Photo-induced toxicity in early life stage fiddler crab (Uca longisignalis) following exposure to Deepwater Horizon oil

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Abstract

The 2010 explosion of the *Deepwater Horizon* (DWH) oil rig led to the release of millions of barrels of oil in the Gulf of Mexico. Oil in aquatic ecosystems exerts toxicity through multiple mechanisms, including photo-induced toxicity following co-exposure with UV radiation. The timing and location of the spill coincided with both fiddler crab reproduction and peak yearly UV intensities, putting early life stage fiddler crabs at risk of injury due to photo-induced toxicity. The present study assessed sensitivity of fiddler crab larvae to photo-induced toxicity during co-exposure to a range of environmentally relevant dilutions of high-energy water accommodated fractions of DWH oil, and either <10, 50, or 100% ambient sunlight, achieved with filters that allowed for variable UV penetration. Solar exposures (duration: 7-h per day) were conducted for two consecutive days, with a dark recovery period (duration: 17-h) in between. Survival was significantly decreased in treatments the presence of >10% UV and relatively low concentrations of oil. Results of the present study indicate fiddler crab larvae are sensitive to photo-induced toxicity in the presence of DWH oil. These results are of concern, as fiddler crabs play an important role as ecosystem engineers, modulating sediment biogeochemical processes via burrowing action. Furthermore, they occupy an important place in the food web in the Gulf of Mexico.

Keywords Polycyclic aromatic hydrocarbon · Fiddler crab · Deepwater Horizon · Photo-induced toxicity

Introduction

Polycyclic aromatic hydrocarbons (PAH) are pervasive organic contaminants released by petrochemical activities, and incomplete combustion of organic materials (Huang et al. 1997; Ravindra et al. 2008). Toxic effects of PAH exposure may include, but are not limited to, narcosis, decreased reproduction, teratogenicity, DNA damage, and tumor formation in aquatic organisms (Xue and

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Warshawsky 2005). The presence of ultraviolet (UV) radiation during PAH exposure dramatically increases toxicity to aquatic organisms, a phenomenon known as photo-induced, or photo-enhanced toxicity (Roberts et al. 2017). Sensitivity to photo-induced toxicity has been demonstrated in zooplankton, microbes, various fish species, and sediment-associated organisms (aquatic vegetation, bivalves, and benthic arthropods) (Alloy et al. 2015, 2017; Huang et al. 1997; Lampi et al. 2007; Newsted and Giesy 1987; Pelletier et al. 1997; Sweet et al. 2017). Numerous studies have also reported photo-induced toxicity of PAH as an important mode of toxic action following oil releases (Alloy et al. 2015; Incardona et al. 2012; Sweet et al. 2017).

On 20 April 2010, the *Deepwater Horizon* (DWH) oil rig exploded, resulting in a massive oil spill in the Gulf of Mexico (GoM) which oiled shorelines from Texas to Florida (DWH NRDA 2016). Shorelines are important reproductive habitats for many aquatic organisms, including fiddler crabs (*Uca longisignalis*), which are widely distributed throughout the GoM (Mouton and Felder 1995; Thurman 1982; Zengel et al. 2016). Fiddler crabs modulate



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biogeochemical processes in sediment via burrowing action, serving as important ecosystem engineers, in addition to comprising an important part of the food web in the Gulf of Mexico (Franco et al. 2017).

The timing of shoreline oiling coincided with peak annual UV intensities (April-July) and the fiddler crab reproductive season, which spans the spring and summer months with peak reproduction in June (Bridges et al. in press; Christy 1983; Mouton and Felder 1995; Villafane et al. 2004; Zengel et al. 2016). Fiddler crabs burrow in the shallow waters of salt marshes, where oiled sediment may increase PAH exposure and bioaccumulation in eggs (Pelletier et al. 1997). Approximately 14 days after mating, freeswimming zoea hatch from external egg masses and are released into the water during nocturnal ebb tides. This planktonic stage of development may last anywhere from a few weeks to several months, depending on the species, during which time they remain in shallow waters where the likelihood of UV exposure is very high (Mouton and Felder 1995; Pelletier et al. 1997). Larvae then molt to a juvenile, terrestrial stage, where they remain until maturity (Thurman 1982).

In general, early life stage (ELS) aquatic organisms exhibit increased sensitivity to PAH photo-induced toxicity (Roberts et al. 2017). A study performed by Finch and Stubblefield (2016) demonstrated a direct relationship between translucence (which generally decreases with increasing age) and sensitivity to photo-induced toxicity. Like many other early life stage aquatic organisms, zoea lack sufficient pigmentation during larval stages, allowing UV to penetrate the organism and interact with photodynamic PAHs (Alloy et al. 2015; Diamond 2003; Roberts et al. 2017). Additionally, they are positively phototactic, and therefore inhabit surface waters where exposure to UV radiation may be intense (Alloy et al. 2017; Brodie et al. 2007). Given the timing of the DWH oil release in the GoM, it is very likely that larval fiddler crabs experienced co-exposure to PAH and UV radiation. Therefore, the goal of the present study is to determine the sensitivity of ELS fiddler crabs to photo-induced toxicity, following exposure to DWH oil.

Methods and materials

Experimental design

Fiddler crab larvae were co-exposed to dilutions of highenergy water accommodated fractions (HEWAFs) from two weathered oil slicks (discussed below in Test Solutions and HEWAF Preparation), and three intensities of UV radiation in a factorial design. The exposure photoperiods for both studies included two repeating cycles of: a 17-h (overnight) HEWAF exposure in the dark, followed by a 7-h natural sunlight exposure as a source of UV, for a total test duration of 48-h. Eighty percent static renewals of test solutions were performed once, following the first UV exposure period. Mortality was assessed at the end of each UV exposure. The test apparatus consisted of a water table with continuous flow, designed to maintain a uniform temperature in all dishes (organisms were not in contact with flow through water). All test dishes (250 mL Pyrex crystallization dishes, Fisher Scientific, Pittsburgh, PA) were suspended at a uniform depth in the water table using polystyrene float boards. The desired UV intensities were achieved using plastic shielding with varying UV transparencies overlaid above the dishes (plastic shields did not come in contact with test dishes).

Fiddler crab culture

Gravid adult female fiddler crabs were collected from a reference site (not oiled by the DWH oil release) on the Alabama coastline near Bayou La Batre (~30°22'46.11"N, 88°18'23.27"W). Organisms were transported to Auburn University in 142-L coolers (Igloo Products Corporation, Houston, TX) containing Pentair Bio Balls (Cary, NC), sediment, and estuarine water from the collection site. Gravid females were kept in a recirculating culture system, in individual 3-L tanks, until eggs hatched. The recirculating system contained artificial seawater, made from deionized water and Instant Ocean Sea Salt (United Pet Group, Blacksburg, VA) (salinity 20-22 ppt, mean pH 7.7, temperature 22-25 °C, dissolved oxygen 7 ppm). Females were checked at least twice daily (in the morning, and evening), to determine if eggs had hatched. Newly hatched larvae (<24 h) were collected in filter cups at the outflow of two tanks, and pooled for all toxicity tests. Larvae were transferred to test dishes containing 200-mL of test solution with a transfer pipette after the evening collection. Each test dish was loaded with 20 larvae and kept in the laboratory until the following morning.

Test solutions and HEWAF preparation

Synthetic seawater used for the control treatment solution and in HEWAF dilution preparation was prepared using Instant Ocean Sea Salt (United Pet Group, Blacksburg, VA) and DI water. Seawater parameters were as follows: 20 ppt salinity, 33.2 mS conductivity, 7.2 ppm dissolved oxygen, pH 8.3, with a mean temperature of 27 °C. Two separate field-collected oils, referred to as either slick A or slick B, were used to conduct the photo-induced toxicity testing described herein. Slick A was collected on 29 July 2010, from the hold of barge number CTC02404 (which received surface slick oil from various skimmer vessels working on the DWH response), while Slick B is a slightly more weathered surface oil by comparison (Forth et al. 2017a, b; Morris et al. 2015). Forth et al. (2017a, b) characterized the extent of weathering in both oil types, with Slick A showing a 68% loss of tPAH50 (sum of 50 PAHs) relative to hopane, compared with 85% tPAH50 loss in Slick B. Additional details on oil collection, chemistry, HEWAF preparation, and PAHs included in tPAH50 sums can be found in Forth et al. (2017a, b).

Each of these oil types were routinely used in bioassays performed as part of the DWH Natural Resource Damage Assessment (Forth et al. 2017b). The HEWAF used in exposure solutions was prepared by blending 1-g of oil, in 1-L of control seawater in a Waring CB15 blender (Waring Commercial Products, Stamford, CT) on low power for 30 s. The resulting HEWAF was transferred to a glass separatory funnel, covered in aluminum foil to prevent photodegradation, and allowed to settle for 1 h. Following the settling time, the lower 200-mL of unfiltered HEWAF was drained from the separatory funnel and discarded. The next 700-mL of remaining HEWAF was used to prepare all dilutions used in the present study, which included 5 target nominal PAH concentrations (0, 0.25, 0.5, 1, 2% slick A HEWAF; 0, 0.75, 1.5, 3, 6% slick B HEWAF) for both oil types. Target nominal concentrations corresponded with measured tPAH₅₀ concentrations of 0.1-26.7 µg/L for slick A, and $0.1-9.3 \,\mu\text{g/L}$ for slick B (Table 1). All treatments (defined as each unique combination of [PAH] and %UV) included 5 replicate dishes, each of which contained 200mL of test solution and 20 zoea. The same stock HEWAF was used to prepare renewal dilutions the second day of the study. Subsamples of HEWAF stocks were collected for chemical analysis during both the initial and renewal dilution preparations.

Table 1 Nominal WAF dilutions and initial $t{\rm PAH}_{50}$ concentrations used in Slick A and Slick B studies

	% HEWAF (nominal)	Initial tPAH ₅₀ (μg/L)
Slick A	0	0.063
	0.25	2.91
	0.5	6.39
	1	13.17
	2	26.67
Slick B	0	0.053
	0.75	1.15
	1.5	2.17
	3	4.54
	6	9.29

UV measurement and screening

Testing was conducted on a sunny summer day at Auburn University (Auburn, AL, USA), using full-spectrum natural sunlight as a source of UV radiation. UV intensities used in this study were approximately 100, 50, and <10% ambient intensity. To attain the UV gradations, three types of screening materials were used to filter the UV radiation. allowing UV control dishes to be maintained under the same conditions as the 50 and 100% UV treatments, thereby minimizing environmental variation. For the 100% ambient UV treatment, a UV transparent plastic sheet (>90% transparent, KNF Corporation, Tamagua, PA, USA) was suspended over the corresponding replicate dishes in the cooling water table. To obtain a 50% ambient UV gradation, a stainless-steel wire cloth (McMaster-Carr, Atlanta, GA, USA) was used in combination with high transparency plastic. A <10% transparent plastic (GAM products, ROSCO Laboratories Inc., Stamford, CT, USA) was used as a control. The water table was placed in an unobstructed location to prevent variations in UV exposure due to shade. Measurements of UV ($\lambda = 380$ nm, hereafter referred to as UV_{380}) irradiance were collected at regular 15–20 min intervals, using a JAZ handheld UV-Vis radiometer (Ocean Optics, Dunedin, FL). The UV₃₈₀ intensity was expressed as milliwatts per square centimeter (mW/cm²). Following the first 7-h UV exposure period, test dishes were taken indoors where mortality was assessed and solutions were renewed. Dishes were kept in a secure, dark location until the next outdoor UV exposure commenced. Dissolved oxygen, pH, conductivity, and salinity were monitored using YSI meters (YSI Incorporated, Yellowsprings, OH) at the initiation of solar exposures, mortality assessments, and before renewals, and temperature was monitored continually using Onset HOBO[®] Pendant Temperature Loggers (Onset, Bourne, MA).

Analytical chemistry

Samples of stocks and dilutions were taken with every preparation (initial and renewal) and shipped (4 °C) to ALS Environmental (Kelso, Washington) for chemical analysis. All PAH analytes were quantified via gas chromatographymass spectrometry in single ion monitoring mode (GC/MS-SIM), based on EPA method 8270D. The sum concentrations of 50 PAH analytes are reported and hereafter referred to as tPAH₅₀ (Forth et al. 2017b).

Phototoxic dose

All tests were performed outdoors, using natural sunlight as a source of UV. Thus, tests not performed simultaneously received different cumulative doses (the integration over the

443

test duration expressed in mW s/cm²) of UV₃₈₀. To account for differences in UV₃₈₀ exposure, a phototoxic dose was calculated using methods described by Oris and Giesy (1987), later modified by Alloy et al. (2015, 2017). Fourteen PAHs present in the HEWAF preparations were used in the calculations: anthracene, benzo[a]anthracene, benzo [e]pyrene, benzo[g,h,i]pervlene, chrysene, fluoranthene, fluorene (as well as C1 and C2 alkylated homologs), phenanthrene (as well as C1, C2, and C3 alkylated homologs), and pyrene. The aqueous concentration of each PAH was calculated as a molar value and multiplied by its relative photodynamic activity (RPA) compared to anthracene. These values were then used to calculate the sum concentration of anthracene equivalents. The anthracene equivalent concentration is multiplied by the UV_{380} dose, vielding phototoxic units expressed in µM/L mW s/cm². Bridges et al. (in press), recently demonstrated that attenuation of UV by oil type, HEWAF preparation, and/or test chamber is negligible under test conditions similar to those described here.

Statistical analysis

Survival data was arcsine transformed and a two-way ANOVA (using HEWAF concentration and UV exposure as factors) followed by a Tukey's post hoc test was run using JMP 11 (SAS Institute, Cary, NC) to compare survival among PAH and UV treatments. Results were considered statistically significant if p < 0.05. A phototoxic dose LC₅₀ using combined data from all three UV treatments was calculated using a least-square nonlinear regression with the logistic equation as the model shape in R using the *drc* package (version 3.1.2).

Results

Water chemistry and UV exposure

Initial concentrations of tPAH₅₀ in HEWAF dilutions made using slick A or slick B ranged from 0.06 to 26.7 µg/L and 0.05 to 9.3 µg/L, respectively (Table 1). A higher percent of slick B HEWAF was required to achieve tPAH₅₀ concentrations similar to those used in the slick A study (Table 1). As previously mentioned, the use of natural sunlight as a source of UV₃₈₀ introduced variations in UV exposure, and consequently, phototoxic doses between slick A and slick B exposures. The UV₃₈₀ dose was slightly more intense during the exposures using slick B HEWAF. For the slick A exposures, mean ambient UV₃₈₀ intensity (±1 SD) was 39.5 ± 3.72 mW s/cm² with an integrated dose of 1039 mW s/cm² for day 1 and 37.9 ± 13.2 mW s/cm² with an integrated dose of 1044 mW s/cm² for day 2. For the slick B exposure,



Fig. 1 Mean percent survival (± 1 SD) of fiddler crab larvae following exposure to slick A HEWAF after **a** first 7-h solar exposure, **b** second 7-h solar exposure. PAH concentrations are listed as the initial tPAH₅₀ in the HEWAF. Nominal UV exposures are based on percent of ambient UV. Asterisk denotes significantly different from the 10% UV

mean UV₃₈₀ intensity (± 1 SD) was 47.8 ± 11.9 mW s/cm² with an integrated dose of 1371 mW s/cm² on day 1 and 33.2 ± 17.3 mW s/cm² with an integrated dose of 1126 mW s/cm² on day 2.

treatment ($\alpha = 0.05$) at the same tPAH₅₀ exposure concentration

Slick A larval survival

Larval survival was dependent on both UV₃₈₀ dose and PAH concentration (p < 0.01, F = 65.12, DF = 14). After the first UV₃₈₀ exposure, survival in the 100% UV₃₈₀ treatment was significantly reduced (p < 0.01, F = 245.7, DF = 9) compared to the <10% UV₃₈₀ treatments at tPAH₅₀ concentrations $\ge 6 \mu g/L$ (Fig. 1a), yielding a LC₅₀ value of 5.12 (95% confidence interval = 4.47–5.81) $\mu g/L$ tPAH₅₀. Larval survival was significantly decreased in the 50% UV₃₈₀ treatment relative to the <10% treatment (p < 0.01, F = 82.7, DF = 9) in treatments with tPAH₅₀ concentrations $\ge 13 \mu g/L$ (Fig. 1a). The LC₅₀ value was determined to be 9.45 (95% confidence interval = 8.16–10.8) $\mu g/L$ tPAH₅₀. No significant effect of tPAH₅₀ concentration on larval survival was observed in the <10% UV₃₈₀ treatments, even at the highest tPAH₅₀ concentrations tested. The 24-h phototoxic



Fig. 2 Fitted curve of percent survival by phototoxic dose following **a** the first 7-h solar exposure and **b** the second 7-h solar exposure to slick A oil

dose LC₅₀ (Fig. 2a) for the slick A test was 2.96 μ M/L mW s/cm² (95% confidence interval = 2.76–3.17 μ M/L mW s/ cm²). After the second UV₃₈₀ exposure, significant mortality was observed in all treatments with tPAH₅₀ concentrations \geq 2.9 μ g/L (Fig. 1b) in both the 50% (p < 0.01, F = 1027.7, DF = 9), and 100% UV₃₈₀ treatments (p < 0.01, F = 290.7, DF = 9) relative to the <10% UV₃₈₀ treatments. Again, no effects on survival were observed in the <10% UV₃₈₀ treatments, even at the highest tPAH₅₀ concentrations tested.

Slick B larval survival

Similarly, larval survival in slick B tests was dependent on both UV₃₈₀ dose and PAH concentration (p < 0.01, F = 90.5,



Fig. 3 Mean percent survival (±1 SD) of fiddler crab larvae following exposure to slick B HEWAF after **a** first and **b** second UV exposures. PAH concentrations are listed as the initial tPAH₅₀ in the first HEWAF. Asterisk denotes significantly different from the 10% UV treatment ($\alpha = 0.05$)

DF = 14). After the first UV₃₈₀ exposure, survival in the 100% UV₃₈₀ treatment was significantly reduced (p < 0.01, F = 89.4, DF = 14) compared to the <10% UV₃₈₀ treatment at tPAH₅₀ concentrations ≥4.5 µg/L (Fig. 3a), yielding a tPAH₅₀ 24-h LC₅₀ of 6.55 (95% confidence interval = 5.91–7.31) µg/L. The 24-h phototoxic dose LC₅₀ (Fig. 4a) for slick B was calculated was 5.34 µM/L mW s/cm² (95% confidence interval = 4.65–6.30 µM/L mW s/cm²). Concentration of tPAH₅₀ showed no significant effects on larval survival in the <10% treatment. Likewise, survival was not significantly affected in the 50% UV₃₈₀ treatments (p = 0.07, F = 1.99, DF = 14) after the first UV₃₈₀ exposure.

Following the second UV₃₈₀ exposure, larval survival was significantly reduced (p < 0.01, F = 169.7, DF = 14) in all test concentrations $\ge 1.2 \ \mu g/L$ in the 100% UV₃₈₀ treatment. In the 50% UV₃₈₀ treatment, survival was significantly reduced (p < 0.01, F = 100.8, DF = 14) in all treatments $\ge 4.5 \ \mu g/L$ relative to the <10% UV₃₈₀ treatment (Fig. 3b). At 48-h the LC₅₀ was determined to be 2.72 (95% confidence interval = 2.12–3.32) $\mu g/L$ tPAH₅₀, and no



Fig. 4 Fitted curve of percent survival by phototoxic dose following **a** the first 7-h solar exposure and **b** the second 7-h solar exposure to slick B oil

significant effects on larval survival were observed in any of the <10% UV₃₈₀ treatments.

Discussion

Standard, laboratory-based toxicity testing may drastically underestimate the toxicity of environmental PAH exposure by failing to account for interactions with natural stressors, such as UV radiation (Alloy et al. 2015, 2017). Fiddler crab larval survival in the <10% UV₃₈₀ treatments was in excess of 80%, even at the highest tPAH₅₀ concentrations (slick A: 26.7 µg/L, slick B: 9.3 µg/L) after 48-h of exposure. Organisms receiving simultaneous exposure to 100% ambient UV radiation at the same concentrations showed a ~50% reduction in survival within 7 h. Alloy et al. (2015) reported a similar response in larval blue crab (*Callinectes sapidus*) following co-exposure to DWH oil and UV radiation, using comparable exposure scenarios. Survival of larval blue crab was significantly reduced at environmentally relevant concentrations of DWH oil in the presence of UV light, with a phototoxic LC₅₀ of 9.5 μ M/L mW s/cm² (slick A). By comparison, fiddler crab larvae displayed increased sensitivity to photo-induced PAH toxicity when compared with data from blue crab studies, as indicated by a lower phototoxic LC₅₀ value (2.96 μ M/L mW s/cm² for slick A).

Life history, behavioral and habitat factors likely contribute to the differential sensitivity between blue and fiddler crab larvae. In separate studies, Boese et al. (1997) and Spehar et al. (1999) found differential sensitivity between a number of invertebrate species following co-exposures to fluoranthene and UV radiation. Interestingly, the study by Boese et al. (1997) was able to show an inverse relationship between the sensitivity of seven marine invertebrate species to photo-induced fluoranthene toxicity, and potential for sunlight exposure in the organism's natural habitat. The authors credited this relationship to evolutionary differences in the development of UV protection and repair mechanisms (Boese et al. 1997; Karentz 1994). Although both species are bottom dwelling organisms, the blue crab is a member of the swimming crab family, Portunidae, which migrate based on salinity preferences during reproduction (Epifanio 1995; Mouton and Felder 1995). It is possible that species-specific behaviors and reproductive strategies which increase UV exposure have contributed to the evolution of more robust UV repair mechanisms, and thus decreased sensitivity in blue crab larvae.

Studies investigating the effects of photo-enhanced toxicity of DWH oil to several ecologically and commercially important fish species also found dramatic increases in PAH toxicity to ELS fish in the presence of UV radiation. Mahi mahi (Coryphaena hippurus) embryos showed significantly reduced hatching effects using similar exposure scenarios, with a phototoxic EC_{50} of 6.77 μ M/L mW s/cm² (slick A HEWAF) (Alloy et al. 2017). Similarly, the phototoxic LC₅₀ for red drum larvae (Sciaenops ocellatus) was $1.41 \,\mu\text{M/L}\,\text{mW}\,\text{s/cm}^2$ (slick A HEWAF) following a single 5.5-h solar exposure, while speckled seatrout (Cynoscion nebulosus) larvae demonstrated even greater sensitivity following a single, 5.5-h solar exposure, with a phototoxic LC_{50} of 0.516 μ M/L mW s/cm² (slick A HEWAF) (Alloy et al. 2017). The range of phototoxic LC_{50} values reported in these studies speak to variations in sensitivity to photoenhanced PAH toxicity across species. Data from the present study indicate fiddler crab larvae fall within the midrange of phototoxic LC50 values for the species tested.

In spite of cross species variations, the combined results of these studies indicate exposure to PAHs from oil spill events may have a significant impact on ELS organisms across a wide range of ecologically important aquatic organisms at much lower exposure concentrations than previously thought.

Penetration of UV radiation in aquatic systems is highly variable, ranging from less than one meter in turbid coastal waters to greater than thirty meters in clear ocean waters (Tedetti and Sempéré 2006). Fiddler crab larvae are carried via tidal flow and upwelling to offshore waters, with the majority of early developmental stages found at depths ranging from 3.9 to 21 m during tidal flood (Epifanio et al. 1988). These depths fall within the UV penetration ranges in marine environments, though attenuation of UV radiation varies depending on physico-chemical properties (turbidity, suspended solids, dissolved organic carbon) at the site (Jeffrey et al. 1996; Roberts et al. 2017; Tedetti and Sempéré 2006; Vasilkov et al. 2001). However, given the wide distribution of fiddler crabs in the GoM, the large area of oiled shoreline, and the timing of the spill relative to fiddler crab reproduction, it is likely that ELS fiddler crabs were impacted by photo-induced toxicity of DWH oil.

Significant increases in mortality were observed in larval fiddler crabs co-exposed to UV as natural sunlight and relatively low concentrations of PAH. Additionally, these decreases in survival were often observed within the first 7h of UV exposure. Given the high likelihood that fiddler crab larvae were simultaneously exposed to DWH and UV, these data suggest significant impacts on survival of larval fiddler crabs due to photo-induced toxicity are possible. Furthermore, these data add to the growing body of evidence establishing that natural stressors, such as UV radiation, are important components to consider when assessing the impacts of oil spills and other petroleum hydrocarbon releases in the environment. Data generated here may be used in future impact assessments following oil spills into marine environments.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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