

SHORT COMMUNICATION

Long-lasting generalization triggered by a single trial event in the invasive crayfish *Procambarus clarkii*

Andrea Dissegna, Andrea Caputi and Cinzia Chiandetti*

ABSTRACT

Behavioural flexibility allows adaptation to environmental changes, a situation that invasive species have often to face when colonizing new territories. Such flexibility arises from a set of cognitive mechanisms among which generalization plays a key role, as it allows the transfer of past solutions to solve similar new problems. By means of a habituation paradigm, we studied generalization in the invasive crayfish *Procambarus clarkii*. Once crayfish had habituated their defensive response to a specific water jet, we tested whether habituation transferred to a new type of water jet. Although habituation did not generalize when the new stimulus was initially presented, it surprisingly emerged 15 and 45 days later. Hence, remarkably, in *P. clarkii*, a single presentation of a new event was sufficient to trigger a long-lasting form of learning generalization from previous similar stimuli, a cognitive ability that may concur in providing adaptive advantages to this invasive species.

KEY WORDS: Behavioural flexibility, Habituation, Learning, Invasive species, Defensive response

INTRODUCTION

Humans may unintentionally spread invasive species while transporting animals, for economic or decorative reasons, outside their native geographical range (Blackburn et al., 2011). Invasive species damage the new ecosystem (Ehrenfeld, 2010) and harm human health and activities (Pejchar and Mooney, 2009), affecting the local economy as well (Olson, 2006). Investigating the common characteristics of invasive species, therefore, may help us to anticipate future human-mediated invasions, and to reduce their occurrence and negative impact (Kolar and Lodge, 2001; Blackburn et al., 2011). In particular, increasing evidence attests that many invasive species share similar behavioural traits (Chapple et al., 2012; Sih et al., 2012). Many of them, for example, show enhanced interspecific and intraspecific aggressiveness, which in turn is positively associated with their boldness, foraging ability and exploratory activity (e.g. Rehage and Sih, 2004; Duckworth and Badyaev, 2007; Pintor et al., 2008). Hence, being aggressive may facilitate invasive species when exploring the new environment, discovering and collecting resources and displacing locals.

Behavioural flexibility is another key trait of invasive species (Sol, 2003; Pintor et al., 2009; Wright et al., 2010). This represents an animal's capacity to accommodate its behaviour to environmental changes, therefore assisting invasive species to

disperse and establish outside their ecological niche (Wright et al., 2010). Wright and colleagues (2010) suggested that behavioural flexibility arises from two mechanisms: animals can 'copy' an adaptive behaviour from others, via social learning; or animals can 'invent' an adaptive behaviour, via innovation. While both these mechanisms have been linked to behavioural flexibility (Audet and Lefebvre, 2017), so far their presence has been demonstrated in few species (e.g. Caldwell and Whiten, 2002; Brosnan and Hopper, 2014). Furthermore, innovation and social learning may represent complex behavioural traits entailing other simpler processes (Heyes, 2012; Griffin and Guez, 2014). Hence, other basic mechanisms may have a more direct effect on behavioural flexibility. In invasive crayfish, for example, these mechanisms include a broader stimulus sensitivity to alarm cues of heterospecifics, and a superior memory capacity in associative learning tasks than native species (Hazlett, 2000; Hazlett et al., 2002, 2003).

Generalization could be another basic mechanism underlying behavioural flexibility. This comprises the animal's capacity to use past solutions in present situations regarded as similar. In the context of predation, for example, animals generalize their antipredator response to a new predator by exploiting the recurrent cues that anticipate a threat across different situations (like the sudden appearance of an odour, a noise or a looming shadow) (Ferrari et al., 2007). Generalization can be considered a basic form of learning because it is universal across animals and is independent from the context, the stimulus modality or the type of response (learned or innate) (Ghirlanda and Enquist, 2003). But, to the best of our knowledge, it has never been studied in relation to the invasive potential of a species.

With this hypothesis in mind, we tested individuals of *Procambarus clarkii* (Girard 1852), an invasive decapod that was introduced in Spain from the southern regions of the USA and Mexico and spread quickly all over Europe (Gherardi, 2006, 2010; Souty-Grosset et al., 2016). Specifically, it arrived in Italy in 1987 for breeding purposes and the first reproductive population was documented in 1989 in the Piedmont region (Del Mastro, 1992; Aquiloni et al., 2010). Hence, it represents an ideal model to study the relationship between behavioural flexibility and the invasive potential of a species. One previous study (Chiandetti and Caputi, 2017) addressed generalization ability in the visual domain in *P. clarkii*, but without providing conclusive evidence. In the present experiment, we used a similar habituation paradigm to test whether in crayfish this ancestral form of learning generalizes between a familiar and a novel water jet. Furthermore, we tested the duration of habituation for up to 45 days.


MATERIALS AND METHODS

Subjects

Red swamp crayfish (*P. clarkii*: $n=14$, 3 males, 11 females) were collected from an artificial pond (Bonifica del Brancolo, 45°46'N, 13°30'E, Province of Gorizia, Italy) and transported to our

Department of Life Sciences, University of Trieste, 34127 Trieste, Italy.

*Author for correspondence (cchiandetti@units.it)

 A.D., 0000-0002-4333-3353; A.C., 0000-0002-4183-2538; C.C., 0000-0002-7774-6068

Received 29 April 2020; Accepted 8 October 2020

laboratory. On their arrival, crayfish were housed in individual plastic tanks (10×14×12 cm) filled with clean water. The walls of the tanks were opaque to limit their visual experience with the external environment. Illumination was provided following a 12 h:12 h dark:light cycle and water temperature was kept constant at 21°C. Crayfish rostrum to telson length ranged between 9.06 and 11.05 cm (mean±s.e.m. 9.94±0.09 cm).

Ethics

The experiment complied with the European Community and Italian laws on animal experiments. After completion of the experiments, crayfish were not returned to their natural habitat but were instead killed by hypothermia because the law forbids the release of invasive species (L.R. 7/2005).

Apparatus

Crayfish were tested within an opaque dry rectangular arena (as used in Chianchetti and Caputi, 2017). An LCD flat screen illuminated the inside of the arena with a white diffused light. On the opposite side, two water sprayers were attached to the wall at about 20 cm from the floor and 2 cm apart from each other. The exploratory space where the crayfish could freely walk was surrounded by a Poliplack partition in a semi-circular shape. The whole arena was covered by a black curtain hanging from above that further isolated the tank from the external environment and the movements of the experimenter. The experiment was recorded at a frame rate of 29 frames s⁻¹ by a camera placed above the arena.

Procedure

Crayfish were tested in 3 individual sessions (sessions 1–3) at 1, 15 and 45 days. At the beginning of each session, one crayfish at a time was placed at the centre of the arena and confined in a clear cylinder; once released, the crayfish could freely explore the new environment for 10 min. Then, the test started: whenever the crayfish faced the wall with the monitor, a stimulus was administered. After reacting to the stimulus, the crayfish started to explore the environment again and, after about 2 min or whenever it was in front of the wall with the monitor again, another stimulus was administered. The unrestrained conditions allowed an ecological administration of the stimuli and prevented the animal from associating a specific place within the arena or a side of its body axis (left or right) with the stimulation. The habituation test consisted of 5 repetitions of the same stimulus, i.e. the habituation stimulus (trials 1–5, 7–11 and 13–17), followed by a novel stimulus (trials 6, 12 and 18). The habituation stimulus was a jet of vaporized water (VAP); the novel stimulus was a direct jet of water (JET). The stimulation was manually delivered by the experimenter and never directed towards the crayfish's body, although of course droplets of water could fill the whole testing area. We scored the time to reach the defence posture by counting the number of frames per second from the moment at which the crayfish reacted to the stimulus by lifting its claws until the moment of freezing (Kelly and Chapple, 1990; Glantz, 1974). Stimulus administration started only while the animal was engaged in locomotor activity with the claws close to the ground, independently of whether the claws were bent or outstretched and still or in motion. The fact that crayfish increased their exploratory activity (i.e. they continued to walk) while learning to ignore the irrelevant water stimulus guaranteed that what we observed (i.e. the decrement of the response) was the outcome of true habituation and not the result of motor fatigue.

We presented all animals with a VAP–JET as opposed to a JET–VAP sequence of stimulation to optimize the testing procedure

to the number of available animals on the basis of previous literature. If the VAP stimulus elicited a response that habituated over time, then the recovery of the response for the JET would have been even stronger. Also, if we could observe a spontaneous recovery of the response for the weaker stimulus (i.e. VAP), we would expect a recovery also for the more intense one (i.e. JET), as proposed by Thompson and Spencer (1966). We acknowledge the lack of a measurement of the baseline response to the JET stimulus, although it is well established that habituation also occurs in response to both stronger stimuli, within the same stimulus modality but at a slower rate (see for instance Davis and Wagner, 1968), and relevant stimuli (see for instance Daniel et al., 2019).

Statistical analyses

We analysed both habituation and generalization, scored by counting the time (frames s⁻¹) needed to show the defensive response, using non-parametric statistics. We used an overall Friedman test and a pairwise Wilcoxon signed rank test to demonstrate the presence of habituation to the VAP stimulus. We used a Wilcoxon signed rank test to demonstrate the presence of generalization of the habituation to the JET stimulus and the response recovery between consecutive sessions. We provide effect sizes (Cohen's *d*) for all statistical comparisons, both significant and non-significant. We also provide the Bayes factor (BF) for the alternative hypothesis being true compared with the null hypothesis to support that our study had enough power to detect all the effects, instead of simply having too few subjects to be sensitive. Data were analysed in Jamovi (v.1.1.9, <https://www.jamovi.org/>) and are available from the Open Science Framework (OSF: doi:10.17605/OSF.IO/FX67G, <https://osf.io/fx67g/>).

RESULTS AND DISCUSSION

Session 1

The crayfish response to the VAP stimulus changed from trial 1 to 5: $\chi^2_4=15.8$, $P=0.003$, $BF_{10}=4.70$ (Fig. 1A). In particular, the response was shorter in trial 5 than in trial 1: mean difference 13.3±3.24 frames, $W=96$, $P=0.007$, $d=1.07$, $BF_{10}=27.42$. Stimulus specificity was attested by a significant increment in crayfish response to the JET stimulus: trial 5 versus trial 6: mean difference -11.7±5.97 frames, $W=15$, $P=0.036$, $d=-0.60$, $BF_{10}=3.83$.

Session 2

Crayfish recovered their response to the VAP stimulus when tested after 15 days; trial 5 versus trial 7: mean difference -10.8±3.94 frames, $W=18$, $P=0.033$, $d=-0.61$, $BF_{10}=3.86$ (Fig. 1A). They decreased their response to the subsequent repetitions of the stimulus: $\chi^2_4=14.4$, $P=0.006$, $BF_{10}=10.44$; trial 7 versus trial 11: mean difference 13.7±4.17 frames, $W=67$, $P=0.031$, $d=0.65$, $BF_{10}=2.37$. This time, crayfish generalized the response decrement to the JET stimulus: trial 11 versus trial 12, mean difference 3.33±5.20 frames, $W=58$, $P=0.40$, $d=0.09$, $BF_{10}=0.28$.

Session 3

The crayfish response to the VAP stimulus significantly recovered after 30 days; trial 11 versus trial 13: mean difference -20.30±3.42 frames, $W=1$, $P<0.001$, $d=-1.58$, $BF_{10}=523$ (Fig. 1A). The decrement of their response from trial 13 to trial 17 was significant: $\chi^2_4=21.3$, $P<0.001$, $BF_{10}=2759.15$; trial 13 versus trial 17: mean difference 21.2±4.69 frames, $W=89$, $P=0.003$, $d=1.16$, $BF_{10}=48.20$. Again, crayfish generalized the response decrement to the JET stimulus: trial 17 versus trial 18, mean difference -1.16±3.68 frames, $W=46.5$, $P=0.73$, $d=-0.01$, $BF_{10}=0.27$.

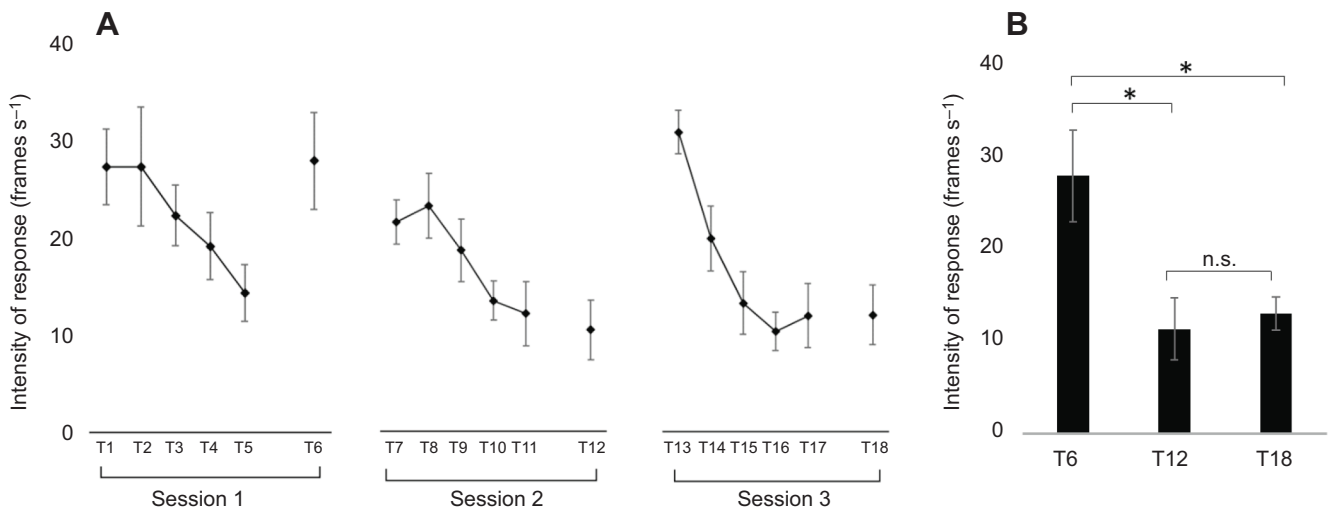


Fig. 1. Defensive response of crayfish to the vaporized water jet (VAP) and novel direct water jet (JET) stimuli. (A) The average defensive response of the 14 crayfish to the VAP and JET stimuli in the 3 sessions. The VAP stimulus was used in trials (T)1–5, 7–11 and 13–17, and the JET stimulus in T6, 12 and 18. Wilcoxon signed-rank tests revealed that crayfish decreased the duration of their defensive response to the VAP stimulus in each session (T1>T5, T7>T11, T13>T17; $P<0.05$). But they recovered their response to the JET during the first session only (T5<T6; $P<0.05$). (B) Summary of the response to the JET stimulus. A set of *post hoc* Durbin–Conover tests showed that the response to the JET was higher during the first session than in the second and third sessions (T6>T14, T6>T18; $P<0.05$). All the P -values are from two-tailed tests with $\alpha=0.05$ (asterisks indicate significance). Bars represent ± 1 s.e.m.

The crayfish response to the JET stimulus changed across the trials 6, 12 and 18 (Fig. 1B): $\chi^2_4=21.3$, $P<0.001$, $BF_{10}=32.44$. Their response to trial 6 was greater than their response to trial 12 and 18 (*post hoc* Durbin–Conover: $T=5.29$, $P<0.001$; $T=3.44$, $P=0.002$).

Conclusions

We demonstrated that *P. clarkii* habituated its defensive response to a repeated vaporized water jet, but then the response recovered when a direct water jet was introduced. However, whereas the response to the vaporized jet showed spontaneous recovery across the different sessions, with time, the crayfish were able to generalize the habituated response between the two types of stimuli. In addition, it is remarkable that the generalization occurred after one learning trial and persisted for up to 45 days.

This is the first experiment demonstrating a generalization ability in *P. clarkii*. Indeed, a previous study by Chiandetti and Caputi (2017) addressed a similar question in this species by adopting the same paradigm but without providing conclusive evidence of generalization. When looming shapes were used to elicit a defensive response, crayfish showed discrimination between ‘curvy’ and ‘spiky’ shapes, as they responded stronger when the ‘curvy’ shape was presented following habituation to the ‘spiky’ shape. But when a rotated version of the ‘spiky’ shape was presented, the habituated response did not recover, showing either generalization or, more parsimoniously, a failure to discriminate between the rotated versions of the same shape as a result of low visual acuity. In the present study, however, the results are clear cut in showing that *P. clarkii* discriminated between the two water jets (session 1). Hence, the lack of response recovery to the water jet observed in sessions 2 and 3 can reliably be attributed to a learning process, namely to a long-lasting form of generalization.

Whether this phenomenon is prototypical for crayfish or distinctive of invasive species like *P. clarkii* is an open question. The lack of investigations on generalization abilities in other crayfish hinders any comparison of performance across different species of crayfish. *Procambarus cubensis* was shown to increase the retention time of the testing chamber characteristics

following longer exposure times (Shuranova et al., 2005) when locomotor exploration was recorded. However, the retention time tested in that study (24 h) can be barely compared with that used in the current study. Invasive species living in changing environments might benefit from behavioural flexibility more than species living in stable environments because the risk of facing unknown stimuli is higher. Therefore, a mechanism could have evolved in these species to transfer innate or learned behaviours to new situations. Accordingly, Hazlett (2000) and Hazlett and colleagues (2002, 2003) suggested that invasive species have developed the ability to recognize and respond to a wider range of stimuli than species living in isolation because they have had the opportunity to experience a greater range of habitats in their evolutionary history, which points to rooted flexibility in invasive species. Alternatively, invasive species could learn through experience how to cope with the challenges posed by the current environment. In this case, behavioural flexibility may entail the capacity to invent new solutions (innovation) or learn these solutions from others (social learning). But animals can also exploit past solutions that have worked in similar situations and that can be generalized to the present one. This solution may be more efficient when innovations and social learning cannot be accomplished rapidly. Furthermore, crayfish do possess some core social abilities, but they seem more a solitary species in which social forms of learning occur in a limited range of situations (Gherardi et al., 2012). Therefore, we propose that crayfish transferred their defensive response between two different stimuli through a generalization mechanism, thereby acquiring an evident adaptive advantage to solve problems in a new and unknown area. Indeed, the distances they can cover, even on land, expose them to mutable contexts and environmental clutter in which they have the chance to exploit such an ability more than native species (Barbaresi et al., 2004; see also Mugan and MacIver, 2020). Whether invasive crayfish have a superior generalization capacity to that of native species remains unexplored, but our habituation paradigm can be reliably used to address this question in future research.

Acknowledgements

We thank Massimo Zanetti, Romero Iacuzzo and the Ente Tutela Pesca Friuli Venezia Giulia and its volunteers for helping with crayfish collection.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.C.; Methodology: A.C., C.C.; Formal analysis: A.D.; Investigation: A.C.; Writing - original draft: A.D.; Writing - review & editing: A.D., C.C.; Supervision: C.C.; Funding acquisition: C.C.

Funding

This work was partially supported by a Fondo per la Ricerca di Ateneo (FRA) from the University of Trieste to C.C.

Data availability

Data are available from the Open Science Framework (doi:10.17605/OSF.IO/FX67G): <https://osf.io/xf67g/>

References

- Aquiloni, L., Tricarico, E. and Gherardi, F. (2010). Crayfish in Italy: distribution, threats and management. *Int. Aquat. Res.* **2**, 1-14.
- Audet, J.-N. and Lefebvre, L. (2017). What's flexible in behavioral flexibility? *Behav. Ecol.* **28**, 943-947. doi:10.1093/beheco/axx007
- Barbaresi, S., Santini, G., Tricarico, E. and Gherardi, F. (2004). Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *J. Nat. Hist.* **38**, 2821-2832. doi:10.1080/00222930410001663308
- Blackburn, T. M., Pyšek, P., Bacher, S., Carlton, J. T., Duncan, R. P., Jarošík, V., Wilson, J. R. U., Richardson, D. M. et al. (2011). A proposed unified framework for biological invasions. *Trends Ecol. Evol.* **26**, 333-339. doi:10.1016/j.tree.2011.03.023
- Brosnan, S. F., Hopper, L. M. (2014). Psychological limits on animal innovation. *Anim. Behav.* **92**, 325-332. doi:10.1016/j.anbehav.2014.02.026
- Caldwell, C. and Whiten, A. (2002). Evolutionary perspectives on imitation: is a comparative psychology of social learning possible? *Anim. Cogn.* **5**, 193-208. doi:10.1007/s10071-002-0151-x
- Chapple, D. G., Simmonds, S. M. and Wong, B. B. M. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends Ecol. Evol.* **27**, 57-64. doi:10.1016/j.tree.2011.09.010
- Chiandetti, C. and Caputi, A. (2017). Visual shape recognition in crayfish as revealed by habituation. *Anim. Behav. Cognit.* **4**, 242-251. doi:10.26451/abc.04.03.04.2017
- Daniel, M. J., Koffinas, L. and Hughes, K. A. (2019). Habituation underpins preference for mates with novel phenotypes in the guppy. *Proc. R. Soc. B* **286**, 20190435. doi:10.1098/rspb.2019.0435
- Davis, M. and Wagner, A. R. (1968). Startle responsiveness after habituation to different intensities of tone. *Psychon. Sci.* **12**, 337-338. doi:10.3758/BF03331339
- Del Mastro, G. B. (1992). Sull'acclimatazione del gambero della Louisiana *Procambarus clarkii* (Girard, 1852) nelle acque dolci italiane (Crustacea: Decapoda: Cambaridae). *Pianura* **4**, 5-10.
- Duckworth, R. A. and Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proc. Natl. Acad. Sci. USA* **104**, 15017-15022. doi:10.1073/pnas.0706174104
- Ehrendfeld, J. G. (2010). Ecosystem consequences of biological invasions. *Annu. Rev. Ecol. Syst.* **41**, 59-80. doi:10.1146/annurev-ecolsys-102209-144650
- Ferrari, M. C. O., Gonzalo, A., Messier, F., Chivers, D. P. (2007). Generalization of learned predator recognition: an experimental test and framework for future studies. *Proc. R. Soc. B* **274**, 1853-1859. doi:10.1098/rspb.2007.0297
- Gherardi, F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Mar. Freshw. Behav. Phys.* **39**, 175-191. doi:10.1080/10236240600869702
- Gherardi, F. (2010). Invasive crayfish and freshwater fishes of the world. *Rev. Sci. Tech.* **29**, 241. doi:10.20506/rst.29.2.1973
- Gherardi, F., Aquiloni, L. and Tricarico, E. (2012). Revisiting social recognition systems in invertebrates. *Anim. Cogn.* **15**, 745-762. doi:10.1007/s10071-012-0513-y
- Ghirlanda, S. and Enquist, M. (2003). A century of generalization. *Anim. Behav.* **66**, 15-36. doi:10.1006/anbe.2003.2174
- Glantz, R. M. (1974). The visually evoked defense reflex of the crayfish: Habituation, facilitation, and the influence of picrotoxin. *J. Neurobiol.* **5**, 263-280. doi:10.1002/neu.480050307
- Griffin, A. S. and Guez, D. (2014). Innovation and problem solving: a review of common mechanisms. *Behav. Proc.* **109**, 121-134. doi:10.1016/j.beproc.2014.08.027
- Hazlett, B. A. (2000). Information use by an invading species: do invaders respond more to alarm odors than native species? *Biol. Invasions.* **2**, 289-294. doi:10.1023/A:1011450702514
- Hazlett, B. A., Acquistapace, P. and Gherardi, F. (2002). Differences in memory capabilities in invasive and native crayfish. *J. Crustac. Biol.* **22**, 439-448. doi:10.1163/20021975-99990251
- Hazlett, B. A., Burba, A., Gherardi, F., Acquistapace, P. (2003). Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biol. Invasions.* **5**, 223-228. doi:10.1023/A:1026114623612
- Heyes, C. (2012). What's social about social learning? *J. Comp. Psychol.* **126**, 193. doi:10.1037/a0025180
- Kelly, T. M. and Chapple, W. D. (1990). Kinematic analysis of the defense response in crayfish. *J. Neurophysiol.* **64**, 64-76. doi:10.1152/jn.1990.64.1.64
- Kolar, C. S. and Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends Ecol. Evol.* **16**, 199-204. doi:10.1016/S0169-5347(01)02101-2
- Mugan, U. and MacIver, M. A. (2020). Spatial planning with long visual range benefits escape from visual predators in complex naturalistic environments. *Nat. Commun.* **11**, 3057. doi:10.1038/s41467-020-16102-1
- Olson, L. J. (2006). The economics of terrestrial invasive species: a review of the literature. *Agric. Econ. Res. Rev.* **35**, 178-194. doi:10.1017/S1068280500010145
- Pejchar, L. and Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. *Trends Ecol. Evol.* **24**, 497-504. doi:10.1016/j.tree.2009.03.016
- Pintor, L. M., Sih, A. and Bauer, M. L. (2008). Differences in aggression, activity and boldness between native and introduced populations of an invasive crayfish. *Oikos* **117**, 1629-1636. doi:10.1111/j.1600-0706.2008.16578.x
- Pintor, L. M., Sih, A. and Kerby, J. L. (2009). Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. *Ecol.* **90**, 581-587. doi:10.1890/08-0552.1
- Rehage, J. S. and Sih, A. (2004). Dispersal behavior, boldness, and the link to invasiveness: a comparison of four *Gambusia* species. *Biol. Invasions* **6**, 379-391. doi:10.1023/B:BINV.0000034618.93140.a5
- Shuranova, Z., Burmistrov, Y. and Abramson, C. I. (2005). Habituation to a novel environment in the crayfish *Procambarus cubensis*. *J. Crustac. Biol.* **25**, 488-494. doi:10.1651/C-2556
- Sih, A., Cote, J., Evans, M., Fogarty, S., Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecol. Lett.* **15**, 278-289. doi:10.1111/j.1461-0248.2011.01731.x
- Sol, D. (2003). Behavioural flexibility: a neglected issue in the ecological and evolutionary literature. In *Animal Innovation* (ed. S. M. Reader and K. N. Laland), pp. 63-82. Oxford, UK: Oxford University Press.
- Souty-Grosset, C., Anastácio, P. M., 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. *Limnologica* **58**, 78-93. doi:10.1016/j.limno.2016.03.003
- Thompson, R. F. and Spencer, W. A. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73**, 16-43. doi:10.1037/h0022681
- Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L., Russello, M. A. (2010). Behavioral flexibility and species invasions: the adaptive flexibility hypothesis. *Ethol. Ecol. Evol.* **22**, 393-404. doi:10.1080/03949370.2010.505580