Sandeman, D., 2000. Crayfish (*Cherax destructor*) use tactile cues to detect and learn topographical changes in their environment. Ethology 106, 247–259.

Bateson, P.P.G., 1983. Mate Choice. Cambridge University Press, Cambridge Cambridgeshire; New York.

Beermann, J., Dick, J.T., Thiel, M., 2015. Social Recognition in Amphipods: an Overview, Social Recognition in Invertebrates. Springer, pp. 85–100.

Belanger, R.M., Moore, P.A., 2006. The use of the major chelae by reproductive male crayfish (*Orconectes rusticus*) for discrimination of female odours. Behaviour 143, 713–731.

Beltran-Bech, S., Richard, F.J., 2014. Impact of infection on mate choice. Anim. Behav. 90, 159–170.

Bergman, D.A., Kozlowski, C., McIntyre, J.C., Huber, R., Daws, A.G., Moore, P.A., 2003. Temporal dynamics and communication of winner-effects in the crayfish, *Orconectes rusticus*. Behaviour 140, 805–825.

Berry, F.C., 2008. The Behavioural Function of Pheromones in Crayfish. University of Hull.

Berry, F.C., Breithaupt, T., 2010. To signal or not to signal? Chemical communication by urine-borne signals mirrors sexual conflict in crayfish. BMC Biol. 8, 25.

Bierbower, S.M., Shuranova, Z.P., Viele, K., Cooper, R.L., 2013. Comparative study of environmental factors influencing motor task learning and memory retention in sighted and blind crayfish. Brain Behav. 3, 4–13.

Bradbury, J.W., Vehrencamp, S.L., 1998. Principles of Animal Communication. Sinauer Associates, Sunderland, MA.

Brandley, N.C., Speiser, D.I., Johnsen, S., 2013. Eavesdropping on visual secrets. Evol. Ecol. 27, 1045–1068.

Brantley, R.K., Bass, A.H., 1994. Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). Ethology 96, 213–232.

Breithaupt, T., 2011. Chemical communication in crayfish. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 257–276.

Breithaupt, T., Thiel, M., 2011. Chemical Communication in Crustaceans. Springer, New York.

Breithaupt, T., Gherardi, F., Aquiloni, L., Tricarico, E., 2016. Chemical ecology of crayfish. Biol. Ecol. Crayfish 132.

Bretman, A., Newcombe, D., Tregenza, T., 2009. Promiscuous females avoid inbreeding by controlling sperm storage. Mol. Ecol. 18, 3340–3345.

Briffa, M., 2013. Contests in crustaceans: assessments, decisions and their underlying mechanisms. Animal Contests 86–112.

Brusca, R.C., Brusca, G.J., 2003. Invertebrates, second ed. Sinauer Associates, Sunderland, Mass.

Bruski, C.A., Dunham, D.W., 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus* .1. An analysis of Bout dynamics. Behaviour 103, 83–107.

Buřič, M., Fořt, M., Bláha, M., Veselý, L., Kozák, P., Kouba, A., 2016. Crayfish bury their own exuviae: a newly discovered behavioral pattern in decapods. SpringerPlus 5, 1674.

Capowiez, Y., Bérard, A., 2006. Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. Ecotoxicol. Environ. Saf. 64, 198–206.

Chapin, K.J., Hebets, E.A., 2016. The behavioral ecology of amblypygids. J. Arachnol. 44, 1–14.

Chenoweth, S.F., Blows, M.W., 2006. Dissecting the complex genetic basis of mate choice. Nat. Rev. Genet. 7, 681–692.

Chibucos, K., Wofford, S.J., Moore, P.A., 2015. Hierarchical decision making: resource distribution exhibits stronger effect on crayfish dominance relationships and shelter occupation than prior social experience and resource ownership. Behaviour 152, 1063–1082.

Christy, J.H., Backwell, P.R.Y., Goshima, S., Kreuter, T., 2002. Sexual selection for structure building by courting male fiddler crabs: an experimental study of behavioral mechanisms. Behav. Ecol. 13, 366–374.

Churcher, A.M., Hubbard, P.C., Marques, J.P., Canario, A.V.M., Huertas, M., 2015. Deep sequencing of the olfactory epithelium reveals specific chemosensory receptors are expressed at sexual maturity in the European eel *Anguilla anguilla*. Mol. Ecol. 24, 822–834.

Crandall, K.A., Buhay, J.E., 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae-Decapoda) in freshwater. Hydrobiologia 595, 295–301.

Crowley, P.H., Travers, S.E., Linton, M.C., Cohn, S.L., Sih, A., Sargent, R.C., 1991. Mate density, predation risk, and the seasonal sequence of mate choices - a dynamic game. Am. Nat. 137, 567–596.

Cummings, M.E., Jordao, J.M., Cronin, T.W., Oliveira, R.F., 2008. Visual ecology of the fiddler crab, *Uca tangeri*: effects of sex, viewer and background on conspicuousness. Anim. Behav. 75, 175–188.

Dalosto, M.M., Palaoro, A.V., Santos, S., 2012. Mother-offspring relationship in the neotropical burrowing crayfish *Parastacus pilimanus* (Von Martens, 1869) (Decapoda, Parastacidae). Crustaceana 85, 1305–1315.

Dawkins, R., Krebs, J.R., 1978. Animal signals: information or manipulation. Behav. Ecol. Evol. Approach 2, 282–309.

De Tomaso, A.W., Nyholm, S.V., Palmeri, K.J., Ishizuka, K.J., Ludington, W.B., Mitchel, K., Weissman, I.L., 2005. Isolation and characterization of a protochordate histocompatibility locus. Nature 438, 454–459.

Delgado-Morales, G., Hernandez-Falcon, J., Ramon, F., 2004. Agonistic behaviour in crayfish: the importance of sensory inputs. Crustaceana 77, 1–24.

Díaz, E.R., Thiel, M., 2004. Chemical and visual communication during mate searching in rock shrimp. Biol. Bull. 206, 134–143.

Dill, L.M., 1987. Animal decision making and its ecological consequences: the future of aquatic ecology and behavior. Can. J. Zool. 65, 803–811.

Dishaw, L.J., Litman, G.W., 2009. Invertebrate allorecognition: the origins of histocompatibility. Curr. Biol. 19, R286–R288.

Dornhaus, A., Chittka, L., 2004. Why do honey bees dance? Behav. Ecol. Sociobiol. 55, 395–401.

Evans, M.L., Dionne, M., Miller, K.M., Bernatchez, L., 2012. Mate choice for major histocompatibility complex genetic divergence as a bet-hedging strategy in the Atlantic salmon (*Salmo salar*). P Roy. Soc. B Biol. Sci. 279, 379–386.

Fero, K.C., Moore, P.A., 2014. Shelter availability influences social behavior and habitat choice in crayfish, *Orconectes virilis*. Behaviour 151, 103–123.

Figler, M.H., Cheverton, H.M., Blank, G.S., 1999. Shelter competition in juvenile red swamp crayfish (*Procambarus clarkii*): the influences of sex differences, relative size, and prior residence. Aquaculture 178, 63–75.

Figler, M.H., Blank, G.S., Peeke, H.V.S., 2001. Maternal territoriality as an offspring defense strategy in red swamp crayfish (*Procambarus clarkii*, Girard). Aggress. Behav. 27, 391–403.

Figler, M.H., Blank, G.S., Peeke, H.V.S., 2005. Shelter competition between resident male red swamp crayfish *Procambarus clarkii* (Girard) and conspecific intruders varying by sex and reproductive status. Mar. Freshw. Behav. Physiol. 38, 237–248.

Fureder, L., Reynolds, J.D., 2003. Is *Austropotamobius pallipes* a good bioindicator? B Fr Peche Piscic 157–163.

Galeotti, P., Bernini, G., Locatello, L., Sacchi, R., Fasola, M., Rubolini, D., 2012. Sperm traits negatively covary with size and asymmetry of a secondary sexual trait in a freshwater crayfish. PLoS One 7, e43771.

Galipaud, M., Gauthey, Z., Turlin, J., Bollache, L., Lagrue, C., 2015. Mate choice and male-male competition among morphologically cryptic but genetically divergent amphipod lineages. Behav. Ecol. Sociobiol. 69, 1907–1916.

Gherardi, F., 2002. Behaviour. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, USA and Canada. Iowa State University Press, Oxford, England; Ames, Iowa, pp. 258–290.

Gherardi, F., Daniels, W.H., 2003. Dominance hierarchies and status recognition in the crayfish *Procambarus acutus acutus*. Can. J. Zool. 81, 1269–1281.

Gherardi, F., Tricarico, E., Atema, J., 2005. Unraveling the nature of individual recognition by odor in hermit crabs. J. Chem. Ecol. 31, 2877–2896.

Gherardi, F., Renai, B., Galeotti, P., Rubolini, D., 2006. Nonrandom mating, mate choice, and male-male competition in the crayfish *Austropotamobius italicus*, a threatened species. Arch. Hydrobiol. 165, 557–576.

Gherardi, F., Aquiloni, L., Dieguez-Uribeondo, J., Tricarico, E., 2011. Managing invasive crayfish: is there a hope? Aquat. Sci. 73, 185–200.

Gherardi, F., Aquiloni, L., Tricarico, E., 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: an imperfect map is better than no map. Curr. Zool. 58, 567–579.

Goessmann, C., Hemelrijk, C., Huber, R., 2000. The formation and maintenance of crayfish hierarchies: behavioral and self-structuring properties. Behav. Ecol. Sociobiol. 48, 418–428.

Gwynne, D.T., 1985. Role-reversal in katydids: habitat influences reproductive behaviour (Orthoptera, Tettigoniidae, *Metaballus* sp). Behav. Ecol. Sociobiol. 16, 355–361.

Haddaway, N.R., Wilcox, R.H., Heptonstall, R.E.A., Griffiths, H.M., Mortimer, R.J.G., Christmas, M., Dunn, A.M., 2012. Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. PLoS One 7, e32229.

Haddock, S.H.D., Moline, M.A., Case, J.F., 2010. Bioluminescence in the sea. Annu. Rev. Mar. Sci. 2, 443–493.

Hallberg, E., Skog, M., 2011. Chemosensory sensilla in crustaceans. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 103–121.

Halliday, T., 1983. The study of mate choice. Mate Choice 1, 462.

Hardege, J.D., Jennings, A., Hayden, D., Muller, C.T., Pascoe, D., Bentley, M.G., Clare, A.S., 2002. Novel behavioural assay and partial purification of a female-derived sex pheromone in *Carcinus maenas*. Mar. Ecol. Prog. Ser. 244, 179–189.

Havlicek, J., Roberts, S.C., 2009. MHC-correlated mate choice in humans: a review. Psychoneuroendocrinology 34, 497–512.

Hay, M.E., 2011. Crustaceans as powerful models in aquatic chemical ecology. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 41–62.

Hock, K., Huber, R., 2009. Models of winner and loser effects: a cost-benefit analysis. Behaviour 146, 69–87.

Holdich, D., 2002. Background and functional morphology. Biol. Freshw. Crayfish 3–29.

Holdich, D., Reeve, I., 1988. Functional morphology and anatomy. In: Holdich, D.M., Lowery, R.S. (Eds.), Freshwater Crayfish: Biology Management and Exploitation. Croom Helm, London, pp. 11–52.

Holdich, D.M., Harlioglu, M.M., Firkins, I., 1997. Salinity adaptations of crayfish in British waters with particular reference to *Austropotamobius pallipes, Astacus leptodactylus* and *Pacifastacus leniusculus*. Estuar. Coast Shelf Sci. 44, 147–154.

Holdich, D.M., Reynolds, J.D., Souty-Grosset, C., Sibley, P.J., 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowl. Manag. Aquat. Ecosyst. 11.

Hopkins, C.D., 1988. Social communication in the aquatic environment. In: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (Eds.), Sensory Biology of Aquatic Animals. Springer, pp. 233–268.

Hu, D.L., Chan, B., Bush, J.W.M., 2003. The hydrodynamics of water strider locomotion. Nature 424, 663–666.

Hudina, S., Hock, K., 2012. Behavioural determinants of agonistic success in invasive crayfish. Behav. Process. 91, 77–81.

Hughes, M., 1996. Size assessment via a visual signal in snapping shrimp. Behav. Ecol. Sociobiol. 38, 51–57.

Hunt, J., Breuker, C.J., Sadowski, J.A., Moore, A.J., 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J. Evol. Biol. 22, 13–26.

Huxley, T.H., 1880. The Crayfish; an Introduction to the Study of Zoology. D. Appleton and Company, New York.

Ilmonen, P., Stundner, G., Thoss, M., Penn, D.J., 2009. Females prefer the scent of outbred males: good-genes-as-heterozygosity? BMC Evol. Biol. 9, 104.

Immelmann, K., Pröve, E., Sossinka, R., 1996. Einführung in die Verhaltensforschung. 4., neubearbeitete Auflage. Pareys Studientexte Nr, vol. 13. Blackwell Wissenschafts-Verlag, Berlin.

Jakobsson, S., Brick, O., Kullberg, C., 1995. Escalated fighting behavior incurs increased predation risk. Anim. Behav. 49, 235–239.

Jelić, M., Klobučar, G., Bláha, M., Maguire, I., 2013. Noble crayfish and narrow-clawed crayfish hybridisation experiment in natural habitat. In: Regional European Crayfish Meeting–craycro. Book of Abstracts. September 26–29, 2013 (Rovinj, Croatia).

Jennions, M.D., Petrie, M., 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol. Rev. 72, 283–327.

Johansson, B.G., Jones, T.M., 2007. The role of chemical communication in mate choice. Biol. Rev. 82, 265–289.

Johnstone, K.A., Lubieniecki, K.P., Koop, B.F., Davidson, W.S., 2012. Identification of olfactory receptor genes in Atlantic salmon *Salmo salar*. J. Fish. Biol. 81, 559–575.

Jurcak, A.M., Moore, P.A., 2014. Behavioral decisions in sensory landscapes: crayfish use chemical signals to make habitat use choices. J. Crustac Biol. 34, 559–564.

Karavanich, C., Atema, J., 1998. Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus*. Behaviour 135, 719–730.

Karplus, I., Zoran, M., Milstein, A., Harpaz, S., Eran, Y., Joseph, D., Sagi, A., 1998. Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel - III. Survival in earthen ponds under ambient winter temperatures. Aquaculture 166, 259–267.

Klocker, C.A., Strayer, D.L., 2004. Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). Northeast. Nat. 11, 167–178.

Knowlton, N., 1980. Sexual selection and dimorphism in two demes of a symbiotic, pair-bonding snapping shrimp. Evolution 34, 161–173.

Kouba, A., Tíkal, J., Císař, P., Veselý, L., Fořt, M., Přiborský, J., Patoka, J., Buřič, M., 2016. The significance of droughts for hyporheic dwellers: evidence from freshwater crayfish. Sci. Rep. Uk 6, 26569.

Kravitz, E.A., Huber, R., 2003. Aggression in invertebrates. Curr. Opin. Neurobiol. 13, 736–743.

Krebs, J.R., Davies, N.B., 1997. Behavioural Ecology: an Evolutionary Approach, fourth ed. Blackwell Science, Cambridge, Mass.

Land, M.F., 2004. Eyes and vision. Treatise Zool. Crustac. 1, 257–300.

Land, M.F., 2005. The optical structures of animal eyes. Curr. Biol. 15, R319–R323.

Lande, R., 1981. Models of speciation by sexual selection on polygenic traits. Proc. Natl. Acad. Sci. Unit. States Am. 78, 3721–3725.

Landry, C., Garant, D., Duchesne, P., Bernatchez, L., 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). Proc. R. Soc. Lond. B Biol. Sci. 268, 1279–1285.

Lima, S.L., 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv. Stud. Behav. 27, 215–290.

Linsenmair, E.K.v., 1965. Optische Signalisierung der Kopulationshohle bei der Reiterkrabbe Ocypode saratan Forsk. Naturwissenschaften 52, 256–257.

Little, E.E., 1975. Chemical communication in maternal behavior of crayfish. Nature 255, 400–401.

Little, E.E., 1976. Ontogeny of maternal behavior and brook pheromone in crayfish. J. Comp. Physiol. 112, 133–142.

Lohse, D., Schmitz, B., Versluis, M., 2001. Snapping shrimp make flashing bubbles. Nature 413, 477–478.

Longrie, N., Poncin, P., Denoel, M., Gennotte, V., Delcourt, J., Parmentier, E., 2013. Behaviours associated with acoustic communication in Nile tilapia (*Oreochromis niloticus*). PLoS One 8, e61467.

Maguire, I., Špelić, I., Jelić, M., Klobučar, G., 2013. Is it possible to detect narrow-clawed and noble crayfish probable hybrids using multivariate discriminant analysis of morphometric data? Freshw. Crayfish 19, 219–227.

Malavasi, S., Torricelli, P., Lugli, M., Pranovi, F., Mainardi, D., 2003. Male courtship sounds in a teleost with alternative reproductive tactics, the grass goby, *Zosterisessor ophiocephalus*. Environ. Biol. Fish. 66, 231–236.

Manning, A., Dawkins, M.S., 2012. An Introduction to Animal Behaviour, sixth ed. Cambridge University Press, Cambridge; New York.

Masters, W.M., Aicher, B., Tautz, J., Markl, H., 1982. A new type of water vibration receptor on the crayfish antenna. 2. Model of receptor function. J. Comp. Physiol. 149, 409–422.

Mathews, L.M., 2011. Mother-offspring recognition and kin-preferential behaviour in the crayfish *Orconectes limosus*. Behaviour 148, 71–87.

Mcfarland, D.J., 1977. Decision-making in animals. Nature 269, 15–21.

McMahon, A., Patullo, B.W., Macmillan, D.L., 2005. Exploration in a T-maze by the crayfish *Cherax destructor* suggests bilateral comparison of antennal tactile information. Biol. Bull. 208, 183–188.

Mellan, D., Warren, A., Buckholt, M.A., Mathews, L.M., 2014. Sexual history affects mating behavior and mate choice in the crayfish *Orconectes limosus*. Ethology 120, 681–692.

Moller, A.P., Milinski, M., 1998. The concept of stress and its relevance for animal behavior - Introduction. Adv. Stud. Behav. 27, Xiii–Xv.

Monteclaro, H.M., Anraku, K., Uno, S., Koyama, J., Matsuoka, T., Yan, H.Y., 2011. Sensitivity of crayfish *Procambarus clarkii* antennules to hydrodynamic stimuli is reduced during diazinon exposure. Zool. Stud. 50, 145–154.

Neumann, C., Duboscq, J., Dubuc, C., Ginting, A., Irwan, A.M., Agil, M., Widdig, A., Engelhardt, A., 2011. Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating. Anim. Behav. 82, 911–921.

Ober, C., Weitkamp, L.R., Cox, N., Dytch, H., Kostyu, D., Elias, S., 1997. HLA and mate choice in humans. Am. J. Hum. Genet. 61, 497–504.

Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B., Wittsell, H., 2003. Major histocompatibility complex and mate choice in sand lizards. P Roy. Soc. B Biol. Sci. 270, S254–S256.

Palacios, A.G., Goldsmith, T.H., Bernard, G.D., 1996. Sensitivity of cones from a cyprinid fish (*Danio aequipinnatus*) to ultraviolet and visible light. Vis. Neurosci. 13, 411–421.

Palmer, A.R., 2009. Animal asymmetry. Curr. Biol. 19, R473–R477.

Partridge, L., 1980. Mate choice increases a component of offspring fitness in fruit flies. Nature 283, 290–291.

Payne, R.J.H., 1998. Gradually escalating fights and displays: the cumulative assessment model. Anim. Behav. 56, 651–662.

Penn, D.J., 2002. The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology 108, 1–21.

Pintor, L.M., Sih, A., Bauer, M.L., 2008. Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. Oikos 117, 1629–1636.

Popper, A.N., Salmon, M., Horch, K.W., 2001. Acoustic detection and communication by decapod crustaceans. J. Comp. Physiol. 187, 83–89.

Porter, M.L., Zhang, Y.F., Desai, S., Caldwell, R.L., Cronin, T.W., 2010. Evolution of anatomical and physiological specialization in the compound eyes of stomatopod crustaceans. J. Exp. Biol. 213, 3473–3486.

Radford, A.N., Kerridge, E., Simpson, S.D., 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav. Ecol. 25, 1022–1030.

Rahman, N., Dunham, D.W., Govind, C.K., 2004. Mate choice in the big-clawed snapping shrimp, *Alpheus Heterochaelis* say, 1818. Crustaceana 77, 95–111.

Reynolds, J., Holdich, D., 2002. Growth and reproduction. Biol. Freshw. Crayfish 152–191.

Roberts, S.C., Gosling, L.M., 2003. Genetic similarity and quality interact in mate choice decisions by female mice. Nat. Genet. 35, 103–106.

Robertson, D.N., Butler, M.J., 2013. Mate choice and sperm limitation in the spotted spiny lobster, *Panulirus guttatus*. Mar. Biol. Res. 9, 69–76.

Rosa, S.F.P., Powell, A.E., Rosengarten, R.D., Nicotra, M.L., Moreno, M.A., Grimwood, J., Lakkis, F.G., Dellaporta, S.L., Buss, L.W., 2010. Hydractinia allodeterminant alr1 resides in an immunoglobulin superfamily-like gene complex. Curr. Biol. 20, 1122–1127.

Rosenthal, G.G., Ryan, M.J., 2000. Visual and acoustic communication in non-human animals: a comparison. J. Biosci. 25, 285–290.

Ryan, E.P., 1966. Pheromone - evidence in a decapod crustacean. Science 151, 340–341.

Ryan, M.J., 1985. The Túngara Frog: a Study in Sexual Selection and Communication. University of Chicago Press.

Sandeman, R., Sandeman, D., 2003. Development, growth, and plasticity in the crayfish olfactory system. Microsc. Res. Tech. 60, 266–277.

Sandeman, D., Sandeman, R., Derby, C., Schmidt, M., 1992. Morphology of the brain of crayfish, crabs, and spiny lobsters: a common nomenclature for homologous structures. Biol. Bull. 183, 304–326.

Schaller, D., 1978. Antennal sensory system of *Periplaneta americana* L. Cell Tissue Res. 191, 121–139.

Schein, H., 1977. Role of snapping in *Alpheus heterochaelis* Say, 1818, big-clawed snapping shrimp. Crustaceana 33, 182–188.

Schneider, R.A.Z., Schneider, R.W.S., Moore, P.A., 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. J. Chem. Ecol. 25, 781–794.

Scholtz, G., Braband, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F., Vogt, G., 2003. Ecology - parthenogenesis in an outsider crayfish. Nature 421, 806-806.

Seebacher, F., Wilson, R.S., 2007. Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. Biol. Lett. 3, 471–474.

Skurdal, J., Taugbøl, T., 2002. Astacus. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, USA and Canada. Iowa State University Press, Oxford England; Ames, Iowa, pp. 467–510.

Snedden, W.A., 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus* - chela size and sperm competition. Behaviour 115, 100–113.

Souty-Grosset, C., Holdich, D.M., Noël, P.Y., Reynolds, J., Haffner, P., 2006. Atlas of Crayfish in Europe. Muséum national d'Histoire naturelle.

Staaterman, E.R., Claverie, T., Patek, S.N., 2010. Disentangling defense: the function of spiny lobster sounds. Behaviour 147, 235–258.

Stabell, O.B., 1984. Homing and olfaction in salmonids: a critical review with special reference to the Atlantic salmon. Biol. Rev. Camb. Philos. Soc. 59, 333–388.

Stacey, N., Cardwell, J., Liley, N., Scott, A., Sorensen, P., 1994. Hormones as sex pheromones in fish. Perspect. Comp. Endocrinol. 438–448.

Stebbing, P.D., Bentley, M.G., Watson, G.J., 2003. Mating behaviour and evidence for a female released courtship pheromone in the signal crayfish *Pacifastacus leniusculus*. J. Chem. Ecol. 29, 465–475.

Sutherland, D.L., Hogg, I.D., Waas, J.R., 2007. Is size assortative mating in *Paracalliope fluviatilis* (Crustacea : Amphipoda) explained by male-male competition or female choice? Biol. J. Linn. Soc. 92, 173–181.

Tautz, J., 1987. Water vibration elicits active antennal movements in the crayfish, *Orconectes limosus*. Anim. Behav. 35, 748–754.

Tautz, J., Muller-Tautz, R., 1983. Antennal neuropile in the brain of the crayfish: morphology of neurons. J. Comp. Neurol. 218, 415–425.

Taylor, C., 2002. Taxonomy and conservation of native crayfish stocks. Biol. Freshw. Crayfish 236, 257.

Thiel, M., Breithaupt, T., 2011. Chemical communication in crustaceans: research challenges for the twenty-first century. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 3–22.

Tobler, M., Riesch, R., de Leon, F.J.G., Schupp, I., Plath, M., 2008. New and morphologically distinct population of cavernicolous *Poecilia mexicana* (Poeciliidae: Teleostei). Environ. Biol. Fish. 82, 101–108.

Tricarico, E., 2015. Behavior: behavioral ecology of crayfish: its contribution to conservation and management. In: Kawai, T., Faulkes, Z., Scholtz, G. (Eds.), Freshwater Crayfish: a Global Overview. CRC Press, p. 221.

Ueda, H., Shoji, T., 2002. Physiological mechanisms of homing migration in salmon. Fish. Sci. 68, 53–56.

Vainikka, A., Rantala, M.J., Niemela, P., Hirvonen, H., Kortet, R., 2011. Boldness as a consistent personality trait in the noble crayfish, *Astacus astacus*. Acta Ethol. 14, 17–25.

Van Den Hurk, R., Resink, J., 1992. Male reproductive system as sex pheromone producer in teleost fish. J. Exp. Zool. 261, 204–213.

Van der Velden, J., Zheng, Y., Patullo, B.W., Macmillan, D.L., 2008. Crayfish recognize the faces of fight opponents. PloS One 3, e1695.

Versluis, M., Schmitz, B., von der Heydt, A., Lohse, D., 2000. How snapping shrimp snap: through cavitating bubbles. Science 289, 2114–2117.

Veselovský, Z., 2005. Etologie: Biologie Chování Zvířat. Academia Praha.

Veselý, L., Buřič, M., Kouba, A., 2015. Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures? Sci. Rep. Uk 5, 16340.

Villanelli, F., Gherardi, F., 1998. Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. Freshw. Biol. 40, 305–315.

Vogt, G., 2002. Functional anatomy. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, USA and Canada. Iowa State University Press, Oxford England; Ames, Iowa, pp. 53–151.

Vogt, G., 2013. Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. Biol. Rev. 88, 81–116.

Vogt, G., Tolley, L., 2004. Brood care in freshwater crayfish and relationship with the offspring's sensory deficiencies. J. Morphol. 262, 566–582.

Wada, S., Arashiro, Y., Takeshita, F., Shibata, Y., 2011. Male mate choice in hermit crabs: prudence by inferior males and simple preference by superior males. Behav. Ecol. 22, 114–119.

Walker, D., Porter, B.A., Avise, J.C., 2002. Genetic parentage assessment in the crayfish *Orconectes placidus*, a high-fecundity invertebrate with extended maternal brood care. Mol. Ecol. 11, 2115–2122.

Wedekind, C., Seebeck, T., Bettens, F., Paepke, A.J., 1995. Mhc-dependent mate preferences in humans. P Roy. Soc. B Biol. Sci. 260, 245–249.

Wenner, A.M., 2002. The elusive honey bee dance "language" hypothesis. J. Insect Behav. 15, 859–878.

Whitmore, N., Huryn, A.D., 1999. Life history and production of *Paranephrops zealandicus* in a forest stream, with comments about the sustainable harvest of a freshwater crayfish. Freshw. Biol. 42, 467–478.

Wiese, K., 1993. Sensory Systems of Arthropods. Birkhäuser Verlag, Basel; Boston.

Wyatt, T.D., 2011. Pheromones and behavior. In: Breithaupt, T., Thiel, M. (Eds.), Chemical Communication in Crustaceans. Springer, pp. 23–38.

Zeil, J., Zanker, J.M., 1997. A glimpse into crabworld. Vis. Res. 37, 3417–3426.

Zeil, J., Sandeman, R., Sandeman, D., 1985. Tactile localization – the function of active antennal movements in the crayfish *Cherax destructor*. J. Comp. Physiol. 157, 607–617.

CHAPTER 2

FEMALES BET ON THE KNOWN: CRAYFISH FEMALES RECOGNIZE AND PREFER MALES FROM FAMILIAR POPULATION, MALES ARE NOT PICKY

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My share on this work was about 50 %.

FEMALES BET ON THE KNOWN: CRAYFISH FEMALES RECOGNIZE AND PREFER MALES FROM FAMILIAR POPULATION, MALES ARE NOT PICKY

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Abstract

Reproductive behaviour is a central pattern in animals and understanding mate choice is among major challenges in behavioural and ecological studies. Mate choice is often based on recognition of conspecifics, their physiological state and social experiences, which play important roles in decision making. In crayfish, females are choosier than males because of their higher energy costs for reproduction (oogenesis, incubation, parental care). We analysed mate preferences of the spiny-cheek crayfish (*Faxonius limosus*) where the choosing crayfish was faced with two similarly sized mature counterparts or different origin (three geographically separated populations were used). Both sexes were presented either with a single familiar and unfamiliar counterpart or with two unfamiliar ones, reflecting the source population. Our results suggest that females are able to recognize and prefer mates from the familiar population, whereas males do not exhibit any specific preference. Chemical communication is the most possible driver but the mechanism of recognition at population level is not known. The specific population microbiome may be contributing to this pattern.

Keywords: *invertebrates, decapoda, communication, mate choice, population, chemical ecology*

Research Highlights

- evidence of differences in reproductive strategies of both sexes
- we demonstrate that females prefer males from familiar population
- support for segregation theory of new individuals in population
- our study extends the knowledge of mate choice for future research in this field

Introduction

Ever since Darwin's idea of female preferences for male ornaments, research into sexual selection continues to be a rapidly growing field (Danchin & Cézilly 2008). Evidence of many different traits affecting mate choice has been described from a range of animals, from small invertebrates to large mammals. Identification of particular traits of reproductive behaviour that influence mate choice is a major challenge in behavioural ecology and evolutionary biology (Aquiloni et al. 2008; Danchin & Cézilly 2008). Andersson (1994) reviewed sexual selection in the animal world as a way to maximize reproductive success. Mating entails costs and potential benefits to both sexes, therefore it is necessary to make optimal choices from among available potential mates (Mellan et al. 2014).

Females are generally choosier than males, because they invest more energy into production of relatively low number of big gametes. Males producing large numbers of relatively low-cost sperm are usually limited only by the frequency of mating (Aquiloni & Gherardi 2008c) and sperm maturation (Vogt 2002). For these reasons, males are less choosy than females in mate choice (Trivers 1972). Individual selectivity is a specific function of many factors such as mate quality, availability of potential mates, degree of investment into offspring or ability to defend an important resource e.g. shelter, food (Bateson 1983; Mellan et al. 2014; Rahman et al. 2004; Tricarico 2015). A range of preferences that influence decision making in mate choice is known in crustaceans. The studies of Rubolini et al. (2006), Aquiloni & Gherardi (2008b) and Aquiloni & Gherardi (2008c) showed higher preference for mates having larger body size in both sexes. Similarly, crayfish females preferred dominant males when choosing between similarly sized known males (Aquiloni et al. 2008; Aquiloni & Gherardi 2008b). On the other hand, males are able to recognize and prefer virginity in females (Aquiloni & Gherardi 2008c). Recognition of functional state of crayfish, or of an individual crayfish, is possible mainly through chemical communication (Berry & Breithaupt 2010; Simon & Moore 2007) processed by well-developed sensory organs (Hallberg & Skog 2011; Vogt 2002). This is supported by many studies examining the ability of individual recognition among crustaceans e.g. in the hermit crabs *Pagurus longicarpus* Say, 1817 (Gherardi & Tiedemann 2004), in the American lobster *Homarus americanus* H. Milne Edwards, 1837 (Karavanich & Atema 1998b), in the big-clawed snapping shrimp *Alpheus heterochaelis* Say, 1818 (Ward et al. 2004), and in yabby *Cherax destructor* Clark, 1936 (Van der Velden et al. 2008). Aquiloni et al. (2009) also investigated sex identification and recognition in red swamp crayfish *Procambarus clarkii* Girard, 1852during female-male interactions via chemical cues only.

In the current study we investigated if crayfish could recognize mate conspecifics from their own (familiar) or geographically distinct (unfamiliar) population. When the study was designed, we supposed that if there is a preference, it would probably be for unfamiliar mates, to increase heterozygosity of the population.

Materials and methods

Collection and Maintenance of Animals

Crayfish collections were planned and designed to capture on small area (up to 100 m of river bank) at each locality. This methodology would be appropriate to fulfill of familiarity (previously met) assumptions. All used crayfish were adults that have travel radius within a tens of meters for foraging, social contact and mating. Males and females of the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) were captured by hand from three separate localities in the Czech Republic: 1) the Elbe River (50° 45′ 45′′ N; 14° 23′ 65′′ E), 2) Hrejkovický brook where it enters the Orlík water reservoir (49° 40′ 38′′ N; 14°25′ 37′′ E), and 3) the Malše River at its mouth to the Vltava River in České Budějovice (48° 97′ 28′′ N; 14°46′ 99′′ E) (see Fig. 1). All localities belong to the Elbe River catchment and have been highly invaded by spiny-cheek crayfish for more than 10 years (Petrusek et al. 2006). Crayfish were collected from July to August 2015, before the reproductive season. In the laboratory, crayfish were held individually in floating plastic boxes placed in flow-through channels under natural ambient light and temperature conditions for at least 5 weeks until the onset of breeding. Crayfish from all three population can smell one another but any tactile or visual contact was prevented.

Both sexes were examined for sexual maturity before the experiment. Sexual maturity of males was assessed using the shape of gonopods, and only sexually active FI males were used (Buřič et al. 2010b; Hamr 2002); and by the presence of developed glair glands in females (Buřič et al. 2010a; Hamr 2002). In all used crayfish we measured carapace length and length and width of both chelae to the nearest 0.1 mm using Vernier callipers. Wet weight was measured to the nearest 0.1 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Thereafter each measured crayfish was assigned a unique box with identification code. Only crayfish without visible damage (lost or regenerating chelae or other appendages) were selected for experimentation.

Figure 1*. Map of the Czech main river network showing source localities of the spiny-cheek crayfish. E – the Elbe River; O – the Orlík reservoir; M – the Malše River*

Experiment design

The experiment was conducted in the reproductive period between 15th and 28th October 2015. Experimental triads were made from two electors (two males or two females) and one chooser of the opposite sex. Electors were chosen as one familiar and one unfamiliar, or as two unfamiliar individuals (control). On the basis of studies by Aquiloni & Gherardi (2008b) and Aquiloni & Gherardi (2008c), we tried to eliminate size-preference effects. Electors in each single triad were therefore chosen using a size-matching strategy where differences in carapace length and chelae size (both length and width) were always < 5%. Used crayfish males and females were encoded to not be influenced by unconscious biases of the observer. We set up 12 trials with familiar and unfamiliar and 6 trials with two unfamiliars per population and sex; in total we observed 108 trials.

For the experiments we used the modified experimental apparatus from Aquiloni & Gherardi (2008c) which consisted of an elliptical plastic arena (60 × 40 cm, water level 10 cm). Water for experiments was from the same source as for flow troughs with crayfish. On the opposite sides of the arena were two elector's areas (radius of 15 cm marked by small points) (Fig. 2), where each elector was carefully attached around the thorax (between the 3rd and 4th pair of pereopods) using a fine fishing line, enabling free movement in a defined area. After the electors were attached, the chooser was placed into the middle of the arena and covered by a 15 cm opaque circular perforated container for 10 min acclimatization, during which only chemical stimuli were possible. After that, the mate choice experiment began with the removal of the container, when the chooser was free to move in the entire arena. The behaviour of the stocked crayfish was then observed for 10 min in each trial. Selected time range is appropriate for animal communication and decision making in mate choice. To avoid pseudo-replication, each chooser crayfish was used only once. Between each trial, the experimental arena was thoroughly washed with clean tap water and new water was provided for a subsequent trial.

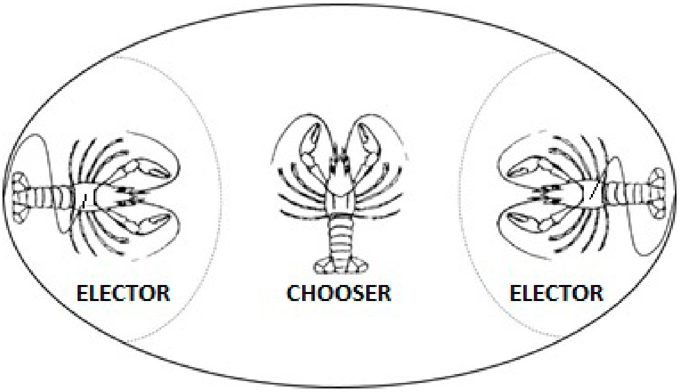

Figure 2. *Illustrated schematic drawing of the experimental arena (dimensions are not to scale). Elector crayfish attached by fishing line; chooser crayfish is freely moving; dotted line – marked elector's area.*

Collecting of data

Each trial was video-taped using a Sony digital camera (HDR-CX240E). Each record was examined by an experienced observer. During the mate choice experiments, the observer recorded parameters of the chooser's selection such as the first choice, time spent and numbers of pre-copulatory contacts, the last choice and mating. Records were processed using the software program EthoVision XT® 13 (Noldus Information Technology b.v., Netherlands) for time spent in each elector's area.

Statistical analyses

Data were tested for normality using the Kolmogorov-Smirnov test. Variables such as time spent in area (carapace centre of chooser crossed the elector's area border), and spent time and number of contacts (chooser actively grasped the elector by claw), fulfilled assumptions of parametric test, were analysed using two-tailed Student's tests (t) for dependent samples; if not, the Wilcoxon matched paired test (z) was used. Other variables such as first and last choices, and mating were tested using Pearson's contingency table chi-squared test. The null hypothesis was rejected at $\alpha = 0.05$ using the software program Statistica version 13.2 for Windows (StatSoft).

Results

Chooser – female

In general, females visited both males about three times during mate choice period, only in four trials females visited just one male. Females did not show preference for males from any unfamiliar population in time spent in elector's areas, time spent and numbers of pre-copulatory contacts, and first and last choices (Table 1). Female choosers from all three populations spent significantly more time in the familiar elector's area and made more contacts with them. Females approached familiar males more often than unfamiliar in comparison of time spent in elector's area (Fig. 3), spent time in contact (Fig. 4), and in numbers of contacts (Fig. 5). First and last choices of choosers were without significant preference to familiar or unfamiliar mates (Table 2).

Female choosers copulated in 19 trials (35.2 %) out of 54 trials. Females showed preference for copulation with familiar males in 6 trials. In some cases (8 trials) the unfamiliar male grasped a female passing them. As this act was involuntary, these copulations were not included in results as the decisive factor for mate choice. No females were observed copulating with more than one male in each trial.

Table 1. ***Statistical analysis of female preferences for both unfamiliar electors (M-E-O).*** *M – the Malše River; E – the Elbe River; O – the Orlík water reservoir; z – Wilcoxon test; ct – number of crayfish trials; f – frequencies; s – seconds; n – numbers.*

	Population					
	M (ct=6)		**E (ct=6)**		**O (ct=6)**	
Variable	statistic	*P* value	statistic	*P* value	statistic	*P* value
First choice (f)	χ^2=0.00	1.000	χ^2=1.33	0.248	χ^2=1.33	0.248
Time spent in elector area (s)	*z*=0.94	0.345	*z*=1.57	0.116	*z*=0.52	0.600
Time spent in contact (s)	*z*=1.15	0.249	*z*=1.57	0.116	*z*=0.11	0.916
Number of contacts (n)	*z*=0.00	1.000	*z*=0.13	0.893	*z*=1.21	0.225
Last choice (f)	χ^2=1.33	0.248	χ^2=1.33	0.248	χ^2=1.33	0.248

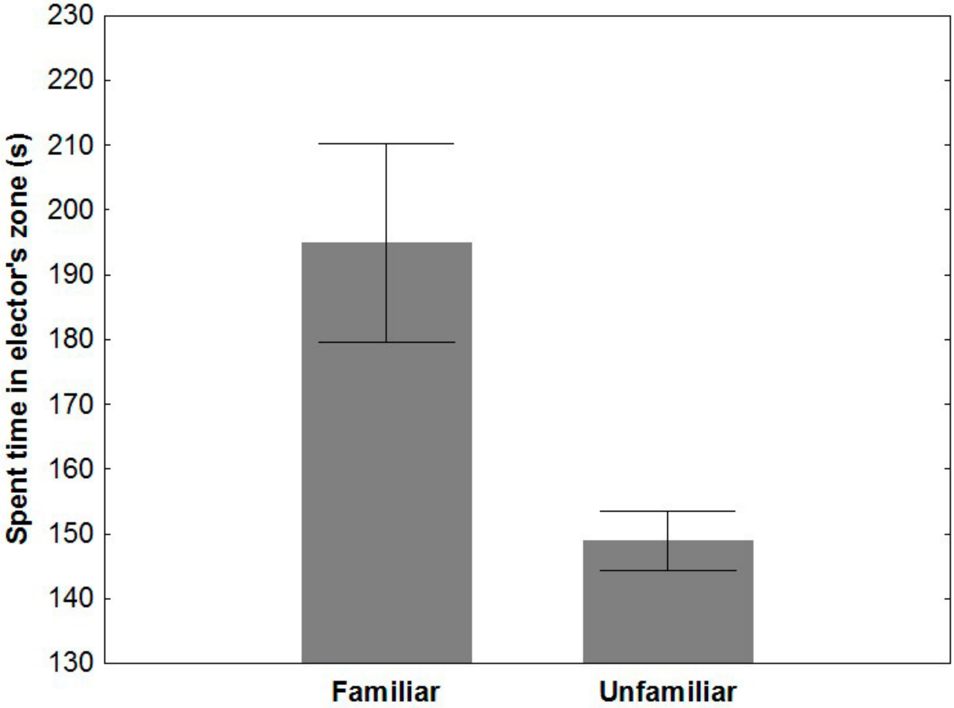

Figure 3. *Average time spent by female chooser in familiar and unfamiliar elector's zone. Data are mean ± SE. Significant difference between familiar and unfamiliar group P < 0.05.*

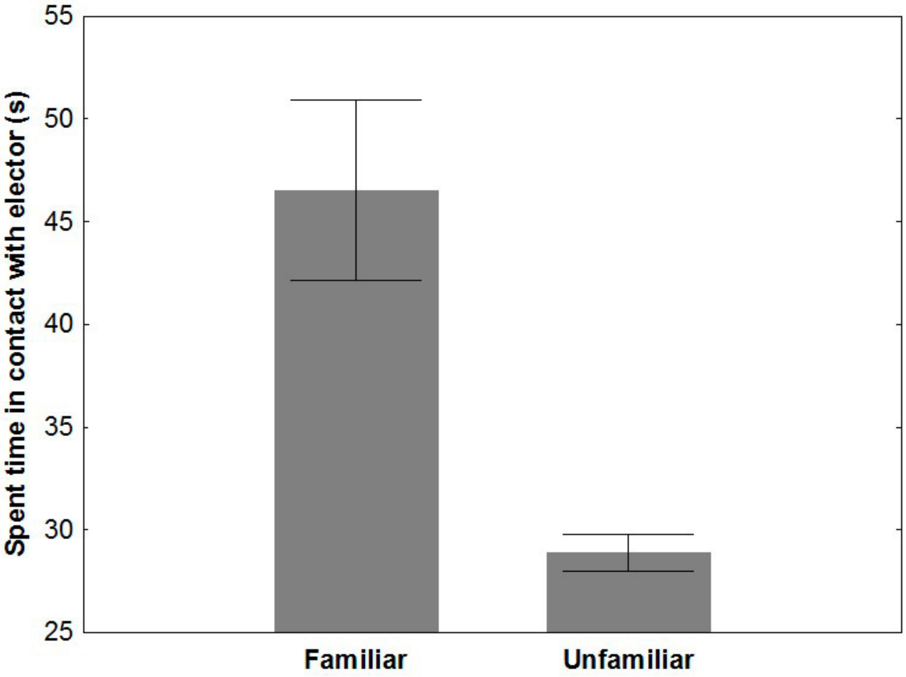

Figure 4. *Average time spent by female chooser in familiar and unfamiliar elector's zone. Data are mean ± SE. Significant difference between familiar and unfamiliar group P < 0.05.*

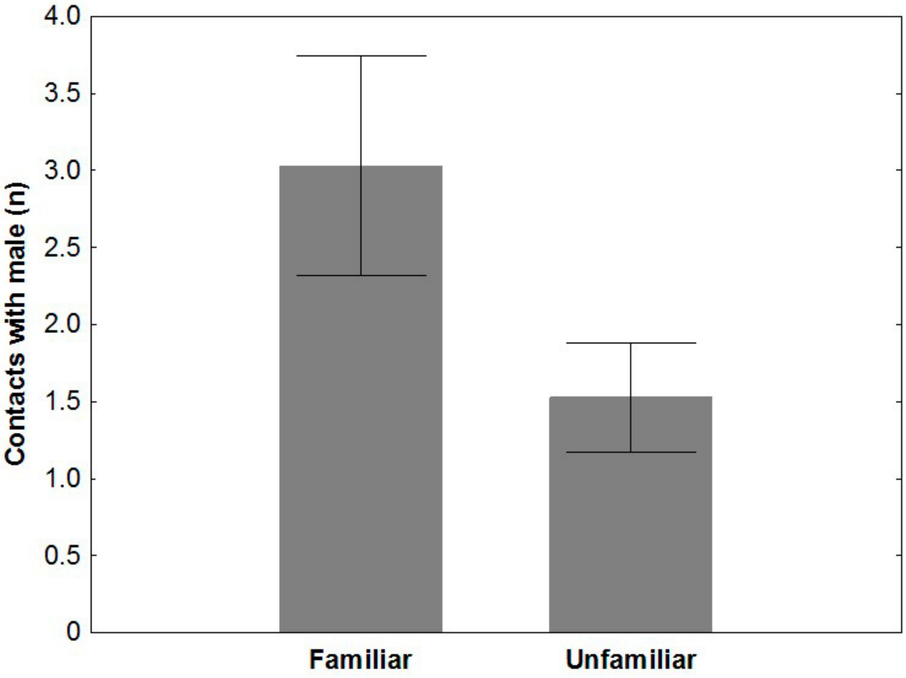

Figure 5. *Average number of female chooser's contacts with familiar and unfamiliar males. Data are mean ± SE. Significant difference between familiar and unfamiliar group P < 0.05.*

Table 2. ***Statistical analysis results of variables in preferences for familiar or unfamiliar males.*** *z – Wilcoxon paired test; t – Student's test; ct – number of crayfish trials; f – frequencies; s – seconds; n – numbers.*

	Familiar vs. Unfamiliar (ct=36)	
Variable	statistic	*P* value
First choice (f)	χ^2=0.89	0.346
Time spent in elector area (s)	t_{35}=-2.71	0.010
Time spent in contact (s)	z=3.03	0.002
Number of contacts (n)	z=2.73	0.006
Last choice (f)	χ^2=0.00	1.000
Mating (f)	χ^2=0.09	0.759

Chooser – male

Total number of copulated male choosers was 34 individuals (63.0 %) out of 54 trials. Males did not exhibit interest in any specific population, either familiar or unfamiliar. Their choices were independent on origin of females. No significant preferences were observed in time spent in elector's areas, time spent and numbers of pre-copulatory contacts, first and last choices and copulations (Table 3). Similar non-significant results were obtained in comparison of male first choice, times spent and contacts with familiar and unfamiliar females (Table 4). An exception was observed only for the last choice, where males significantly preferred unfamiliar females ($P < 0.05$). No females copulating with more than one male were observed.

Table 3. ***Statistical analysis of male preferences for both unfamiliar electors (M-E-O).*** *M – the Malše River; E – the Elbe River; O – the Orlík water reservoir; z – Wilcoxon test; ct – number of crayfish trials; f – frequencies; s – seconds; n – numbers.*

	Population					
	M (ct=6)		**E (ct=6)**		**O (ct=6)**	
Variable	statistic	*P* value	statistic	*P* value	statistic	*P* value
First choice (f)	χ^2=1.33	0.248	χ^2=1.33	0.248	χ^2=1.33	0.248
Time spent in elector area (s)	z=0.52	0.600	z=1.78	0.075	z=0.32	0.753
Time spent in contact (s)	z=0.32	0.753	z=1.78	0.075	z=0.73	0.463
Number of contacts (n)	z=0.99	0.382	z=0.51	0.605	z=0.91	0.412
Last choice (f)	χ^2=1.33	0.248	χ^2=1.33	0.248	χ^2=0.00	1.000

Table 4. ***Statistical analysis results of variables in preferences for familiar or unfamiliar males.*** *z – Wilcoxon paired test; t – Student's test; ct – number of crayfish trials; f – frequencies; s – seconds; n – numbers.*

	Familiar vs. Unfamiliar (ct=36)	
Variable	statistic	*P* value
First choice (f)	$\chi^2=3.56$	0.059
Time spent in elector area (s)	$t_{35}=0.14$	0.889
Time spent in contact (s)	$t_{35}=0.11$	0.913
Number of contacts (n)	$z=0.01$	0.992
Last choice (f)	$\chi^2=18.00$	$<0.10^{-3}$
Mating (f)	$\chi^2=2.36$	0.125

Discussion

Recognition of individuals by crayfish has been studied in recent decades. Several studies demonstrate capabilities of individual recognition in crayfish from different families (Aquiloni & Gherardi 2008a; Aquiloni et al. 2009; Seebacher & Wilson 2007). However, there are no reports if crayfish are able to distinguish between animals from geographically distinct areas. Previous studies mainly focused on social interactions such as hierarchy levels, mate size, winner and loser cognition, and gender and conspecific recognition (Acquistapace et al. 2002; Aquiloni et al. 2008; Van der Velden et al. 2008).

Our results show that at least females of spiny-cheek crayfish are able to recognize males from their familiar population and prefer them to ones from a distant unfamiliar population. Newly introduced animals can therefore theoretically be discriminated in reproduction in accordance to female choice. On the other hand, males did not show any recognition-preference in the study, which confirms the previous findings of Villanelli & Gherardi (1998). They found males not to be choosy, targeting mainly the quantity of mates rather than quality, despite the described preference for large size or virginity in males (Acquistapace et al. 2002; Ameyaw-Akumfi & Hazlett 1975; Aquiloni & Gherardi 2008c). Hence males are very active, in order to find as many mates as possible during the mating season (Buřič et al. 2009; Gherardi & Barbaresi 2000) especially when they can remove the sperm of a previously mated male, as in Cambaridae (Snedden 1990) and Astacidae (Villanelli & Gherardi 1998). In contrast, females consider their choices more because of higher investment in oocyte development and future incubation and parental care (Thiel 2000; Thiel 2003).

However, some questions still persist, e.g. why females prefer, and how do they detect the difference between familiar and unfamiliar individuals. The reason for our original hypothesis was because all spiny-cheek populations in Europe are probably based only on 90 individuals introduced at the end of the 19th century and underwent many bottlenecks during their man-made translocations (Filipová et al. 2011). But crayfish are probably not as capable and resourceful as to manage their population diversity. A more realistic answer as to why to choose between a familiar and unfamiliar mate can be reached from literature where recognition and preference of familiar individuals for shoaling are well-documented in many fish species, e.g. Griffiths & Magurran (1997), Brown (2002), Ward & Hart (2003). Mate choice towards familiars was also detected in other crustaceans (Caldwell 1992; Johnson 1977; Karavanich & Atema 1998a). A familiarity mechanism of natural selection can potentially reduce costly aggressive behaviour as reported in fish (Hojesjo et al. 1998), Seppa et al. (2001) and also discussed in crayfish in light of decreased agonistic behaviour, reduced costs of competition (Bovbjerg 1953; Daws et al. 2011) and shortening of the courtship period. Such advantages can play an important role in determining why females preferentially select mates from the same locality.

However, the mechanism by which females make their decision is unknown. Due to origins of used experimental animals, we were unable to tell if the effect was based on familiarity rather than kinship (Griffin & West 2003; Mathews 2011). Obviously, mate preference is not based on long-term memory or full differentiation of known individuals. Moreover, Van der Velden et al. (2008) described cognition ability of crayfish, although based only on three individuals. In addition, these crayfish were stored individually for at least 5 weeks and to our knowledge there is no report in the literature about such long-term memory in crayfish, regardless of the stimuli (visual or chemical).

It can be assumed that the recognition is rather based on chemical stimuli, as crayfish are aquatic animals mostly dependent on chemical signals from the environment, predators, prey, food or heterospecifics and conspecifics (Breithaupt 2011; Gherardi 2002). Olfactory cues are therefore widely used for circumstances of evaluation and decision making, so we can consider specific odours actively or passively produced by males. It seems too unlikely to imagine that males actively report about their place of origin, but the cues may be a function of previous cohabitation at a locality which could lead to a special "odour of the locality". This odour can probably be connected with the similar microbiome of animals living in a similar locality (Arbuthnott et al. 2016; Ezenwa et al. 2012), with minimal impact on metabolic, functional and virulence potential during the change of environmental conditions (Van Bonn et al. 2015). Specific chemical, physical and biological conditions in different localities can lead to significant differentiation of microfloral settlement and therefore to different odour scent (Lize et al. 2013). The concrete mechanism of familiar population recognition and its functioning is a challenge for future research.

Conclusions

This study demonstrated different reproductive strategies of females and males, from observations that females rely on some familiarity of sexual partners as a reliable guarantee for successful mating, to confirmation of a different male reproductive priority for securing successful mating which includes as many mating acts as possible. This study raised the question of the importance of familiarity in mating in mixed wildlife populations where there are significant differences in adult size. Is familiarity of a partner stronger than a larger migrating super-male? And finally, how can females recognize conspecifics from the same populations? Future studies are expected to solve these issues.

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References

Acquistapace, P., L. Aquiloni, B. A. Hazlett & F. Gherardi, 2002. Multimodal communication in crayfish: sex recognition during mate search by male *Austropotamobius pallipes*. Can J Zool 80 (11): 2041–2045 doi:10.1139/Z02-171.

Ameyaw-Akumfi, C. & B. A. Hazlett, 1975. Sex recognition in the crayfish *Procambarus clarkii*. Science 190 (4220): 1225–1226.

Andersson, M. B., 1994. Sexual selection. Princeton University Press, Princeton, N.J.

Aquiloni, L., M. Buřič & F. Gherardi, 2008. Crayfish females eavesdrop on fighting males before choosing the dominant mate. Curr Biol 18 (11): R462-R463 doi:10.1016/j.cub.2008.04.006.

Aquiloni, L. & F. Gherardi, 2008a. Assessing mate size in the red swamp crayfish *Procambarus clarkii*: effects of visual versus chemical stimuli. Freshwater Biol 53 (3): 461–469 doi:10.1111/j.1365-2427.2007.01911.x.

Aquiloni, L. & F. Gherardi, 2008b. Evidence of female cryptic choice in crayfish. Biol Letters 4 (2): 163-165 doi:10.1098/rsbl.2007.0590.

Aquiloni, L. & F. Gherardi, 2008c. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J Zool 274 (2): 171–179 doi:10.1111/j.1469-7998.2007.00370.x.

Aquiloni, L., A. Massolo & F. Gherardi, 2009. Sex identification in female crayfish is bimodal. Naturwissenschaften 96 (1): 103–110 doi:10.1007/s00114-008-0458-9.

Arbuthnott, D., T. C. Levin & D. E. L. Promislow, 2016. The impacts of Wolbachia and the microbiome on mate choice in *Drosophila melanogaster*. J Evolution Biol 29 (2): 461–468 doi:10.1111/jeb.12788.

Bateson, P. P. G., 1983. Mate choice. Cambridge University Press, Cambridge Cambridgeshire; New York.

Berry, F. C. & T. Breithaupt, 2010. To signal or not to signal? Chemical communication by urine-borne signals mirrors sexual conflict in crayfish. Bmc Biol 8 (1): 25 doi:10.1186/1741-7007-8-25.

Bovbjerg, R. V., 1953. Dominance order in the crayfish *Orconectes Virilis* (Hagen). Physiol Zool 26 (2): 173-178 doi:10.1086/physzool.26.2.30154514.

Breithaupt, T., 2011. Chemical communication in crayfish. In Breithaupt, T. & M. Thiel (eds) Chemical communication in crustaceans. Springer, 257-276.

Brown, C., 2002. Do female rainbowfish (*Melanotaenia* spp.) prefer to shoal with familiar individuals under predation pressure? J Ethol 20 (2): 89–94 doi:10.1007/s10164-002-0059-6.

Buřič, M., A. Kouba & P. Kozák, 2009. Spring mating period in *Orconectes limosus*: the reason for movement. Aquat Sci 71 (4): 473–477 doi:10.1007/s00027-009-0102-6.

Buřič, M., A. Kouba & P. Kozák, 2010a. Intra-sex dimorphism in crayfish females. Zoology 113 (5): 301-307 doi:10.1016/j.zool.2010.06.001.

Buřič, M., A. Kouba & P. Kozák, 2010b. Molting and growth in relation to form alternations in the male spiny-cheek crayfish *Orconectes limosus*. Zool Stud 49 (1): 28–38.

Caldwell, R. L., 1992. Recognition, signaling and reduced aggression between former mates in a stomatopod. Anim Behav 44 (1): 11–19 doi:10.1016/S0003-3472(05)80749-3.

Danchin, E. & F. Cézilly, 2008. Sexual selection: another evolutionary process. In Danchin, E., L.-A. Giraldeau & F. Cézilly (eds) Behav Ecol. Oxford University Press, Oxford, 363–427 p.

Daws, A. G., K. Hock & R. Huber, 2011. Spatial structure of hierarchical groups: testing for processes of aggregation, clustering, and spatial centrality in crayfish (*Orconectes rusticus*). Mar Freshw Behav Phy 44 (4): 209–222 doi:10.1080/10236244.2011.622097.

Ezenwa, V. O., N. M. Gerardo, D. W. Inouye, M. Medina & J. B. Xavier, 2012. Animal behavior and the microbiome. Science 338 (6104): 198-199 doi:10.1126/science.1227412.

Filipová, L., D. A. Lieb, F. Grandjean & A. Petrusek, 2011. Haplotype variation in the spiny-cheek crayfish *Orconectes limosus*: colonization of Europe and genetic diversity of native stocks. Journal of the North American Benthological Society 30 (4): 871–881 doi:10.1899/10-130.1.

Gherardi, F., 2002. Behaviour. In Holdich, D. M. (ed) Biology of freshwater crayfish. Blackwell Science, USA and Canada, Iowa State University Press, Oxford, England; Ames, Iowa, 258–290.

Gherardi, F. & S. Barbaresi, 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). Arch Hydrobiol 150 (1): 153–168.

Gherardi, F. & J. Tiedemann, 2004. Binary individual recognition in hermit crabs. Behav Ecol Sociobiol 55 (6): 524-530 doi:10.1007/s00265-003-0734-9.

Griffin, A. S. & S. A. West, 2003. Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. Science 302 (5645): 634-636 doi:10.1126/science.1089402.

Griffiths, S. W. & A. E. Magurran, 1997. Schooling preferences for familiar fish vary with group size in a wild guppy population. P Roy Soc B-Biol Sci 264 (1381): 547-551 doi:10.1098/rspb.1997.0078.

Hallberg, E. & M. Skog, 2011. Chemosensory sensilla in crustaceans. In Breithaupt, T. & M. Thiel (eds) Chemical communication in crustaceans. Springer, 103–121.

Hamr, P., 2002. Orconectes. In Holdich, D. M. (ed) Biology of freshwater crayfish. Blackwell Science, USA and Canada, Iowa State University Press, Oxford, England; Ames, Iowa, 585–608.

Hojesjo, J., J. I. Johnsson, E. Petersson & T. Jarvi, 1998. The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). Behav Ecol 9 (5): 445-451 doi:10.1093/beheco/9.5.445.

Johnson, V. R., 1977. Individual recognition in banded shrimp *Stenopus hispidus* (Olivier). Anim Behav 25 (May): 418–428 doi:10.1016/0003-3472(77)90017-3.

Karavanich, C. & J. Atema, 1998a. Individual recognition and memory in lobster dominance. Anim Behav 56: 1553-1560 doi:10.1006/anbe.1998.0914.

Karavanich, C. & J. Atema, 1998b. Olfactory recognition of urine signals in dominance fights between male lobster, *Homarus americanus*. Behaviour 135: 719–730 doi:10.1163/156853998792640440.

Lize, A., R. Mckay & Z. Lewis, 2013. Gut microbiota and kin recognition. Trends Ecol Evol 28 (6): 325–326 doi:10.1016/j.tree.2012.10.013.

Mathews, L. M., 2011. Mother-offspring recognition and kin-preferential behaviour in the crayfish *Orconectes limosus*. Behaviour 148 (1): 71–87 doi:10.1163/000579510X548600.

Mellan, D., A. Warren, M. A. Buckholt & L. M. Mathews, 2014. Sexual history affects mating behavior and mate choice in the crayfish *Orconectes limosus*. Ethology 120 (7): 681–692 doi:10.1111/eth.12241.

Petrusek, A., L. Filipova, Z. Duris, I. Horka, P. Kozak, T. Policar, M. Stambergova & Z. Kucera, 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present. B Fr Peche Piscic (380-81): 903–917 doi:10.1051/kmae:2006030.

Rahman, N., D. W. Dunham & C. K. Govind, 2004. Mate choice in the big-clawed snapping shrimp, *Alpheus heterochaelis* Say, 1818. Crustaceana 77: 95–111 doi:10.1163/156854004323037919.

Rubolini, D., P. Galeotti, G. Ferrari, M. Spairani, F. Bernini & M. Fasola, 2006. Sperm allocation in relation to male traits, female size, and copulation behaviour in a freshwater crayfish species. Behav Ecol Sociobiol 60 (2): 212–219 doi:10.1007/s00265-005-0158-9.

Seebacher, F. & R. S. Wilson, 2007. Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. Biol Letters 3 (5): 471–474 doi:10.1098/rsbl.2007.0289.

Seppa, T., A. Laurila, N. Peuhkuri, J. Piironen & N. Lower, 2001. Early familiarity has fitness consequences for Arctic char (*Salvelinus alpinus*) juveniles. Can J Fish Aquat Sci 58 (7): 1380–1385 doi:10.1139/cjfas-58-7-1380.

Simon, J. L. & P. A. Moore, 2007. Male-female communication in the crayfish *Orconectes rusticus*: The use of urinary signals in reproductive and non-reproductive pairings. Ethology 113 (8): 740–754 doi:10.1111/j.1439-0310.2007.01387.x.

Snedden, W. A., 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus* - chela size and sperm competition. Behaviour 115: 100–113 doi:10.1163/156853990X00301.

Thiel, M., 2000. Extended parental care behavior in crustaceans: a comparative overview. Crustacean Issues 12: 211–226.

Thiel, M., 2003. Extended parental care in crustaceans - an update. Rev Chil Hist Nat 76 (2): 205-218.

Tricarico, E., 2015. Behavior: Behavioral Ecology of Crayfish: Its Contribution to Conservation and Management. In Kawai, T., Z. Faulkes & G. Scholtz (eds) Freshwater Crayfish: A Global Overview. CRC Press, 221.

Trivers, R., 1972. Parental investment and sexual selection. Sexual Selection & the Descent of Man, Aldine de Gruyter, New York: 136–179.

Van Bonn, W., A. LaPointe, S. M. Gibbons, A. Frazier, J. Hampton-Marcell & J. Gilbert, 2015. Aquarium microbiome response to ninety-percent system water change: Clues to microbiome management. Zoo Biol 34 (4): 360–367 doi:10.1002/zoo.21220.

Van der Velden, J., Y. Zheng, B. W. Patullo & D. L. Macmillan, 2008. Crayfish recognize the faces of fight opponents. Plos One 3 (2): e1695 doi:10.1371/journal.pone.0001695.

Villanelli, F. & F. Gherardi, 1998. Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. Freshwater Biol 40 (2): 305–315 doi:10.1046/j.1365-2427.1998.00355.x.

Vogt, G., 2002. Functional anatomy. In Holdich, D. M. (ed) Biology of freshwater crayfish. Blackwell Science, USA and Canada, Iowa State University Press, Oxford England; Ames, Iowa, 53–151.

Ward, A. J. W. & P. J. B. Hart, 2003. The effects of kin and familiarity on interactions between fish. Fish Fish 4 (4): 348–358 doi:10.1046/j.1467-2979.2003.00135.x.

Ward, J., N. Saleh, D. W. Dunham & N. Rahman, 2004. Individual discrimination in the big-clawed snapping shrimp, *Alpheus heterochelis*. Mar Freshw Behav Phy 37 (1): 35–42 doi: 10.1080/10236240420000199908.

CHAPTER 3

STILL WATERS RUN DEEP: MARBLED CRAYFISH DOMINATES OVER RED SWAMP CRAYFISH IN AGONISTIC INTERACTIONS

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Still waters run deep: marbled crayfish dominates over red swamp crayfish in agonistic interactions

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Abstract Intra- and interspecific interactions contribute to the successful establishment and consequent spreading of species in the environment, which became particularly apparent in the context of ongoing biological invasions. The parthenogenetic marbled crayfish, *Procambarus virginalis*, Lyko 2017 is recently recognized as an emerging invader due to its high adaptability, fast growth, early maturation, and high fecundity. The present study explored the interaction patterns of size-matched (including 15 body parts morphometry evaluation) pairs of marbled crayfish and red swamp crayfish *Procambarus clarkii*, a well-known highly aggressive and widely distributed invader of freshwater ecosystems. Despite this, marbled crayfish won significantly more fights and establish dominancy in more cases in both premature and mature experimental trials. Premature red swamp crayfish pairs were more active in contact and fight initiation than mature. In mature, the dominance over female red swamp crayfish was 100%, in males it reached 60%. Premature marbled crayfish dominated in more than 75% pairs. Agonistic behaviour and intensity of fights significantly dropped after establishment of dominance in particular (size and sex) pairs. Therefore, we confirmed that sex and age (size) have effects on agonistic behaviour in crayfish as well as the dominance of marbled crayfish within similarly sized specimens. Despite described behavioural patterns, we can expect that the situation in the potential sympatric occurrence of both species will not be as clear as found in experimental conditions due to greater maximal size of red swamp crayfish.

Keywords Competition · Dominance · Interaction · *Procambarus virginalis* · *Procambarus clarkii*

Introduction

Agonistic interactions (i.e. antagonism and aggression) are the key aspects of animal behaviour (Parker 1974; Manning and Dawkins 2012) contributing to a success of species in the environment. Aggressive behaviour occurs during the entire life span of crayfish, and is associated with an invasion success (Sih et al. 2012). Their importance often arise up when animals forage in overlapping ecological niches and

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use identical resources, e.g. food and shelter (Wilson 2000; Huntingford 2013). Agonistic behaviour is primarily demonstrated by increased aggressiveness between participants of conflict (Gherardi 2002) and boosts success in coping with novel predators and competitors (Duckworth 2008; Hudina and Hock 2012). The non-native species that persist, establish, and become invasive can cause negative effects on economy, native biota and ecosystem functioning (Kolar and Lodge 2001).

The mysterious, obligate apomictic parthenogenetic marbled crayfish *Procambarus virginalis*, Lyko 2017 which has no male individuals (Martin et al. 2007; Vogt et al. 2008) and native range (Taylor et al. 1996; Martin et al. 2016) were firstly discovered in the German aquarist trade in the mid-1990s (Scholtz et al. 2003). Their fast growth, early maturation, high fecundity and frequent spawning (Seitz et al. 2005), tolerance to various environmental conditions facilitate their spread over many localities over the Europe during the last decade through human translocation (Hossain et al. 2018). Due to unintentional or intentional releases by hobbyists marbled crayfish have been reported from natural conditions in the Netherlands (Souty-Grosset et al. 2006), Germany (Chucholl et al. 2012; Chucholl 2015), Italy (Nonnis-Marzano et al. 2009; Vojkovská et al. 2014), Czech Republic (Patoka et al. 2016), Slovakia (Janský and Mutkovič 2010; Lipták et al. 2016), Hungary (Lőkkös et al. 2016), Sweden (Bohman et al. 2013), Madagascar (Jones et al. 2009), Japan (Kawai and Takahata 2010), Croatia (Samardžić et al. 2014), Ukraine (Novitsky and Son 2016), Hungary (Lőkkös et al. 2016), Estonia (Estonian Research Council 2018) and Romania (Pârvulescu et al. 2017).

Marbled crayfish have higher potential to be invasive than expected in the past (Kawai et al. 2016) being able to withstand desiccation by burrowing (Kouba et al. 2016) and winter temperatures of temperate climate (Veselý et al. 2015), which could pose a great threat for native crayfish and other representatives of freshwater biota (Faulkes et al. 2012; Kawai et al. 2016). However, ecological impacts are hypothesized rather than demonstrated. It is only assumed that marbled crayfish can be a perfect invader for its high reproductive performance (Jones et al. 2009) and ecological adaptability (Kawai et al. 2016). Marbled crayfish engaged in intraspecific fight (Farca Luna et al. 2009), in groups or in resource less environment, and establish dominance hierarchies like other decapods of sexual species (Bovbjerg 1953; Huber and Kravitz 1995; Issa et al. 1999; Herberholz et al. 2007) although exhibit less aggressiveness than other crayfish (Vogt et al. 2004).

Recently, Jimenez and Faulkes (2011) showed that the marbled crayfish is able to compete with the red swamp crayfish *Procambarus clarkii,* the utmost successful crayfish invader around the world (Souty-Grosset et al. 2016). Nevertheless, the authors examined nearly adult marbled crayfish (in accordance with size used) interaction with only intermolt premature group of red swamp crayfish. Moreover, the pairs used were only roughly size-matched where difference was about 10–20% in size. Hence, this study aims on the revision of observed patterns together with gaining results from two size groups of interacting pairs (using size-matched individuals with detailed morphometry of used animals) and evaluation of more behavioural characteristics. The present study is therefore designed to clear discover the interaction patterns of the emerging invasive crayfish with highly aggressive and widely established invader.

Materials and methods

Experimental animals and measurements

Marbled crayfish and red swamp crayfish originated from our own laboratory culture reared at the same environment, having the similar social experience from their monospecific culture tanks (temperature was maintained at 20 °C and crayfish were fed daily in excess by commercial dry feed for aquarium fish enriched with algae). For experimental work, we selected two size groups representing premature (yet non-reproducing and without developed glair glands) and mature (reproducing) of both species in January 2017. In juveniles or premature animals are the morphological and behavioural differences between sexes determined commonly as minimal or unimportant (Gherardi 2002; Reynolds 2002). Only mature red swamp crayfish were therefore paired considering also the sex since it has significant effect on dominant status in adults (Bovbjerg 1956; Pavey and Fielder 1996). The size of individuals used in interaction pairs was matched prior experiment to be at lower difference than 5% of wet weight (Aquiloni and Gherardi

2008; Momohara et al. 2013) despite the fact that some previous studies were less strict in this regard (Hudina et al. 2011; Jimenez and Faulkes 2011). The carapace length (CL) and weight of live crayfish used in experiments are shown in Table 1. The values did not differ in both, premature (*t* test, $t_{CL} = 0.53$, $P_{CL} = 0.60$; $t_W = -0.12$, $P_W = 0.91$) and mature animals (*t* test, $t_{CL} = 1.09$, $P_{CL} = 0.28$; $t_W = -0.03$, $P_W = 0.98$).

Acclimation and experimental set-up

In order to avoid the effects of previous hierarchical status in the source stocks, all selected specimens were kept individually in separated plastic boxes for 7 days (individuals cannot see each other during this acclimatization period) prior the experiment. During this period, the water temperature was maintained at 20 °C, and crayfish were fed daily in excess by commercial dry feed for aquarium fish enriched with algae (Sera Granugreen, Sera GmbH, and Germany).

After acclimation period, crayfish pairs were stocked into the rectangular experimental arenas (Steele et al. 2018). Smaller arena for premature animals (265 × 185 × 70 mm in size) was filled by 2 l of aged tap water (~ 20 °C) and 0.2 l (376 g of dry weight) of fine sand (particle size < 1 mm). Arena for mature animals (400 × 250 × 200 mm in size) was filled by 6 l of aged tap water (~ 20 °C) and 0.4 l (752 g of dry weight) of fine sand. Crayfish were stocked under transparent perforated plastic cups to acclimate to experimental water conditions for 5 min (Buřič et al. 2018). Then perforated cups were removed to enable crayfish to move and interact freely with each other in experimental arena. The experimental period lasted for 15 min (Steele et al. 2018). Crayfish in experimental arenas were recorded using digital video camera (Sony HDR-CX240, Sony, Japan). In total, 10 and 17 size-matched pairs were recorded for premature and mature animals, respectively. In mature animals, 9 males and 8 females of red swamp crayfish were used.

Data were evaluated later using both visual and automatic analysis of obtained recordings. Visual evaluation was carried out to evaluate the number of contacts, threats, the number of fights, fight duration, to recognize fight initiator, fight winner, avoidance behaviour, and tail flips (Table 2). The dominance establishment was determined as the time where the last underwent agonistic event lead to further

Table 1 Carapace length (CL) and weight (*W*) of premature and mature *P. virginalis* and *P. clarkii* used in interaction pairs

Experimental group	Species	CL (mm)	*W* (g)	*D* (%)
Premature	Marbled crayfish	16.07 ± 0.53 (13.65–18.13)	0.96 ± 0.09 (0.54–1.35)	2.65 ± 0.57 (0–4.65)
	Red swamp crayfish	16.45 ± 0.50 (14.38–19.25)	0.94 ± 0.08 (0.55–1.27)	
Mature	Marbled crayfish	27.37 ± 1.36 (22.32–34.47)	4.81 ± 0.94 (2.28–11.38)	2.13 ± 0.49 (0.24–4.60)
	Red swamp crayfish—males	29.01 ± 1.59 (23.78–38.35)	4.84 ± 0.95 (2.39–11.6)	
	Marbled crayfish	30.68 ± 1.11 (24.93–35.57)	5.66 ± 0.53 (3.94–7.93)	2.45 ± 0.73 (0.19–4.68)
	Red swamp crayfish—females	32.03 ± 0.91 (27.94–35.93)	5.67 ± 0.53 (4.12–7.90)	
	Marbled crayfish	28.93 ± 0.95 (22.32–35.57)	5.41 ± 0.74 (2.28–11.38)	2.28 ± 0.42 (0.19–4.68)
	Red swamp crayfish—in total	30.43 ± 0.99 (23.78–38.35)	5.39 ± 0.73 (2.39–11.60)	

Last column shows the average percent weight difference (*D*) between individuals in particular pairs. Data are presented as mean ± SE and as the range (minimum–maximum) in parentheses

Table 2 Definition of agonistic interaction pattern of *P. virginalis* and *P. clarkii.* Modified from Sato and Nagayama (2012), Aquiloni et al. (2008) and (Fort et al. 2019)

Event	Definition
Contact	Touch with claws, body (carapace, abdomen) rather than antennae and antennules
Avoidance	Retreat from an approaching crayfish that has not exhibited threatening behaviour
Threat	Approach with signs of warning—mainly raising, waving, and shaking claws
Winner	The individual that did not retreat from the fight or that left the opponent that assumed a body down posture or remained motionless. In the very few cases when both individuals retreated simultaneously no winner was scored
Fight	Crayfish make contact with pushing or slight pinching with claws or direct contact and biting with clear intent to inflict injury
Fight initiator	Crayfish which one start first the fight activity recognize as a fight initiator
Tail flip	Rapid abdominal flexions results powerful backward swimming stroke

persistent avoidance of one opponent to fight, contact or stay in the proximity (one body length) of the dominant crayfish. To support this determination, the number of fights won and fights initiated were also taken into account.

Ethical statement

The study did not involve endangered or protected species. No specific permissions were required for the locations and activities involved in this study. It must be mentioned that both species used are involved to a species list of European Commission Regulations (EU Regulation No. 1143/2014 and Commission Implementing Regulation No. 2016/1141). That means that the import, trade, keeping and breeding of marbled crayfish is prohibited in the European Union (EU). Keeping for research is allowed but requires permission and should be done in proper way to avoid possible escapes into the natural environment (Hossain et al. 2018). However, Czech Republic still not has the national implementation of regulations mentioned above, and there are no permissions needed for research organisations yet. All experimental manipulations (rearing, capture and measurements) were conducted according to the principles of the Ethical Committee for the Protection of Animals in Research of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Research Institute of Fish Culture and Hydrobiology, Vodňany, based on the EU harmonized animal welfare act of Czech Republic. The above-named Institutional Animal Care and Use Committee (IACUC) specifically approved this study. The principles of laboratory animal care and the national laws 246/1992 and regulations on animal welfare were followed (Ref. No. 22761/2009-17210).

Statistical analysis

Statistical analyses were performed using Statistica 13.2 software (StatSoft Inc., Tulsa, USA). Chi-square (χ^2) test was used to test expected frequencies of interactions outcomes with observed values (contact initiator, fight initiator, avoidance, winner and dominance establishment) according to hypotheses of experiment. Fight number, duration and latency to first fight were analysed using student t test. Tailflip and retreat were analysed through nonparametric Mann–Whitney U test. The null hypothesis was rejected at $\alpha < 0.05$ in all tests of this study. Data were presented as mean ± SE.

Results

Dominance establishment

There were no significant differences were observed among the measured parameters in the species compared except the abdomen width (Table S1). In premature animals, the dominance was clearly established in 90% of pairs observed. In this group, marbled crayfish dominated over red swamp crayfish significantly (χ^2 test = 30.91; $P < 10^{-3}$). In mature animals group, 52.9% of pairs showed no defined dominancy over each other. While among the rest (i.e. 8 pairs where dominance was established) in mature animals, marbled crayfish governed a dominating role in greater percentage than red swamp crayfish (χ^2 test = 25.0; $P < 10^{-4}$). When the sex of red swamp crayfish is taken into account, marbled crayfish dominate in all female–female pairs (χ^2 test = 100.0; $P < 10^{-4}$). In the case of male–female pairs, the situation was more balanced, but the overweight of marbled crayfish was still evident (χ^2 test = 4.0; $P = 0.045$). Data are described in more details in Table 3.

Latency to start first fight was similar among experimental groups (t test = 0.52; $P = 0.600$), though mature pairs tend to initiate fight slightly earlier than prematures. However, total fights duration (t test = −2.85; $P = 0.010$) and number of fights per pair (t test = −2.46; $P = 0.020$) differed significantly between premature and mature animals. Though the mean fight duration showed large variation between the groups, statistically there was no significant variation (t test = −1.82; $P = 0.08$) (Table 4). The difference in crayfish behaviour was detected in the pairs after the dominance establishment (Table 5) with the number of fights significantly dropped down in both premature (t test = 9.44; $P < 10^{-3}$) and mature animals (t test = 3.71; $P = 0.003$). The mean fight duration was significantly lower after dominance establishment in premature (t test = 3.04; $P = 0.010$) and mature animals (t test = 2.36; $P = 0.040$).

Agonistic encounters and contacts

In the premature animals, red swamp crayfish was significantly more active in contact initialization (χ^2 test = 4.796; $P = 0.029$), while in fight initialization, it also showed higher number while not significant (χ^2 test = 0.34; $P = 0.560$). On the other hand, marbled crayfish won significantly more fights over red swamp crayfish (χ^2 test = 20.04; $P < 10^{-3}$); thus the avoidance to meet the opponent was higher in the latter (χ^2 test = 6.44; $P = 0.010$).

In mature animals, the situation was different in the case of contact (χ^2 test = 0.52; $P = 0.470$) and fight initialization (χ^2 test = 5.29; $P = 0.020$), where marbled crayfish were significantly more active. Similarly as in premature, marbled crayfish were more successful in the fights won (χ^2 test = 30.78; $P < 10^{-4}$), with significantly more avoidance events in the mature red swamp crayfish (χ^2 test = 9.85; $P = 0.002$). In addition, mature marbled crayfish also won significantly more fight, when grouped and analysed with considering the male and female of red swamp crayfish (χ^2 test$_{\text{Male}}$ = 232.79; $P < 10^{-4}$ and χ^2 test$_{\text{Female}}$ = 208.84; $P < 10^{-4}$). These differences are shown in Figs. 1, 2 for premature and mature group (sex together) and for mature group when sexes are considered, respectively. Tailflip (premature: 1.7 ± 0.3; adult: 1.1 ± 0.1) ($U = 13.5$, $P = 0.2$) and retreat (premature: 2.0 ± 0.6; adult: 2.6 ± 0.7) ($U = 16.0$, $P = 0.6$) in interacting pair were not significantly vary among premature and adult groups.

Table 3 Percentage of premature and mature *P. virginalis* and *P. clarkii* pairs with established and not defined dominance, and percentage of dominant specimens of each species in pairs where dominance was established

Group	Species	Established dominance (%, n)	Not defined (%, n)	Dominant from established (%)
Premature	Marbled crayfish	70.0 (7)	10.0 (1)	77.8^{a}
	Red swamp crayfish	20.0 (2)		22.2^{b}
Mature	Marbled crayfish	33.3 (3)	44.5 (4)	60.0^{a}
	Red swamp crayfish—males	22.2 (2)		40.0^{b}
	Marbled crayfish	37.5 (3)	62.5 (5)	100.0^{a}
	Red swamp crayfish—females	0.00 (0)		0.0^{b}
	Marbled crayfish	35.3 (6)	52.9 (9)	75.0^{a}
	Red swamp crayfish—in total	11.8 (2)		25.0^{b}

Number in parentheses indicates the no. of crayfish pairs. Different superscripts in the same column shows the statistically significant differences at $\alpha < 0.05$ between used species within the groups

Table 4 Total number of fights per pair, time to start first fight, total fights duration per pair and mean fight duration in premature and mature *P. virginalis* and *P. clarkii*

Group	Total no. of fights	Time to start fight (s)	Total fights duration (s)	Mean fight duration (s)
Premature	3.4 ± 0.7^{b}	257.9 ± 65.0^{a}	57.4 ± 15.5^{b}	16.9 ± 3.9^{a}
Mature (male–female)	5.1 ± 0.7^{a}	228.1 ± 56.1^{a}	163.0 ± 39.6^{a}	31 ± 6.6^{a}
Mature (female–female)	6.0 ± 0.9^{a}	218.4 ± 39.5^{a}	272.8 ± 74.3^{a}	53.6 ± 20.3^{a}
Mature (total)	5.6 ± 0.5^{a}	223.3 ± 32.2^{a}	217.9 ± 41.8^{a}	42.3 ± 10.4^{a}

Values are presented as mean ± SE. Different superscripts in the same column highlight the statistically significant differences at $\alpha < 0.05$

Table 5 Percentage of fights and mean fight duration till the dominant/subordinate status of crayfish was established and after that in interacting premature and mature pairs of *P. virginalis* and *P. clarkii*

Group	Percentage of fights		Mean fight duration (s)	
	Till dominance establishment	After dominance establishment	Till dominance establishment	After dominance establishment
Premature	88.2 ± 5.7^{a}	11.8 ± 5.4^{b}	19.8 ± 5.6^{a}	1.6 ± 0.7^{b}
Mature (male–female)	70.4 ± 7.2^{a}	29.6 ± 7.7^{b}	36.8 ± 9.2^{a}	12.1 ± 3.9^{b}
Mature (female–female)	81.0 ± 11.7^{a}	19.0 ± 11.7^{b}	140.4 ± 32.2^{a}	8.3 ± 5.1^{b}
Mature (total)	74.9 ± 9.5^{a}	25.1 ± 6.1^{b}	81.2 ± 19.1^{a}	10.5 ± 2.8^{b}

Values are presented as mean ± SE. Different superscripts in the same row highlight the statistically significant differences at $\alpha < 0.05$ between the same parameters

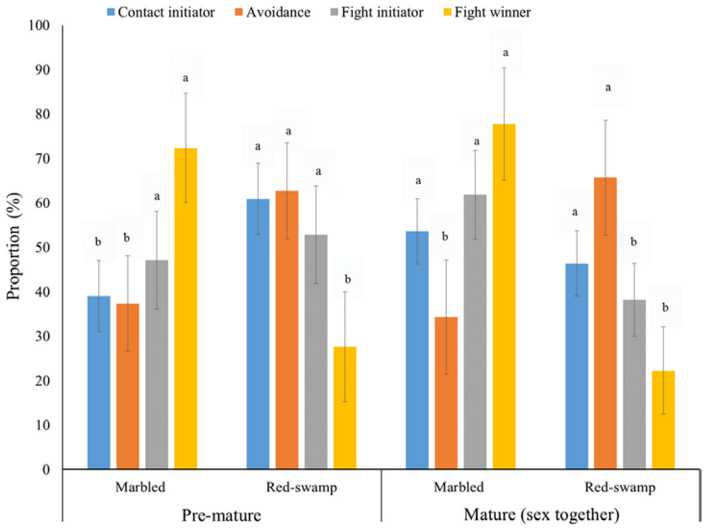

Fig. 1 Comparison of percentage of contact initialization, avoidance events, fight initialization and fight wins between premature and mature *P. virginalis* and *P. clarkii*. Values are presented as mean ± SE. Different superscripts within the column colures (groups apart) highlight the statistically significant differences at $\alpha < 0.05$. (Color figure online)

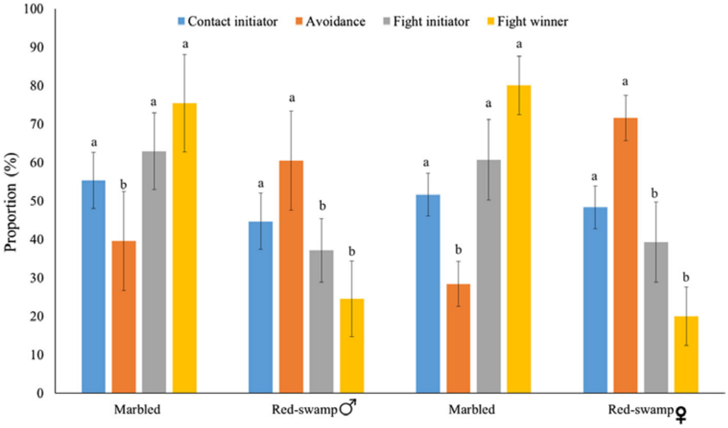

Fig. 2 Comparison of percentage of contact initialization, avoidance events, fight initialization and fight wins between mature *P. virginalis* and *P. clarkii* in respect to sex of *P. clarkii*. Values are presented as mean ± SE. Different superscripts within the column colours (groups apart) indicates the statistically significant differences at $\alpha < 0.05$. (Color figure online)

Discussion

Successful crayfish invaders are basically determined, aside from ecological strategies and adaptability (Lindqvist and Huner 1999; Holdich et al. 2009) through agonistic behaviour during interspecific interactions (Gherardi 2002; Hudina et al. 2016). The importance of marbled crayfish is increasing due to its current spreading in natural habitats worldwide (Kawai et al. 2016; Patoka et al. 2016). The information about the competitiveness of marbled crayfish with other species, including other already established aliens, is scarce (Jimenez and Faulkes 2010; Kawai et al. 2016). Generally, marbled crayfish is considered more placid in stress conditions (Kawai et al. 2016) often stimulated by mechanical stimuli only (Kasuya and Nagayama 2016). Despite its reputation for a calm disposition, it has the previously dominance over size-matched spiny cheek crayfish caught from wild populations in Germany (Linzmaier et al. 2018) and very active and aggressive red swamp crayfish, which is probably an utmost successful introduced species worldwide (Jimenez and Faulkes 2011). In some previous research, some (Jimenez and Faulkes 2011) trials were not performed on size-matched individuals, which resulted in potential bias in results since size is one of the major determinants of dominance in agonistic interactions in crayfish. In addition, from the size of marbled and red swamp crayfish can be supposed that nearly adult marbled crayfish, especially when 8–10 months old (Seitz et al. 2005), fought with juveniles of red swamp crayfish. However, this stock was assessed as premature but unfortunately without evidence of glair glands development consideration (Faulkes, personal communication). We therefore decided to revise these results to a greater extent using two size groups tightly matched for weight and with detailed morphometric evaluation of specimens used. Moreover, two observation methods (including visual and automatic software evaluation) were used to see deeply to the patterns accompanied with dominance establishment.

Our results indicate that marbled crayfish might be a potent challenger in interspecific competition even with highly aggressive and widely distributed alien crayfish. Marbled crayfish significantly more successfully established dominance in interspecific pairs with red swamp crayfish in both premature and mature group. In addition, the status of hierarchy remained not defined in about 50% pairs in mature group, whereas in premature, it was only 10%. The mature group seems to require more time to establish dominancy. More time to establish dominance could be due to prolonged sequential assessment of the opponent due to pair matching. Moreover, the pattern of more rapid formation of social hierarchy in small size crayfish than in larger has been already reported (Sato and Nagayama 2012). In the mature group, marbled crayfish dominated in female–female pairs. In the female–male pairs, the dominance of marbled crayfish was less pronounced (60% with male and 100% with female) probably due to more aggressive nature of males (Gherardi 2002). Males are usually also advantaged with resource-holding potential (RHP) over females (Parker 1974; Shine 1989; Wilson 1992) because males primarily compete for access to female (Draud et al. 2004), while female spent most time and energy in reproduction (including vitellogenesis) and carrying young (Trivers 1972). Male and female crayfish also use different strategies during agonistic interactions (Wofford 2013). Mature crayfish males are more aggressive than females, but in premature animals, their agonistic features are not pronounced by sex (Bovbjerg 1956) though dominant–subordinate relationship is formed between premature as early as the third developmental stage (Issa et al. 1999).

The morphometric comparison did not reveal any significant differences in strategic body parts, as chelae and carapace dimensions, abdomen or total length (Bovbjerg 1953; Stein 1976; Gherardi 2002), and the differences of body dimensions therefore probably did not play important role in our study. The only evident difference was significantly wider abdomen of red swamp crayfish in premature group and conversely wider abdomen of marbled crayfish in mature group, which can hypothetically be at the utmost the precursor of better stability of marbled crayfish during fights (Buřič et al. 2010; Hossain, Buřič, unpublished work). However, other variables such as plasma protein level and exoskeleton calcium concentrations (Vye et al. 1997) should be considered in future studies being physiological and biochemical status has effect on aggressive motivation (Kravitz 1988).

Size and developmental stage are important factors related to agonistic behaviour of crayfish (Bovbjerg 1956) with an elevated level of aggression and more fighting events occurred as juvenile (premature) crayfish became larger (Sato and Nagayama 2012). In our experiment, we found that total fight number per pair, fight duration and mean fight duration was significantly higher in mature group. Schroeder and Huber (2001) also showed that duration of fights (fights escalated more rapidly) is shorter and resolved generally at lower intensities in small crayfish. Premature red swamp crayfish was more active in contact and fight initiation than marbled crayfish. On the other hand, their attacks (contact and fights initialization) was in the majority of cases unsuccessful which led to more established dominant marbled crayfish in experimental pairs. Therefore, lower rate of success of red swamp in fights might be due to continuous locomotion in experimental period compared to less active marbled crayfish.

Crayfish aggressive behaviour is mediated by visual, tactile and chemical communication (Breithaupt and Eger 2002; Bergman et al. 2005; Bergman and Moore 2005) with body vital statistics as possible predictors of dominance (Pavey and Fielder 1996). During direct interaction or conflict, fighters of many animal taxa are thought to evaluate an opponent's relative fighting ability, comparing it with their own (Parker 1974; Whitehouse 1997). The subordinate one avoids the dominant one and also the activity of contact initiation drops down (Rubenstein and Hazlett 1974; Höjesjö et al. 1998; Issa et al. 1999). Chemical signals are usually responsible for this social recognition, and crayfish have therefore the ability to recognize even unfamiliar dominant and subordinate individuals through chemical cues (Schneider et al. 1999). We therefore performed a prolonged 7-day lasting acclimation where crayfish were reared individually to reset their previous social status in original communal stocks. In crayfish, use of antennules as chemosensory organs has been well documented in agonistic interaction (Rutherford et al. 1996; Smith and Dunham 1996). In our experiment, in most of cases, both crayfish avoided each other or engaged in fight before communicated with their antennules. It is also noticeable that the subordinate crayfish also avoided the dominant one after seeing raised claws at a distance. This behaviour is therefore result of visual and chemical communication. Subordinate crayfish often avoid dominate individuals from possible contact through tailflip or retreat. In present study, tailflips frequency were higher value in premature animals, whereas retreat frequency was elevated in mature group which was also true for American lobster *Homarus americanus* with the increasing size escape behaviour through tailflip replaced by a defensive strategy (Lang et al. 1977). Sato and Nagayama (2012) also observed that with increasing body size, the probability of tailflips decreased, while retreats increased as the submissive behavioural act of subordinate animals. This contrast in the repertoire of submissive behavioural acts between smaller and larger crayfish could be due to a different level of activation of the neural circuitry underlying tailflip (Edwards et al. 1994a, b).

Conclusion

Finally, it can be the assumed that the marbled crayfish is able to dominate to similarly sized premature, mature females as well as mature males of red swamp crayfish despite its less activity in initialization of contact and fights in premature animals. Marbled crayfish seems to be thrifty in terms of energy expenditure, but is able to effectively react of the challenges. When dominance is established, both crayfish species lost the interest to initialize other contacts and fights. However, the interaction pattern would not be always straightforward, because in natural conditions, the population structure is not balanced as in experimental conditions, and crayfish usually have to face to different-sized specimens in more complex habitat and predation risk. In addition, red swamp crayfish have faster growth than marbled crayfish and disproportionate claw size in large male adults (Holdich et al. 2006). On the other hand, marbled crayfish being all female specimens have moderately sized claw also in larger specimens (Seitz et al. 2005). These would limit the success of marbled crayfish in natural habitats. Either way, marbled crayfish was confirmed as successful competitor in interactions with the probably most aggressive alien crayfish species (Gherardi and Cioni 2004; Gherardi and Daniels 2004; Jimenez and Faulkes 2011). Moreover, the successful invader is not determined only by aggressive behaviour and dominancy in the fights but also in other specifics like reproduction strategy and outputs together with an ability to adapt in different conditions (Lindqvist and Huner 1999). In this view, marbled crayfish is recently known as highly adaptable, early maturating and fecund species which can reproduce more than once per year via apomictic parthenogenesis (Martin et al. 2007; Kawai et al. 2016; Kouba et al. 2016). Moreover, marbled crayfish seems to have an ability to spare the energy and be more active only in the inevitable conditions. Hence, together with the found abilities to directly outcompete even such aggressive species as red swamp crayfish, marbled crayfish pose a substantial threat to native ecosystems and biodiversity of aquatic environment which can establish not only in crayfish free habitats, but is able to compete with resident species. One way or another, future studies are needed for example to evaluate the competition for strategic resources like food or shelter in basic as well as complex experimental conditions and in established populations.

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References

Aquiloni L, Gherardi F (2008) Extended mother-offspring relationships in crayfish: the return behaviour of juvenile *Procambarus clarkii*. Ethol 114:946–954

Aquiloni L, Buřič M, Gherardi F (2008) Crayfish females eavesdrop on fighting males before choosing the dominant mate. Curr Biol 18:462–463

Bergman DA, Moore PA (2005) The role of chemical signals in the social behavior of crayfish. Chem Senses 30:305–306

Bergman DA, Martin AL, Moore PA (2005) Control of information flow through the influence of mechanical and chemical signals during agonistic encounters by the crayfish, *Orconectes rusticus*. Anim Behav 70:485–496

Bohman P, Edsman L, Martin P, Scholtz G (2013) The first Marmorkrebs (Decapoda: Astacida: Cambaridae) in Scandinavia. BioInvasion Rec 2:227–232. https://doi.org/10.3391/bir.2013.2.3.09

Bovbjerg RV (1953) Dominance order in the crayfish Orconectes virilis (Hagen). Physiol Zool 26:173–178

Bovbjerg RV (1956) Some factors affecting aggressive behavior in crayfish. Physiol Zool 29:127–136

Breithaupt T, Eger P (2002) Urine makes the difference. J Exp Biol 205:1221–1231

Buřič M, Kouba A, Kozák P (2010) Intra-sex dimorphism in crayfish females. Zoology 113:301–307

Buřič M et al (2018) Environmentally relevant concentrations of tramadol and citalopram alter behaviour of an aquatic invertebrate. Aquat Toxicol 200:226–232

Chucholl C (2015) Marbled crayfish gaining ground in Europe: the role of the pet trade as invasion pathway. In: Tadashi Kawai ZF, Scholtz G (eds) Freshwater crayfish: a global overview. CRC Press, New York, pp 83–114

Chucholl C, Morawetz K, Groß H (2012) The clones are coming–strong increase in Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*] records from Europe. Aquat Invasions 7:511–519

Draud M, Macías-Ordóñez R, Verga J, Itzkowitz M (2004) Female and male Texas cichlids (Herichthys cyanoguttatum) do not fight by the same rules. Behav Ecol 15:102–108

Duckworth RA (2008) Adaptive dispersal strategies and the dynamics of a range expansion. Am Nat 172:4–17

Edwards DH, Fricke RA, Barnett LD, Yeh S-R, Leise EM (1994a) The onset of response habituation during the growth of the lateral giant neuron of crayfish. J Neurophysiol 72:890–898

Edwards DH, Yeh S-R, Barnett LD, Nagappan PR (1994b) Changes in synaptic integration during the growth of the lateral giant neuron of crayfish. J Neuophysiol 72:899–908

Estonian Research Council (2018) Invasive marbled crayfish found in Narva power plant cooling canal. https://phys.org/news/2018-06-invasive-marbled-crayfishnarva-power.html. Accessed 8 June 2018

Farca Luna AJ, Hurtado-Zavala JI, Reischig T, Heinrich R (2009) Circadian regulation of agonistic behavior in groups of parthenogenetic marbled crayfish, *Procambarus* sp. J Biol Rhythms 24:64–72

Faulkes Z, Feria TP, Muñoz J (2012) Do marmorkrebs, *Procambarus fallax* f. *virginalis*, threaten freshwater Japanese ecosystems? Aquat Biosyst 8:13

Fort M, Hossain MS, Kouba A, Buric M, Kozak P (2019) Agonistic interactions and dominance establishment in three crayfish species non-native to Europe. Limnologica 74:73–79

Gherardi F (2002) Behavior. In: Holdich DM (ed) Biology of Freshwater Crayfish. Blackwell Science, Oxford, pp 258–290

Gherardi F, Cioni A (2004) Agonism and interference competition in freshwater decapods. Behaviour 141:1297–1324

Gherardi F, Daniels WH (2004) Agonism and shelter competition between invasive and indigenous crayfish species. Can J Zool 82:1923–1932

Herberholz J, McCurdy C, Edwards DH (2007) Direct benefits of social dominance in juvenile crayfish. Biol Bull 213:21–27

Höjesjö J, Johnsson JI, Petersson E, Järvi T (1998) The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). Behav Ecol 9:445–451

Holdich D, Haffner P, Noël P (2006) Species files. In: Souty-Grosset C, Holdich D, Noel PY, Reynolds JD, Haffner P (eds) Atlas of crayfish in Europe, vol 64. Muséum national d'Histoire naturelle, Paris Patrimoines naturels, Paris, pp 50–129

Holdich D, Reynolds J, Souty-Grosset C, Sibley P (2009) A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. Knowl Manag Aquat Ecosyst 11:394–395

Hossain MS, Patoka J, Kouba A, Buřič M (2018) Clonal crayfish as biological model: a review on marbled crayfish. Biologia 73:841–855. https://doi.org/10.2478/s11756-018-0098-2

Huber R, Kravitz E (1995) A quantitative analysis of agonistic behavior in juvenile American lobsters (Homarus americanus L.). Brain Behav Evol 46:72–83

Hudina S, Hock K (2012) Behavioural determinants of agonistic success in invasive crayfish. Behav Processes 91:77–81

Hudina S, Galić N, Roessink I, Hock K (2011) Competitive interactions between co-occurring invaders: identifying asymmetries between two invasive crayfish species. Biol Invasions 13:1791–1803

Hudina S, Hock K, Radović A, Klobučar G, Petković J, Jelić M, Maguire I (2016) Species-specific differences in dynamics of agonistic interactions may contribute to the competitive advantage of the invasive signal crayfish (*Pacifastacus leniusculus*) over the native narrow-clawed crayfish (*Astacus leptodactylus*). Mar Freshw Behav Physiol 49:147–157

Huntingford FA (2013) Animal conflict. Chapman & Hall Animal Behaviour Series. Springer, New York

Issa FA, Adamson DJ, Edwards DH (1999) Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. J Exp Biol 202:3497–3506

Janský V, Mutkovič A (2010) Rak Procambarus sp. (Crustacea: Decapoda: Cambaridae)–Prvŷ Nález na Slovensku. Acta Rerum Naturalium Musei Nationalis Slovaci Bratislava 56:64–67

Jimenez SA, Faulkes Z (2010) Establishment and care of a colony of parthenogenetic marbled crayfish, Marmorkrebs. Invertebr Rearing 1:10–18

Jimenez SA, Faulkes Z (2011) Can the parthenogenetic marbled crayfish Marmorkrebs compete with other crayfish species in fights? J Ethol 29:115–120

Jones JP et al (2009) The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. Biol Invasions 11:1475–1482

Kasuya A, Nagayama T (2016) Habituation of Backward Escape Swimming in the Marbled Crayfish. Zool Sci 33:6–12

Kawai T, Takahata M (eds) (2010) Biology of crayfish. Hokkaido University Press, Sapporo, Japan

Kawai T, Faulkes Z, Scholtz G (2016) Freshwater crayfish: a global overview. CRC Press, New York

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

Kouba A et al (2016) The significance of droughts for hyporheic dwellers: evidence from freshwater crayfish. Sci Rep 6:1–7

Kravitz EA (1988) Hormonal control of behavior: amines and the biasing of behavioral output in lobsters. Science 241:1775–1781

Lang F, Govind C, Costello WJ, Greene SI (1977) Developmental neuroethology: changes in escape and defensive behavior during growth of the lobster. Science 197:682–685

Lindqvist OV, Huner J (1999) Life history characteristics of crayfish: What makes some of them good colonizers? In: Gherardi F, Holdich DM (eds) Crayfish in Europe as Alien Species: How to make the best of the bad situation?. A. A Balkema, Rotterdam, pp 23–30

Linzmaier SM, Goebel LS, Ruland F, Jeschke JM (2018) Behavioral differences in an over-invasion scenario: marbled vs. spiny-cheek crayfish. Ecosphere 9:e02385

Lipták B, Mrugała A, Pekárik L, Mutkovič A, Gruľa D, Petrusek A, Kouba A (2016) Expansion of the marbled crayfish in Slovakia: beginning of an invasion in the Danube catchment? J Limnol 75:305–312

Lőkkös A, Müller T, Kovács K, Várkonyi L, Specziár A, Martin P (2016) The alien, parthenogenetic marbled crayfish (Decapoda: Cambaridae) is entering Kis-Balaton (Hungary), one of Europe's most important wetland biotopes. Knowl Manag Aquat Ecosyst 417:1–9

Manning A, Dawkins MS (2012) An introduction to animal behaviour, 5th edn. Cambridge University Press, Cambridge

Martin P, Kohlmann K, Scholtz G (2007) The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. Naturwissenschaften 94:843–846

Martin P, Thonagel S, Scholtz G (2016) The parthenogenetic Marmorkrebs (Malacostraca: Decapoda: Cambaridae) is a triploid organism. J Zool Syst Evol Res 54:13–21

Momohara Y, Kanai A, Nagayama T (2013) Aminergic control of social status in crayfish agonistic encounters. PLoS One 8:e74489

Nonnis-Marzano F, Scalici M, Chiesa S, Gherardi F, Piccinini A, Gibertini G (2009) The first record of the marbled crayfish adds further threats to fresh waters in Italy. Aquat Invasions 4:401–404

Novitsky RA, Son MO (2016) The first records of Marmorkrebs [*Procambarus fallax* (Hagen, 1870) f. *virginalis*](Crustacea, Decapoda, Cambaridae) in Ukraine. Ecol Monten 5:44–46

Parker GA (1974) Assessment strategy and the evolution of fighting behaviour. J Theor Biol 47:223–243

Pârvulescu L, Togor A, Lele S-F, Scheu S, Şinca D, Panteleit J (2017) First established population of marbled crayfish *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Decapoda, Cambaridae) in Romania. BioInvasions Rec 6:357–362

Patoka J et al (2016) Predictions of marbled crayfish establishment in conurbations fulfilled: evidences from the Czech Republic. Biologia 71:1380–1385

Pavey C, Fielder D (1996) The influence of size differntial on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). J Zool 238:445–457

Reynolds J (2002) Growth and reproduction. In: Holdich DM (ed) Biology of freshwater crayfish. Blackwell Science, Oxford, pp 152–191

Rubenstein DI, Hazlett BA (1974) Examination of the agonistic behaviour of the crayfish *Orconectes virilis* by character analysis. Behaviour 50:193–215

Rutherford P, Dunham D, Allison V (1996) Antennule use and agonistic success in the crayfish Orconectes rusticus (Girard, 1852) (Decapoda, Cambaridae). Crustaceana 69:117–122

Samardžić M, Lucić A, Maguire I, Hudina S (2014) The first record of the marbled crayfish (*Procambarus fallax* (Hagen, 1870) f. *virginalis*) in Croatia. Crayfish News 36:4

Sato D, Nagayama T (2012) Development of agonistic encounters in dominance hierarchy formation in Juvenile crayfish. J Exp Biol 215:1210–1217

Schneider RAZ, Schneider RW, Moore PA (1999) Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. J Chem Ecol 25:781–794

Scholtz G et al (2003) Ecology: parthenogenesis in an outsider crayfish. Nature 421:806

Schroeder L, Huber R (2001) Fight strategies differ with size and allometric growth of claws in crayfish, Orconectes rusticus. Behaviour 138:1437–1449

Seitz R, Vilpoux K, Hopp U, Harzsch S, Maier G (2005) Ontogeny of the Marmorkrebs (marbled crayfish): a parthenogenetic crayfish with unknown origin and phylogenetic position. J Exp Zool A Ecol Genet Physiol 303:393–405

Shine R (1989) Ecological causes for the evolution of sexual dimorphism: a review of the evidence. Q Rev Biol 64:419–461

Sih A, Cote J, Evans M, Fogarty S, Pruitt J (2012) Ecological implications of behavioural syndromes. Ecol Lett 15:278–289

Smith MR, Dunham DW (1996) Antennae mediate agonistic physical contact in the crayfish Orconectes rusticus (Girard, 1852) (Decapoda, Cambaridae). Crustaceana 69:668–674

Souty-Grosset C, Holdich DM, Noël PY (2006) Atlas of crayfish in Europe. Collection patrimoines naturels. Muséum national d'histoire naturelle, Paris

Souty-Grosset C, Anastácio PM, Aquiloni L, Banha F, Choquer J, Chucholl C, Tricarico E (2016) The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-being. Limnol Ecol Manag Inland Waters 58:78–93

Steele AN, Belanger RM, Moore PA (2018) Exposure through runoff and ground water contamination differentially impact behavior and physiology of crustaceans in fluvial systems. Arch Environ Contam Toxicol 75:436–448

Stein RA (1976) Sexual dimorphism in crayfish chelae: functional significance linked to reproductive activities. Can J Zool 54:220–227

Taylor CA, Warren ML Jr, Fitzpatrick J Jr, Hobbs HH III, Jezerinac RF, Pflieger WL, Robison HW (1996) Conservation status of crayfishes of the United States and Canada. Fisheries 21:25–38

Trivers R (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man. Aldine, Chicago, pp 136–179

Veselý L, Buřič M, Kouba A (2015) Hardy exotics species in temperate zone: can "warm water" crayfish invaders establish regardless of low temperatures? In: Scientific reports 5

Vogt G, Tolley L, Scholtz G (2004) Life stages and reproductive components of the Marmorkrebs (marbled crayfish), the first parthenogenetic decapod crustacean. J Morphol 261:286–311

Vogt G, Huber M, Thiemann M, van den Boogaart G, Schmitz OJ, Schubart CD (2008) Production of different phenotypes from the same genotype in the same environment by developmental variation. J Exp Biol 211:510–523

Vojkovská R, Horká I, Tricarico E, Ďuriš Z (2014) New record of the parthenogenetic marbled crayfish *Procambarus fallax* f. *virginalis* from Italy. Crustaceana 87:1386–1392

Vye C, Cobb J, Bradley T, Gabbay J, Genizi A, Karplus I (1997) Predicting the winning or losing of symmetrical contests in the American lobster Homarus americanus (Milne-Edwards). J Exp Mar Biol Ecol 217:19–29

Whitehouse ME (1997) Experience influences male–male contests in the spider *Argyrodes antipodiana* (Theridiidae: Araneae). Anim Behav 53:913–923

Wilson JD (1992) Correlates of agonistic display by great tits Parus major. Behaviour 121:168–214

Wilson EO (2000) Sociobiology. 25th Anniversary Edition edn. Harvard University Press, Cambridge

Wofford SJ (2013) Sex and fighting: male and female crayfish utilize different assessment strategies during agonistic behavior. Bowling Green State University, Bowling Green

CHAPTER 4

OXAZEPAM ALTERS THE BEHAVIOUR OF CRAYFISH AT DILUTED CONCENTRATIONS, VENLAFAXINE DOES NOT

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Oxazepam Alters the Behavior of Crayfish at Diluted Concentrations, Venlafaxine Does Not

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Abstract: Pharmaceutically active compounds are only partially removed from wastewaters and hence may be major contaminants of freshwaters. Direct and indirect effects on aquatic organisms are reported at dilute concentrations. This study was focused on the possible effects of environmentally relevant concentrations (~1 µg L^{-1}) of two psychoactive compounds on the behavior of freshwater crayfish. Experimental animals exposed to venlafaxine did not show any behavioral alteration. Crayfish exposed to the benzodiazepine oxazepam exhibited a significant alteration in the distance moved and activity, and the effects were different when individuals were ready for reproduction. Results suggested that even the low concentration of selected psychoactive pharmaceuticals could alter the behavioral patterns of crayfish, as reported for other pharmaceuticals. These results provide new information about the possible adverse effects of pharmaceuticals at dilute concentrations. From previous knowledge and our results, it is obvious that different compounds have different effects and the effects are even specific for different taxa. Detailed studies are therefore needed to assess the possible ecological consequences of particular substances, as well as for their mixtures.

Keywords: environmental pollution; pharmaceuticals; freshwaters; crayfish

1. Introduction

Pharmaceutically active compounds (PhAC) are considered emerging contaminants in aquatic environments [1,2]. PhACs originate mainly from human or animal excretion or runoff from hospitals [3,4] and penetrate freshwaters via effluents of sewage treatment plants (STPs) which are ineffective in their removal [5]. The residues have several non-lethal effects on aquatic organisms and, through them, on whole ecosystems [6,7]. Psychotropic substances are present often at much lower concentrations in surface waters [8–10] than, for example, antibiotics or hypertension drugs, [11,12] but they have important effects in very diluted concentrations as well [13,14].

The psychotropic substances venlafaxine and oxazepam alter the state of the brain by flooding it with the neurotransmitter serotonin (5-HT) or act on benzodiazepine receptors, having direct inhibitory effects on the central nervous system [15,16]. Invertebrates (including crayfish) have similar receptors for psychotropic compounds as mammals [17], even with the potential for bioaccumulation [14,18], which increases the probability of the apparent PhACs' effects on these animals.

Some psychoactive PhACs are also bioactive and can persist in the sediments of surface waters [19], enabling their transfer via the food-web [20]. They are developed to modify behavioral patterns, so a behavioral alteration in aquatic organisms is likely [21,22]. However, the behavioral effects of these psychotropic compounds still remain less understood than eco-toxicity tests [23]. Behavioral effects, from an ecological point of view, can affect the survival of an individual, as well as the long-term sustainability of a population [24]. Crayfish seem to be good model organisms, having well known social and spatial behavior [25,26] and being similarly susceptible to the behavioral changes induced by PhACs [13,27,28].

In this study, the behavioral patterns of a clonal species, the marbled crayfish (*Procambarus virginalis*, Lyko 2017), were assessed using an ethological software where control animals and those exposed to environmentally relevant concentrations of venlafaxine and oxazepam were used. We hypothesized about the possible behavioral changes associated with the pollutants used at concentrations commonly detected in surface waters, as confirmed in our previous study with other PhACs.

2. Materials and Methods

2.1. Chemicals

Venlafaxine (VEN) and oxazepam (OXA) were obtained from AK Scientific (Union City, CA, USA) and Lipomed (Cambridge, MA, USA), respectively. Stock solutions of both compounds (concentration of 10 mg L^{-1}) were prepared using ultra-pure water (AquaMax Basic 360 Series and Ultra 370 Series instrument, Young Lin, Anyang, Republic of Korea) and were stored at 4 °C. The exposure solutions of 1 μg L^{-1} were prepared by dilution of the stock solution in aged tap water. Concentration testing was utilized to evaluate reported [29] environmentally relevant concentrations.

Isotopically labeled venlafaxine (D6-VEN) and oxazepam (D5-OXA; both from Lipomed (USA)) were used as internal standards for the analyses of water samples. Ultra-pure water and acetonitrile (LC/MS grade purity, Merck, Kenilworth, NJ, USA), both acidified with formic acid (Sigma-Aldrich, Darmstadt, Germany), were used as the mobile phases in liquid chromatography (LC).

2.2. Experimental Animals

Marbled crayfish (with a carapace length of 16–22 mm, measured using a vernier caliper to the nearest 0.1 mm) were randomly selected from our laboratory culture. Crayfish weight (to the nearest 0.1 g) was obtained using an electronic balance (Kern & Sohn GmbH, Balingen, Germany). The mean lengths and weights of the animals used (Table 1) did not differ between the control and exposed groups.

Table 1. The mean carapace length (CL) and weight (W) of marbled crayfish (*Procambarus virginalis*, Lyko 2017) animals used in the experimental groups in either the presence or absence of shelter. Data are presented as mean ± standard deviation. The *t*-test values and *p*-values are shown to demonstrate no differences between experimental groups.

Tested Compound	Group	Shelter	CL (mm)	*t*-test	*p*	W (g)	*t*-test	*p*
Venlafaxine	exposed	no	19.0 ± 1.8	−0.13	0.897	2.0 ± 0.6	−0.14	0.889
	control	no	18.8 ± 2.1			1.9 ± 0.7		
	exposed	yes	18.3 ± 2.1	0.39	0.695	1.8 ± 0.7	0.39	0.699
	control	yes	18.4 ± 2.0			1.8 ± 0.7		
Oxazepam	exposed	no	20.1 ± 3.0	1.12	0.269	2.3 ± 0.9	1.39	0.171
	control	no	19.3 ± 2.3			2.0 ± 0.8		
	exposed	yes	19.1 ± 2.8	0.36	0.717	2.1 ± 0.9	0.43	0.672
	control	yes	18.8 ± 2.7			2.0 ± 0.9		

2.3. Experimental Design

The exposition and experimental work was conducted in November (VEN) and December (OXA) 2017. In total, 55 crayfish were exposed to a concentration of ~1 μg L^{-1} of VEN for 21 days and 60 animals to an OXA compound for 7 days, respectively. The concentration was chosen based on previously reported environmental concentrations [9,14,18,30,31]. The exposure times were chosen in relation to the mode of action of the selected compounds. VEN acts when a steady-state plasma concentration is achieved (3–4 weeks) [32], while OXA acts immediately [16]. Crayfish maintained in aged tap water were used as controls, with the same handling as the exposed groups. The crayfish were held individually in transparent plastic boxes (190 × 140 × 75 mm) with 0.5 L of exposure solution or aged tap water. The numbers of animals that molted, spawned, or died during the exposure period were recorded.

During the exposure period, crayfish were fed ad libitum with fish pellets (Sera Granugreen, Sera, Heinsberg, Germany). Boxes were cleaned during exposure to the solution and the water exchange (every 48 h). The control group was always handled first to avoid its contamination. Crayfish which molted or spawned were discarded from the experiment. Water temperature was measured by an alcohol thermometer (to the nearest 0.1 °C) and did not differ ($p > 0.05$) between the control and the exposed group in both VEN and OXA studies. Water temperature ranged between 19.3 and 20.6 °C.

The real concentrations of VEN and OXA in the exposure solution, as well as in the control group's water, was checked using liquid chromatography with a tandem mass spectrometer (LC-MS/MS, Research Institute of Fish Culture and Hydrobiology, Vodňany, Czech Republic) four (VEN) and three times (OXA) during the exposure period. The concentrations of the compounds were analyzed in freshly prepared solutions (at time 0) and after 48 h, when the used solution was exchanged (time 48). Collected samples were filtered (0.20 μm regenerated cellulose, Labicom, Olomouc, Czech Republic) and stored in a freezer at −20 °C until analysis. After thawing and the addition of the internal standards, the samples were measured using the 10 min method on a Hypersil Gold aQ column (50 × 2.1 mm; 5 mm particles) coupled with an Accela 1250 LC pump and a TSQ Quantum Ultra Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The concentrations of the tested compounds in the analyzed water samples from the exposed boxes at time 0 and time 48 did not differ. The concentrations of VEN and OXA in water samples from the control group were below the limit of detection (see Table 2).

Table 2. The concentration of VEN and OXA in the water at time 0 (control, exposed) and after 48 h of exposure (control, exposed) ($\alpha = 0.05$). Data are presented as mean ± standard deviation.

Tested Compound	Group	n	Time 0 (μg L^{-1})	Time 48 (μg L^{-1})	Paired *t*-test	*p*
Venlafaxine	exposed	4	0.7 ± 0.0	0.7 ± 0.0	0.927	0.42
	control	4	<0.02	<0.02	---	---
Oxazepam	exposed	3	1.3 ± 0.3	1.2 ± 0.2	0.983	0.43
	control	3	<0.01	<0.01	---	---

2.4. Behavioral Data Acquisition

The exposed crayfish were individually placed in circular plastic tanks (280 mm in diameter), with 2 L of aged tap water and 200 mL of fine sand (<1 mm). In total, 110 and 120 crayfish were used for video-tracking in the VEN and OXA experiments, respectively. Stocked crayfish were video-tracked using a digital video camera (Sony HDR-CX240, Sony, Tokyo, Japan) in trials of 20 parallel tracked tanks (10 control and 10 exposed animals), i.e., 6 trials were done for each compound. Half of the trials were made without shelter, while the other half were conducted with shelter (consisting of halved ceramic plant pots of a 60 mm entry width and a depth of 50 mm). Shelter is an essential resource of crayfish, being nocturnal animals which are usually only active for a period throughout the day, and affecting shelter use can make crayfish more prone to predation or cannibalism. After the video recording, the presence of glair glands (a mark of readiness for reproduction) was also recorded in the used crayfish, due to the possible consequences of upcoming reproduction on their behavior.

The video-recording lasted for 4 h. Light was provided, as permanent indirect illumination, by fluorescent tubes (daylight, 2310 lm). Video-recordings were analyzed later using EthoVision® XT

13.0 software (Noldus Information Technology by Wageningen, The Netherlands) with a multiple-arena module. The distance moved (cm), activity (percentage of time when crayfish locomotion was detected), and velocity (cm s^{-1}) were evaluated. When conditions of shelter were present, the software also revealed results about the time spent inside/outside the shelter.

2.5. Statistical Analysis

A chi-square test was used for numbers of molted and spawned crayfish in comparison with control ones. The Kolmogorov-Smirnov normality test was done for the entire data set. The homogeneity of variances was tested using the Bartlett test for the parameters of behavioral patterns. The concentrations of the tested compound at time 0 and time 48 were compared through paired *t*-tests. A *t*-test for independent samples was used to compare the size and weight of the animals used in the exposed and control groups. The distance moved, velocity, activity, and time spent outside the shelter (replicate groups as a random factor, exposure as a fixed factor), were analyzed through factorial ANOVA. The null hypothesis was rejected at $\alpha = 0.05$. The data were statistically analyzed by Statistica 12.0 software (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Venlafaxine

No significant differences were detected in VEN-exposed crayfish in comparison with control animals in set-ups both with and without available shelter (Table 3). The only effect detected was that of developed glair glands in the set-up with shelter on activity ($F_{1,44} = 6.95$, $p = 0.012$) and time spent outside the shelter ($F_{1,44} = 4.94$, $p = 0.031$). No values are recorded for crayfish without shelter due to the absence of glair glands (Table 4). Data are shown in Tables 3 and 4.

Table 3. The values of the distance moved, velocity, activity, and time spent outside the shelter in crayfish exposed to venlafaxine and in control crayfish, in set-ups with and without available shelter. Data are shown as mean ± standard deviation.

Compound	Shelter Available	Distance Moved (cm)	Velocity (cm s^{-1})	Activity (%)	Time Spent Outside the Shelter (%)
Venlafaxine	no	5184 ± 367	0.39 ± 0.03	69.9 ± 1.8	---
Control	no	5195 ± 382	0.38 ± 0.03	67.8 ± 2.2	---
Venlafaxine	yes	1569 ± 339	0.65 ± 0.07	59.3 ± 5.9	27.0 ± 5.5
Control	yes	1618 ± 363	0.67 ± 0.05	66.0 ± 5.5	24.9 ± 6.5

Table 4. The values of the distance moved, velocity, activity, and time spent outside the shelter in crayfish exposed to venlafaxine and in control crayfish in accordance with the presence of glair glands, in set-ups with and without available shelter. Data are shown as mean ± standard deviation.

Compound	Shelter Available	Glair Glands	Distance Moved (cm)	Velocity (cm s^{-1})	Activity (%)	Time Spent Outside the Shelter (%)
Venlafaxine	no	yes	---	---	---	---
		no	5184 ± 367	0.39 ± 0.03	69.9 ± 1.8	---
Control		yes	---	---	---	---
		no	5195 ± 382	0.38 ± 0.03	67.8 ± 2.2	---
Venlafaxine	yes	yes	1397 ± 571	0.78 ± 0.10	76.0 ± 5.3	14.8 ± 6.1
		no	1676 ± 542	0.62 ± 0.17	48.9 ± 8.0	34.7 ± 7.6
Control		yes	1279 ± 693	0.92 ± 0.17	76.4 ± 7.8	12.3 ± 8.3
		no	1777 ± 660	0.51 ± 0.07	61.1 ± 7.5	30.7 ± 8.5

3.2. Oxazepam

In the set-up without available shelter, crayfish moved longer distances ($F_{1,56}$ = 4.17, p = 0.046) and showed higher activity ($F_{1,56}$ = 4.75, p = 0.034) when exposed to OXA, compared to the control group. The effect of glair glands was observed only in the control animals in both the distance moved ($F_{1,56}$ = 5.73, p = 0.024) and activity ($F_{1,56}$ = 6.92, p = 0.013). In OXA-exposed animals, this effect was not so obvious and no differences in either the distance moved ($F_{1,56}$ = 0.20, p = 0.656) or activity ($F_{1,56}$ = 1.36, p = 0.248) were revealed. Similarly, no significant difference was revealed in the velocity, either between the control and exposed animals or between the animals with and without glair glands (Figures 1 and 2).

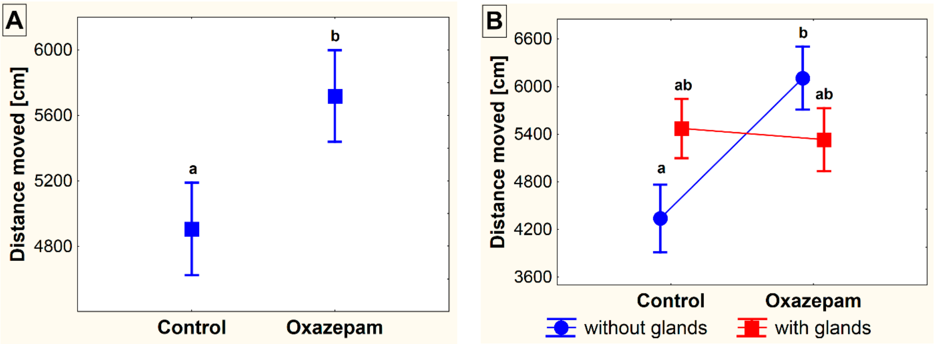

Figure 1. The distance moved (in cm) of marbled crayfish (*Procambarus virginalis*, Lyko 2017) exposed to oxazepam (~1 μg L^{-1}) and of the control animals in the conditions without available shelter (**A**). The differences detected between the groups of crayfish and in accordance with the presence of developed glair glands (**B**). The different superscripts show significant differences (α = 0.05) among groups. Data are presented as mean ± standard error of mean.

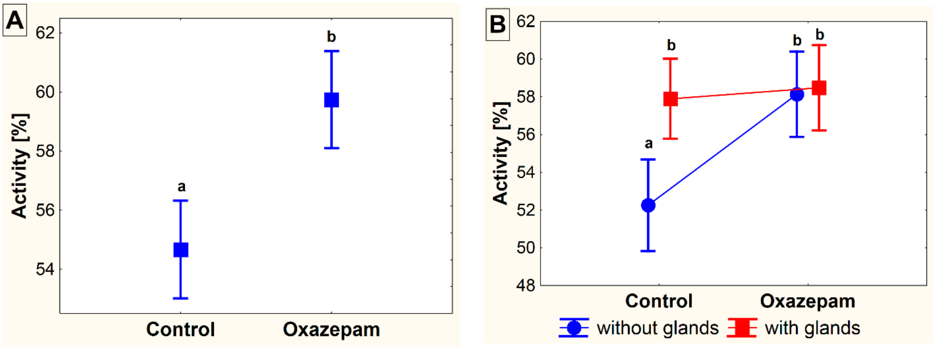

Figure 2. The activity (in %) of marbled crayfish (*Procambarus virginalis*, Lyko 2017) exposed to oxazepam (~1 μg L^{-1}) and control crayfish in the conditions without available shelter (**A**). The differences detected between the groups of crayfish in accordance with the presence of developed glair glands (**B**). The different superscripts show significant differences (α = 0.05) among groups. Data are presented as mean ± standard error of mean.

In the set-up with available shelter, no differences were detected between the control and OXA-exposed animals. Only the effect of glair glands was detected in all of the parameters observed; the distance moved ($F_{1,56}$ = 8.74, p = 0.005), velocity ($F_{1,56}$ = 4.24, p = 0.044), activity ($F_{1,56}$ = 9.47, p = 0.003), and the time spent outside the shelter ($F_{1,56}$ = 6.51, p = 0.014).

Data are summarized in Tables 5 and 6.

Table 5. The values of the distance moved, velocity, activity, and time spent outside the shelter in crayfish exposed to oxazepam and in control crayfish, in set-ups with and without available shelter. Data are shown as mean ± standard deviation.

Compound	Shelter Available	Distance Moved (cm)	Velocity (cm s^{-1})	Activity (%)	Time Spent Outside the Shelter (%)
Oxazepam	yes	1848.1 ± 312.7	0.39 ± 0.03	18.7 ± 2.2	29.3 ± 3.2
Control	yes	1852.6 ± 256.8	0.43 ± 0.03	18.6 ± 2.6	28.9 ± 3.9

Table 6. The values of the distance moved, velocity, activity, and time spent outside the shelter in crayfish exposed to oxazepam and in control crayfish in accordance with the presence of glair glands, in set-ups with and without available shelter. Data are shown as mean ± standard deviation.

Compound	Shelter Available	Glair Glands	Distance Moved (cm)	Velocity (cm s^{-1})	Activity (%)	Time Spent Outside the Shelter (%)
Oxazepam	no	yes	5515.2 ± 360.2	0.39 ± 0.02	59.5 ± 2.9	---
		no	5967.7 ± 642.7	0.43 ± 0.05	60.2 ± 2.4	---
Control		yes	5471.6 ± 378.1	0.40 ± 0.03	52.3 ± 1.8	---
		no	4570.5 ± 160.8	0.35 ± 0.02	57.9 ± 2.1	---
Oxazepam	yes	yes	2382.9 ± 606.3	0.42 ± 0.04	22.8 ± 3.9	34.1 ± 5.4
		no	1380.2 ± 210.7	0.35 ± 0.03	15.1 ± 2.2	25.1 ± 3.6
Control		yes	2483.9 ± 405.1	0.47 ± 0.04	24.7 ± 4.4	36.8 ± 6.7
		no	1221.2 ± 228.4	0.38 ± 0.03	12.5 ± 2.0	21.0 ± 3.0

3.3. Life History Traits

During the exposure period, the number of molted crayfish did not vary significantly in both VEN ($\chi^2 = 0.08$, $p = 0.783$) and OXA ($\chi^2 = 0.72$, $p = 0.398$) exposed crayfish compared to control ones. However, the number of crayfish which spawned eggs during the exposure period was slightly lower but not statistically significant ($\chi^2 = 2.81$, $p = 0.094$) in the VEN-exposed group compared to the control group. The number of spawned animals in the OXA-exposed group did not differ from the controls ($\chi^2 = 0.15$, $p = 0.703$). There was no reported mortality (Table 7).

Table 7. The total number of molted, spawned, and dead crayfish in the control and exposed groups during the exposure period to venlafaxine and oxazepam.

Pharmaceutical	Group	Molted (n)	Spawned (n)	Dead (n)
Venlafaxine	control	61	4	0
	exposed	58	1	0
Oxazepam	control	12	3	0
	exposed	9	4	0

4. Discussion

Pharmaceuticals accessing natural ecosystems via sewage water treatment plant effluents [5,33] are reported as drivers of ecological changes [18,34], and psychotropic drugs are often confirmed as inducing behavioral changes in fish and other aquatic invertebrates [13,35]. Knowledge about the behavioral endpoint of these drugs is still too scarce to summarize their ecological consequences [13].

The present study assessed the behavioral effects of two psychoactive compounds, VEN and OXA, on clonal marbled crayfish exposed to dilute concentrations which can be found in natural conditions. The study also follows up on previous studies [13,36] exploring the compound-specific effects on the behavior of a model invertebrate in comparable, defined conditions. The results observed again confirmed that the low concentrations observed in natural conditions can have important consequences. Exploratory behavior, expressed as the activity and distance moved, should affect the wasting of energy, leading to a shorter life span in more active individuals [37]. In addition, the visibility of an animal to predators, especially in conditions of invaded ecosystems, can have effects on food resources, which are under greater pressure due to the higher activity [38,39]. Such alterations can then change the ecosystem's functioning [40,41]. The elevated activity, followed by altered foraging behavior can, therefore, lead to the breakdown of food chains, loss of biodiversity, and ecosystem instability [41,42].

Our experimental data helped to identify the environmental risks of OXA, but surprisingly no behavioral changes have been observed for the antidepressant VEN. Venlafaxine effects are reported at higher concentrations [43] compared to other antidepressants, citalopram [13] and sertraline [36], which were tested at the same (or even lower concentrations in the case of fluoxetine and sertraline) [44].

Earlier research demonstrated different, or even opposite results among similar compounds or compounds with similar modes of action, as well as among different taxa observed [21,35,45]. This can be due, in part, to different experimental conditions, ways of application (injection, oral application, passively from a water solution), doses (lower than in environmentally detected, environmentally relevant, elevated), and approaches used to determine the behavioral effects. We, therefore, tried to minimize the variables and use similar, relatively easy approaches for the observation, an application imitating the environmental intoxication from the dilute solutions, and the use of genetically uniform marbled crayfish, to erase the effect of different genotypes. However, the results presented, compared with previous ones produced with the same methodology, reveal again the differences among individual compounds. In fact, there is a need to investigate the mechanisms of action of these substances in detail and to elaborate on studies dealing with different mixtures of pharmaceuticals as they act simultaneously on aquatic biota [46].

The present study can be helpful for the set-up of new studies aimed not only at behavioral patterns but at life history traits, including reproduction. Behavioral alterations provide the potential to assess the risk of ecological effects of pharmaceutical products, thus it might be useful for the generalization of the impacts on the aquatic environment. In our present study, we also observed changed behavior in crayfish with developed glair glands, indicative of the preparation for reproduction [37,47]. To safeguard their future offspring, crayfish limit their activity, which was expressed as a lower distance moved and less activity. In the exposed animals, this pattern was missing, which can affect the reproductive success in a population due to a higher risk of predation. Life history traits and affected reproduction have also been confirmed from other studies with several psychoactive drugs on different aquatic taxa [36,43,48].

To summarize, our results together with those previously published show high variability in the type, strength, and direction of the effects of pharmaceuticals on aquatic biota. The use of passive application due to exposure through diluted, environmentally relevant concentrations of tested compounds seems to be appropriate for the assessment of their environmental effects. The main pollutants should also be tested individually, as well as their mixtures as found in field sampling [46]. The large number of compounds in surface waters and their different/specific modes of action is motivation for the better understanding of their real ecological impact on ecosystems.

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Ethical code: No specific permissions were required for the locations and activities involved in this study. All experiments were conducted according to the principles of the Ethics Committee for the Protection of Animals in Research of the University of South Bohemia in České Budějovice, FFPW, Vodňany, based on the EU-harmonized Animal Welfare Act of the Czech Republic, No. 53100/2013-MZE-17214.

References

1. Burkina, V.; Zlabek, V.; Zamaratskaia, G. Effects of pharmaceuticals present in aquatic environment on Phase I metabolism in fish. *Environ. Toxicol. Pharmacol.* **2015**, *40*, 430–444. [CrossRef] [PubMed]
2. Ebele, A.J.; Abdallah, M.A.-E.; Harrad, S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg. Contam.* **2017**, *3*, 1–6. [CrossRef]
3. Collado, N.; Rodriguez-Mozaz, S.; Gros, M.; Rubirola, A.; Barcelo, D.; Comas, J.; Rodriguez-Roda, I.; Buttiglieri, G. Pharmaceuticals occurrence in a WWTP with significant industrial contribution and its input into the river system. *Environ. Pollut.* **2014**, *185*, 202–212. [CrossRef] [PubMed]
4. Frederic, O.; Yves, P. Pharmaceuticals in hospital wastewater: Their ecotoxicity and contribution to the environmental hazard of the effluent. *Chemosphere* **2014**, *115*, 31–39. [CrossRef] [PubMed]
5. Golovko, O.; Kumar, V.; Fedorova, G.; Randak, T.; Grabic, R. Removal and seasonal variability of selected analgesics/anti-inflammatory, anti-hypertensive/cardiovascular pharmaceuticals and UV filters in wastewater treatment plant. *Environ. Sci. Pollut. Res.* **2014**, *21*, 7578–7585. [CrossRef] [PubMed]
6. Boxall, A.B.A.; Rudd, M.A.; Brooks, B.W.; Caldwell, D.J.; Choi, K.; Hickmann, S.; Innes, E.; Ostapyk, K.; Staveley, J.P.; Verslycke, T.; et al. Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? *Environ. Health Perspect.* **2012**, *120*, 1221–1229. [CrossRef] [PubMed]
7. Huerta, B.; Rodriguez-Mozaz, S.; Barcelo, D. Pharmaceuticals in biota in the aquatic environment: Analytical methods and environmental implications. *Anal. Bioanal. Chem.* **2012**, *404*, 2611–2624. [CrossRef]
8. Schulz, M.; Iwersen-Bergmann, S.; Andresen, H.; Schmoldt, A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Crit. Care* **2012**, *16*, R136. [CrossRef]
9. Fedorova, G.; Golovko, O.; Randak, T.; Grabic, R. Storage effect on the analysis of pharmaceuticals and personal care products in wastewater. *Chemosphere* **2014**, *111*, 55–60. [CrossRef]
10. Yadav, M.K.; Short, M.D.; Aryal, R.; Gerber, C.; van den Akker, B.; Saint, C.P. Occurrence of illicit drugs in water and wastewater and their removal during wastewater treatment. *Water Res.* **2017**, *124*, 713–727. [CrossRef]
11. Marti, E.; Huerta, B.; Rodriguez-Mozaz, S.; Barcelo, D.; Marce, R.; Balcazar, J.L. Abundance of antibiotic resistance genes and bacterial community composition in wild freshwater fish species. *Chemosphere* **2018**, *196*, 115–119. [CrossRef] [PubMed]
12. Stankiewicz, A.; Giebultowicz, J.; Stankiewicz, U.; Wroczynski, P.; Nalecz-Jawecki, G. Determination of selected cardiovascular active compounds in environmental aquatic samples—Methods and results, a review of global publications from the last 10 years. *Chemosphere* **2015**, *138*, 642–656. [CrossRef] [PubMed]
13. Buric, M.; Grabicova, K.; Kubec, J.; Kouba, A.; Kuklina, I.; Kozak, P.; Grabic, R.; Randak, T. Environmentally relevant concentrations of tramadol and citalopram alter behaviour of an aquatic invertebrate. *Aquat. Toxicol.* **2018**, *200*, 226–232. [CrossRef] [PubMed]
14. Fong, P.P.; Ford, A.T. The biological effects of antidepressants on the molluscs and crustaceans: A review. *Aquat. Toxicol.* **2014**, *151*, 4–13. [CrossRef]
15. Hyttel, J. Pharmacological Characterization of Selective Serotonin Reuptake Inhibitors (SSRIs). *Int. Clin. Psychopharm.* **1994**, *9*, 19–26. [CrossRef]
16. Skerritt, J.H.; Johnston, G.A. Enhancement of GABA binding by benzodiazepines and related anxiolytics. *Eur. J. Pharmacol.* **1983**, *89*, 193–198. [CrossRef]

17. Rosi-Marshall, E.J.; Snow, D.; Bartelt-Hunt, S.L.; Paspalof, A.; Tank, J.L. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. *J. Hazard. Mater.* **2015**, *282*, 18–25. [CrossRef] [PubMed]
18. Grabicova, K.; Grabic, R.; Blaha, M.; Kumar, V.; Cerveny, D.; Fedorova, G.; Randak, T. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. *Water Res.* **2015**, *72*, 145–153. [CrossRef]
19. Klaminder, J.; Brodin, T.; Sundelin, A.; Anderson, N.J.; Fahlman, J.; Jonsson, M.; Fick, J. Long-Term Persistence of an Anxiolytic Drug (Oxazepam) in a Large Freshwater Lake. *Environ. Sci. Technol.* **2015**, *49*, 10406–10412. [CrossRef]
20. Lagesson, A.; Fahlman, J.; Brodin, T.; Fick, J.; Jonsson, M.; Bystrom, P.; Klaminder, J. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web—Insights from a field experiment. *Sci. Total. Environ.* **2016**, *568*, 208–215. [CrossRef]
21. Brodin, T.; Piovano, S.; Fick, J.; Klaminder, J.; Heynen, M.; Jonsson, M. Ecological effects of pharmaceuticals in aquatic systems-impacts through behavioural alterations. *Philos. Trans. R. Soc. B* **2014**, *369*. [CrossRef] [PubMed]
22. Brodin, T.; Nordling, J.; Lagesson, A.; Klaminder, J.; Hellstrom, G.; Christensen, B.; Fick, J. Environmental relevant levels of a benzodiazepine (oxazepam) alters important behavioral traits in a common planktivorous fish, (*Rutilus rutilus*). *J. Toxicol. Environ. Health A* **2017**, *80*, 963–970. [CrossRef] [PubMed]
23. Pal, R.; Megharaj, M.; Kirkbride, K.P.; Naidu, R. Illicit drugs and the environment—A review. *Sci. Total Environ.* **2013**, *463*, 1079–1092. [CrossRef] [PubMed]
24. Nielsen, S.V.; Kellner, M.; Henriksen, P.G.; Olsen, H.; Hansen, S.H.; Baatrup, E. The psychoactive drug Escitalopram affects swimming behaviour and increases boldness in zebrafish (*Danio rerio*). *Ecotoxicology* **2018**, *27*, 485–497. [CrossRef] [PubMed]
25. Hossain, M.S.; Patoka, J.; Kouba, A.; Buric, M. Clonal crayfish as biological model: A review on marbled crayfish. *Biologia* **2018**, *73*, 841–855. [CrossRef]
26. Kubec, J.; Kouba, A.; Buřič, M. Communication, behaviour, and decision making in crayfish: A review. *Zool. Anz.* **2018**, *278*, 28–37. [CrossRef]
27. Barry, M.J. Effects of fluoxetine on the swimming and behavioural responses of the Arabian killifish. *Ecotoxicology* **2013**, *22*, 425–432. [CrossRef]
28. Jonsson, M.; Fick, J.; Klaminder, J.; Brodin, T. Antihistamines and aquatic insects: Bioconcentration and impacts on behavior in damselfly larvae (Zygoptera). *Sci. Total Environ.* **2014**, *472*, 108–111. [CrossRef]
29. Cunha, D.L.; Mendes, M.P.; Marques, M. Environmental risk assessment of psychoactive drugs in the aquatic environment. *Environ. Sci. Pollut. Res.* **2019**, *26*, 78–90. [CrossRef]
30. Grabic, R.; Fick, J.; Lindberg, R.H.; Fedorova, G.; Tysklind, M. Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry. *Talanta* **2012**, *100*, 183–195. [CrossRef]
31. Sehonova, P.; Svobodova, Z.; Dolezelova, P.; Vosmerova, P.; Faggio, C. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: A review. *Sci. Total Environ.* **2018**, *631–632*, 789–794. [CrossRef] [PubMed]
32. Courtney, D.B. Selective serotonin reuptake inhibitor and venlafaxine use in children and adolescents with major depressive disorder: A Systematic Review of Published Randomized Controlled Trials. *Can. J. Psychiatry* **2004**, *49*, 557–563. [CrossRef] [PubMed]
33. Loos, R.; Carvalho, R.; Antonio, D.C.; Cornero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47*, 6475–6487. [CrossRef] [PubMed]
34. Grabicova, K.; Grabic, R.; Fedorova, G.; Fick, J.; Cerveny, D.; Kolarova, J.; Turek, J.; Zlabek, V.; Randak, T. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. *Water Res.* **2017**, *124*, 654–662. [CrossRef] [PubMed]
35. Brodin, T.; Fick, J.; Jonsson, M.; Klaminder, J. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. *Science* **2013**, *339*, 814–815. [CrossRef] [PubMed]
36. Hossain, M.S.; Kubec, J.; Grabicova, K.; Grabic, R.; Randak, T.; Guo, W.; Kouba, A.; Buřič, M. Environmentally relevant concentrations of methamphetamine and sertraline modify the behavior and life history traits of an aquatic invertebrate. *Sci. Total Environ.* **2019**, under review.

37. Gherardi, F. Behaviour. In *Biology of Freshwater Crayfish*; Holdich, D.M., Ed.; Blackwell Science: Oxford, UK, 2002; pp. 258–290.
38. Holdich, D.M. Background and functional morphology. In *Biology of Freshwater Crayfish*; Holdich, D.M., Ed.; Blackwell Science: Oxford, UK, 2002; pp. 3–29.
39. Craddock, N.; Jones, I. Genetics of bipolar disorder. *J. Med. Genet.* **1999**, *36*, 585–594. [CrossRef]
40. Manning, A.; Dawkins, M.S. *An Introduction to Animal Behaviour*, 6th ed.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2012; p. ix, 458p.
41. Schmitz, O.J. Predator diversity and trophic interactions. *Ecology* **2007**, *88*, 2415–2426. [CrossRef]
42. Duffy, J.E.; Cardinale, B.J.; France, K.E.; McIntyre, P.B.; Thebault, E.; Loreau, M. The functional role of biodiversity in ecosystems: Incorporating trophic complexity. *Ecol. Lett.* **2007**, *10*, 522–538. [CrossRef]
43. Fong, P.P.; Bury, T.B.; Dworkin-Brodsky, A.D.; Jasion, C.M.; Kell, R.C. The antidepressants venlafaxine (“Effexor”) and fluoxetine (“Prozac”) produce different effects on locomotion in two species of marine snail, the oyster drill (*Urosalpinx cinerea*) and the starsnail (*Lithopoma americanum*). *Mar. Environ. Res.* **2015**, *103*, 89–94. [CrossRef]
44. Bossus, M.C.; Guler, Y.Z.; Short, S.J.; Morrison, E.R.; Ford, A.T. Behavioural and transcriptional changes in the amphipod *Echinogammarus marinus* exposed to two antidepressants, fluoxetine and sertraline. *Aquat. Toxicol.* **2014**, *151*, 46–56. [CrossRef] [PubMed]
45. Imeh-Nathaniel, A.; Rincon, N.; Orfanakos, V.B.; Brechtel, L.; Wormack, L.; Richardson, E.; Huber, R.; Nathaniel, T.I. Effects of chronic cocaine, morphine and methamphetamine on the mobility, immobility and stereotyped behaviors in crayfish. *Behav. Brain Res.* **2017**, *332*, 120–125. [CrossRef] [PubMed]
46. Di Lorenzo, T.; Castaño-Sánchez, A.; Di Marzio, W.D.; García-Doncel, P.; Martínez, L.N.; Galassi, D.M.P.; Iepure, S. The role of freshwater copepods in the environmental risk assessment of caffeine and propranolol mixtures in the surface water bodies of Spain. *Chemosphere* **2019**, *220*, 227–236. [CrossRef] [PubMed]
47. Reynolds, J.; Holdich, D. Growth and reproduction. In *Biology of Freshwater Crayfish*; Holdich, D.M., Ed.; Blackwell Science: Oxford, UK, 2002; pp. 152–191.
48. Carfagno, G.L.F.; Fong, P.P. Growth Inhibition of Tadpoles Exposed to Sertraline in the Presence of Conspecifics. *J. Herpetol.* **2014**, *48*, 571–576. [CrossRef]

CHAPTER 5

EFFECTS OF S-METOLACHLOR AND ITS DEGRADATION PRODUCT METOLACHLOR OA ON MARBLED CRAYFISH (*PROCAMBARUS VIRGINALIS*)

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Effects of S-metolachlor and its degradation product metolachlor OA on marbled crayfish (*Procambarus virginalis*)

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HIGHLIGHTS

- S-metolachlor and its metabolite metolachlor OA are frequently detected in rivers.
- Their harmful effects were studied on the non-target invertebrate at an environmentally relevant concentration.
- Exposure to these compounds was associated with changes in biochemical parameters, histology, and behaviour in crayfish.
- Damage was not reversed by 28 days recovery in clean water.

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ABSTRACT

Increasing production of energy crops in Europe, mainly maize and rapeseed, has altered patterns of pesticide use in recent decades. The long-term effects of S-metolachlor (S-M) and of its metabolite metolachlor OA (M-OA) at the environmentally relevant concentration of 4.2 $\mu g\ L^{-1}$ and at 42 $\mu g\ L^{-1}$ (tenfold concentration) on marbled crayfish (*Procambarus virginalis*) were evaluated in a 28-day exposure and after a subsequent 28-day recovery period. Indicators assessed were behaviour; biochemical haemolymph profile; oxidative and antioxidant parameters of gill, hepatopancreas, and muscle; and histology of hepatopancreas and gill. Results showed biochemical haemolymph profile (lactate, alanine aminotransferase, aspartate aminotransferase, inorganic phosphate), lipid peroxidation in hepatopancreas, and antioxidant parameters (catalase, reduced glutathione, glutathione S-transferase) of hepatopancreas and gill of crayfish exposed to S-M and M-OA to significantly differ from controls ($P < 0.01$). Antioxidant biomarker levels remained different from controls after a 28-day recovery period. Differences in behaviour including speed of movement and velocity, and histopathological damage to gill and hepatopancreas were associated with S-M and M-OA exposure and persisted after 28 days in S-M- and M-OA-free water. Results suggest harmful effects of low concentrations of S-M and its metabolite M-OA on non-target organisms and provide information for assessing their effects at environmentally relevant concentrations.

1. Introduction

Recent decades, with increasing urbanization and population and rapid economic development, have seen frequent incidents of pollution and deterioration of water quality with impacts on the aquatic ecosystem and the whole environment (Yao et al., 2015; Aliko et al., 2018; Kong et al., 2018; Faggio et al., 2018; Sehonova et al., 2018). The most common synthetic pollutants entering the water are pharmaceuticals, pesticides, chemical fertilizers, detergents, petrochemicals, and plastics (Fazio et al., 2012; Faggio et al., 2016; Pagano et al., 2016; Savorelli et al., 2017; White, 2017; Burgos Aceves et al., 2018a; b; Guzzetti et al., 2018;

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Hodkovicova et al., 2019).

Chloroacetanilide herbicides are commonly used for control of broadleaf grasses and annual weeds in maize, sunflowers, and sugar beets (Chromcova et al., 2015; Fiorino et al., 2018; Plhalova et al., 2017). S-metolachlor (S-M) introduced in the late 1990s is one of the ten most commonly used pre- and post-emergent herbicides in Europe and the United States (Yun et al., 2006; EPA, 2011; Chen et al., 2017). S-metolachlor is physically and chemically equivalent to metolachlor, but is enriched with the S-isomer that increases herbicidal activity (Zemolin et al., 2014). It is highly water soluble (480 mg L^{-1} at 20 °C), has a half-life of 70 days with exposure to sunlight and 30 days at pH 5–9, and is considered persistent. Its high use and relatively low adsorption in soils result in considerable leaching into groundwater, threatening water quality (Guelfi et al., 2018). Metolachlor OA (M-OA) is the main metabolite of metolachlor and S-M (Arthur et al., 2000).

S-metolachlor and M-OA are frequently detected in natural waters (Maronic et al., 2018) and have been reported at concentrations from 0.01 to 5.30 μg L^{-1} in European surface waters (Trask et al., 2003; Roubeix et al., 2012; CHMI , 2018). In the United States, concentrations have been reported at up to 143 μg L^{-1} in streams and rivers (Battaglin et al., 2000). Metolachlor OA had been detected at concentrations from 0.10 to 4.20 μg L^{-1} in surface waters of Europe (CHMI , 2018).

Even at environmentally relevant concentrations, S-M and M-OA may pose a potential hazard to aquatic organisms (Maronic et al., 2018). Most studies have described acute toxicity at high doses, usually mg L^{-1} (Liu and Xiong, 2006; Dobsikova et al., 2011; Neves et al., 2015; Maazouzi et al., 2016; Quintaneiro et al., 2017, 2018). Studies assessing subchronic and chronic exposure to low concentrations of S-M and M-OA on aquatic organisms are scarce. Velisek et al. (2018a) assessed chronic exposure (45 days) to M-OA at 4.2, 42, and 420 μg L^{-1} of early life stages of marbled crayfish. Long-term exposure to low concentrations of pesticides has been associated with behaviour dysfunction, physiological disturbance, histological damage, haematological alterations, biochemical changes, immune suppression, hormone disruption, diminished learning, reproductive abnormalities, and cancer (Faggio et al., 2016; Marino et al., 2016; Srivastava et al., 2016; Velisek et al., 2018a, b).

The goals of the present study were to assess the effects of S-metolachlor and its metabolite metolachlor OA at an environmentally relevant concentration, and a higher concentration, on the behaviour, haemolymph biochemical profile, enzymes involved in the oxidative stress response, and antioxidant biomarkers in gill, hepatopancreas, and muscle of marbled crayfish (*Procambarus virginalis*) as well as to evaluate changes to gill and hepatopancreas tissue and to assess recovery from exposure.

2. Materials and methods

2.1. Chemicals and chemical analysis

S-metolachlor (2-Chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]acetamide; purity 98.2%) and metolachlor OA (2-[(2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl) amino]-2-oxoacetic acid; purity ≥ 98.0%) were purchased from Sigma-Aldrich Corporation (USA). Agreement between nominal and actual S-M and M-OA concentrations in water was ensured using liquid chromatography tandem mass spectrometry (LC-MS/MS) according to De Oliveira Arias et al. (2017). In the water samples, the limit of quantification (LOQ) of S-M and M-OA was 0.01–0.02 μg L^{-1}. The concentration of S-M and M-OA in dechlorinated tap water used for crayfish prior to exposure and for control crayfish during the trial was below the LOQ. The concentration of S-M and M-OA in water over the course of 72 h as mean of the five sampling dates compared to the nominal value for all test groups is presented in Table 1.

2.2. Experimental animals

One-hundred-eighty marbled crayfish (*Procambarus virginalis*) mean weight 4.72 ± 1.22 g and carapace length 25.45 ± 2.23 mm, were obtained by laboratory culture. Twelve crayfish were randomly selected and placed into each of fifteen aquaria (5 experimental groups in triplicate) containing 50 L water. They were held for a 14-day acclimation period prior to starting the experiment. The study was conducted in compliance the principles of the Ethical Committee for the Protection of Animals in Research of the University of South Bohemia, FFPW Vodnany.

2.3. Experimental protocol

After acclimatization, crayfish were exposed to the selected concentrations of S-M and M-OA for 28 days. At the conclusion of the exposure and sampling, the remaining crayfish were held for a recovery period of 28 days in fresh water without S-M or M-OA.

Two concentrations of S-M, two concentrations of M-OA, and a control held in fresh tap water were used:

S-M1: 4.2 μg L^{-1} (environmentally relevant concentration, Trask et al., 2003);

S-M2: 42 μg L^{-1} (ten-fold environmentally relevant concentration);

M-OA1: 4.2 μg L^{-1} (environmentally relevant concentration, CHMI , 2018);

M-OA2: 42 μg L^{-1} (ten-fold environmentally relevant concentration);

Control: dechlorinated water only.

The experiment was semistatic, with the bath and exposure solutions changed twice weekly 1 h after feeding. Water quality was monitored daily to maintain dissolved oxygen at 96.5 ± 2.8%, temperature of 21.8 ± 1.8 °C, and pH of 8.1 ± 0.21. The photoperiod was 12L:12D. Crayfish were fed a commercial diet (Crabs Natural, Sera GmbH, Germany) at 1% body weight per day.

Three crayfish from each of the three replicates of each treatment were randomly sampled for analyses on days 14 and 28 of exposure. A third sampling was conducted after 28 days in S-M and M-OA-free water.

2.4. Behaviour

For each tested compound, randomly selected crayfish were subjected to an analysis of behaviour on days 14 and 28 of exposure and after 28 days in pesticide-free water. The trial was conducted in triplicate.

Crayfish (n = 9 per treatment) were individually placed in white cylindrical plastic tanks (diameter 280 mm) with 200 mL fine sand (<1 mm) and 2 L of the appropriate water solution. Animals were video-recorded for 1 h using a video camera (Sony HDR-CX240E, Sony, Japan) and behaviour was evaluated using EthoVision® XT 13 software (Noldus Information Technology, Wageningen, Netherlands). A multiple-arenas model detected simultaneous patterns of crayfish locomotion. Distance moved (cm), velocity (cm s^{-1}), and activity (percentage of time that crayfish locomotion was detected) were recorded.

2.5. Biochemical haemolymph profiles

Following analysis of behaviour patterns, the nine crayfish from each treatment were subjected to biochemical haemolymph profile

Table 1
Concentration of S-metolachlor (S-M) and metolachlor OA (M-OA) in chronic toxicity tests on marbled crayfish (*Procambarus virginalis*).

Group	Sample time (h)	S-metachlor μg L^{-1}	Metachlor-OA μg L^{-1}
Tap water	–	< LOQ	< LOQ
Control	0	< LOQ	< LOQ
	24	< LOQ	< LOQ
	48	< LOQ	< LOQ
	72	< LOQ	< LOQ
S-M1 (4.2 μg L^{-1})	0	4.32 ± 0.15	< LOQ
	24	4.11 ± 0.21	< LOQ
	48	4.08 ± 0.29	< LOQ
	72	3.84 ± 0.18	< LOQ
S-M2 (42 μg L^{-1})	0	41.76 ± 1.22	< LOQ
	24	41.62 ± 1.30	< LOQ
	48	41.02 ± 1.16	< LOQ
	72	40.54 ± 1.06	< LOQ
M-OA1 (4.2 μg L^{-1})	0	< LOQ	4.22 ± 0.11
	24	< LOQ	4.07 ± 0.19
	48	< LOQ	4.00 ± 0.31
	72	< LOQ	3.71 ± 0.16
M-OA2 (42 μg L^{-1})	0	< LOQ	41.06 ± 1.58
	24	< LOQ	41.00 ± 0.98
	48	< LOQ	40.32 ± 1.02
	72	< LOQ	39.28 ± 1.44

Concentrations were measured at the stated sample times immediately post-exchange. The values are expressed as mean ± SD (standard deviation), n = 5. LOQ = limit of quantification.

analyses. Haemolymph was collected from the ventral part of abdomen by syringe with 0.01 mL sodium heparin (Heparin inj., Leciva, Prague, Czech Republic) per 1 mL haemolymph. Haemolymph was centrifuged for 10 min at 10 000×*g* at 4 °C. The biochemical variables measured included glucose (GLU), total protein (TP), ammonia (NH_3), triacylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), lactate (LACT), creatine (CREA), calcium (Ca), magnesium (Mg), inorganic phosphate (PHOS), and alkaline phosphatase (ALP). Measurement of biochemical haemolymph parameters was conducted using the VETTEST 8008 analyser (IDEXX Laboratories Inc., Maine, USA) as described by Kolarova and Velisek (2012).

2.6. Oxidative stress and antioxidant biomarkers

Immediately following haemolymph sampling, the crayfish were killed by ice anaesthesia, and hepatopancreas, abdominal muscle, and gill were removed for the determination of lipid peroxidation and antioxidant biomarkers from each nine crayfish from each group which were used for biochemical haemolymph profile. Samples of hepatopancreas and gill from six crayfish of each group were placed in 10% buffered formalin for histology. Remaining samples were immediately frozen at −80 °C until biochemical analysis. Prior to analysis, the frozen samples were weighed and 50 mM phosphate buffer saline (PBS: 0.8% NaCl; 0.02% KCl; 0.29% $Na_2HPO_4 \cdot 12H_2O$; 0.02% KH_2PO_4 in deionized water, pH 7.2) was added at 1 mL to 100 mg of tissue, and samples were homogenized in a ball homogenizer (TissueLyser II QIAGEN) on ice. This homogenate sample was divided into three parts: 30 μL for protein and 250 μL for lipid peroxidation with the remainder divided for assessment of antioxidant biomarkers.

The level of lipid peroxidation (LPO) was measured by thiobarbituric acid reactive species (TBARS) according to Uchiama and Mihara (1978) modified for micro-plate design with absorbance measured spectrophotometrically at 550 nm (Infinite M200, TECAN) and the concentrations (nmol TBARS mg^{-1} protein) calculated according to the standard calibration generated with malondialdehyde (MDA).

To obtain the post-mitochondrial supernatant for antioxidant biomarker assessment, homogenate was centrifuged at 30 000×*g* for 30 min and 4 °C for superoxide dismutase (SOD) and catalase (CAT) analyses and at 10 000×*g* for 15 min at 4 °C for other antioxidant parameters. Total SOD (SOD; EC 1.15.1.1) activity was determined according to Ewing and Janero (1995) based on inhibition of nitro blue tetrazolium reduction and production of superoxides by nicotinamide adenine dinucleotide and phenazine methosulfate, measured spectrophotometrically at 560 nm and expressed as the amount of enzyme per milligram of protein. Catalase (EC 1.11.1.6) activity was assayed by spectrophotometric measurement at 240 nm of the decomposition rate of hydrogen peroxide (H_2O_2) by CAT according to Aebi (1984), expressed as μmol H_2O_2 per min per mg protein. Glutathione S-transferase (GST; EC 2.5.1.18) activity was measured according to Habig et al. (1974) based on the detection of a conjugate between reduced glutathione and 1-chloro-2,4-dinitrobenzene, a substrate common to all glutathione-transferase isoforms. The reaction was measured at 340 nm and expressed as nmoles of formed product per minute per milligram protein. Reduced glutathione (GSH) level was assayed using the methods of Ellmann (1959) based on the reaction of the thiol-selective Ellmann reagent (2,2-dinitro-5,5-dithiobenzoic acid) with free -SH groups to form a coloured product measured at 420/680 nm with concentration (nmol GSH mg^{-1} protein) calculated according to standard calibrations. These methods were performed with slight modifications by Stara et al. (2016, 2018) for crayfish.

The protein concentration according to Bradford (1976) was determined using bovine serum albumin as a standard for protein calibration with concentration measured spectrophotometrically at 562 nm.

2.7. Histology

Gill and hepatopancreas were fixed in 10% formalin for 24 h, placed in 70% ethanol, and subsequently processed in a Histomaster 2052/1.5 (MDS-group, Germany). Sections were cut from paraffin blocks on a rotary microtome, stained with haematoxylin-eosin in and automatic slide staining system (TISSUE-TEK® DRS™ 2000, SEKURA). Tissues were examined under a light microscope combined with camera system type E-600 (Olympus BX51, Japan).

2.8. Statistical analysis

Data of behaviour parameters were evaluated by the Bartlett test for homogeneity of variances. We analysed differences in distance moved, velocity, and activity level on observation days during exposure and after depuration using Kruskal-Wallis test for non-parametric post-hoc comparisons for all behaviour parameters as normal distribution was not assumed. Differences among the tested groups were evaluated using Statistica v. 12.0 for Windows (StatSoft).

3. Results

3.1. Behaviour

Crayfish showed no significant differences or deviations in assessed behaviour throughout the trial compared with control. Behaviour at days 14 and 28 of exposure and 28 days depuration was summarized for comparison (Table 2). Control crayfish showed no significant differences in assessed behaviours at the three sampling times (Fig. 1A).

Exposure to S-M at 4.2 and 42 µg L^{-1} was not associated with ($P > 0.05$) differences in behaviour between days 14 and 28, but we observed significantly lower values ($P < 0.05$) in distance moved and velocity after 28 days in S-M-free water compared to the trial period (Fig. 1B and C).

Crayfish exposed to M-OA at 4.2 µg L^{-1} and 42 µg L^{-1} showed no significant differences in distance moved, velocity, or activity level between days 14 and 28 of exposure. Both concentrations were associated with a significant decrease ($P < 0.05$) in distance moved and velocity after 28 days in M-OA-free water compared to during the trial period (Fig. 1D and E).

3.2. Biochemical haemolymph profile

Results of haemolymph biochemical profiling are given in Table 3. The S-M exposure at 4.2 µg L^{-1} showed no differences from controls. S-metolachlor at 42 µg L^{-1} was associated with significantly higher ($P < 0.01$) levels of LACT, ALT, and AST and significantly lower ($P < 0.01$) PHOS than observed in controls after 14 and 28 days exposure.

The M-OA1 group showed significantly higher ($P < 0.01$) ALT, AST, and NH_3 levels after 28 days than was observed in controls, at 14 days exposure, and after the 28-day recovery period. Crayfish in this group had significantly higher ($P < 0.01$) LACT and significantly lower ($P < 0.01$) PHOS than controls at 14 and 28 days exposure and at the conclusion of the recovery period. Crayfish in group M-OA2 showed significantly higher ($P < 0.01$) GLU after 28 days compared to control, 14 days exposure, and the 28 day recovery period. They contained significantly higher ($P < 0.01$) LACT, ALT, AST, and NH_3 with significantly lower ($P < 0.01$) PHOS at 14 days and 28 days compared to controls and after the 28 day recovery period.

The remaining indicators, LDH, CK, TP, TAG, CREA, ALP, Mg, and Ca were similar among groups throughout the test. Biochemical haemolymph parameters were at values comparable to control after 28 days in S-M and M-OA-free water.

3.3. Oxidative stress and antioxidant responses

Exposure to S-M and M-OA was associated with significant differences ($P < 0.01$) in oxidative stress and antioxidant biomarkers of hepatopancreas and gill (Table 4). An effect on muscle was not observed. The S-M1 group exhibited significant differences ($P < 0.01$) from controls in CAT activity in hepatopancreas at 14 days and 28 days exposure and in the gill after the recovery period. The GSH level was significantly lower ($P < 0.01$) in hepatopancreas at 14 and 28 days compared to control. Experimental group S-M2 demonstrated a significantly ($P < 0.01$) higher CAT activity in hepatopancreas at 28 days and significantly lower GSH level in hepatopancreas after 14 and 28 days as well as in gill after the recovery period compared to controls and exposed samples. Exposure to M-OA at 4.2 µg L^{-1} led to significantly lower ($P < 0.01$) CAT activity in gill after 14 days compared with control. The GSH level in M-OA1 was significantly lower ($P < 0.01$) than controls in hepatopancreas after 14 and 28 days. The GST activity was significantly lower ($P < 0.01$) in gill after 28 days exposure and the 28-day recovery period compared to activity level at 14 days. The M-OA2 group exhibited significantly higher ($P < 0.01$) LPO in hepatopancreas at 28 days compared with control, the level at 14 days, and after the recovery period. The M-OA2 exposure led to significant differences ($P < 0.01$) in CAT activity in hepatopancreas throughout the trial compared to controls. In gill, CAT activity was found to be lower than controls and exposed crayfish after 28 days. The GSH level and GST activity in gill were significantly reduced ($P < 0.01$) in all tested groups at the end the recovery period compared to exposure sampling times. No significant differences were found in SOD activity.

3.4. Histology

No differences were observed in structure of hepatopancreas of controls and experimental group S-M1 sampled on day 14 of exposure (Fig. 2A). Marked differences from controls including enlargement of the intertubular space packed with eosinophilic substance and haemolytic infiltration were recorded in group S-M1 on day 28 of exposure as well as in group S-M2 on day 14 of exposure (Fig. 2B). The most pronounced abnormalities were observed in group S-M2 on day 28 of exposure. Observations included focal disorganisation to disintegration of the tubular epithelial structure (Fig. 2C). Replication of embryonic and fibrillar cells, characteristic of tissue reparation, were detected in both S-M1 and S-M2 after the recovery period (Fig. 2D). Similar

Table 2
Behaviour parameters of the marbled crayfish (*Procambarus virginalis*), summary of the trial period at 14 and 28 days exposure, and after 28 days in S-metolachlor (S-M) and metolachlor OA (M-OA)-free water. H – Kruskal-Wallis test; n = 27.

Group	Behaviour parameters					
	Distance moved (cm)		Velocity (cm s^{-1})		Activity (%)	
	statistic H	*P*-value	statistic H	*P*-value	statistic H	*P*-value
Control	2.53	0.283	0.67	0.716	3.27	0.195
S-M1 (4.2 µg L^{-1})	7.57	0.023	7.11	0.029	1.37	0.504
S-M2 (42 µg L^{-1})	10.39	0.006	9.23	0.010	5.89	0.053
M-OA1 (4.2 µg L^{-1})	38.26	$<10^{-4}$	35.37	$<10^{-4}$	5.60	0.061
M-OA2 (42 µg L^{-1})	40.08	$<10^{-4}$	33.89	$<10^{-4}$	5.69	0.058

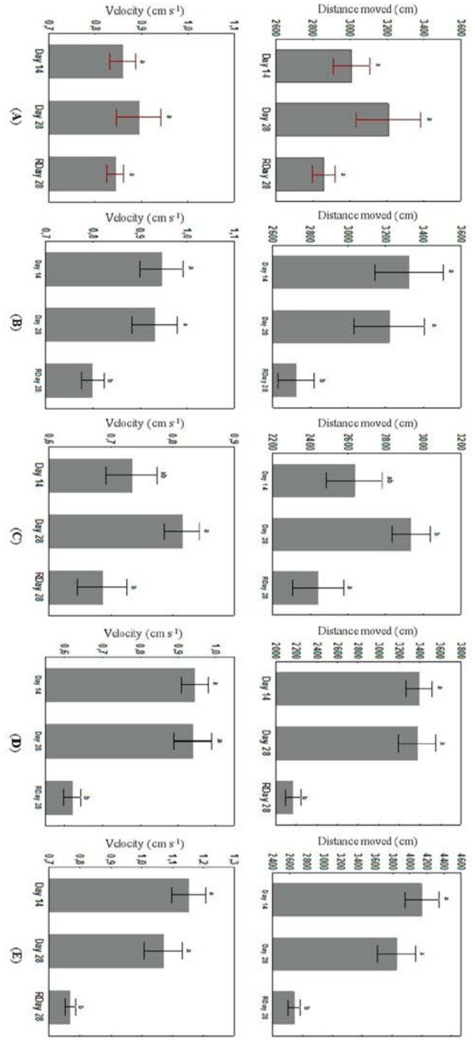

Fig. 1. Mean distance moved and velocity of marbled crayfish (*Procambarus virginalis*) after 14 and 28 days exposure and at 28 recovery days (RDay); (A) Control; (B) 4.2 μg L^{-1} S-metolachlor (S-M1); (C) 42 μg L^{-1} S-metolachlor (S-M2); (D) 4.2 μg L^{-1} metolachlor OA (M-OA1); (E) 42 μg L^{-1} metolachlor OA (M-OA2). Data are mean ± SE (standard error), n = 27. ^{a} no significant difference from other sampling days ($P > 0.05$), ^{b} significant difference compared to other sampling days ($P < 0.05$).

hepatopancreatic pathologies were observed in crayfish exposed to M-OA, including enlargement of intertubular space in group M-OA1 on day 14 of exposure.

Gill showed similar histology in controls and experimental group S-M1 on day 14 (Fig. 3A). Gill of crayfish in the S-M2 group at day 14 exhibited fragmentation with marked dilatation of lamellae (Fig. 3B). This was accompanied by extensive destruction of vascular tissue in groups S-M1 and S-M2 sampled day 28 of exposure, more pronounced in S-M2 (Fig. 3C). Slight damage to gill epithelium including detachment of lamella cuticle was recorded in groups S-M1 and S-M2 sampled after the 28-day recovery period. Gill showed signs of reorganisation (Fig. 3D). Similar trends were observed in crayfish exposed to M-OA, with slight dilatation of lamellae and detachment of lamella cuticle similar to that observed in group S-M2 on day 14 of exposure.

4. Discussion

4.1. Behaviour

Our tested crayfish did not show significant difference from controls in distance moved, velocity, or activity at either exposure time. This is similar to the trend of increased movement in crayfish exposed to metolachlor reported by Velisek et al. (2018a). Herbicides such as metolachlor influence chemical stimulus perception (Wolf and Moore, 2002) and reduce response (Cook and Moore, 2008), and affected crustaceans show reduced orientation and environmental cognition leading to higher movement.

Crayfish exposed to the S-M and M-OA exhibited significant decrease in distance moved, velocity, and activity after 28 days depuration compared to that observed during exposure. This indicated regression to lower mobility values and detoxification of exposed crayfish or to sensory receptor impairment, not stabilization of crayfish behaviour to normal levels, at least within 28 recovery days. Behaviour values were not significantly different from controls after 28 days depuration due to a wide range of S-M and M-OA crayfish behaviour values, nevertheless, behaviour patterns of exposed crayfish showed lower values than recorded in controls. Exposure to both herbicides was associated with physical impairments requiring energy for recovery. Pesticide-exposed or stressed crayfish exhibit reduced movement and spend more time in shelters (Buric et al., 2018), leading to slow escape reflex and reduced access to food. In the wild, foraging to compensate for energy loss could be a challenge for crayfish exposed to the S-M and M-OA. The survival of an individual does not depend only on the health status but also on indirect consequences such as behaviour patterns (Kubec et al., 2019).

4.2. Haemolymph biochemical profiles

Biochemical profile of haemolymph in crayfish, similar to blood plasma in fish, provides important information about the internal environment of the organism (Sevcikova et al., 2016; Stara et al., 2018). Exposure to an environmentally relevant concentration (4.2 μg L^{-1}) of M-OA and to 42 μg L^{-1} of M-OA and S-M was associated with high levels of GLU, LACT, ALT, AST, and NH_3 and deficiency in PHOS after 14 and 28 days. These parameters were comparable to those of control crayfish after the 28-day recovery period. In crayfish, as in other animals, changes to these parameters can indicate damage, stress, fatigue, pain, or morbidity.

Glucose haemostasis is controlled by the crustacean hyperglycaemic hormone, synthesized and stored in the eyestalk X-organ prior to release into the sinus gland. The crustacean eyestalks play a crucial role in the mobilization of glucose from glycogen stores of the muscles and digestive gland. Pollution, including by pesticides, can lead to rapid hyperglycaemia in crayfish (Sepici-Dincel et al., 2013; Benli, 2015). Lactate is an anaerobic glycolysis product and usually increases in parallel with GLU (Sepici-Dincel et al., 2013). Increased levels of GLU and LACT in haemolymph were detected in narrow clawed crayfish (*Astacus leptodactylus*) exposed 96 h to

Table 3
Biochemical characteristics of haemolymph of marbled crayfish (*Procambarus virginalis*) following exposure to S-metolachlor (S-M) and metolachlor OA (M-OA).

Indices	Exposure time (days)	Test groups				
		Control	S-M1 4.2 μg L^{-1}	S-M2 42 μg L^{-1}	M-OA1 4.2 μg L^{-1}	M-OA2 42 μg L^{-1}
GLU (mmol L^{-1})	14	1.36 ± 0.24^{a}	1.34 ± 0.15^{a}	1.36 ± 0.15^{a}	1.33 ± 0.18^{a}	1.39 ± 0.07^{a}
	28	1.35 ± 0.23^{a}	1.38 ± 0.17^{a}	1.39 ± 0.14^{a}	1.51 ± 0.09^{a}	$1.78 \pm 0.16^{b*}$
	RDay 28	1.30 ± 0.20^{a}	1.28 ± 0.19^{a}	1.31 ± 0.17^{a}	1.28 ± 0.19^{a}	1.32 ± 0.14^{a}
LACT (mmol L^{-1})	14	3.06 ± 0.51^{a}	3.13 ± 0.36^{a}	$5.92 \pm 0.85^{b*}$	$5.68 \pm 0.61^{b*}$	$6.30 \pm 0.50^{b*}$
	28	3.27 ± 0.23^{a}	3.31 ± 0.43^{a}	$6.37 \pm 0.54^{b*}$	$5.90 \pm 0.35^{b*}$	$6.66 \pm 0.40^{b*}$
	RDay 28	3.26 ± 0.62^{a}	3.24 ± 0.68^{a}	3.21 ± 0.58^{a}	3.27 ± 0.58^{a}	3.20 ± 0.62^{a}
ALT (μkat L^{-1})	14	1.19 ± 0.19^{a}	1.23 ± 0.14^{a}	1.25 ± 0.18^{a}	1.28 ± 0.16^{a}	$2.18 \pm 0.43^{b*}$
	28	1.27 ± 0.13^{a}	1.29 ± 0.10^{a}	$2.36 \pm 0.42^{b*}$	$2.02 \pm 0.16^{b*}$	$2.54 \pm 0.44^{b*}$
	RDay 28	1.21 ± 0.31^{a}	1.26 ± 0.26^{a}	1.24 ± 0.21^{a}	1.27 ± 0.39^{a}	1.24 ± 0.18^{a}
AST (μkat L^{-1})	14	0.52 ± 0.15^{a}	0.51 ± 0.19^{a}	$0.79 \pm 0.11^{b*}$	0.54 ± 0.10^{a}	$0.88 \pm 0.15^{b*}$
	28	0.57 ± 0.12^{a}	0.54 ± 0.08^{a}	$0.87 \pm 0.13^{b*}$	$0.74 \pm 0.11^{b*}$	$0.91 \pm 0.16^{b*}$
	RDay 28	0.58 ± 0.20^{a}	0.55 ± 0.15^{a}	0.56 ± 0.21^{a}	0.55 ± 0.12^{a}	0.58 ± 0.16^{a}
LDH (μkat L^{-1})	14	3.48 ± 0.95^{a}	3.70 ± 0.86^{a}	3.75 ± 0.59^{a}	3.72 ± 0.73^{a}	3.74 ± 0.65^{a}
	28	3.74 ± 0.47^{a}	3.61 ± 0.46^{a}	3.65 ± 0.26^{a}	3.66 ± 0.36^{a}	3.55 ± 0.44^{a}
	RDay 28	3.66 ± 0.35^{a}	3.68 ± 0.28^{a}	3.71 ± 0.33^{a}	3.67 ± 0.23^{a}	3.65 ± 0.49^{a}
CK (μkat L^{-1})	14	0.24 ± 0.05^{a}	0.25 ± 0.11^{a}	0.26 ± 0.05^{a}	0.22 ± 0.05^{a}	0.25 ± 0.07^{a}
	28	0.27 ± 0.06^{a}	0.25 ± 0.03^{a}	0.26 ± 0.04^{a}	0.25 ± 0.05^{a}	0.26 ± 0.05^{a}
	RDay 28	0.27 ± 0.07^{a}	0.26 ± 0.07^{a}	0.26 ± 0.09^{a}	0.25 ± 0.09^{a}	0.27 ± 0.04^{a}
TP (g L^{-1})	14	25.89 ± 3.14^{a}	24.44 ± 2.27^{a}	24.56 ± 2.79^{a}	24.67 ± 2.75^{a}	24.22 ± 2.44^{a}
	28	26.67 ± 1.83^{a}	25.33 ± 2.16^{a}	25.56 ± 2.01^{a}	25.22 ± 3.22^{a}	25.54 ± 3.27^{a}
	RDay 28	24.67 ± 2.67^{a}	25.56 ± 1.26^{a}	25.00 ± 1.63^{a}	25.11 ± 2.33^{a}	25.44 ± 2.59^{a}
TAG (mmol L^{-1})	14	0.25 ± 0.06^{a}	0.26 ± 0.07^{a}	0.24 ± 0.07^{a}	0.26 ± 0.05^{a}	0.28 ± 0.08^{a}
	28	0.25 ± 0.06^{a}	0.26 ± 0.09^{a}	0.25 ± 0.05^{a}	0.24 ± 0.04^{a}	0.23 ± 0.04^{a}
	RDay 28	0.26 ± 0.04^{a}	0.27 ± 0.05^{a}	0.25 ± 0.06^{a}	0.26 ± 0.07^{a}	0.26 ± 0.06^{a}
NH_3 (μmol L^{-1})	14	123.78 ± 13.73^{a}	127.11 ± 12.83^{a}	129.89 ± 15.37^{a}	131.00 ± 12.98^{a}	$151.00 \pm 13.58^{b*}$
	28	126.44 ± 9.74^{a}	129.56 ± 15.52^{a}	128.00 ± 6.39^{a}	$145.89 \pm 11.24^{b*}$	$152.22 \pm 17.49^{b*}$
	RDay 28	126.89 ± 20.08^{a}	125.44 ± 15.33^{a}	126.11 ± 18.58^{a}	128.00 ± 15.83^{a}	125.48 ± 13.42^{a}
CREA (μmol L^{-1})	14	7.70 ± 0.94^{a}	7.66 ± 0.38^{a}	7.82 ± 0.67^{a}	7.78 ± 0.69^{a}	7.52 ± 0.84^{a}
	28	7.90 ± 0.71^{a}	7.86 ± 0.78^{a}	7.83 ± 0.86^{a}	7.79 ± 1.05^{a}	7.84 ± 0.57^{a}
	RDay 28	7.71 ± 0.78^{a}	7.66 ± 0.88^{a}	7.69 ± 0.54^{a}	7.77 ± 0.28^{a}	7.79 ± 0.44^{a}
ALP (μkat L^{-1})	14	0.18 ± 0.05^{a}	0.20 ± 0.07^{a}	0.19 ± 0.05^{a}	0.21 ± 0.04^{a}	0.22 ± 0.07^{a}
	28	0.18 ± 0.04^{a}	0.22 ± 0.06^{a}	0.19 ± 0.06^{a}	0.19 ± 0.04^{a}	0.18 ± 0.07^{a}
	RDay 28	0.20 ± 0.08^{a}	0.21 ± 0.07^{a}	0.22 ± 0.07^{a}	0.21 ± 0.07^{a}	0.21 ± 0.04^{a}
Mg (mmol L^{-1})	14	1.22 ± 0.17^{a}	1.21 ± 0.20^{a}	1.28 ± 0.18^{a}	1.29 ± 0.18^{a}	1.28 ± 0.14^{a}
	28	1.24 ± 0.13^{a}	1.27 ± 0.17^{a}	1.24 ± 0.08^{a}	1.21 ± 0.19^{a}	1.24 ± 0.28^{a}
	RDayec 28	1.29 ± 0.34^{a}	1.32 ± 0.38^{a}	1.31 ± 0.25^{a}	1.30 ± 0.22^{a}	1.31 ± 0.21^{a}
Ca (mmol L^{-1})	14	2.55 ± 0.24^{a}	2.57 ± 0.20^{a}	2.49 ± 0.12^{a}	2.58 ± 0.12^{a}	2.64 ± 0.37^{a}
	28	2.51 ± 0.32^{a}	2.54 ± 0.23^{a}	2.55 ± 0.26^{a}	2.53 ± 0.18^{a}	2.52 ± 0.29^{a}
	RDay 28	2.57 ± 0.42^{a}	2.54 ± 0.52^{a}	2.53 ± 0.42^{a}	2.55 ± 0.46^{a}	2.54 ± 0.19^{a}
PHOS (mmol L^{-1})	14	0.25 ± 0.05^{a}	0.26 ± 0.07^{a}	$0.17 \pm 0.06^{b*}$	$0.14 \pm 0.04^{b*}$	$0.14 \pm 0.05^{b*}$
	28	0.26 ± 0.05^{a}	0.27 ± 0.11^{a}	$0.14 \pm 0.03^{b*}$	$0.16 \pm 0.03^{b*}$	$0.13 \pm 0.03^{b*}$
	RDay 28	0.26 ± 0.09^{a}	0.27 ± 0.08^{a}	0.29 ± 0.07^{a}	0.27 ± 0.06^{a}	0.28 ± 0.11^{a}

Data are mean ± SD, n = 9. Different superscripts indicate significant differences ($P < 0.01$) among groups at the same sample time. *Denotes significant differences among group values over time ($P < 0.01$). Glucose (GLU), lactate (LACT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), total protein (TP), triacylglycerols (TAG), ammonia (NH_3), creatine (CREA), alkaline phosphatase (ALP), magnesium (Mg), calcium (Ca), inorganic phosphate (PHOS). RDay 28 = 28 days recovery period in S-metolachlor and metolachlor OA -free water.

etofenprox at 0.04 and 0.1 μg L^{-1} (Benli, 2015). Stara et al. (2018) found similar GLU and LACT values in haemolymph of *Cherax destructor* exposed to atrazine (1.21 mg L^{-1}) for 14 days, persisting through a 14 day of recovery period. Increase of LACT in red swamp crayfish (*Procambarus clarkii*) was observed with 14 days exposure to 2.9 μg L^{-1} terbuthylazine-desethyl. Levels remained elevated after 14 days in clean water (Stara et al., 2016).

In general, enzyme activity in plasma reflects the release from the cells into the blood of enzymes important in cellular nitrogen metabolism, oxidation of amino acids, and liver gluconeogenesis. Stress leads to increased activity of enzymes AST and ALT as described in studies of fish after exposure to pesticides (Banaee et al., 2014; Samanta et al., 2014; Mosiichuk et al., 2018). We observed this in the haemolymph of crayfish with M-OA at 4.2 and 42 μg L^{-1} and S-M at 42 μg L^{-1}, suggesting a toxic effect. Enzyme activity was not different from that of unexposed controls after depuration in S-M- and M-OA-free water.

Inorganic phosphate in tissue fluids is an important factor in metabolic processes, particularly in detoxification (Gomes et al., 2012). Changes in PHOS indicate severe kidney damage (Kolarova and Velisek, 2012).

Ammonia is a catabolic product of digestion and metabolism of proteins in aquatic organisms and is extremely toxic. It is ordinarily released by diffusion and eliminated via the gills. An increase in ammonia in haemolymph is an indicator of liver failure or indicates metabolic defects of ammonia in the Krebs cycle (Ip and Chew, 2010; De las Heras et al., 2018). *Cherax destructor* exposed for 14 days at 1.21 mg L^{-1} to atrazine showed increased NH_3. After 14 days in chemical free water, NH_3 production was comparable to control crayfish (Stara et al., 2018).

Pesticide effects on haemolymph may require a recovery period of more than 14 days. Our study indicated that 28 days may be sufficient.

4.3. Oxidative stress and antioxidant responses

Biomarkers of oxidative stress in aquatic organisms have been shown to reflect a wide range of pesticides. Most studies use

Table 4
Oxidative stress and antioxidant biomarkers in organ tissue of marbled crayfish (*Procambarus virginalis*) following exposure to S-metolachlor (S-M) and metolachlor OA (M-OA).

Parameters	Organ	Exposure time (days)	Test groups				
			Control 0.00	S-M1 $4.2\ \mu g\ L^{-1}$	S-M2 $42\ \mu g\ L^{-1}$	M-OA1 $4.2\ \mu g\ L^{-1}$	M-OA2 $42\ \mu g\ L^{-1}$
LPO (nmol mg^{-1} protein)	Hepatopancreas	14	0.64 ± 0.14^{a}	0.77 ± 0.24^{a}	0.75 ± 0.25^{a}	0.99 ± 0.29^{a}	0.77 ± 0.29^{a}
		28	0.84 ± 0.17^{a}	1.03 ± 0.29^{ab}	1.01 ± 0.26^{ab}	0.89 ± 0.16^{ab}	$1.26 \pm 0.24^{b*}$
		RDay 28	0.76 ± 0.31^{a}	0.78 ± 0.23^{a}	0.73 ± 0.25^{a}	0.75 ± 0.30^{a}	0.66 ± 0.25^{a}
	Muscle	14	0.36 ± 0.11^{a}	0.31 ± 0.06^{a}	0.35 ± 0.02^{a}	0.34 ± 0.03^{a}	0.38 ± 0.04^{a}
		28	0.35 ± 0.08^{a}	0.35 ± 0.06^{a}	0.38 ± 0.03^{a}	0.38 ± 0.03^{a}	0.39 ± 0.04^{a}
		RDay 28	0.36 ± 0.03^{a}	0.34 ± 0.04^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.06^{a}
	Gill	14	0.19 ± 0.02^{a}	0.18 ± 0.01^{a}	0.18 ± 0.02^{a}	0.18 ± 0.03^{a}	0.18 ± 0.03^{a}
		28	0.21 ± 0.04^{a}	0.22 ± 0.03^{a}	0.21 ± 0.04^{a}	0.19 ± 0.03^{a}	0.21 ± 0.03^{a}
		RDay 28	0.15 ± 0.02^{a}	0.17 ± 0.02^{a}	0.16 ± 0.03^{a}	0.16 ± 0.02^{a}	0.16 ± 0.03^{a}
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	Hepatopancreas	14	0.51 ± 0.15^{a}	0.33 ± 0.13^{b}	0.60 ± 0.27^{a}	0.55 ± 0.37^{a}	0.34 ± 0.19^{b}
		28	0.66 ± 0.16^{a}	$0.72 \pm 0.21^{ab*}$	$0.73 \pm 0.24^{ab*}$	0.64 ± 0.27^{a}	$0.98 \pm 0.21^{b*}$
		RDay 28	0.64 ± 0.16^{a}	0.47 ± 0.30^{ab}	0.59 ± 0.36^{a}	0.40 ± 0.22^{ab}	0.33 ± 0.08^{b}
	Muscle	14	0.25 ± 0.09^{a}	0.21 ± 0.09^{a}	0.25 ± 0.09^{a}	0.27 ± 0.09^{a}	0.23 ± 0.02^{a}
		28	0.25 ± 0.09^{a}	0.21 ± 0.04^{a}	0.23 ± 0.02^{a}	0.23 ± 0.28^{a}	0.23 ± 0.20^{a}
		RDay 28	0.27 ± 0.11^{a}	0.25 ± 0.10^{a}	0.29 ± 0.10^{a}	0.28 ± 0.11^{a}	0.34 ± 0.19^{a}
	Gill	14	0.62 ± 0.06^{a}	0.52 ± 0.12^{ab}	0.53 ± 0.08^{ab}	0.50 ± 0.05^{b}	0.53 ± 0.10^{ab}
		28	0.50 ± 0.07^{a}	0.54 ± 0.18^{a}	0.52 ± 0.08^{a}	0.44 ± 0.07^{ab}	$0.36 \pm 0.14^{b*}$
		RDay 28	$0.37 \pm 0.05^{a*}$	$0.37 \pm 0.13^{a*}$	0.41 ± 0.09^{a}	0.40 ± 0.10^{a}	0.42 ± 0.07^{a}
SOD (nmol NBT min^{-1} mg^{-1} protein)	Hepatopancreas	14	0.15 ± 0.05^{a}	0.16 ± 0.07^{a}	0.12 ± 0.04^{a}	0.15 ± 0.07^{a}	0.11 ± 0.06^{a}
		28	0.18 ± 0.07^{a}	0.21 ± 0.08^{a}	0.15 ± 0.06^{a}	0.13 ± 0.08^{a}	0.12 ± 0.07^{a}
		RDay 28	0.21 ± 0.07^{a}	0.17 ± 0.05^{a}	0.18 ± 0.07^{a}	0.14 ± 0.06^{a}	0.15 ± 0.04^{a}
	Muscle	14	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		28	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
		RDay 28	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
	Gill	14	0.13 ± 0.07^{a}	0.11 ± 0.03^{a}	0.11 ± 0.06^{a}	0.17 ± 0.06^{a}	0.09 ± 0.06^{a}
		28	0.17 ± 0.06^{a}	0.14 ± 0.05^{a}	0.18 ± 0.08^{a}	0.14 ± 0.09^{a}	0.15 ± 0.03^{a}
		RDay 28	0.12 ± 0.07^{a}	0.10 ± 0.05^{a}	0.16 ± 0.03^{a}	0.20 ± 0.06^{a}	0.12 ± 0.07^{a}
GSH (nmol GSH mg^{-1} protein)	Hepatopancreas	14	416.28 ± 147.63^{a}	276.85 ± 65.21^{b}	281.99 ± 92.98^{b}	266.08 ± 179.95^{b}	151.66 ± 60.69^{c}
		28	$656.13 \pm 197.56^{a*}$	$495.12 \pm 193.04^{b*}$	$433.82 \pm 180.18^{b*}$	$339.65 \pm 120.44^{b*}$	$546.96 \pm 162.14^{b*}$
		RDay 28	281.37 ± 84.99^{a}	233.45 ± 104.66^{ab}	271.38 ± 174.53^{ab}	188.66 ± 72.31^{ab}	165.55 ± 34.74^{b}
	Muscle	14	99.48 ± 36.11^{a}	85.42 ± 29.83^{a}	78.09 ± 10.48^{a}	91.71 ± 11.12^{a}	100.38 ± 15.96^{a}
		28	99.63 ± 35.07^{a}	94.83 ± 33.28^{a}	99.78 ± 35.11^{a}	86.17 ± 14.73^{a}	91.91 ± 13.61^{a}
		RDay 28	89.11 ± 35.07^{a}	93.72 ± 42.37^{a}	109.12 ± 35.34^{a}	99.50 ± 14.24^{a}	94.40 ± 29.04^{a}
	Gill	14	89.37 ± 30.67^{a}	69.87 ± 17.82^{a}	72.79 ± 22.79^{a}	69.82 ± 16.16^{a}	78.10 ± 20.28^{a}
		28	63.87 ± 19.97^{a}	65.16 ± 16.92^{a}	59.66 ± 11.51^{a}	57.36 ± 10.27^{a}	62.07 ± 10.13^{a}
		RDay 28	$46.70 \pm 12.89^{a*}$	$49.81 \pm 10.36^{a*}$	$48.44 \pm 13.13^{a*}$	$50.74 \pm 11.56^{a*}$	$48.39 \pm 13.39^{a*}$
GST (nmol min^{-1} mg^{-1} protein)	Hepatopancreas	14	5.43 ± 1.73^{a}	4.47 ± 1.68^{a}	5.69 ± 1.76^{a}	6.64 ± 1.53^{a}	4.87 ± 2.52^{a}
		28	$7.36 \pm 1.66^{a*}$	5.86 ± 2.06^{a}	5.90 ± 1.80^{a}	6.42 ± 2.44^{a}	$9.19 \pm 2.61^{b*}$
		RDay 28	4.92 ± 1.60^{a}	5.24 ± 1.10^{a}	4.81 ± 1.18^{a}	5.64 ± 1.28^{a}	5.35 ± 1.01^{a}
	Muscle	14	1.81 ± 0.36^{a}	1.16 ± 0.47^{a}	1.99 ± 0.86^{a}	1.55 ± 0.46^{a}	1.86 ± 1.17^{a}
		28	1.66 ± 0.86^{a}	1.37 ± 0.45^{a}	1.14 ± 0.56^{a}	1.59 ± 0.52^{a}	1.62 ± 0.57^{a}
		RDay 28	1.26 ± 0.41^{a}	1.38 ± 0.40^{a}	1.49 ± 0.29^{a}	2.23 ± 0.74^{a}	1.47 ± 0.38^{a}
	Gill	14	4.62 ± 0.96^{a}	3.97 ± 0.65^{a}	3.74 ± 0.72^{a}	4.08 ± 1.13^{a}	4.23 ± 1.10^{a}
		28	3.43 ± 1.17^{a}	3.56 ± 1.17^{a}	3.32 ± 1.12^{a}	$2.72 \pm 0.43^{a*}$	3.22 ± 0.70^{a}
		RDay 28	$2.11 \pm 0.53^{a*}$	$2.32 \pm 0.54^{a*}$	$2.25 \pm 0.69^{a*}$	$2.23 \pm 0.74^{a*}$	$2.16 \pm 062^{a*}$

Data are mean ± SD, n = 9. Different superscripts indicate significant differences ($P < 0.01$) among groups at the same sampled time. *Denotes significant differences among group values over time ($P < 0.01$). Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione S-transferase (GST). RDay 28 = 28 days recovery period in S-metolachlor and metolachlor OA -free water.

pesticide concentrations higher than those found in the environment (Semren et al., 2018; Stara et al., 2019). Generally, oxidative stress in organisms occurs when antioxidant defence mechanisms are overcome, exhausted, or damaged by reactive oxygen species (ROS), which can be generated through pesticide exposure (Alavanja et al., 2013). The ROS readily react with lipids, proteins, carbohydrates, and nucleic acids and affect the structure and function of cellular components (Semren et al., 2018). The most common ROS are superoxide anion, molecular and singlet oxygen, hydrogen peroxide, hydroxyl radicals, and some of their derivatives (Lushchak, 2011). Lipid peroxidation is a commonly cited biomarker of oxidative stress. Alteration in LPO concentration reflects severe oxidative damage and is used as a biomarker of toxicity, and MDA is a naturally occurring product of LPO that acts as its indicator (Abhijith et al., 2016). Exposure to S-M and M-OA, also at environmentally relevant concentrations, led to increased levels of LPO in hepatopancreas after 28 days that returned to levels comparable to control crayfish after a 28-day depuration period. Stara et al. (2016) found high levels of LPO in red swamp crayfish exposed to terbuthylazine-desethyl at 2.9 and 580 μg L^{-1} for 14 days, returning to control levels after a 14-day recovery period. Thus, it is likely that 14 days were sufficient to reduce the overproduction of ROS formation in exposed crayfish. Arceo et al. (2015) and Benli et al. (2016) observed increased levels of LPO in crayfish hepatopancreas exposed to pesticides. Lidova et al. (2016) and Velisek et al. (2018a) found reduced LPO in crayfish exposed to cypermethrin and metolachlor OA, respectively. The latter studies did not report recovery time.

Antioxidant biomarkers protect proteins, lipids, and nucleic acids and demonstrate a synergistic effect in protection against oxidative stress and tissue damage. The antioxidant defence system is divided into the enzymatic (CAT, SOD, GST) and the non-enzymatic (GSH, vitamins, carotenoids) (Abdel-Latif and Euony, 2016). Catalase and SOD are considered the first step in defence

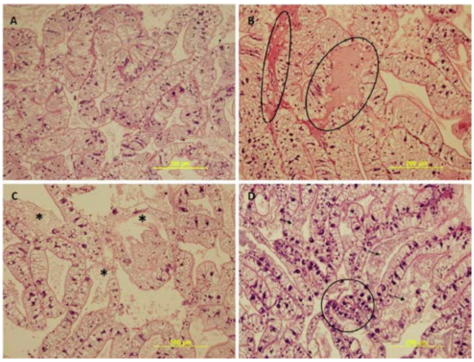

Fig. 2. Transverse section of hepatopancreas of marbled crayfish (*Procambarus virginalis*) exposed to S-metolachlor (S–M); (A) control with typical structure and uniform arrangement of epithelial cells; (B) S-M2 (42 μg L^{-1}) group sampled day 14 of exposure; (C) S-M2 (42 μg L^{-1}) group sampled day 28 of exposure; (D) S-M2 (42 μg L^{-1}) group sampled after 28 days depuration. Ovals indicate enlarged intertubular space filled with eosinophilic substance; * damaged areas of hepatopancreatic tubules; circle indicates the location of replicated embryonic and fibrillar cells; arrows indicate granular content of enlarged tubules. (H&E).

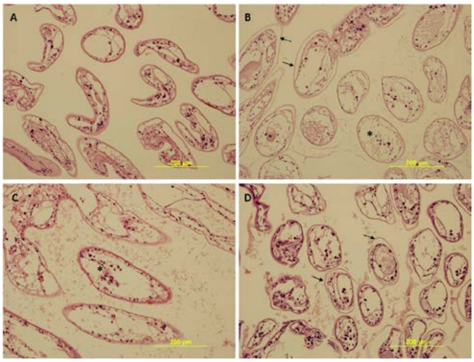

Fig. 3. Transverse section of gill lamellae of marbled crayfish (*Procambarus virginalis*) exposed to S-metolachlor (S–M); (A) control group with typical structure and uniform arrangement; (B) S-M2 (42 μg L^{-1}) group sampled day 14 of exposure; (C) S-M2 (42 μg L^{-1}) group sampled day 28 of exposure; (D) S-M2 (42 μg L^{-1}) group sampled after 28 day depuration period, showing damaged lamellae. Arrows indicate separation of lamella cuticle; * damaged lamella structures. (H&E).

against oxidative stress. Superoxide dismutase catalyses dismutation of two superoxide radicals into molecular oxygen, and CAT decomposes hydrogen peroxide (Semren et al., 2018). Changes in GSH levels and GST activity are components of the second phase of biotransformation involved in disrupting the conjugation of glutathione to various compounds and the transportation and removal of reactive compounds that have additional indirect antioxidant functions (Livingstone, 2003; Lushchak, 2011). Antioxidants demonstrate coordinated action, but changes in their levels may tip the balance toward oxidative stress (Semren et al., 2018). Our study demonstrated that long-term exposure of crayfish to S-M and M-OA can lead to anomalies in CAT, GST, and GSH in hepatopancreas and gill that remain detectable after the 28-day recovery period, while depuration time led to a reduction in ROS formation to control levels. Stara et al. (2016) described similar results, sub-chronic exposure of terbuthylazine-desethyl at 580 μg L^{-1} affected GSH levels persisting after 14 days recovery time. The antioxidant biomarkers show, as stated by Abhijith et al. (2016), that, under stress, aquatic animals adapt and alter metabolic and antioxidant function. The hepatopancreas regulates overall body metabolism, therefore in this tissue also be attributed to more and high metabolic reactions (Torre et al., 2013a, b). The gill is the initial organ encountering pesticides and is vulnerable to damage (Schlenk, 2005; Messina et al., 2014; Abhijith et al., 2016). Studies of Decapoda exposed to pesticides have described changes in antioxidant systems without accompanying oxidative damage (Lavarias and Garcia, 2015; Stara et al., 2018) or, when detected, oxidative damage was not present after a depuration period (Stara et al., 2014; Lidova et al., 2016; Koutnik et al., 2017; Velisek et al., 2018a). Antioxidant defence includes a number of enzymes, the balance of which may be affected by factors such as species, habitat, feeding, behaviour, and temperature (Stara et al., 2014). Although it seems that 28 days is sufficient time to reduce LPO, a longer time is required for stabilization of antioxidant biomarkers after chronic exposure to pesticides.

4.4. Histology

Crayfish hepatopancreas is involved in a variety of physiological processes including the secretion of digestive juices, absorption and storage of digested food, and detoxification of xenobiotics (Icely and Noot, 1992). Ability to degrade xenobiotics might be overwhelmed by high concentrations of these substances and lead to failure of regulatory mechanisms and pathological damage (Velmurugan et al., 2007). Exposed crayfish exhibited damage to tubular epithelium characterized by enlargement of intertubular space and focal disorganisation and disintegration of tubular epithelial structure. These findings corresponded with increased levels of ALT and AST in haemolymph of exposed groups as important clinical indicators of hepatocellular injury (Solter et al., 2000).

The gills are in direct contact with the environment and are involved in gas exchange and ion flux regulation. Crayfish exposed to of S-M and M-OA at 42 μg L^{-1} exhibited gill anomalies including dilatation of lamellae and destruction of vascular tissue, which influenced gill function. Similar changes were found by Stara et al. (2018) in crayfish *Cherax destructor* exposed to atrazine at 6.86 μg L^{-1} and 1.21 mg L^{-1} for 14 days. Structure of gill and hepatopancreas was not different from controls following a 14-day recovery period. We observed signs of reparative processes, especially in hepatopancreas, characterized by replication of embryonic and fibrillar cells after 28 days depuration.

5. Conclusions

This study suggest that non-target aquatic animals are damaged through prolonged exposure to concentrations of S-M and M-OA reported in the aquatic environment. We observed changes in behaviour, haemolymph parameters, and oxidative stress biomarkers as well as histopathological tissue damage. Some observed effects persisted after treatment in toxicant-free water and physiological state of body were not steady. Results suggest that assessment of haemolymph, hepatopancreas, and gill of crayfish exposed to pesticides can be suitable parameters to evaluate toxicity. Due to the widespread use of S-M herbicides it is important to carry out further investigations of its potential effects in order to provide information on whether S-M and its metabolite M-OA may pose a risk to the aquatic environment.

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References

Abdel-Latif, H.M.R., Euony, O.I.E., 2016. Effects of rotenone on liver functions, antioxidants and lipid peroxidation of nile tilapia fingerlings. Alex. J. Vet. Sci. 51, 186–193.
Abhijith, B.D., Ramesh, M., Poopal, R.K., 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of *Catla catla* during methyl parathion exposure. J. Basic Appl. Zool. 77, 31–40.
Aebi, H., 1984. Catalase invitro. Meth. Enzymol. 105, 121–126.
Alavanja, M.C., Ross, M.K., Bonner, M.R., 2013. Increased cancer burden among pesticide applicators and others due to pesticide exposure. Ca - Cancer J. Clin. 63, 120–142.
Aliko, V., Qirjo, M., Sula, E., Morina, V., Faggio, C., 2018. Antioxidant defense system, immune response and erythron profile modulation in gold fish, *Carassius auratus*, after acute manganese treatment. Fish Shellfish Immunol. 76, 101–109.
Arceo, S.D.B., Martinez-Tabche, L., Alvarez-Gonzalez, I., Lopez, E.L., Madrigal-Bujaidar, E., 2015. Toxicity induced by dieldrin and chlorpyrifos in the freshwater crayfish *Cambarellus montezumae* (Cambaridae). Rev. Biol. Trop. 63, 83–96.
Arthur, E.L., Perkovich, B.S., Anderson, T.A., Coats, J.R., 2000. Degradation of an atrazine and metolachlor herbicide mixture in pesticide-contaminated soils from two agrochemical dealerships in Iowa. Water, Air, Soil Pollut. 119, 75–90.
Banaee, M., Sureda, A., Zohiery, F., Hagi, B.N., Garanzini, D.S., 2014. Alterations in biochemical parameters of the freshwater fish, *Alburnus mossulensis*, exposed to sub-lethal concentrations of Fenpropathrin. Int. J. Aquat. Biol. 2, 58–68.
Battaglin, W.A., Furlong, E.T., Burkhardt, M.R., Peter, C.J., 2000. Occurrence of sulfonylurea,sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Sci. Total Environ. 248, 123–133.
Benli, A.C.K., 2015. The influence of etofenprox on narrow clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823): acute toxicity and sublethal effects on histology, hemolymph parameters, and total hemocyte counts. Environ. Toxicol. 30, 887–894.
Benli, A.C.K., Sahin, D., Selvi, M., Sarikaya, R., Memmi, B.K., Dincel, A.S., 2016. The sublethal effects of (2,4-Dichlorophenoxy) acetic acid (2,4-D) on narrow-clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823). Arh. Hig. Rada. Toksikol. 67, 289–296.
Bradford, M.M., 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. Anal. Biochem. 72, 248–254.
Burgos Aceves, M.A., Cohen, A., Paolella, G., Lepretti, M., Smith, Y., Faggio, C., Lionetti, L., 2018a. Modulation of mitochondrial functions by xenobiotic-induced microRNA: from environmental sentinel organisms to mammals. Sci. Total Environ. 645, 79–88.
Burgos Aceves, M.A., Cohen, A., Smith, Y., Faggio, C., 2018b. MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. Ecotoxicol. Environ. Saf. 148, 995–1000.
Buric, M., Grabicova, K., Kubec, J., Kouba, A., Kuklina, I., Kozak, P., Grabic, R., Randak, T., 2018. Environmentally relevant concentrations of tramadol and citalopram alter behaviour of an aquatic invertebrate. Aquat. Toxicol. 200, 226–232.
Chen, Z., Chen, Y., Vymazal, J., Kule, L., Koželuh, M., 2017. Dynamics of chloroacetanilide herbicides in various types of mesocosm wetlands. Sci. Total Environ. 577, 386–394.
CHMI (Czech Hydrometeorological Institute), 2018. On-line Water Duality Database. http://hydro.chmi.cz/. (Accessed 11 June 2018).
Chromcova, L., Blahova, J., Zivna, D., Plhalova, L., Casuscelli Di Tocco, F., Divisova, L., Prokes, M., Faggio, C., Tichy, F., Svobodova, Z., 2015. NeemAzal T/S – toxicity to early-life stages of common carp (*Cyprinus carpio* L.). Vet. Med. 60, 23–30.
Cook, M.E., Moore, P.A., 2008. The effects of the herbicide metolachlor on agonistic behavior in the crayfish, *Orconectes rusticus*. Arch. Environ. Contam. Toxicol. 55, 94–102.
De las Heras, J., Aldamiz-Echevarria, L., Martinez-Chantar, M.L., Delgado, T.C., 2018. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. Expert Opin. Drug Metabol. Toxicol. 13, 439–448.
De Oliveira Arias, J.L., Schneider, A., Batista-Andrade, J.A., Vieira, A.A., Gehrke, V.R., Camargo, E.R., Caldas, S.S., Primel, E.G., 2017. Evaluation of dilute-and-shoot and solid-phase extraction methods for the determination of S-metolachlorhlor and metolachlor-OA in runoff water samples by liquid chromatography tandem mass spectrometry. Anal. Meth. 9, 5777–5783.
Dobsikova, R., Blahova, J., Modra, H., Skoric, M., Svobodova, Z., 2011. The effect of acute exposure to herbicide Gardoprim Plus Gold 500 SC on haematological and biochemical indicators and histopathological changes in common carp (*Cyprinus carpio* L.). Acta Vet. 80, 359–363.
Ellmann, G.L., 1959. Tissue sulfhydryl group. Arch. Biochem. Biophys. 82, 70–79.
EPA US (Environmental Protection Agency United States), 2011. In: Agency, E.P. (Ed.), Pesticide Industry Sales and Usage: 2006 and 2007 Market Estimates. Washington (DC).
Ewing, J.F., Janero, D.R., 1995. Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. Anal. Biochem. 232, 243–248.
Faggio, C., Pagano, M., Alampi, R., Vazzana, I., Felice, M.R., 2016. Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternium-15 in *Mytilus galloprovincialis*. Aquat. Toxicol. 180, 258–265.
Faggio, C., Tsarpali, V., Dailianis, S., 2018. Mussel digestive gland as a model for assessing xenobiotics: an overview. Sci. Total Environ. 613, 20–229.
Fazio, F., Faggio, C., Marafioti, S., Torre, A., Sanfilippo, M., Piccione, G., 2012. Comparative study of haematological profile on *Gobius niger* in two different habitat sites: Faro Lake and Tyrrhenian Sea. Cah. Biol. Mar. 53, 213–219.
Fiorino, E., Sehonova, P., Plhalova, L., Blahova, J., Svobodova, Z., Faggio, C., 2018. Effects of glyphosate on early life stages: comparison between *Cyprinus carpio* and *Danio rerio*. Environ. Sci. Pollut. Res. 25, 8542–8549.
Gomes, F.M., Carvalho, D.B., Peron, A.C., Saito, K., Miranda, K., Machado, E.A., 2012. Inorganic polyphosphates are stored in spherites within the midgut of *Anticarsia gemmatalis* and play a role in copper detoxification. J. Insect Physiol. 58, 211–219.
Guelfi, D.R.V., Gozzi, F., Machulek, A., Sires, I., Brillas, E., de Oliveira, S.C., 2018. Degradation of herbicide S-metolachlor by electrochemical AOPs using a boron-doped diamond anode. Catal. Today 313, 182–188.
Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: environmental and toxicological effects. Environ. Toxicol. Pharmacol. 64, 164–171.
Habig, W.M., Pabst, M.J., Jakoby, W.B., 1974. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.
Hodkovicova, N., Chmelova, L., Sehonova, P., Blahova, J., Doubkova, V., Plhalova, L., Fiorino, E., Vojtek, L., Vicenova, V., Siroka, Z., Enevova, V., Berlinska, J., Faldyn, M., Svobodova, Z., Faggio, C., 2019. The effects of a therapeutic formalin bath on selected immunological and oxidative stress parameters in common carp (*Cyprinus carpio*). Sci. Total Environ. 653, 1120–1127.
Icely, J.D., Noot, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harison, F.W. (Ed.), Microscopic Anatomy Invert, vol. 10, pp. 147–201.
Ip, Y.K., Chew, S.F., 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. Front. Physiol. Aquat. Physiol. 1, 20. https://doi.org/10.3389/fphys.2010.00134.
Kolarova, J., Velisek, J., 2012. Determination and evaluation of the biochemical profile of blood fish. FROV JU Vodnany Meth. 135, 54 (In Czech).
Kong, P., Cheng, X., Sun, R., Chen, L., 2018. The synergic characteristics of surface water pollution and sediment pollution with heavy metals in the Haihe river basin, Northern China. Water 73, 17. https://doi.org/10.3390/w10010073.
Koutnik, D., Stara, A., Zuskova, E., Kouba, A., Velisek, J., 2017. The chronic effects of terbuthylazine-2-hydroxy on early life stages of marbled crayfish (*Procambarus fallax* f. *virginalis*). Pestic. Biochem. Physiol. 136, 29–33.
Kubec, J., Kouba, A., Buric, M., 2019. Communication, behaviour, and decision making in crayfish: a review. Zool. Anzeig. 278, 28–37.
Lavarias, S., Garcia, C.F., 2015. Acute toxicity of organophosphate fenitrothion on biomarkers in prawn *Palaemonetes argentinus* (Crustacea: Palaemonidae). Environ. Monit. Assess. 187, 65.
Lidova, J., Stara, A., Kouba, A., Velisek, J., 2016. The effects of cypermethrin on oxidative stress and antioxidant biomarkers in marbled crayfish (*Procambarus fallax* f. *virginalis*). Neuroendocrinol. Lett. 37, 53–59.
Liu, H., Xiong, M., 2006. Comparative toxicity of racemic metolachlor and S-metolachlor to *Chlorella pyrenoidosa*. Aquat. Toxicol. 93, 100–106.
Livingstone, D.R., 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Revue Méd. Vét. 154, 427–430.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.
Maazouzi, C., Coureau, C., Piscart, C., Saplairoles, M., Baran, N., Marmonier, P., 2016. Individual and joint toxicity of the herbicide S-metolachlor and a metabolite, deethylatrazine on aquatic crustaceans: difference between ecological groups. Chemosphere 165, 118–125.
Marino, F., Di Caro, G., Gugliandolo, C., Spano, A., Faggio, C., Genovese, G., Morabito, M., Russo, A., Barreca, D., Fazio, F., Santulli, A., 2016. Preliminary study on the *in vitro* and *in vivo* effects of *Asparagopsis taxiformis* bioactive phycoderivates on Teleosts. Front. Physiol. 7, 459.
Maronic, D.S., Camagajevac, I.S., Horvatic, J., Pfeiffer, T.Z., Stevic, F., Zarkovic, N., Waeg, G., Jaganjac, M., 2018. S-metolachlor promotes oxidative stress in green microalga *Parachlorella kessleri* - a potential environmental and health risk for higher organisms. Sci. Total Environ. 637–638, 41–49.
Messina, C.M., Faggio, C., Laudicella, V.C., Sanfilippo, M., Trischitta, F., Santulli, A., 2014. Effect of sodium dodecyl sulfate (SDS) on stress response in the Mediterranean mussel (*Mytilus galloprovincialis*): regulatory volumedecrease (Rvd) and modulation of biochemical markers related tooxidative stress. Aquat.

Toxicol. 157, 94–100.

Mosiichuk, N.M., Maksymiv, I.V., Husak, V.V., Storey, J.M., Storey, K.B., Lushchak, V.L., 2018. Effect of prometryn-containing herbicide gesagard on hematological profiles and biochemical parameters in goldfish liver and plasma. Turk. J. Fish. Aquat. Sci. https://doi.org/10.4194/1303-2712-v18_10_04.

Neves, M., Castro, B.B., Vidal, T., Vieira, R., Marques, J.C., Coutinho, J.A.P., Goncalves, F., Goncalves, A.M.M., 2015. Biochemical and populational responses of an aquatic bioindicator species, *Daphnia longispina*, to a commercial formulation of a herbicide (Primextra® Gold TZ) and its active ingredient (S-metolachlor). Ecol. Indicat. 53, 220–230.

Pagano, M., Capillo, G., Sanfilippo, M., Palato, S., Trischitta, F., Manganaro, A., Faggio, C., 2016. Evaluation of functionality and biological responses of mytilus galloprovincialis after exposure to Quaternium-15 (Methenamine 3-Chloroallylochloride). Molecules 21, 144.

Plhalova, L., Blahova, J., Divisova, L., Enevova, V., Casuscelli Di Tocco, F., Faggio, C., Tichy, F., Vecerek, V., Svobodova, Z., 2017. The effects of subchronic exposure to NeemAzal T/S on zebrafish (*Danio rerio*). Chem. Ecol. 21, 302–307.

Quintaneiro, C., Patricio, D., Novais, S.C., Soares, A.M.V.M., Monteiro, M.S., 2017. Endocrine and physiological effects of linuron and S-metolachlor in zebrafish developing embryos. Sci. Total Environ. 586, 390–400.

Quintaneiro, C., Soares, A.M.V.M., Monteiro, M.S., 2018. Effects of the herbicides linuron and S-metolachlor on Perez's frog embryos. Chemosphere 194, 595–601.

Roubeix, V., Fauvelle, V., Tison-Rosebery, J., Mazzella, N., Coste, M., Delmas, F., 2012. Assessing the impact of chloroacetanilide herbicides and their metabolites on periphyton in the Leyre River (SWFrance) via short termgrowth inhibition tests on autochthonous diatoms. J. Environ. Monit. 14, 1655–1663.

Samanta, P., Pal, S., Mukherjee, A.K., Ghosh, A.R., 2014. Evaluation of metabolic enzymes in response to Excel Mera 71, a glyphosate-based herbicide, and recovery pattern in freshwater teleostean fishes. BioMed Res. Int. 2014, 425159, 6 p.

Savorelli, F., Manfra, L., Croppo, M., Tornambè, A., Palazzi, D., Canepa, S., Trentini, P.L., Cicero, A.M., Faggio, C., 2017. Fitness evaluation of *Ruditapes philippinarum* exposed to nickel. Biol. Trace Elem. Res. 177, 384–393.

Sehonova, P., Svobodova, Z., Dolezelova, P., Vosmerova, P., Faggio, C., 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Sci. Total Environ. 631–632, 789–794.

Semren, T.Z., Zunec, S., Pizent, A., 2018. Oxidative stress in triazine pesticide toxicity: a review of the main biomarker findings. Arh. Hig. Rada. Toksikol. 69, 109–125.

Sepici-Dincel, A., Alparslan, Z.N., Benli, A.C.K., Selvi, M., Sarikaya, R., Ozkul, I.A., Erkoc, F., 2013. Hemolymph biochemical parameters reference intervals and total hemocyte counts of narrow clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823). Ecol. Indicat. 24, 305–309.

Sevcikova, M., Modra, H., Blahova, J., Dobsikova, R., Plhalova, L., Zitka, O., Hynek, D., Kizek, R., Skoric, M., Svobodova, Z., 2016. Biochemical, haematological and oxidative stress responses of common carp (*Cyprinus carpio* L.) after subchronic exposure to copper. Vet. Med. 61, 35–50.

Schlenk, D., 2005. Chapter 6 Pesticide biotransformation in fish. Biochem. Mol. Biol. Fishes 6, 171–190.

Solter, P., Liu, Z., Guzman, R., 2000. Decreased hepatic ALT synthesis is an outcome of subchronic microcystin-LR toxicity. Toxicol. Appl. Pharmacol. 164, 216–220.

Srivastava, P., Singh, A., Pandey, A.K., 2016. Pesticides toxicity in fishes: biochemical, physiological and genotoxic aspects. Biochem. Cell. Arch. 16, 199–218.

Stara, A., Kouba, A., Velisek, J., 2014. Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in red swamp crayfish (*Procambarus clarkii*). BioMed Res. Int. 2014, 680131, 6 p.

Stara, A., Zuskova, E., Kouba, A., Velisek, J., 2016. Effects of terbuthylazine-desethyl, a terbuthylazine degradation product, on red swamp crayfish (*Procambarus clarkii*). Sci. Total Environ. 566–567, 733–740.

Stara, A., Kouba, A., Velisek, J., 2018. Biochemical and histological effects of subchronic exposure to atrazine in crayfish *Cherax destructor*. Chem. Biol. Interact. 291, 95–102.

Stara, A., Bellinvia, R., Velisek, J., Strouhova, A., Kouba, A., Faggio, C., 2019. Acute exposure of common yabby (*Cherax destructor*) to the neonicotinoid pesticide. Sci. Total Environ. 665, 718–723.

Torre, A., Trischitta, F., Faggio, C., 2013a. Effect of $CdCl_2$ on regulatory volume decrease (RVD) in *Mytilus galloprovincialis* digestive cells. Toxicol. Vitro 27, 1260–1266.

Torre, A., Trischitta, F., Corsaro, C., Mallamace, D., Faggio, C., 2013b. Digestive cells from *Mytilus galloprovincialis* show a partial regulatory volume decrease following acute hypotonic stress through mechanisms involving inorganic ions. Cell Biochem. Funct. 31, 489–495.

Trask, J.R., Harbourt, C.M., Johnson, L.C., Ball, R.H., 2003. S-metolachlor Monitoring Data for Community Water Systems in 43 States in 2002. Syngenta Crop Protection Report T001 596-03. Inc., Greensboro, NC.

Uchiama, M., Mihara, M., 1978. Determination of malonyldialdehyd precursor in tissue by thiobarbituric acid test. Anal. Biochem. 86, 271–278.

Velmurugan, B., Selvayagam, M., Cengiz, E.I., Ulmu, E., 2007. The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*. J. Environ. Sci. Health 42, 157–163.

Velisek, J., Stara, A., Zuskova, E., Kubec, J., Buric, M., Kouba, A., 2018a. Chronic toxicity of metolachlor OA on growth, ontogenetic development, antioxidant biomarkers and histopathology of early life stages of marbled crayfish. Sci. Total Environ. 643, 1456–1463.

Velisek, J., Stara, A., Zuskova, E., Kubec, J., Buric, M., Kouba, A., 2018b. Effects of s-metolachlor on early life stages of marbled crayfish. Pestic. Biochem. Physiol. (in press).

White, S., 2017. Impact of Air and Water Pollution on the Environment and Public Health. House of Lords Library Briefing, p. 21.

Wolf, M.C., Moore, P.A., 2002. The effects of the herbicide metolachlor on the perception of chemical stimuli by *Orconectes rusticus*. J. North Am. Benthol. Soc. 21, 457–467.

Yao, H., Li, W., Qian, X., 2015. Identification of major risk sources for surface water pollution by risk indexes (RI) in the multi-provincial boundary region of the Taihu basin, China. Int. J. Environ. Res. Public Health 12, 10150–10170.

Yun, M.A., Wei-Ping, L.I.U., Yue-Zhong, W.E.N., 2006. Enantioselective degradation of Rac-metolachlor and S-metolachlor in soil. Pedosphere 16, 489–494.

Zemolin, C.R., Avila, L.A., Cassol, G.V., Massey, J.H., Camargo, E.R., 2014. Environmental fate of S-metolachlor: a review. Planta Daninha 32, 655–664.

CHAPTER 6

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

GENERAL DISCUSSION

Decisive processes are an integral part of all animal life, solving essential problems connected with development, food acquisition, social behaviour and reproduction (Danchin et al., 2008). One of the most important, and most studied, is sexual selection. During mate selection, individuals follow the possible benefits of reproduction, expressed as the best offspring fitness by choosing the best available mate on the basis of visual, chemical, and behavioural cues (Alcock, 2001; Roberts and Gosling, 2003). Sexual history, dominance status, size of body parts, investment in offspring, and fitness are advantageous characteristics in sexual selection (Jennions and Petrie, 1997). During mate selection, individuals often engage in agonistic interactions with competitors (Alcock, 2001; Hunt et al., 2009). Crayfish are no exception. Male competition for a female has been observed in crayfish, however, the female may choose to receive or refuse either male, regardless of the outcome of the battle (Gherardi, 2002; Mellan et al., 2014). An important aspect of successful reproduction is recognition by both partners. Males can recognize whether the distant females are receptive or non-receptive based on pheromone signalling (Breithaupt and Thiel, 2011; Gherardi, 2002). Recent studies focused on social interaction hierarchies, winner and loser cognition, and gender and conspecific recognition demonstrate the capabilities of individual recognition in crayfish such as Cambaridae (Aquiloni and Gherardi, 2008; Aquiloni et al., 2009), Parastacidae (Seebacher and Wilson, 2007; Van der Velden et al., 2008) and Astacidae (Acquistapace et al., 2002).

The present study on female spiny cheek crayfish mate selection proved that reproductively active females can distinguish between familiar (originating from the same population) and unfamiliar males (from different, geographically distant population). Female's choosers demonstrated a preference for selecting familiar mates based on the frequency of premating contacts and time spent in the male's area. While recognition of familiar and unfamiliar females by males may not lead to preferred mates from the same geographical locality. Clearly, mate preference is not based on long-term memory or fully differentiated individuals, Van der Velden et al. (2008) limited cognition ability of crayfish only up to three individuals. Crayfish are mostly dependent on chemical signals for circumstances evaluation and decision making. However, some questions persist, e.g. why should females prefer familiars, and how do they detect the difference between familiars and individuals from distant localities. It could be importance of familiar chemical cues from social interactions at the locality as described in vertebrates. Recognition and preference of familiars in shoaling are well documented in fish species such as guppies (Griffiths and Magurran, 1997), the rainbow fish (Brown, 2002), cyprinids (Barber and Wright, 2001; Ward et al., 2003) and the sticklebacks (Frommen and Bakker, 2004; Mehlis et al., 2008; Ward et al., 2005).

Mate choice towards familiars was over studied in invertebrates such as the American lobster (Karavanich and Atema, 1998), the red band shrimp (Johnson, 1977), the mantis shrimp (Caldwell, 1992), the big-claw snapping shrimp (Rahman et al., 2004), and the spiny-cheek crayfish in present study. A mechanism of natural selection according to familiarity is not unusual and reduces potentially costly aggressive behaviour. The familiarity effects stabilize the group hierarchy and increases feeding and fitness levels, e.g. in the case of sea trout (Hojesjo et al., 1998). Similarly, Seppa et al. (2001) examined the influence of familiarity on increased fitness of familiar groups in the arctic charr. Subsequently, crayfish chemical cue of familiarity could be a beneficial investment into offspring (fitness), or dominance status cognition (Gherardi and Daniels, 2004), decreased agonistic behaviour, reduced cost of competition (Bovbjerg, 1953; Daws et al., 2011), visual and chemical cue attractiveness or just shortening courtship period due to high investment cost into gametes. Previous cohabitation could lead to a microbiome-like effect of animals living in a similar locality (Ezenwa et al., 2012; Arbuthnott et al., 2016). These advantages may explain why female mate selection favours mates from the same locality.

On the other hand, males did not prefer any certain locality-specific females. It can be assumed that crayfish males are able to discriminate between females (Acquistapace et al., 2002; Ameyaw-Akumfi and Hazlett, 1975), but they do not show a strong relationship for mating with familiar or unfamiliar females. This behavioural pattern could be based on evolved strategies for allocating as much as possible sperm to increase male's probability of spreading their offspring, and hence maximize survival of their genetic material. The studies of Villanelli and Gherardi (1998) and Galeotti et al. (2007) described the intentional removal of spermatophores from females. Even in Cambaridae, the mating plug of a previous male could be removed (Snedden, 1990). Stimulus to mate with every female or everything (Stebbing et al., 2003) could be more substantial than long-term decision making for the right mate. This conclusion suggests that male selectors showed more agonistic behaviour against females and grasped females to mate independently on their preferences. This demonstrates the different reproductive strategies of both sexes. Females rely on some familiarity of sexual partners as a reliable guarantee for successful mating. This study raised the question on the importance of familiarity in mating in mixed wildlife populations, where significant differences present in size of adults.

Agonistic behaviour is characteristic of all crayfish life stages. It is driven by different factors, including direct competition for resources and space (inter- and intra-specific), mates or for the establishment of hierarchy (Gherardi, 2002; Krebs and Davies, 1997). Agonistic behaviour is primarily demonstrated by increased aggression. The use of chemical or visual communication signals to intimidate an opponent can be a substitute for combat avoiding the unnecessary higher costs of physical aggression (Breithaupt and Thiel, 2011). Agonistic inter- and intraspecific interactions occur when a stranger invades a crayfish's territory (Bruski and Dunham, 1987). Successful invaders mostly exhibit agonistic behaviour using higher aggression during interspecific interactions (Gherardi, 2002). The red swamp crayfish is a well-known invader showing higher activity and aggression towards other species, even conspecifics (Jimenez and Faulkes, 2011). Nowadays, freshwater ecosystems are faced with a new invader, which is considered to be more placid in stress or unfavourable conditions (Kawai et al., 2006) and is often stimulated only by a mechanical stimuli (Kasuya and Nagayama, 2016). The marbled crayfish, has spread to natural habitats worldwide, posing a competitive threat to other species, despite its reputation for a calm disposition.

We provide new evidence that marbled crayfish could be a potent challenger towards native crayfish species or even already established aliens, with higher levels of activity and aggression. This parthenogenetic species was able to establish dominance over the red swamp crayfish in both developmental categories (juveniles and adults). In addition, the hierarchy remained undefined in about 50% of pairs in mature groups, whereas in the premature groups, it was only 10%. The mature individuals seem to be more reserved to fight for establishing dominancy. Crayfish need more time to establish dominance due to prolonged cognition and assessment of the size-matched opponent. Sato and Nagayama (2012) reviewed effect of faster formation of social hierarchy in small crayfish. Mature marbled crayfish dominated in female–female pairs, while the dominance of marbled crayfish was less pronounced (60% with male and 100% with female) in the female–male pairs. This is probably caused by the more aggressive nature of males, or perhaps by their proportionally larger chelae (Gherardi, 2002). Male and female crayfish use different strategies during agonistic interactions (Wofford, 2013). Males are usually skilled in resource-holding potential (RHP) (Shine, 1989; Wilson, 1992), especially for competing in mate selection (Draud et al., 2004). While females spent most of their time and energy in reproduction (including vitellogenesis) and carrying young

(Trivers, 1972). Mature crayfish males are more aggressive than females, but in premature animals, their agonistic features are not pronounced by sex (Bovbjerg, 1956). While hierarchy is formed between premature crayfish as early as the third developmental stage (Issa et al., 1999).

The agonistic behaviour, and its patterns, are closely related to the size and the age of crayfish (Bovbjerg, 1956). Mature individuals showed significant differences in their total number of fights and their duration, while pre-mature fights did not. Previous study of Schroeder and Huber (2001) described the fight duration of small crayfish as less intense as they escalated more rapidly, was shorter and resolved more rapidly. Our results suggest premature red swamp crayfish are more active in contact and initiation of fights, despite many cases of unsuccessful swipes, which helped with the formation of dominance hierarchies by marbled crayfish.

Actually, chemo-, visual, and tactile cues are commonly associated with body fitness and serve as potential predictors of dominance (Breithaupt and Eger, 2002; Pavey and Fielder, 1996). Animals need to compare and evaluate all their surrounding events during interaction for their own benefit (Parker, 1974; Whitehouse, 1997), including avoiding dominant individuals (Hojesjo et al., 1998). Naturally, the factor of social cognition and previous hierarchical status should be disrupted by a prolonged acclimation of separately held crayfish (Schneider et al., 1999). Experimental animals avoid each other or fight, starting earlier than communication became, and subordinate crayfish bypassed to dominant ones raising claws at a distance. This behaviour is clearly based on visual and chemical communication and commonly with a tail flip retreat, which is considered to be a defensive strategy to reduce energy losses (Lang et al., 1977).

In conclusion, intra- and interspecific interactions provide a wide spectrum of new knowledge towards our understanding of behavioural patterns and impact estimation of invasive species on native ones, or for comparison with more invasive species. The parthenogenesis, high fecundity, potential to overpower the more aggressive species, and other abilities previously mentioned make the marbled crayfish a substantial threat to native ecosystems and the biodiversity of freshwater systems.

The biodiversity and functioning of aquatic ecosystems is based, not only on the species composition, but also on water quality. The effluents from water treatment plants are a source of pharmaceutical compounds (PhACs), which cause ecological changes (Grabicová et al., 2015; Grabicová et al., 2017). Pollution by psychoactive drugs influences the behaviour of aquatic vertebrates, and even invertebrates (Brodin et al., 2013; Buřič et al., 2018). Insight into the behavioural endpoints of PhAC occurrence in freshwater systems is helpful to investigate their ecological consequences. The use of behaviour as a criterion for pharmacological risk assessment has great potential (Brodin et al., 2014; Klaminder et al., 2014). E.g. Tierney (2016) and Buřič et al. (2018) observed reduced locomotion in crayfish exposed to fluoxetine and citalopram at environmentally relevant concentrations. Similar studies have shown that fluoxetine (at higher concentrations) affected movement and burrowing behaviour of freshwater mussels (Hazelton et al., 2014) and decreased locomotion in marine snails through foot detachment (Fong et al., 2015; Fong and Molnar, 2013). In conclusion, our work showed behavioural effects of psychoactive drugs on marbled crayfish exposed to dilute concentrations which are currently detectable in natural conditions (Fedorova et al., 2014). Our results confirm various consequences of low concentration of PhACs on the aquatic environment. Locomotory behaviour (described as the activity and travel distance) can result in higher energy costs and induce shorter life spans in the more active individuals (Gherardi, 2002). Most psychoactive compounds exhibit SSRI (Selective Serotonin Reuptake Inhibitor) effects that change serotonin levels in exposed organisms (Lillesaar, 2011). In crustaceans, the increased serotonin level could influence other hormones, e.g. the crustacean hyperglycaemic hormone, to increase locomotor activity (McPhee and Wilkens, 1989) and exploration behaviour. Buřič et al. (2018) suggests an increase in boldness and activity of crayfish after SSRI exposure. Boldness is associated with aggression (Huntingford, 2013), but leads to higher risk of predation. Boldness, or reduction in shelter seeking behaviour, are also linked to increased anxiety through elevated brain serotonin levels (Fossat et al., 2015; Fossat et al., 2014). Anxiety, high aggression, and higher vulnerability to predators have repercussions for ecosystem functioning and needs to be investigated further with alarm or predator cue.

Our results identified the environmental risks of Oxazepam, but surprisingly no behavioural alterations have been observed for the antidepressant Venlafaxine. The effects of Venlafaxine are reported at higher concentrations (Fong et al., 2015) compared to other antidepressants, such as citalopram (Buřič et al., 2018) and sertraline (Hossain et al., 2019). We attempted to minimize the variables and use similar approaches for observation. Pharmaceutical applications imitated the environmental intoxication from dilute solutions and genetically uniform marbled crayfish to minimise the genotype bias. Mainly the mechanism of action in Venlafaxin is needed to investigate in detail and to examine simultaneously effect of these compounds in aquatic ecosystem (Di Lorenzo et al., 2019). With consideration of previous literature we show wide variability in type, strength, and direction of PhAC effects. These effects can be considered dependent on species tested, concentration tested, exposure time, exposure technique as well as experimental conditions and observations. Tested pharmaceuticals could alter aquatic invertebrate behaviour at the dilute concentrations currently detected in environmental conditions (Grabicová et al., 2015). The effects of environmentally relevant concentrations of pollutants remain to be elucidated, despite their widespread presence worldwide (Petrie et al., 2016).

Bioindication is a practical way for assessing xenobiotic-driven changes in the ecosystem. The main advantage of bioindicators is their fast detection of pollutants even at low concentrations. (Kuklina et al., 2013; Parmar et al., 2016). One of the most important pollutants in this group are pesticides. Our results showed stimulation of sensory-mediated behaviour of locomotion. These changes were detected even in tested environmental concentrations. Marbled crayfish, subjected to prolonged exposure of s-metolachlor and metolachlor OA metabolite at environmental and sub-lethal concentrations, were subsequently exposed to herbicide-free-water to investigate behaviour during their recovery period. Environmental concentrations of herbicides do not directly induce mortality, but they have the potential to induce behavioural changes in aquatic organisms (Velíšek et al., 2018). Exposed crayfish did not exhibit significant differences in the evaluated parameters compared to the control at either exposure time. Still, there are trends of increased crayfish movement and velocity following the study of Velíšek et al. (2019). Pesticides mostly influence crayfish perception of chemical cues (Wolf and Moore, 2002). Crayfish have higher behavioural sensitivity to water-borne chemicals at sub-lethal concentrations. General effects of s-metolachlor and metolachlor OA led to increased movement caused by reduced orientation and environmental cognition (Cook and Moore, 2008). Obvious alterations in distance moved, velocity, and activity were observed after 28 days depuration compared to that observed during exposure. Regression to lower mobility parameters and detoxification, or to sensory receptor impairment probably caused decreased behavioural values rather than stabilization of crayfish, at least within 28 recovery days. Exposure to both herbicides was associated with physical impairments requiring energy for recovery. Survival of crayfish relies heavily on spatial sensory information for avoiding predation and finding mates (Hazlett, 1990; Keller and Moore, 1999; Giri & Dunham, 2000; Belanger & Moore, 2006), detecting food (Moore et al., 1991), responding to alarm cues (Wolf & Moore, 2002), determine social status (Zulandt Schneider et al., 2001), and palpitation changes (Kuklina et al., 2018). Our findings might have some implications for survival due to inadequate behavioural responses to environmental stimuli. We should consider behavioural alternations as early warning signals of herbicide effects in aquatic ecosystem (Velíšek et al., 2019).

Reviewed experimental studies investigated intended goals of this thesis. These findings contribute to our understanding of crayfish reproduction and paves the way for further research. Similarly, interspecific interactions of invasive species suggest a potential risks of rather overlooked species for which no further progress was anticipated.

References

Acquistapace, P., Aquiloni, L., Hazlett, B.A., Gherardi, F., 2002. Multimodal communication in crayfish: sex recognition during mate search by male *Austropotamobius pallipes*. Can J Zool 80 (11): 2041–2045.

Alcock, J., 2001. Animal Behavior: an Evolutionary Approach, seventh ed. Sinauer Publishers, Sunderland, Mass.

Ameyaw-Akumfi, C., Hazlett, B.A., 1975. Sex recognition in the crayfish *Procambarus clarkii*. Science 190 (4220): 1225–1226.

Aquiloni, L., Gherardi, F., 2008. Evidence of female cryptic choice in crayfish. Biol. Lett. 4, 163-165.

Aquiloni, L., Massolo, A., Gherardi, F., 2009. Sex identification in female crayfish is bimodal. Naturwissenschaften 96 (1): 103–110.

Arbuthnott, D., Levin, T.C., Promislow, D.E.L., 2016. The impacts of Wolbachia and the microbiome on mate choice in *Drosophila melanogaster*. J Evolution Biol 29 (2): 461–468.

Barber, I., Wright, H.A., 2001. How strong are familiarity preferences in shoaling fish? Anim Behav 61:975-979.

Belanger, R.M., Moore, P.A., 2006. The use of the major chelae by reproductive male crayfish (*Orconectes rusticus*) for discrimination of female odours. Behaviour 143, 713–731.

Bovbjerg, R.V., 1956. Some factors affecting aggressive behavior in crayfish. Physiol Zool 29:127–136.

Bovbjerg, R. V., 1953. Dominance order in the crayfish *Orconectes virilis* (Hagen). Physiol Zool 26 (2): 173-178.

Breithaupt, T., Eger, P., 2002. Urine makes the difference. J Exp Biol 205:1221–1231.

Breithaupt, T., Thiel, M., 2011. Chemical Communication in Crustaceans. Springer, New York.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339, 814–815.

Brodin, T., Piovano, S., Fick, J., Klaminder, J., Heynen, M., Jonsson, M., 2014. Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. Philos. Trans. R. Soc. B 369, 20130580.

Brown, C., 2002. Do female rainbowfish (*Melanotaenia* spp.) prefer to shoal with familiar individuals under predation pressure? J Ethol 20 (2): 89–94.

Bruski, C.A., Dunham, D.W., 1987. The importance of vision in agonistic communication of the crayfish *Orconectes rusticus* .1. An analysis of Bout dynamics. Behaviour 103, 83–107.

Buřič, M., Grabicová, K., Kubec, J., Kouba, A., Kuklina, I., Kozák, P., Grabic, R., Randák, T., 2018. Environmentally relevant concentrations of tramadol and citalopram alter behaviour of an aquatic invertebrate. Aquat. Toxicol. 200, 226–232.

Caldwell, R. L., 1992. Recognition, signaling and reduced aggression between former mates in a stomatopod. Anim Behav 44 (1): 11–19.

Cook, M.E., Moore, P.A., 2008. The effects of the herbicide metolachlor on agonistic behavior in the Crayfish, *Orconectes rusticus*. Arch. Environ. Contam. Toxicol. 55, 94–102.

Danchin, E., Giraldeau, L. A., Cézilly, F., 2008. Behavioural ecology (No. Sirsi) i9780199206292).

Daws, A.G., Hock, K., Huber, R., 2011. Spatial structure of hierarchical groups: testing for processes of aggregation, clustering, and spatial centrality in crayfish (*Orconectes rusticus*). Mar Freshw Behav Phy 44 (4): 209–222.

Di Lorenzo, T., Castaño-Sánchez, A., Di Marzio, W.D., García-Doncel, P., Martínez, L.N., Galassi, D.M.P., Iepure, S., 2019. The role of freshwater copepods in the environmental risk assessment of caffeine and propranolol mixtures in the surface water bodies of Spain. Chemosphere 2019, 220, 227–236.

Draud, M., Macías-Ordóñez, R., Verga, J., Itzkowitz, M., 2004. Female and male Texas cichlids (*Herichthys cyanoguttatum*) do not fight by the same rules. Behav Ecol 15:102–108.

Ezenwa, V.O., Gerardo, N.M., Inouye, D.W., Medina, M., Xavier, J.B., 2012. Animal behavior and the microbiome. Science 338 (6104): 198–199.

Fedorova, G., Golovko, O., Randak, T., Grabic, R., 2014. Storage effect on the analysis of pharmaceuticals and personal care products in wastewater. Chemosphere 111, 55–60.

Fong, P.P., Bury, T.B., Dworkin-Brodsky, A.D., Jasion, C.M., Kell, R.C., 2015. The antidepressants venlafaxine ("Effexor") and fluoxetine ("Prozac") produce different effects on locomotion in two species of marine snail, the oyster drill (*Urosalpinx cinerea*) and the starsnail (*Lithopoma americanum*). Mar Environ Res 103: 89–94.

Fong, P.P., Molnar, N., 2013. Antidepressants cause foot detachment from substrate in five species of marine snail. Mar Environ Res 84: 24–30.

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Cattaert, D., Delbecque, J-P., 2015. Serotonin, but not dopamine, controls the stress response and anxiety-like behavior in the crayfish *Procambarus clarkii*. J Exp Biol 218: 2745–2752.

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J-P., Cattaert, D., 2014. Anxiety-like behavior in crayfish is controlled by serotonin. Science 344: 1293-1297.

Frommen, J.G., Bakker, T.C.M., 2004. Adult three-spined sticklebacks prefer to shoal with familiar kin. Behaviour 141:1401–1409.

Galeotti, P., Pupin, F., Rubolini, D., Sacchi, R., Nardi, P.A., Fasola, M., 2007. Effects of female mating status on copulation behaviour and sperm expenditure in the freshwater crayfish *Austropotamobius italicus*. Behavioral Ecology and Sociobiology, 61(5), 711–718.

Gherardi, F., 2002. Behaviour. In: Holdich, D.M. (Ed.), Biology of Freshwater Crayfish. Blackwell Science, USA and Canada. Iowa State University Press, Oxford, England; Ames, Iowa, pp. 258–290.

Gherardi, F, Daniels, W.H., 2004. Agonism and shelter competition between invasive and indigenous crayfish species. Can J Zool 82:1923–1932.

Giri, T., Dunham, D.W., 2000. Female crayfish (*Procambarus clarkii* (Girard, 1852)) use both antennular rami in the localization of male odour. Crustaceana 73, 447–458.

Grabicová, K., Grabic, R., Bláha, M., Kumar, V., Červený, D., Fedorova, G., Randák, T., 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Res. 72, 145–153.

Grabicová, K., Grabic, R., Fedorova, G., Fick, J., Červený, D., Kolářová, J., Turek, J., Žlábek, V., Randák, T., 2017. Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream. Water Res. 124, 654–662.

Griffiths, S.W., Magurran, A.E., 1997. Schooling preferences for familiar fish vary with group size in a wild guppy population. P Roy Soc B-Biol Sci 264 (1381): 547–551.

Hazelton, P.D., Du, B., Haddad, S.P., Fritts, A.K., Chambliss, C.K., Brooks, B.W., Bringolf, R.B., 2014. Chronic fluoxetine exposure alters movement and burrowing in adult freshwater mussels. Aquat Toxicol 151: 27–35.

Hazlett, B.A., 1990. Source and nature of disturbance-chemical system in crayfish. J. Chem. Ecol. 16, 2263–2275.

Hazlett, B., Rubenstein, D., Rittschof, D., 1975. Starvation, energy reserves, and aggression in the crayfish *Orconectes virilis* (Hagen, 1870) (Decapoda, Cambaridae). Crustaceana, 11–16.

Hojesjo, J., Johnsson, J.I., Petersson, E., Jarvi, T., 1998. The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). Behav Ecol 9 (5): 445–451.

Hossain, M.S., Kubec, J., Grabicová, K., Grabic, R., Randák, T., Guo, W., Kouba, A., Buřič, M., 2019. Environmentally relevant concentrations of methamphetamine and sertraline modify the behavior and life history traits of an aquatic invertebrate. Aquatic Toxicology 213: 105222.

Hunt, J., Breuker, C.J., Sadowski, J.A., Moore, A.J., 2009. Male-male competition, female mate choice and their interaction: determining total sexual selection. J. Evol. Biol. 22, 13–26.

Huntingford, F.A., 2013. Animal conflict: Springer Science & Business Media.

Issa, F.A., Adamson, D.J., Edwards, D.H., 1999. Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. J Exp Biol 202: 3497–3506.

Jennions, M.D., Petrie, M., 1997. Variation in mate choice and mating preferences: a review of causes and consequences. Biol. Rev. 72, 283–327.

Jimenez, S.A, Faulkes, Z., 2011. Can the parthenogenetic marbled crayfish marmorkrebs compete with other crayfish species in fights? J Ethol 29:115–120.

Johnson, V. R., 1977. Individual recognition in banded shrimp *Stenopus hispidus* (Olivier). Anim Behav 25 (May): 418–428.

Karavanich, C., Atema, J., 1998. Individual recognition and memory in lobster dominance. Anim Behav 56: 1553–1560.

Kasuya, A., Nagayama, T., 2016. Habituation of backward escape swimming in the Marbled crayfish. Zool Sci 33:6–12.

Kawai, T., Faulkes, Z., Scholtz, G., 2016. Freshwater crayfish: a global overview. CRC Press, New York.

Keller, T.A., Moore, P.A., 1999. Effects of ontogeny and odors on behavior: the influence of crayfish size and fish odors on crayfish movement. Mar. Freshw. Behav. Physiol. 33, 35–50.

Klaminder, J., Jonsson, M., Fick, J., Sundelin, A., Brodin, T., 2014. The conceptual imperfection of aquatic risk assessment tests: highlighting the need for tests designed to detect therapeutic effects of pharmaceutical contaminants. Environ. Res. Lett. 9, 084003.

Krebs, J.R., Davies, N.B., 1997. Behavioural ecology: an evolutionary approach, fourth ed. Blackwell Science, Cambridge, Mass.

Kuklina, I., Kouba, A., Kozák, P., 2013. Real-time monitoring of water quality using fish and crayfish as bio-indicators: a review. Environ. Monit. Assess. 185, 5043–5053.

Kuklina, I., Ložek, F., Císař, P., Kouba, A., Kozák, P., 2018. Crayfish can distinguish between natural and chemical stimuli as assessed by cardiac and locomotor reactions. Environ. Sci. Pollut. Res.

Lang, F., Govind, C., Costello, W.J., Greene, S.I., 1977. Developmental neuroethology: changes in escape and defensive behavior during growth of the lobster. Science 197:682–685.

Lillesaar, C., 2011. The serotonergic system in fish. J. Chem. Neuroanat. 41, 294–308.

McPhee, M., Wilkens, J., 1989. Serotonin, but not dopamine or octopamine, modifies locomotor and phototaxic behavior of the crab, *Carcinus maenas*. Can J Zool 67: 391–393.

Mehlis, M., Bakker, T.C.M, Frommen, J.G., 2008. Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. Anim Cogn 11(4):643–650.

Mellan, D., Warren, A., Buckholt, M.A., Mathews, L.M., 2014. Sexual history affects mating behavior and mate choice in the crayfish *Orconectes limosus*. Ethology 120, 681–692.

Moore, P.A., Scholz, N., Atema, J., 1991. Chemical orientation of lobsters, *Homarus americanus*, in turbulent odor plumes. J. Chem. Ecol. 17, 1293–1307.

Parker, G.A., 1974. Assessment strategy and the evolution of fighting behaviour. J Theor Biol 47:223–243.

Parmar, T.K., Rawtani, D., Agrawal, Y.K., 2016. Bioindicators: the natural indicator of environmental pollution. Front. Life Sci. 9, 110–118.

Pavey, C., Fielder, D., 1996. The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda: Parastacidae). J Zool 238:445–457.

Petrie, B., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2016. Multi-residue analysis of 90 emerging contaminants in liquid and solid environmental matrices by ultra-high performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1431, 64–78.

Rahman, N., Dunham, D.W., Govind, C.K., 2004. Mate choice in the big-clawed snapping shrimp, *Alpheus heterochaelis* Say, 1818. Crustaceana 77: 95–111.

Roberts, S.C., Gosling, L.M., 2003. Genetic similarity and quality interact in mate choice decisions by female mice. Nat. Genet. 35, 103–106.

Sato, D., Nagayama, T., 2012. Development of agonistic encounters in dominance hierarchy formation in Juvenile crayfish. J Exp Biol 215:1210–1217.

Schneider, R.A.Z., Schneider, R.W., Moore, P.A., 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. J Chem Ecol 25:781–794.

Schroeder, L., Huber, R., 2001. Fight strategies differ with size and allometric growth of claws in crayfish, *Orconectes rusticus*. Behaviour 138:1437–1449.

Seebacher, F., Wilson, R.S., 2007. Individual recognition in crayfish (*Cherax dispar*): the roles of strength and experience in deciding aggressive encounters. Biol. Lett. 3, 471–474.

Seppa, T., Laurila, A., Peuhkuri, N., Piironen, J., Lower, N., 2001. Early familiarity has fitness consequences for Arctic char (*Salvelinus alpinus*) juveniles. Can J Fish Aquat Sci 58 (7): 1380–1385.

Shine, R., 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. Q Rev Biol 64: 419–461.

Snedden, W.A., 1990. Determinants of male mating success in the temperate crayfish *Orconectes rusticus* - chela size and sperm competition. Behaviour 115: 100–113.

Stebbing, P.D., Bentley, M.G., Watson, G.J., 2003. Mating behaviour and evidence for a female released courtship pheromone in the signal crayfish *Pacifastacus leniusculus*. Journal of chemical ecology, 29(2), 465–475.

Tierney, A.J., 2016. Effects of fluoxetine on growth and behavior in the crayfish *Orconectes rusticus*. Mar Freshw Behav Physiol v. 49: 133–145.

Trivers, R., 1972. Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man. Aldine, Chicago, pp 136–179.

Van der Velden, J., Zheng, Y., Patullo, B.W., Macmillan, D.L., 2008. Crayfish recognize the faces of fight opponents. PloS One 3, e1695.

Villanelli, F., Gherardi, F., 1998. Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. Freshwater Biol 40 (2): 305–315.

Velíšek, J., Stará, A., Zusková, E., Kubec, J., Buřič, M., Kouba, A., 2019. Effects of s-metolachlor on early life stages of marbled crayfish. Pesticide biochemistry and physiology, 153, 87–94.

Velíšek, J., Stará, A., Zusková, E., Kubec, J., Buřič, M., Kouba, A., 2018. Chronic toxicity of metolachlor OA on growth, ontogenetic development, antioxidant biomarkers and histopathology of early life stages of marbled crayfish. Sci. Total Environ. 643, 1456–1463.

Ward, A.J.W., Axford, S., Krause, J., 2003. Cross-species familiarity in shoaling fishes. P Roy Soc B-Biol Sci 270(1520): 1157–1161.

Ward, A.J.W., Holbrook, R.I., Krause, J., Hart, P.J.B., 2005. Social recognition in sticklebacks: the role of direct experience and habitat cues. Behav Ecol Sociobiol 57(6): 575–583.

Whitehouse, M.E., 1997. Experience influences male–male contests in the spider *Argyrodes antipodiana* (Theridiidae: Araneae). Anim Behav 53: 913–923.

Wilson, J.D., 1992. Correlates of agonistic display by great tits *Parus major*. Behaviour 121:168–214.

Wofford, S.J., 2013. Sex and fighting: male and female crayfish utilize different assessment strategies during agonistic behavior. Bowling Green State University, Bowling Green.

Wolf, M.C., Moore, P.A., 2002. The effects of the herbicide metolachlor on the perception of chemical stimuli by *Orconectes rusticus*. J. North Am. Benthol. Soc. 21, 457–467.

Zulandt Schneider, R.A., Huber, R., Moore, P.A., 2001. Individual and status recognition in the crayfish, *Orconectes rusticus*: the effects of urine release on fight dynamics. Behaviour 138, 137–153.

ENGLISH SUMMARY

Decision making in crayfish: behavioral and reproductive issues

Jan Kubec

This thesis provides an overview of case studies investigating the behaviour of freshwater crayfish, including social and reproductive interactions, decision making, and channels for recognition or processing of environmental stimuli. We described relationships between the environment and the organism and relationships among conspecifics and heterospecifics. These aspects are important, given that every decision and interaction has consequences for crayfish survival and reproduction, i.e. individual and even species success. For ethological studies in aquatic ecosystems, crustaceans, and especially crayfish, represent practical model organisms. Crayfish are advantageous over other invertebrates given their high level of social interaction in both the field and the laboratory. They exhibit unique eco- ethological behaviour in variety of feeding habits, activity cycles, habitat selection, and resource utilization at the level of species, sex, and age. Similar to vertebrates, crayfish demonstrate unique behaviours throughout their life. Crayfish are widely accepted as sensitive and rapid bioindicators of water quality changes. They are considered keystone species in freshwater ecosystems and are important ecosystem engineers. Subsequently, pollution impacts on native crayfish stock can result in ecosystem instability.

The different reproductive strategies of females and males demonstrate that females rely on some familiarity of sexual partners as a reliable guarantee for successful mating. Crayfish females are choosier than males because of their higher energy costs for reproduction (oogenesis, incubation, parental care). While male reproduction prioritises securing their genetic information by searching for as many mates as possible. Crayfish mate preferences was analysed by facing the choosing crayfish with two equal sized mature counterparts from the same or different origin. Both sexes were presented either with a single familiar and unfamiliar counterpart or with two unfamiliar ones, reflecting the source population. Our conclusions could be applied in conservation research, captive breeding, and recovery programs as well as in aquaculture. Native crayfish are usually studied to extend knowledge of habitat requirements and mating behaviour, while the invasive crayfish species are investigated for their reproductive behaviour, plasticity, and for developing methods for their control. Description of mate choice in crayfish in this new view naturally opens new research areas in both, field and laboratory studies.

Given the increasing occurrence of biological invasions, invasive species are now more relevant than ever. The success of biological invaders has been described as the outcome from intra- and interspecific interactions. We provide experimental interaction patterns of two of the most effective invasive crayfish species. The marbled crayfish, known as parthenogenetic species with high adaptability, fast growth, early maturation, and high fecundity, established dominant status in more cases by interspecific combat victories. Fight intensity and aggressiveness decreased after dominance was established in particular pairs. We also reported evidence of sex and size effects on agonistic behaviour as dominance establishment. Observed behavioural patterns do not reflect clear interaction outcomes in natural conditions due to greater maximal size of red swamp crayfish in the wild. However, invader success would not only be determined by agression and hierarchy, but also in other factors, such as reproduction strategy and adaptability/plasticity Marbled crayfish seem to have an ability to conserve energy by increasing activity only at times of danger . Based on known species-specific abilities, the marbled crayfish is capable of outcompeting aggressive

species, such as the red swamp crayfish. This parthenogenetic species poses a substantial threat to native ecosystems and biodiversity of aquatic environments, since it can establish not only in crayfish free habitats, but is also capable of outcompeting resident species.

Recently, dilute concentrations of contaminants such as pharmaceuticals, daily-care products and pesticides have been reported to indirectly affect aquatic organisms. Our research of the pharmaceutical effects at the environmentally relevant levels presents a relatively simple methodology to examine the full polluting impacts on organisms in freshwaters. We found that selected pharmaceuticals can alter the behavioural patterns of crayfish, event at the very low concentration. Based on these results and the literature, the effects are both compound are species specific. The mixture of main pollutants should be investigate at field sampling level and also at experimental level to elicit their possible synergic or antagonistic effect. The risks associated with surface water pollutants stresses the importance of research investigating their ecological impacts and their different/specific mechanisms of action. The pharmaceutical industry, energy crops and intensive agriculture are among the fastest growing industries lead to a changing pesticide management. Newly introduced pesticides belong to more effective compounds with decreased toxicity. Nevertheless, the indirect impacts on non-target aquatic animals were observed in our studies. Prolonged exposure to the low concentrations of two pesticides exhibited changes in crayfish behavioural patterns. Various alterations in locomotion were observed after treatment in pesticide-free water. In the wild, decreased movement and activity can be a challenge for crayfish survival because of predation, even foraging to compensate for energy loss could be very difficult for less active individuals. This study suggests that crayfish behaviour assessment is a practical approach not only for investigating basic biology, or intra- and inter-specific interactions, but also for assessing the risks associated with micro-pollutants.

CZECH SUMMARY

Rozhodovací proces u raků: problematika chování a reprodukce

Jan Kubec

Tato práce poskytuje přehled případových studií zkoumající chování sladkovodních raků, včetně sociálních a reprodukčních interakcí, rozhodovacích procesů a prostředků pro rozpoznávání nebo zpracování environmentálních podnětů. Popsali jsme vztahy mezi prostředím a organizmem, stejně tak i vnitrodruhové a mezidruhové vztahy. Tyto aspekty jsou důležité vzhledem k tomu, že každé rozhodnutí a interakce mají důsledky pro přežití a reprodukci raků, tj. vede k individuálnímu až dokonce i druhovému úspěchu. Pro etologické studie ve vodních ekosystémech představují korýši, a zejména raci, praktické modelové organizmy. Raci jsou vhodnější než ostatní bezobratlí, vzhledem k jejich vysoké úrovni sociální interakce jak v přírodě, tak v laboratorních podmínkách. Vykazují jedinečné eko-etologické chování v různých stravovacích zvyklostech, cyklech aktivity, výběru stanovišť a využívání zdrojů na úrovni druhů, pohlaví či věku. Podobně k obratlovcům, raci demonstrují jedinečné chování během celého jejich života. Raci jsou široce přijímáni jako citliví a rychlí bioindikátoři změn kvality vody. Jsou považováni za klíčové druhy ve sladkovodních ekosystémech a jsou důležitými inženýry ekosystémů. Ovšem, jakékoli znečistění může za následek dopad na původní populaci raků či nestabilitu ekosystému.

Různé reprodukční strategie samic a samců ukazují, že samice se spoléhají na určitou známost sexuálních partnerů jako na spolehlivou záruku úspěšného spáření. Samice raka jsou vybíravější než samci z důvodu vyšších nákladů na reprodukci (oogeneze, inkubace, rodičovská péče). Zatímco samčí reprodukční strategie upřednostňuje zajištění jejich genetických informací hledáním co nejvíce partnerek. Preference raků byly analyzovány tak, že jedinci čelili výběru ze dvou podobných zralých protějšků ze stejným nebo jiným původem. Obě pohlaví byla vystavena buď jednomu známému a neznámému protějšku nebo dvěma neznámým, což odráží zdrojové populace. Naše závěry mohou najít uplatnění v oblasti ochrany přírody, chovu v zajetí a programů obnovy či akvakultury. Původní raci jsou obvykle studováni pro rozšíření znalostí, požadavků na biotop a reprodukční chování, zatímco invazivní druhy raků jsou zkoumány pro jejich reprodukční chování, plasticitu a pro vyvíjející se metody jejich kontroly. Popis výběru partnera u raků v tomto novém pohledu přirozeně otevírá nové výzkumné oblasti jak v terénních, tak laboratorních studiích.

Vzhledem k rostoucímu výskytu biologických invazí jsou nyní invazivní druhy relevantnější než kdy jindy. Úspěch biologických invazivců byl popsán jako výsledek vnitro- či mezidruhových interakcí. Naše studie poskytuje experimentální interakční vzorce dvou nejúspěšnějších invazivních druhů raků. Rak mramorovaný, znám jako parthenogenetický druh s vysokou adaptabilitou, rychlým růstem, časným zráním a vysokou plodností, prokázal dominantní postavení ve více případech mezidruhových bojových vítězství. Intenzita souboje a agresivita se snížily po ustanovení dominance u jednotlivých dvojic. Rovněž jsme uvedli důkazy o vlivu pohlaví a velikosti na agonistické chování jako projev dominance. Pozorované vzorce chování neodrážejí jasné interakční výsledky v přírodních podmínkách v důsledku větší maximální velikosti raka červeného ve volné přírodě. Úspěch invazních druhů by však nebyl předurčen pouze agresí a hierarchií, ale také dalšími faktory, jako je reprodukční strategie a nebo adaptabilita/plasticita. Zdá se tak, že druh raka mramorovaného má schopnost šetřit energii zvyšováním aktivity pouze v případě přímého ohrožení. Na základě známých druhově specifických schopností je rak mramorovaný schopen překonat agresivní druh, jako je rak červený. Tento partenogenetický druh představuje významnou hrozbu pro původní ekosystémy a biologickou rozmanitost vodního prostředí, protože může vytvořit trvalé osídlení nejen v lokalitách postrádajících raky, ale je také schopen překonat rezidentní druhy.

V poslední době bylo zjištěno, že zředěné koncentrace kontaminantů, jako jsou léčiva, přípravky denní péče a pesticidy, nepřímo ovlivňují vodní organizmy. Náš výzkum farmaceutických účinků na úrovni podmínek životního prostředí představuje relativně jednoduchou metodiku pro zkoumání přímých znečišťujících vlivů na organizmy ve sladkých vodách. Zjistili jsme, že vybraná léčiva mohou změnit vzorce chování raka již při velmi nízké koncentraci. Na základě těchto výsledků a literatury bylo popsáno, že účinky sloučenin jsou druhově specifické. Směs hlavních znečišťujících látek by měla být zkoumána na úrovni odběru environmentálních vzorků a také na experimentální úrovni, aby se dosáhlo jejich možného synergického nebo antagonistického účinku. Rizika spojená s látkami znečišťujícími povrchovou vodu zdůrazňují význam našeho výzkumu, který zkoumá jejich ekologické dopady a jejich různé/specifické mechanizmy působení. Mezi nejrychleji rostoucí průmyslová odvětví patří farmaceutický průmysl, energetické plodiny a intenzivní zemědělství, které vedou k měnícímu se managementu pesticidů. Nově zavedené pesticidy patří k účinnějším sloučeninám se sníženou toxicitou. I když v našich studiích byly pozorovány nepřímé dopady na necílové vodní živočichy. Při dlouhodobém vystavení nízkým koncentracím dvou pesticidů vykazovali raci změny ve známých vzorech chování. Po expozici pesticidů prosté vodě byly pozorovány nenadálé změny v lokomoci. Ve volné přírodě může být snížený pohyb a aktivita výzvou pro přežití raků v důsledku predace, a to i pro krmení a kompenzaci energetických ztrát, může být přežití méně aktivních jedinců velmi obtížné. Tato studie podporuje, že hodnocení chování raků je velmi praktickým přístupem nejen pro zkoumání základní biologie, ale i pro vnitro- a mezi-druhové interakce a rovněž pro posuzování rizik spojených s mikropolutanty.

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- GAJU (012/2016/Z), Occurrence and critical evaluation of the effects of contaminants on exposed organisms in aquatic ecosystems and options for prevention and elimination of serious viral diseases in fish culture (2016-2018, responsible leader Assoc. Prof. Vladimír Žlábek)
- GACR 16-06498S, Psychoactive compounds in the aquatic environment and their effects on exposed organisms (2016–2020, responsible leader Prof. Tomáš Randák)

LIST OF PUBLICATIONS

Peer-reviewed journals with IF

Bláha, M., Grabicova, K., Shaliutina, O., **Kubec, J.**, Randák, T., Žlábek, V., Buřič, M., Veselý, L. 2019. Foraging behaviour of top predators mediated by pollution of psychoactive pharmaceuticals and effects on ecosystem stability. Science of the Total Environment, 662: 655–661. (IF 2018 = 5.589)

Buřič, M., Grabicová, K., **Kubec, J.**, Kouba, A., Kuklina, I., Kozák, P., Grabic, R., Randák, T., 2018. Environmentally relevant concentrations of Tramadol and Citalopram alter behavior of an aquatic invertebrate. Aquatic Toxicology 200: 226–232. (IF 2018 = 3.794)

Guo, W., **Kubec, J.**, Hossain, S., Buřič, M., McClain, R., Kouba, A., 2019. High air humidity is sufficient for successful egg incubation and early postembryonic development in the marbled crayfish. Freshwater Biology (accepted). (IF 2018 = 3.404)

Haubrock, P.J., **Kubec, J.**, Veselý, L., Buřič, M., Tricarico, E., Kouba, A., 2019. Water temperature as a hindrance, but not limiting factor for the survival of warm water invasive crayfish introduced in cold periods. Journal of Great Lakes Research (accepted). (IF 2018 = 2.175)

Hossain, Md. S., **Kubec, J.**, Kouba, A., Kozák, P., Buřič, M., 2019. Still waters run deep: marbled crayfish dominates over red swamp crayfish in agonistic interactions. Aquatic Ecology 53: 97–107. (IF 2018 = 2.505)

Hossain, M.S., **Kubec, J.**, Grabicová, K., Grabic, R., Randák, T., Guo, W., Kouba, A., Buřič, M., 2019. Environmentally relevant concentrations of methamphetamine and sertraline modify the behavior and life history traits of an aquatic invertebrate. Aquatic Toxicology 213: 105222. (IF 2018 = 3.794)

Kubec, J., Kouba, A., Buřič, M., 2019. Communication, behaviour and decision making of crayfish: A review. Zoologischer Anzeiger 278: 28–37. (IF 2018 = 1.601)

Kubec, J., Hossain, M. S., Grabicová, K., Randák, T., Kouba, A., Grabic, R., Roje, S., Buřič, M. 2019. Oxazepam alters the behavior of crayfish at diluted concentrations, Venlafaxine does not. Water 11: 196. (IF 2018 = 2.524)

Kubec, J., Kouba, A., Kozák, P., Buřič, M., 2019. Females bet on the known: crayfish females recognize and prefer males from familiar population, males are not picky. Manuscript.

Kubec, J., Evans, T., Hardege, J., Smyth, K., Buřič, M., Breithaupt, T., 2019. Crucial role of alarm odour: a unique anxiety-like behavioural (ethological) model for environmental testing. Manuscript.

Ložek, F., Kuklina, I., Grabicová, K., **Kubec, J.**, Buřič, M., Grabic, R., Randák, T., Císař, P., Kozák, P., 2019. Behaviour and cardiac response to stress in signal crayfish exposed to environmental concentrations of tramadol. Aquatic Toxicology (accepted). (IF 2018 = 3.794)

Stará, A., **Kubec, J.**, Zusková, E., Buřič, M., Faggio, C., Kouba, A., Velíšek, J., 2019. Effects of S-metolachlor and its degradation product metolachlor OA on marbled crayfish (*Procambarus virginalis*). Chemosphere 224: 616–625. (IF 2018 =5.108)

Velíšek, J., Stará, A., Zusková, E., **Kubec, J.**, Buřič, M., Kouba, A. 2019. Effects of s-metolachlor on early life stages of marbled crayfish. Pesticide Biochemistry and Physiology 153: 87–94. (IF 2018 = 2.870)

Velíšek, J., Stará, A., Zusková, E., **Kubec, J.**, Buřič, M., Kouba, A., 2018. Chronic toxicity of metolachlor OA on growth, ontogenetic development, antioxidant biomarkers and histopathology of early life stages of marbled crayfish. Science of the Total Environment 643: 1456–1463. (IF 2018 = 5.589)

Abstracts and conference proceedings

Hossain, M.S., **Kubec, J.**, Kouba, A., Kozák, P., Buřič, M., 2017. Placid but powerful: unexpected success of marbled crayfish over red-swamp crayfish. European Crayfish Conference 2017: The IAA Cruise 2017, Helsinki (Finland) and Stockholm (Sweden), August 16–18, 2017.

Kubec, J., Grabicová, K., Kuklina, I., Kouba, A., Kozák, P., Grabic, R., Randák, T., Buřič, M., 2017. Effects of environmentally relevant concentrations of opioid painkiller and antidepressant drug on crayfish behavior. European Crayfish Conference 2017: The IAA Cruise 2017, Helsinki (Finland) and Stockholm (Sweden), August 16–18, 2017.

Kubec, J., Kouba, A., Kozák, P., Buřič, M., 2016. Mate choice in spiny-cheek crayfish (*Orconectes limosus*, Rafinesque 1817): females prefer males from the same population, males not. In: 21st Symposium of International Association of Astacology (IAA 21), Madrid, Spain, 5–8 September 2016, p. 43.

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Kubec, J., Grabicová, K., Kuklina, I., Kouba, A., Kozák, P., Grabic, R., Randák, T., Buřič, M., 2017. Effects of environmentally relevant concentrations of opioid painkiller and antidepressant drug on crayfish behavior. European Crayfish Conference 2017: The IAA Cruise 2017, Helsinki (Finland) and Stockholm (Sweden), August 16–18, 2017.	2017
Kubec, J., Kouba, A., Kozák, P., Buřič, M., 2016. Mate choice in spiny-cheek crayfish (*Orconectes limosus*, Rafinesque 1817): females prefer males from the same population, males not. In: 21st Symposium of International Association of Astacology (IAA 21), Madrid, Spain, 5–8 September 2016, p. 43.	2016

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