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Colour change impacted by antidepressants in cuttlefish

Antidepressants Modify Cryptic Behaviour in Juvenile Cuttlefish at Environmentally
Realistic Concentrations

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Abstract: Contamination of marine environment by antidepressants may affect neurophysiological processes in non-target organisms, such as the common cuttlefish, *Sepia officinalis*. The present study tested whether environmentally realistic concentrations of antidepressants, *i.e.*, fluoxetine alone (5 ng·L⁻¹) or cumulated with venlafaxine (2.5 or 5 ng·L⁻¹ each), affect camouflage in newly hatched cuttlefish. Results show that antidepressants improve uniform body patterns, while disruptive body patterns were not affected.

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INTRODUCTION

For purposes of communication or camouflage, cuttlefish, *Sepia officinalis*, display various body patterns on their dorsal side of their mantle as well as their arms and head.

This rapid colour change in cuttlefish is effected by nervously controlled chromatophores embedded in the skin. The rapid relaxation of chromatophores is under the control of serotonin (5-hydroxytryptamine, 5-HT), while rapid contraction is brought about by glutamate (Messenger, 2001). Chromatophore expansion and contraction are directly controlled by motoneurons from the chromatophore lobes of the brain and the optic lobes (Messenger, 2001), providing a point of action for antidepressants by changing the levels neurotransmitters.

Although newly-hatched cuttlefish are able to express complex body patterns, they learn to improve cryptic behaviours while their nervous system is maturing during the first month after birth (Hanlon and Messenger, 1988; Poirier et al., 2005). Cuttlefish can express uniform, mottle and disruptive body patterns to achieve camouflage on uniform as well as subtly heterogeneous or strongly contrasted backgrounds, respectively (Hanlon and Messenger, 2018).

The highly efficient and dynamic camouflage allows the cuttlefish to blend into the environment within milliseconds (Messenger, 2001), and to stay hidden from

predators, but it can also be a strategy to ambush their prey. Thus, any modification of the colour change deriving from changes in the levels of neurotransmitters involved in the nervous control of chromatophores, such as 5-HT, for instance, can reduce or increase the chances for survival. Many pollutants detected in marine environments can potentially interfere with the serotonergic system (Mezzelani et al., 2018), but one group of molecules is of particular interest, because it is designed to modify 5-HT levels: antidepressants of the class of selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). Their increasing release in the environment derives from a constant rise of prescriptions (OECD Health Statistics 2019). Consequently, residues of antidepressants from human excretion or direct disposal of expired pills are continuously detected in concentrations ranging from a few $\text{ng}\cdot\text{L}^{-1}$ to several $\mu\text{g}\cdot\text{L}^{-1}$ in fresh- and marine waters (Gonzalez-Rey et al., 2015; Meador et al., 2016; Metcalfe et al., 2010; Klosterhaus et al., 2013; Paíga et al., 2016; Schultz et al., 2010). More specifically, Mínguez *et al.* (2016) reported concentrations of $0.3 \text{ ng}\cdot\text{L}^{-1}$ of the SSRI fluoxetine (FLX) and $1.3 \text{ ng}\cdot\text{L}^{-1}$ of the SNRI venlafaxine (VEN) in the Orne estuary (Normandy, France), which is why, we focused on these two active molecules in the present study, which comprise some of most prescribed and most frequently detected antidepressant drugs (Fong and Ford, 2014). FLX was the first SSRI to be commercialised in 1988 (Pe, 1993) and still is one the most administered antidepressants. It has thus been extensively studied by ecotoxicologists (Fong and Ford, 2014; Sehonova et al., 2018), which is why it served as reference antidepressant in this study. Nearly one decade later, the first SNRIs, with VEN, were commercialised (Zerjav et al., 2009). Both antidepressants are commonly detected in aquatic animals (Fong and Ford, 2014; Schultz et al., 2010; Silva et al., 2016), even if their concentrations in the water are below detection limits (Klosterhaus et al., 2013;

Meador et al., 2016). A number of studies revealed effects of antidepressants, such as FLX and VEN on different behaviours in various marine species: predator and anti-predator behaviour (Bisesi et al., 2014; Peters et al., 2017), locomotion (Fong et al., 2015; Mesquita et al., 2011), agonistic behaviour (Tierney et al., 2016), photo- and geotaxis behaviour (Guler and Ford, 2010). These studies used much higher concentrations than those detected in water bodies, and they tested single antidepressants only. Realistic environmental exposure scenarios are, however, characterised by low $\text{ng}\cdot\text{L}^{-1}$ concentrations of several antidepressants that may cumulate to concentrations in the $\mu\text{g}\cdot\text{L}^{-1}$ range (Metcalf et al., 2010). To date, few studies focused on the effects of environmentally realistic concentration of accumulated antidepressants such as FLX and VEN on different behavioural traits: predator behaviour (Bisesi et al., 2016), predator avoidance (Painter et al., 2009), reproductive behaviour (Schultz et al., 2011), circadian rhythm (Melvin, 2017). Earlier studies from our laboratory demonstrated impacts of FLX and/or VEN at very low concentrations in the range of 1 to 100 $\text{ng}\cdot\text{L}^{-1}$, on behaviour of cuttlefish: learning and memory processes (Di Poi et al., 2013), maturation and learning of sand-digging behaviour (Chabenat et al., 2019), cryptic behaviour and colour change (Bidel et al., 2016a, 2016a; Di Poi et al., 2014).

Juvenile *Sepia officinalis* are developing in coastal areas before starting seasonal offshore migrations. Continuous discharge of various pharmaceutical residues in the vicinity of estuarine outflows may, therefore, influence the physiological and behavioural development of juvenile cuttlefish. As antidepressants never occur as single compounds in the environment, this study tested the hypothesis that environmentally realistic concentrations of cumulated antidepressants present in the coastal environments affect the colour change of newly-hatched cuttlefish. To test this

hypothesis, the camouflage response was investigated on artificial backgrounds in *S. officinalis* exposed to two different concentrations of FLX **alone or combined with** VEN during the first month after hatching, which is essential for the cuttlefish's development.

1. Material and methods

1.1 Animal rearing and experimental conditions.

Clutches from wild animals (*Sepia officinalis*) were collected at various sites in the English Channel off the coast of Luc-sur-mer, France. Eggs from different clutches were combined and incubated in four 1000 L aquaria with natural seawater at the Centre de Recherches en Environnement Côtier (CREC) of the University of Caen Normandie at Luc-sur-mer, France. The aquaria were supplied by a **flow-through system constantly renewing 50% of the water body** ($15\pm 1^\circ\text{C}$). The photoperiod was following to natural summer conditions (~16h light: 8h dark).

At hatching, individual cuttlefish (**n=14-16**) were immediately isolated in glass beakers (\varnothing 11 cm, height 6 cm), henceforth designated as 'home tank', constituting a semi-static system **with a 100% daily water renewal using** 250 mL of activated charcoal filtered seawater (FSW) at a salinity of 35 PSU. Hatchlings were randomly assigned to either (1) FSW (control), or (2) $5\text{ ng}\cdot\text{L}^{-1}$ FLX (FLX5)¹, or (3) $2.5\text{ ng}\cdot\text{L}^{-1}$ FLX and $2.5\text{ ng}\cdot\text{L}^{-1}$ VEN in combination (FLXVEN2.5) as well as (4) $5\text{ ng}\cdot\text{L}^{-1}$ FLX and $5\text{ ng}\cdot\text{L}^{-1}$ VEN (FLXVEN5) and exposed over a period of 29 days post-hatching (dph). The concentrations of FLX and VEN were renewed together with the daily

¹ No animal was exposed to VEN alone in the present experiment. The focus was on cumulating concentrations in order to approach a more environmentally realistic exposure scenario. For logistic limitation reasons, we could not include a VEN alone condition and opted for FLX alone as more experiments in the field have been conducted with FLX. For the testing of single antidepressants at different concentrations in *Sepia officinalis*, the reader is referred to Di Poi et al. 2014 and Bidet et al. 2016."

water changes, which also allowed maintaining appropriate O₂-levels ($\geq 80\%$) and low nitrite and nitrate concentrations ($[\text{NO}_2^-]_{\text{max}}=0.05 \text{ mg}\cdot\text{L}^{-1}$, $[\text{NO}_3^-]_{\text{max}}=0.15 \text{ mg}\cdot\text{L}^{-1}$). Pretests demonstrated that the actual concentrations never fell below 75% of nominal concentrations within 24 h, with VEN being more stable than FLX. Animals were fed daily with one sand shrimp (*Crangon crangon*) until the end of the experiments. Animal rearing and animal experiments were carried out in accordance with the guidelines of the EU Directive 2010/63/EU for animal experiments (Protocol approved by the French National Ethical Committee for Animal Experimentation, Agreement number: 035121.02).

1.2 Chemical contamination.

FLX and VEN concentrations were chosen to approximate surface water concentrations measured off the coast where the eggs were sampled (Minguez et al. 2016). Final concentrations of either 2.5 or 5 ng·L⁻¹ were obtained from stock solutions of fluoxetine hydrochloride (CAS 56296-78-7, Sigma Aldrich, St. Louis, USA) or venlafaxine hydrochloride (CAS 99300-78-4, Sigma Aldrich) dissolved in **filtered distilled water**. Stock solutions were at 10 µg·L⁻¹ of FLX and VEN, respectively, portioned in small aliquots of 0.5, 1 and 2 mL and kept at -80°C until use.

1.3 Behavioural tests.

Camouflage responses of the cuttlefish were assessed through the analysis of body patterns **as described previously** (Barbosa et al., 2007; Di Poi et al., 2014). **Body patterns were** displayed on two different backgrounds: uniform grey or black and white checkerboard. The test apparatus was a custom-made drop-shaped PVC compartment (length 11 cm, height 4 cm, max. width 5 cm). Eight independent compartments were concentrically arranged to allow for the simultaneous observation

of eight individuals, *i.e.*, two animals per exposure condition (Fig. 1). The inside walls and floors of the compartments were either bare medium grey PVC (Fig. 1A), or covered with laminated paper printed with a black and white checkerboard (Fig. 1B) in order to induce a uniform or disruptive pattern response, respectively. The square size of the checkerboards corresponded to 90% of the cuttlefish's dorsal white square (Di Poi et al., 2014). Each cuttlefish passed both behavioural tests at four different ages: at 7, 14, 21, 28 dph (**n= 14 per group**) for uniform background, and 8, 15, 22, 29 dph (**n=16 per group**) for checkerboard background.

Behavioural tests were conducted in the climate chamber where the cuttlefish were reared and exposed to antidepressants. The tests were performed in a custom-made black tent in order to avoid any external stimuli. A circular LED lamp (Neewer®, Guangdong, China) was positioned 60 cm above the test apparatus. To avoid direct light on cuttlefish, the lamp was placed in an oblique position and the inner surfaces of the tent were lined with white fabrics to diffuse light evenly. All the tests were recorded with a Legria HF R506 Camcorder (Canon, Tokyo, Japan).

Each cuttlefish was gently transferred from its home tank into one of the eight compartments previously filled with 50 mL of water from its own home tank to avoid additional stress. For each test session, two cuttlefish per exposure condition (FSW; FLX5; FLXVEN2.5 and FLXVEN5) of exactly the same age were randomly placed in the eight compartments of the experimental apparatus.

The animals were videorecorded during 30 min to evaluate the stability of the body patterns over time.

2.4. Data analysis.

For each video-recording, two still photographs were analysed: one at the beginning of the test, as soon as the cuttlefish was transferred into the compartment and stopped

moving ($T0 \pm 1\text{min}$), and one at the end of the test period ($T30 \pm 1\text{min}$). Insofar as cuttlefish hatchlings hardly express a true uniform body pattern, we choose to use the disruptive score on both uniform and checkerboard background to evaluate background matching, rather than the mean gray value or the heterogeneity index (Bidel et al., 2016a; Di Poi et al., 2014). According to Barbosa et al. (2007), juvenile cuttlefish can express up to 11 body components, all of which may be more or less expressed and, consequently, graded from 0 (not expressed) to 3 (strongly expressed). A final disruptive score ranging between 0 (perfect uniform body pattern, *i.e.*, no component expressed) to 33 (perfect disruptive body pattern, *i.e.*, the 11 components strongly expressed) was assigned to each cuttlefish at T0 (beginning of the test) and at T30 (end of the test, 30 min). The evolution of the calculated disruptive scores according to the developmental stages (from 7 to 28 dph) and the comparison between the exposure conditions (control; FLX5; FLXVEN2.5 and FLXVEN5) were assessed for both, uniform and checkerboard backgrounds. A lower disruptive score on uniform background and a higher disruptive score on checkerboard indicate a more efficient background matching.

All statistical tests were performed using R Studio (Version 1.1.453, RStudio Inc., Boston, USA, <http://www.r-project.org>). As data did not meet the assumptions of normality and homoscedasticity, we used non-parametric permutation test analyses of data from factorial experiments (function ezPerm; “ez” package). The number of iterations was set to 1000. We used mixed within-and-between Ss designs (*i.e.*, repeated factors: time, age; independent factor: experimental groups) to evaluate uniform and disruptive body pattern of juvenile cuttlefish at T0 and T30 between groups and ages. If the null hypothesis was rejected ($p \leq 0.05$), post-hoc permutation tests were performed to highlight any differences between experimental groups and

between ages, using the “RVAidememoire” package. The false discovery rate was adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995).

2. Results

Body pattern on uniform background (Fig. 2)

The disruptive score was significantly different between treatment groups ($p = 0.001$) and between t_0 and t_{30} min ($p = 0.001$), but there was no difference between ages ($p = 0.479$) (Fig. 2). Pairwise comparisons showed a significant difference between each of the exposed groups and the controls (post-hoc permutation test: $p = 0.004$ for each of the treatment groups *vs.* control, respectively). All groups exposed to antidepressants exhibited a better background matching than the control group, *i.e.*, from 7 dph to 28 dph they expressed a lower disruptive score than the control group. Moreover, the score exhibited by the FLXVEN5 group was also significantly different from the two other treatment groups (post-hoc permutation test: FLXVEN5-FLXVEN2.5, $p = 0.027$; FLXVEN5-FLX5, $p = 0.038$). This means that the FLXVEN5 group expressed an even better background matching than the FLXVEN2.5 and FLX5 groups.

In addition, there was a significant interaction between groups and time ($p = 0.016$), *i.e.*, the difference of disruptive score at the beginning and the end of the test was different depending on the group. Pairwise comparisons revealed that no difference of the score over time existed in the control group (post-hoc permutation test: control: control: t_0 -control: t_{30} , $p = 0.343$), that a trend towards a difference over time was found in the FLX5 group (post-hoc permutation test: FLX5: t_0 -FLX5: t_{30} , $p = 0.065$), and a significant difference over time existed in both FLXVEN2.5 and FLXVEN5 exposures (post-hoc permutation test: FLXVEN2.5: t_0 -FLXVEN2.5: t_{30} , $p = 0.004$; FLXVEN5: t_0 -FLXVEN5: t_{30} , $p = 0.036$). While the control group exhibited a fairly stable uniform pattern over time, FLXVEN2.5, FLXVEN5 and most probably FLX5

displayed a more dynamic evolution of their body patterns. Cuttlefish from the exposed groups improved their body pattern over a 30 min period of time towards a more uniform pattern.

Body pattern on checkerboard background (Fig. 3)

The disruptive body pattern was neither significantly different between groups ($p = 0.768$), nor between age ($p = 0.109$), or over the 30 min test period ($p = 0.767$). Overall, juvenile cuttlefish, whether exposed to antidepressants or not, expressed on average the same disruptive score over a 30 min test period during their first month. Nevertheless, it may be noticed that the disruptive score of cuttlefish of 8 dph was generally lower than that of 15, 22 and 29 dph, although no statistically significant difference emerged because of the high intragroup variability ($p = 0.109$). In addition, there was a significant interaction between groups and time ($p = 0.004$) and between ages and time ($p = 0.007$). Yet, pairwise comparisons failed to reveal the statistical differences in both interactions. Again, this may be due to the high intragroup variability. However, regarding the means, at 8 dph, cuttlefish from all groups followed the same tendency of a decreasing disruptive score after 30 min (as for the uniform body pattern, see above). At 15 dph, all cuttlefish exhibited this pattern of decreased disruptive scores after 30 min, except those from FLXVEN2.5 group, which increased their disruptive score. At 22 dph, the control and FLXVEN5 groups slightly decreased their disruptive score after 30 min, but not the FLX5 and FLXVEN2.5 group. Finally, at 29 dph, all cuttlefish, whether exposed or not, displayed an increased disruptive score after 30 min.

DISCUSSION

Like many invertebrate species, cuttlefish hatchlings receive no parental care and must learn on their own how to adapt their behaviours in response to environmental

changes or to threats (Dickel et al., 2006). Although background matching through chromatic colouration is visible on the cuttlefish's body as soon as it hatches, this primary defence is still immature, *i.e.*, hatchlings and early juvenile cuttlefish often display an 'inappropriate' body pattern (Hanlon and Messenger, 1988). Notably, they are more likely to express a disruptive colouration when placed on uniform substrates than older animals (Hanlon and Messenger, 1988). Thus, expressing a uniform pattern for brightness matching is more challenging for cuttlefish hatchlings than producing a disruptive pattern on a contrasted background. The ability to express a uniform pattern improves, however, in the course of the first two months of life according to their early experience (*i.e.*, rearing environment) (Poirier et al., 2005). The disruptive scores obtained in our control group confirm this difficulty for hatchlings to express a uniform pattern on uniform background. The unusual lack of improvement with age may be related to the impoverished rearing conditions, which were constrained in our study by the antidepressant exposure, as compared to the richer natural environment. Indeed, minimised rearing conditions have been shown to slow down the maturation of uniform camouflage abilities in hatchling cuttlefish (Poirier et al., 2005). Our results, therefore, confirm that is useful to evaluate the efficiency of both, uniform and disruptive pattern by the disruptive score, as juvenile cuttlefish did not express uniform body pattern easily.

Cuttlefish exposed to antidepressants at very low environmentally realistic concentrations displayed a less disruptive pattern on a uniform background, which is indicative of a better background matching compared to the control group. This holds particularly true for the highest concentrations of FLX and VEN at $5 \text{ ng}\cdot\text{L}^{-1}$ each, *i.e.*, $10 \text{ ng}\cdot\text{L}^{-1}$ of accumulated antidepressants in total. Moreover, cuttlefish exposed to FLX and VEN distinctly improved this behaviour over the 30 min test period.

Cuttlefish exposed to FLX alone followed this trend, albeit less evidently. On the contrary, the control group displayed a fairly stable, much less dynamic evolution of their body pattern. Whether VEN alone would have equally improved background matching in the present experimental setting cannot be told. Di Poi et al. (2014) found that two weeks-old cuttlefish exposed to FLX at 1 and 100 ng·L⁻¹ improved their uniform body pattern over a 30 min test period, whereas controls did not. This was, however, not the case in newly-hatched cuttlefish exposed to 1 ng·L⁻¹ of FLX until two weeks of age, which displayed a decreased efficiency to express uniform body patterns (Di Poi et al., 2014). Furthermore, cuttlefish exposed to VEN for 19 days to 100 ng·L⁻¹ demonstrated a lower quality of the uniform body pattern compared to the controls, while no difference emerged in cuttlefish exposed to 5 ng VEN ·L⁻¹ for 19 days (Bidel et al., 2016). Finally, embryonic exposure at higher concentrations of FLX, *i.e.*, 1 and 10 µg·L⁻¹, did not induce any noticeable modification of uniform body pattern. Overall, on a 30 min period, concentrations of FLX had an impact on uniform body pattern, whereas VEN did not. In addition, our results show significant effects of the combination of both FLX and VEN towards a better uniform body pattern on medium grey background. In summary, the results of the present and earlier experiments produced inconclusive responses with respect to the effects of antidepressants on achieving uniform body patterns. Those studies differed slightly in their experimental design and concentrations used, but also in the way of assessing camouflage efficiency. Indeed, Bidet et al. (2016a, 2016b) and Di Poi et al. (2014) used the Mean Gray Value and the Heterogeneity Index from ImageJ[®] to evaluate the efficiency of uniform pattern of cuttlefish despite the fact that juvenile cuttlefish more easily display disruptive components and struggle to display a perfect uniform body pattern. Those parameters may be more relevant for adult cuttlefish that master the

uniform body pattern, while the disruptive score may be more appropriate for young cuttlefish. In addition, a growing body of evidence points to a non-monotonic dose-response for antidepressants with greater effects at concentrations in the lower range (Fong and Ford, 2014; Vandenberg et al., 2012). This might explain the variability of responses between different studies.

Unlike uniform pattern, disruptive patterns are easily expressed, by hatchlings and juvenile cuttlefish, even if they may not be perfect. This provides them with an efficient camouflage when their environment comprises contrasted components. No distinct effects of antidepressants on the disruptive body pattern of newly-hatched cuttlefish placed in a checkerboard visual environment emerged from the present study. Previous studies from Bidet et al. (2016a, 2016b) and Di Poi et al. (2014) are in line with these findings. Indeed, when exposed to either FLX at 1 and 100 ng·L⁻¹ or 1 and 10 µg·L⁻¹, or VEN at 5 and 100 ng·L⁻¹, juvenile cuttlefish did not show any modification of the disruptive pattern on a checkerboard background. Despite the fact that its complexity, *i.e.*, the number of chromatic component expressed, can be modulated by environmental conditions (Poirier et al., 2005) and, therefore, is subject to maturation as well, the disruptive pattern seems to be a far more robust and insensitive to waterborne antidepressants, even at the highest concentrations tested or when different antidepressants were combined.

The modification of background matching towards a greater efficiency after an exposure to a SSRI and/or SNRI may be explained by their action on the chromatophores. Both antidepressants used in this study are designed to modulate the levels of 5-HT, which is involved in the retraction of chromatophores muscles (Messenger, 2001). If FLX and/or VEN increase the synaptic concentrations of 5-HT, the exposure to such antidepressants may cause a relaxation of the chromatophore's

muscle crown leading to pigment concentration. This reduces disruptive colour patterning and brings forward the uniform coloration by the leucophores. Accordingly, Florey and Kriebel (1969) demonstrated that 5-HT-application to the skin of *Loligo opalescens* caused a paling due to rapid chromatophore retraction. Loi et al. (1996) obtained similar results with 5-HT causing a more pronounced retraction than in normal state in an *in-vivo* bioassay with cuttlefish skin. Accordingly, such a direct action on chromatophores may explain the effect of FLX alone as well as in combination with VEN in promoting an improved background matching on uniform substrate due to a retraction of all the chromatophores resulting in a decrease in contrasts between the different skin chromatic components. But, if so, a decrease of the expression of the disruptive pattern should have been observed on the checkerboard background as well, which was not the case, neither for the disrupting score in this study, nor for the heterogeneity index in previous studies (Bidel et al., 2016b, 2016a; Di Poi et al., 2014). Furthermore, both Bidet et al. (2016a, 2016b) and Di Poi et al. (2014) suggested that FLX and VEN influences the dopaminergic and noradrenergic system, respectively, rather than the serotonergic system. Some studies also pointed to the effect of antidepressants on glutamatergic system, in particular by modulating N-methyl-D-aspartate (NMDA) receptors (Musazzi et al., 2013, 2011; Wong et al., 1995). Bidet et al. (2016a) investigated if VEN could modulate binding sites of NMDA-like receptors in brain of cuttlefish. Indeed, exposure to either 5 or 100 ng·L⁻¹ of VEN for 20 days post-hatching decreased the number of NMDA-like receptor binding sites. Thus, VEN may have an impact on chromatophore contraction in neuro-muscular junctions, enhancing a uniform body pattern. Yet, this modulation should exert a similar effect on a disruptive background. As stated before, however, no effect could be measured in this condition.

Notwithstanding, the modulation of glutamatergic system may have an effect in brain structures, such as optic lobes in cuttlefish. For instance, in *Sepia pharaonis*, visual development has been linked with the expression of NMDA receptors in optic lobes (Lee et al., 2013). Exposure to either FLX or VEN has been linked with decreased cell proliferation in the optic lobes of juvenile cuttlefish (Bidel et al., 2016b, 2016a). Both antidepressants may, putatively, modulate brain development and functions, such as visual perception or motor control of chromatic skin components and/or inhibition of the disruptive pattern that cuttlefish hatchlings readily express. The possible effects of FLX and VEN on neurotransmitter levels and structural changes in brain may lead to a different processing of visual information and, consequently, to modifications in the expression of uniform body pattern.

Our study reveals an effect of antidepressants on colour change, specifically when a uniform body pattern is required. The expression of uniform body pattern, immature at hatching (Poirier et al., 2005), seemed to be the more sensitive aspect of cryptic colouration. Because its maturation was more pronounced, it was more prone to modification by antidepressants. Maturation in cuttlefish appeared to be affected by antidepressants in an earlier study (Chabenat et al., 2019). In that study, the sand digging behaviour –which is part of the cryptic behaviour– decreased, whilst homochromy at about 80% remained largely unaltered when *S. officinalis* was exposed to concentrations as low as $2.5 \text{ ng}\cdot\text{L}^{-1}$ FLX and VEN combined. This demonstrates that while certain aspects of camouflage may be improved by antidepressants, others may be deteriorated, with the potential to impair overall camouflage abilities. Whether improving uniform body patterns, which is *a priori* a positive effect, could have negative repercussions in other parameters of camouflage is difficult to predict. What becomes evident, however, is that relatively low

concentrations of antidepressants can affect the maturation of camouflage at early periods of cuttlefish development in various, yet unpredictable manners. This implies the possibility that SSRIs/SNRIs at environmental concentrations may also impair complex behavioural traits, such as cryptic behaviour, or that positive effects on one parameter may entail negative trade-offs on other parameters.

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Author contributions statement—A.C performed the experiment and data analyses; A.C, C.B, T.K developed and designed the experiment and data analyses; A.C, C.B, T.K wrote the manuscript; A.C, C.B, T.K proofread the manuscript.

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FIGURE CAPTIONS

Figure 1. Experimental setup of the behavioural tests with uniform (A) and checkerboard (B) background. Individual and independent drop-shaped PVC compartments were arranged concentrically below the video camera.

Figure 2. Evolution of disruptive score on a uniform medium-grey background in four treatment groups of juvenile cuttlefish (control, FLX at 5 ng·L⁻¹, FLX + VEN at 2.5 ng·L⁻¹ each, and FLX + VEN at 5 ng·L⁻¹ each), at four different ages (7, 14, 21, 28 days post-hatching [dph]) and over a 30 min test period each (i.e., from T0 to T30). Results are expressed as means ± SEM of disruptive scores/33, n=14. Post-hoc permutation tests with FDR correction (Benjamini-Hochberg procedure) were performed for inter-group and age comparisons: * indicates a statistically significant difference between control and all antidepressant exposed groups; # indicates a statistically significant difference between FLXVEN5 and both FLX5 and FLXVEN2.5; p≤0.05.

Figure 3. Evolution of disruptive score on checkerboard background in four treatment groups of juvenile cuttlefish (control, FLX at 5 ng·L⁻¹, FLX + VEN at 2.5 ng·L⁻¹ each, and FLX + VEN at 2.5 ng·L⁻¹ each) at four different time points (8, 15, 22, 29 days post-hatching [dph]) and over a 30 min test period each (i.e., from T0 to T30). Results are expressed as means ± SEM of disruptive scores/33, n=16.



