Chemical communication in tilapia: a comparison of *Oreochromis mossambicus* with *O. niloticus*

By

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Abstract

In allopatric speciation species differentiation generally results from different selective pressures in different environments, and identifying the traits responsible helps to understand the isolation mechanism(s) involved. Male Mozambique tilapia (*Oreochromis mossambicus*) use urine to signal dominance; furthermore, 5β-pregnane-3α,17,20β-triol-3α-glucuronide (and its α-epimer, 5β-pregnane-3α,17,20α-triol-3α-glucuronide), in their urine is a potent pheromone, the concentration of which is correlated with social status. The Nile tilapia (*O. niloticus*) is a close relative; species divergence probably resulted from geographical separation around 6 million years ago. This raises the question of whether the two species use similar urinary chemical cues during reproduction. The olfactory potency of urine, and crude extracts, from either species was assessed by the electro-olfactogram and the presence of the steroid glucuronides in urine from the Nile tilapia by liquid-chromatography/mass-spectrometry. Both species showed similar olfactory sensitivity to urine and respective extracts from either species, and similar sensitivity to the steroid glucuronides. 5β-pregnan-3α,17α,20β-triol-3α-glucuronide was present at high concentrations (approaching 0.5 mM) in urine from Nile tilapia, with 5β-pregnan-3α,17α,20α-triol-3α-glucuronide present at lower concentrations, similar to the Mozambique tilapia. Both species also had similar olfactory sensitivity to estradiol-3-glucuronide, a putative urinary cue from females. Together, these results support the idea that reproductive chemical cues have not been subjected to differing selective pressure. Whether these chemical cues have the same physiological and behavioural roles in *O. niloticus* as *O. mossambicus* remains to be investigated.

Key words: cichlid, pheromone, steroid, olfaction, urine, speciation.

Abbreviations:

EOG:	Electro-ofactogram
17,20β-P:	17α,20β-dihydroxypregn-4-en-3-one
20α-P-3-G:	5β-pregnane-3α,17α,20α-triol-3α-glucuronide
20β-P-3-G:	5β-pregnane-3α,17α,20β-triol-3α-glucuronide
20one-P-3-G:	3α,17α-dihydroxy-5β-pregnan-,20-one-3α-glucuronide

Introduction

The Mozambique tilapia (*Oreochromis mossambicus*) and Nile tilapia (*O. niloticus*) are maternal mouth-brooding African cichlids of enormous scientific and economic importance, both in aquaculture and - as a direct consequence - as invasive species (particularly in Asia, Australia and North and South America; Lowe et al., 2012; Russell et al., 2012; Sanches et al., 2012). The Mozambique tilapia has also proven to be an excellent model species for teleost reproduction, due to its widespread availability, robustness and its highly developed courtship and dominance behaviours (Baerends and Baerends van Roon, 1950). During the spawning season, the males congregate in 'leks' wherein they establish a social hierarchy, and dig and defend pits in the substrate; the more dominant males occupy the pits closer to the centre of the lek. Ripe females then visit these leks, choose one or more males with which to spawn, and incubate the fertilized eggs in their mouths away from the males (Turner, 1986). Despite – or, perhaps, because of – the clear importance of vision in cichlid behaviour and speciation (Kocher, 2004; Seehausen et al., 1999; Seehausen et al., 2008), little work has addressed the possible role of chemical cues in these processes.

We have previously shown that male Mozambique tilapia urinate at high frequency immediately before aggressive male-male encounters and during courtship, and that the urine from dominant males is more potent an odorant than that from subordinate males (Barata et al., 2008; Barata et al., 2007; Miranda et al., 2005). The urinary bladders of dominant males are larger and more muscular than those of subordinate males, and females; an apparent adaptation to allow storage of larger volumes of urine for release in the appropriate social context (Keller-Costa et al., 2012). Furthermore, exposure to male urine evokes an increase in 17,20β-P (the oocyte maturation-inducing steroid; Nagahama, 1987; Nagahama, 1997) metabolism in females

(Huertas et al., 2014), whereas prevention of urination results in higher aggression in male-male encounters (Keller-Costa et al., 2012). Together, this evidence strongly suggests that males are signaling both to rival males and potential female mates *via* (a) urinary pheromone(s). This hypothesis has been strengthened by the recent identification of 5β-pregnane-3α,17α,20β-triol-3α-glucuronide and 5β-pregnane-3α,17α,20α-triol-3α-glucuronide in the urine of males, the concentration of which depends on social status of the donor and which also act as potent pheromones on females (Keller-Costa et al., 2014). Steroid glucuronides have been shown to play pheromonal roles in the reproduction of several fish species (reviewed by; Stacey and Sorensen, 2006; Stacey and Sorensen, 2009). However, how - or even if - species specificity is conferred to the pheromonal message largely remains unclear (Levesque et al., 2011; Lim and Sorensen, 2011; Stacey, 2010).

The African cichlids have generated great interest in evolutionary biologists because of the speciation 'explosion' that occurred in this group in the East African lakes around two million years ago (for example, see; Kocher, 2004; Schwarzer et al., 2009; Seehausen et al., 2008). Given their often dazzling colouration and patterning, much of the focus has been on visual signaling as part of speciation and reproductive isolation mechanisms (Seehausen et al., 1999). However, growing attention has recently been paid to the role of olfactory cues in reproductive isolation (for example, see Blais et al., 2007; Plenderleith et al., 2005; Smadja and Butlin, 2009). The Mozambique and Nile tilapia are thought to have diverged, presumably through geographical separation, around 6 million years ago (Genner et al., 2007). It is therefore reasonable to hypothesize that selective pressure to evolve different communication strategies during reproduction must have been weak or absent, and the chemical cues used by the two species are likely to be the same. Specifically, here it was we wished to test: (i) whether

both species have similar olfactory sensitivity to male urine from the other species as their own; (ii) whether urine from male Nile tilapia contains the same steroids as those previously identified in Mozambique tilapia and; (iii) if so, whether the two species have the same olfactory sensitivity to these steroids.

Materials and Methods

Fish

Fish care and experimentation complied with the guidelines of the European Union Council (86/609/EU) and Portuguese legislation for the use of laboratory animals under a "Group-1" license issued by the Veterinary General Directorate of the Ministry of Agriculture, Rural Development and Fisheries of Portugal. Mozambique tilapia (60-200 g) were taken from a self-propagating population kept in the fish-holding facilities at the University of the Algarve. Nile tilapia (200-500 g) were transported from the experimental hatchery of Wageningen University 'De Haar Vissen' (Wageningen, The Netherlands) and kept in similar conditions at the University of the Algarve. Both species were kept at 27ºC under a 12L:12D photoperiod and fed daily with commercial cichlid feed (Sparos Lda., Portugal).

Urine Collection

Social groups of either species (three males and six to eight females) were established. Regular observations (three times per week for two weeks prior to urine collection) were taken to identify the dominant male in each group - black colouration in the Mozambique tilapia, white colouration in the Nile tilapia, occupation and defence of a nest or floor area in both species. Urine samples were then taken from the dominant male from each tank by gently squeezing the abdomen immediately above and anterior to the genital papilla, and collecting the urine directly into a glass vial. Successive samples obtained from each male were frozen until at least 1.0 ml had been taken. A pool of 6 ml was then made using equal volumes from each male, and 3 ml subjected to solid-phase extraction (C18 cartridges Waters 'Sep-Pak®', Waters Corporation,

Milford, MA, USA). Retained substances were eluted with 3 ml methanol and both unretained '(aqueous fraction') and retained ('eluate') were aliquotted and stored at -20ºC until use. The remaining pool of 3 ml of untreated urine from each species was also aliquotted and frozen. Immediately prior to use in EOG recording (see below), samples were thawed and diluted in charcoal-filtered tap-water.

Steroid Glucuronides

5β-Pregnane-3α,17α,20α-triol-3α-glucuronide (20α-P-3-G) and 5β-pregnane-3α,17α,20β-triol-3α-glucuronide (20β-P-3-G) were synthesized from the precursor 3α,17α-dihydroxy-5β-pregnan-20-one as described in Keller-Costa *et al.* (2014). 3α,17α-dihydroxy-5β-pregnan-20-one-3α-glucuronide (20one-P-3-G) and 17β-estradiol-3-glucuronide were bought from Steraloids Inc. (Newport, RI, USA). All steroids (10^{-3}M) were dissolved in ethanol or ethanol:water (50:50) and stored at -20ºC until use. Steroids were diluted to the appropriate dilution in charcoal-filtered tap-water immediately prior to use in electro-olfactogram (EOG) recording (see below). A solution of 10^{-5}M L-serine was similarly prepared from 10^{-3}M aliquots stored at -20ºC.

Recording the Electro-Olfactogram

Mature tilapia, of both sexes, were anaesthetized with $NaHCO_3$-buffered MS222 (3-aminobenzoic acid ethyl ester, Sigma-Aldrich) in water (200 $mg.l^{-1}$), immobilized with 3$mg.kg^{-1}$ gallamine triethiodide (Sigma-Aldrich) and the EOG recorded as previously described in detail (Frade et al., 2002). All odorants were presented as a 4 second pulse in order of increasing concentration with at least one minute between stimuli. The EOG amplitude was measured (in mV) from the baseline to the peak of the initial downward deflection of the trace. This was blank-subtracted (blank water – the same water used to

dilute the stimuli – given as a stimulus) and normalized to the response to 10^{-5}M L-serine, similarly blank-subtracted. For the urine and respective fractions, linear regression was applied to a plot of normalized EOG amplitude against log(dilution), using only concentrations giving responses significantly greater than blanks. The calculated thresholds of detection (intercept on the *x* axis) and slopes were compared by Student's *t* test (paired within species and unpaired between species) and corrected for multiple testing using the False Discovery Rate method ($Q<0.05$) of Benjamini and Hochberg (1995). For the steroids, normalized data were fitted to a three-parameter Hill plot, and the derived I_{max} (maximal response amplitude) and EC_{50} ('half maximal effective concentration', or concentration of odorant required to give a response 50% of the maximum) values compared by Student's *t* test (paired within species and unpaired between species). A *P* value of less than 0.05 was taken as significant. Data are shown as mean ± S.E.M.

Liquid-Chromatography/Mass-Spectrometry

The LC-MS system was an Agilent Technologies 1200 Series LC coupled to a Bruker Daltonics HCT ultra (ion trap), able to carry out MSn, n = 11. The spray and ion optics conditions were the following: ionization, negative polarity; capillary voltage, 3.5 kV; drying gas (nitrogen), 330 ºC at 10 L/min; nebulizer gas pressure, 50 psi; capillary exit voltage, 130 V; skimmer voltage, 40 V. A Hamilton PRP-1 reversed phase LC column (15.0 cm length, 2.1 mm internal diameter, 5 µm average particle diameter), stabilised at 25 °C was used. The eluent system was acetonitrile (A) and water (B), both with 0.1 % of formic acid. The gradient started with 20% of A, followed by a linear increase up to 80% in 20 min. In a second gradient step an increase up 100 % took place in 5 minutes. A final cleaning step using 100% of A during 5 min was made after each run.

190 The eluent was then allowed to recover the initial conditions (20 % of A and 80% of B)
191 in 1 min and then stabilise for an additional six minutes before the next run.
192

Results

Olfactory Responses to Conspecific and Heterospecific Urine

Consistent with previous studies, the urine of dominant male Mozambique tilapia evoked strong EOG responses in males of the same species (Fig. 1), with an estimated threshold of detection of $1{:}10^{6.04\pm0.10}$ (Fig. 1C). However, urine from Nile tilapia evoked similar-sized responses, resulting in a similar concentration-response curve with similar slopes and threshold of detection ($1{:}10^{6.00\pm0.06}$). Conversely, Nile tilapia were slightly less sensitive to conspecific urine than that from Mozambique tilapia ($P<0.05$); the threshold of detection for conspecific urine was $1{:}10^{5.16\pm0.04}$, whereas that of heterospecific urine was $1{:}10^{5.98\pm0.08}$ (Fig. 1D). The slopes could not be compared *between* species, as the relatively smaller response to L-serine in Nile tilapia resulted in larger (approximately two-fold) normalized responses than in Mozambique tilapia.

In the Mozambique tilapia, there were no significant differences of EOG responses to the eluate of conspecific urine and those of the eluate of Nile tilapia urine (Fig. 2A); thresholds of detection were $1{:}10^{6.07\pm0.15}$ for conspecific urine and $1{:}10^{-5.94\pm0.11}$ for heterospecific urine, and slopes were similar. In the Nile tilapia – in contrast to the whole urine – the eluates of both species proved to be equally potent (Fig. 2B); thresholds of detection were $1{:}10^{5.76\pm0.04}$ for conspecific eluate and $1{:}10^{5.83\pm0.09}$ for heterospecific eluate, and slopes were equal (whereas, again, the inter-specific difference was maintained).

However, in the Mozambique tilapia, the aqueous fraction of conspecific urine proved to be slightly more potent than that of the heterospecific aqueous fraction (Fig. 3A); the threshold of detection was $1{:}10^{5.77\pm0.013}$ compared to $1{:}10^{5.39\pm0.05}$ for the aqueous fraction from Nile tilapia urine, although this just failed to reach significance.

The slopes were again equal. Interestingly, this pattern was repeated in the olfactory responses from Nile tilapia (Fig. 3B); the aqueous fraction of urine from Mozambique tilapia was significantly more potent than that of conspecifics. Thresholds of detection were $1{:}10^{4.79\pm0.03}$ for the aqueous fraction of conspecific urine and $1{:}10^{5.21\pm0.11}$ for heterospecific ($P < 0.01$).

Liquid Chromatography/Mass Spectrometry

The urine pool from both species showed a major peak at 9.37 min, showing m/z 511 under negative polarity (Fig. 4). Both species also showed a minor peak at 8.94 corresponding to an isomer compound (also m/z 511). Based on previous studies with Mozambique tilapia and on the analysis of authentic reference compounds, we assign these signals to 20β-P-3-G and 20α-P-3-G, respectively (Fig.4). The estimated concentration for 20β-P-3-G in both species approached 0.5 mM, consistent with previously published data for the Mozambique tilapia (Keller-Costa et al., 2014). This strongly suggests that both stereo-isomers are also present in the urine of male Nile tilapia, at a similar ratio, and at similar concentrations. Although other, minor, peaks were seen in both species, none of these coincided with that of the standard for 20one-P-3-G; indicating this compound is not, therefore, a normal constituent of tilapia urine (Fig. 4).

Olfactory Sensitivity to 20α-P-3-G and 20β-P-3-G

Consistent with our previous study (Keller-Costa et al., 2014), Mozambique tilapia had olfactory sensitivity to both 20α-P-3-G and 20β-P-3-G (Fig. 5A). Both steroids evoked sigmoidal concentration-response curves, with thresholds of detection around 10^{-9}M, and reaching a plateau at 10^{-6}M. In Nile tilapia, similar sigmoidal concentration-

response curves were evoked (Fig. 5B), with similar thresholds and plateaus. In both species, there was a tendency for 20α-P-3-G to evoke a slightly higher apparent I_{max} than 20β-P-3-G (Fig. 5C), but this failed to reach statistical significance. As with urine, the normalized responses were larger in Nile tilapia than in Mozambique tilapia. More importantly, however, the apparent EC_{50} values were similar in both species (Fig. 5D); in both the Mozambique tilapia, the apparent EC_{50} for 20β-P-3-G (21.8 ± 6.1 nM) was significantly lower than that of 20α-P-3-G (153.3 ± 49.1 nM) and in the Nile tilapia the apparent EC_{50} for 20β-P-3-G (46.1 ± 11.8 nM) was significantly lower than that of 20α-P-3-G (158.2 ± 31.1 nM).

Olfactory Sensitivity to 20one-P-3-G and Estradiol-3-G

Although 20one-P-3-G is not present in male urine of either Mozambique or Nile tilapia, it is commercially available, whereas 20α-P-3-G and 20β-P-3-G are not. Nevertheless, the Mozambique tilapia had olfactory sensitivity to it, giving sigmoidal concentration-response curves (Fig. 6A). Estradiol-3-G, another 3-glucuronidated steroid, also evoked sigmoidal concentration-response curves, but never as large amplitude EOGs as the other steroid glucuronides tested. Similar olfactory sensitivity to 20one-P-3-G and estradiol-3-G was seen in the Nile tilapia (Fig. 6B); both evoked sigmoidal concentration-response curves, but the normalized amplitudes of EOG responses were much larger for 20one-P-3-G than estradiol-3-G. In both species, the I_{max} evoked by 20one-P-G was similar to that of 20α-P-3-G and 20β-P-3-G (Fig. 6C), whereas that of estradiol-3-G was significantly lower. Nevertheless, the ratio between the two was similar in the two species. Despite the relatively low amplitude of responses evoked by estradiol-3-G, this steroid was detected with the lowest apparent EC_{50} values (Mozambique, 0.25 ± 0.12 nM; Nile, 0.44 ± 0.16 nM; Fig. 6D). Apparent

268 EC_{50} values for 20one-P-3-G and estradiol-3-G were similar between the two species.
269 The apparent Hill coefficients for all steroids were around one in both species.
270

Discussion

Olfactory Responses to Male Urine

The current study shows that urine taken from dominant males of either Mozambique or Nile tilapia is a potent odorant for conspecifics. For the Mozambique tilapia, this agrees with our previous studies (Barata et al., 2008; Barata et al., 2007; Frade et al., 2002; Keller-Costa et al., 2014). However, this is a novel observation for the Nile tilapia. Furthermore, we have shown that, despite geographic isolation, the urine from one species is equally potent, if not more so, to the other. Solid-phase extracts (the non-polar/hydrophobic components) of male urine from either species evoked similar responses in both. This does not necessarily mean, however, that the active compounds are the same. Conversely, the polar/hydrophilic components remaining in the filtrate proved to be more potent in the urine of the Mozambique tilapia than the Nile tilapia, irrespective of the species of the receiver. This may not mean that the odorants involved are the same, but it is suggestive that urinary odorants released by the two species may differ significantly in this fraction; could this be the fraction wherein cues concerning species identity are found?

Steroid Glucuronides in Tilapia Urine

We have previously identified 20α-P-3-G and 20β-P-3-G as components of the urinary pheromone in male Mozambique tilapia. 20β-P-3-G is more abundant than the 20α-P-3-G, at a ratio of approximately 15:1 (although there is considerable inter-individual variation; Keller-Costa et al., 2014). In dominant males, the urinary concentration can reach as high as 0.5 mM, an exceptionally high concentration for any steroid in any fluid, suggesting an active transport and/or concentrating mechanism in the renal system

of both species. The current study has shown that both steroids are present at similar concentrations and at a similar ratio in the urine from dominant Nile tilapia, suggesting that the olfactory potency of the eluate fraction of both species may be due mainly to these two steroids. The 20keto form is not, apparently, present in the urine from either species, although both species have high olfactory sensitivity to it. This steroid, however, has been identified as a component of the male pheromone of the African catfish (Van den Hurk and Resink, 1992).

Olfactory Sensitivity to Steroid Glucuronides in Tilapia

20α-P-3-G and 20β-P-3-G are potent odorants for the Mozambique tilapia (Keller-Costa et al., 2014). Both evoke sigmoidal concentration-response curves when olfactory activity is assessed by EOG; this is unusual, as most 'conventional' fish odorants, such as amino acids or bile acids, evoke linear or exponential semi-logarithmic concentration-response curves (for example, see; Hara, 1994; Hubbard et al., 2011; Zhang and Hara, 2009). Nevertheless, the dynamic range of olfactory sensitivity to these steroids in tilapia lies approximately between 10^{-9} and 10^{-6} M, corresponding to a dilution of 1:500 – 1:500,000 of crude urine. This fits well with the observed olfactory sensitivity to untreated urine, and its corresponding C18 eluate, and can explain behavioural and physiological pheromonal effects (both during courtship/reproduction and male-male aggression; Barata et al., 2008; Barata et al., 2007; Huertas et al., 2014; Keller-Costa et al., 2014), which typically take place at close range. However, the olfactory sensitivity is insufficient to propose a long-range role for this urinary pheromone, such as that proposed for the sea lamprey (Li et al., 2002; Sorensen et al., 2005). Nevertheless, it is also clear that there are other components in the urine that both species can smell. What are these components, and what is their role?

Given that the two steroid glucuronides are present in similar concentrations in the urine of both species, it is interesting to note that the aqueous filtrate fractions evoke different responses; the urine filtrate from Mozambique tilapia has higher olfactory activity than that of the Nile tilapia, irrespective of the receiver species. It is possible that the two species are sensitive to different components in this fraction, but – given the similarity of the concentration-response curves between the two species – it is more likely that they are detecting the same compounds, and that these compound differ in concentration between the two species. This suggests that species-specificity may be conferred to the pheromonal message by odorants in this fraction (as shown in cyprinids; Levesque et al., 2011; Lim and Sorensen, 2011). Clearly, the identities of these compounds need to be established before this can be tested. However, evidence also suggests that hydrophilic urinary components – possibly trimethylamine – play a role in the communication of social status in the fathead minnow (Martinovic-Weigelt et al., 2012).

Since the work of Crapon de Caprona (1980), chemical cues have been known to be important to cichlids. For example, urination rates increase in different social contexts in male *Astotilapia burtoni* (a mouth-brooding cichlid from Lake Tanganyika) in a similar way to the Mozambique tilapia (Maruska and Fernald, 2012). However, the identity of the odorant(s) involved is not yet known. Using a different approach, Cole and Stacey (2006) showed that *A. burtoni* had olfactory sensitivity to some conjugated steroids (both glucuronides and sulphates at the 3 and 17 positions) and the authors suggest five distinct olfactory receptor mechanisms to account for this. Given that we putatively have identified only two olfactory receptor mechanisms in the Mozambique tilapia, both detecting 3-glucuronide steroids, it is interesting to speculate that the species radiation in Lake Tanganyika (and other African lakes) involved the evolution

of olfactory sensitivity to a greater range of steroid conjugates. Thus, investigations into the role(s) of chemical communication in reproductive isolation and species radiation in African cichlids would be of interest.

In conclusion, the current study has shown that the same urinary steroid glucuronides are present in the urine of male Mozambique and Nile tilapia, and that both species have similar olfactory sensitivity to these steroid glucuronides. Whether the two tilapia species interpret these chemical messages in the same way, however, remains to be investigated. Furthermore, the role of chemical communication in cichlid species radiation should be addressed.

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Figure Legends

Figure 1. Olfactory responses of tilapia to conspecific and heterospecific urine. Typical electro-olfactogram (EOG) responses of (**A**) Mozambique and (**B**) Nile tilapia in response to dilutions of urine pool (diluted 1:10,000) from male Mozambique (red horizontal bars) and Nile (blue horizontal bars) tilapia. Semi-logarithmic plots of normalised EOG responses of the Mozambique tilapia (**C**) and Nile tilapia (**D**) to dilutions of untreated male urine from Mozambique tilapia (red circles) and Nile tilapia (blue circles). Data are shown as mean ± S.E.M. (n = 7); ***P<0.001 comparing thresholds calculated from linear regression of individual semi-logarithmic plots.

Figure 2. Semi-logarithmic plots of normalised EOG responses of the Mozambique tilapia (**A**) and Nile tilapia (**B**) to dilutions of the eluate of solid-phase extracts of male urine (non-polar/hydrophobic fraction) from Mozambique tilapia (red squares) and Nile tilapia (blue squares). Data are shown as mean ± S.E.M. (n = 7); there are no statistical differences between the two stimuli in either species.

Figure 3. Semi-logarithmic plots of normalised EOG responses of the Mozambique tilapia (**A**) and Nile tilapia (**B**) to dilutions of the aqueous fraction of male urine from Mozambique tilapia (red triangles) and Nile tilapia (blue triangles). Data are shown as mean ± S.E.M. (n = 7); **P<0.01 comparing thresholds calculated from linear regression of individual semi-logarithmic plots.

Figure 4. Representative LC/MS traces of male urine (diluted 1:50) from Mozambique tilapia (red) and Nile tilapia (blue) showing the major peaks which coincide with 5β-

pregnan-3α,17α,20α-triol-3β-glucuronide (upper pink trace) and minor peaks that coincide with 5β-pregnan-3α,17α,20α-triol-3α-glucuronide (upper green trace) standards. The chromatogram for the 5β-pregnan-3α,17α,20one-triol-3α-glucuronide (upper purple trace) is also shown; no equivalent peaks are seen in the urine from either species. Numbers in black refer to retention times (mins).

Figure 5. Olfactory sensitivity to urinary steroid glucuronides in the Mozambique and Nile tilapia. Semi-logarithmic plot of normalised EOG amplitude against concentration of 20α-P-3-G (pink circles) and 20β-P-3-G (green circles) in the Mozambique tilapia (**A**) and Nile tilapia (**B**). The apparent I_{max} values are similar for the two steroids, independently of species but larger in the Nile than Mozambique tilapia (**C**), whereas the apparent EC_{50} values are significantly lower for 20β-P-3-G than 20α-P-3-G in both species, but similar between species (**D**). Data are shown as mean ± S.E.M. (N = 7); * P<0.05.

Figure 6. Olfactory sensitivity to steroid glucuronides in the Mozambique and Nile tilapia. Semi-logarithmic plot of normalised EOG amplitude against concentration of 20one-P-3-G (brown circles) and estradiol-3-G (orange circles) in the Mozambique tilapia (**A**) and Nile tilapia (**B**). The apparent I_{max} values markedly different for the two steroids, independently of species but, again, larger in the Nile than Mozambique tilapia (**C**), whereas the apparent EC_{50} values are significantly lower for estradio-3-G than 20one-P-3-G in both species, but similar between the two species (**D**). Data are shown as mean ± S.E.M. (N = 7); * P<0.05.

Figure 1

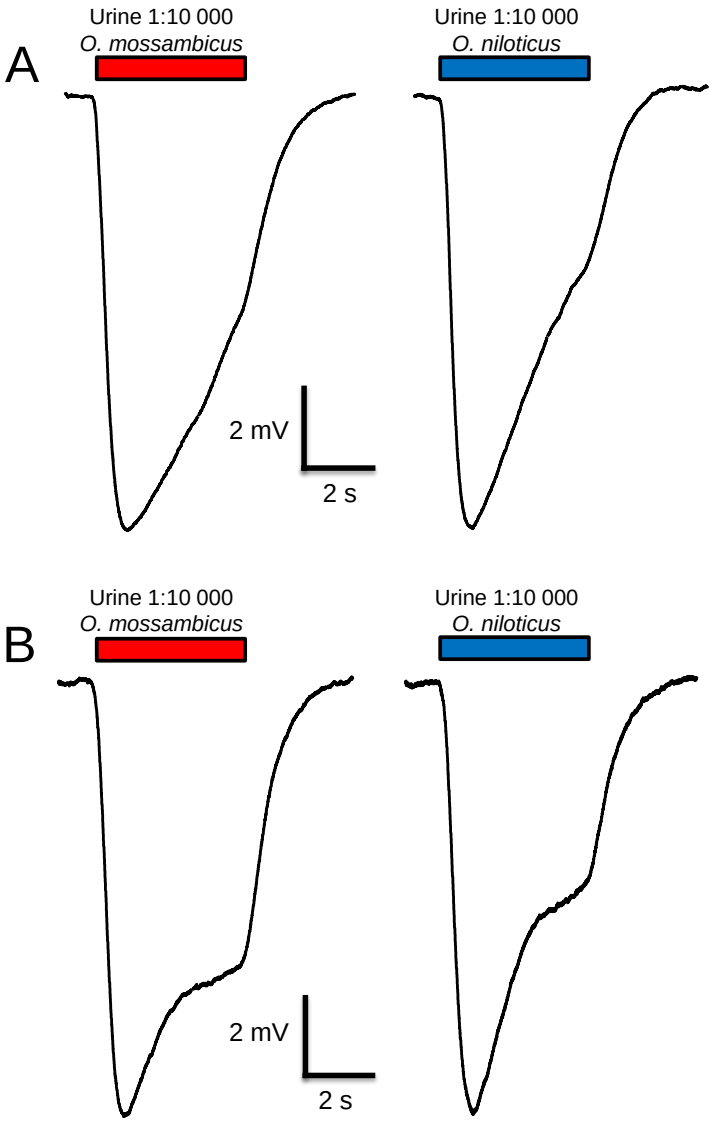

Figure 1A and 1B.

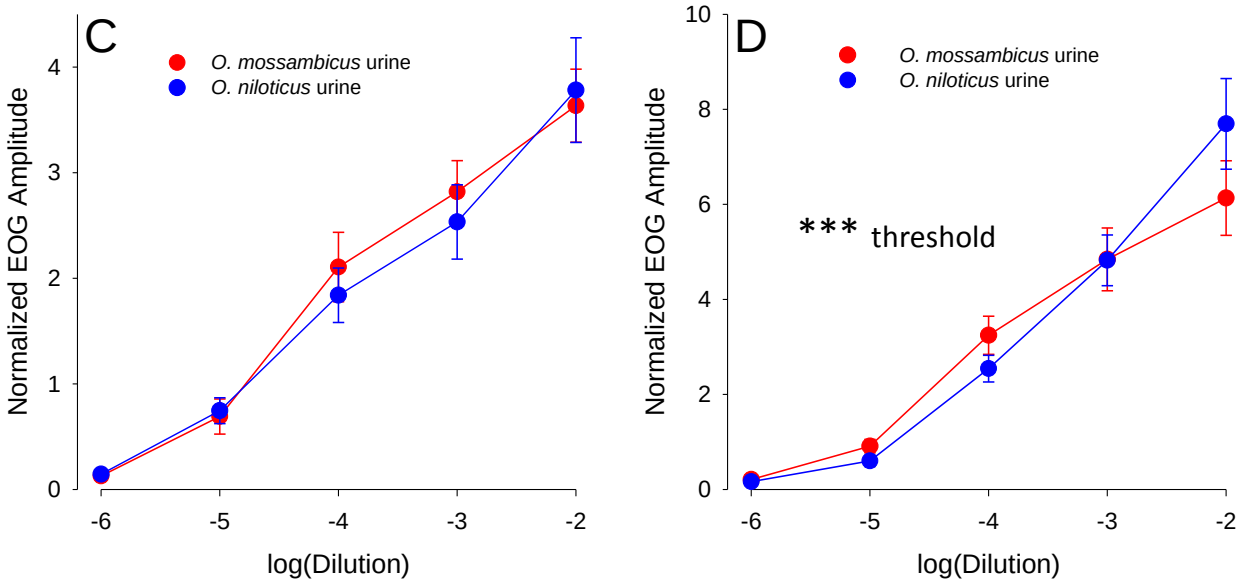

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Figure 2

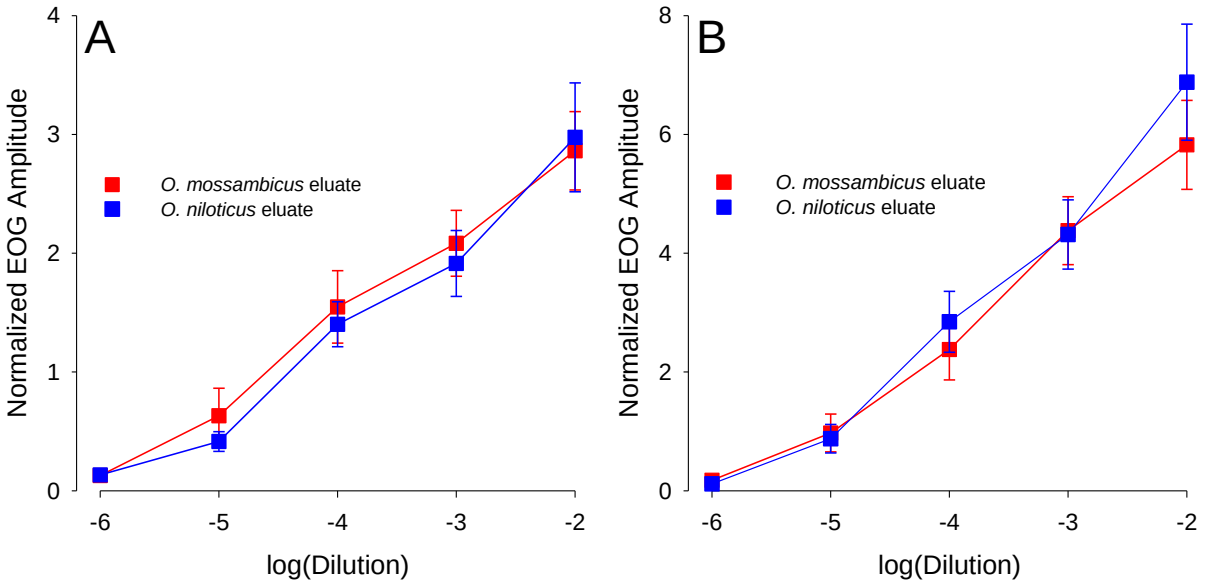

Figure 2.

Figure 3

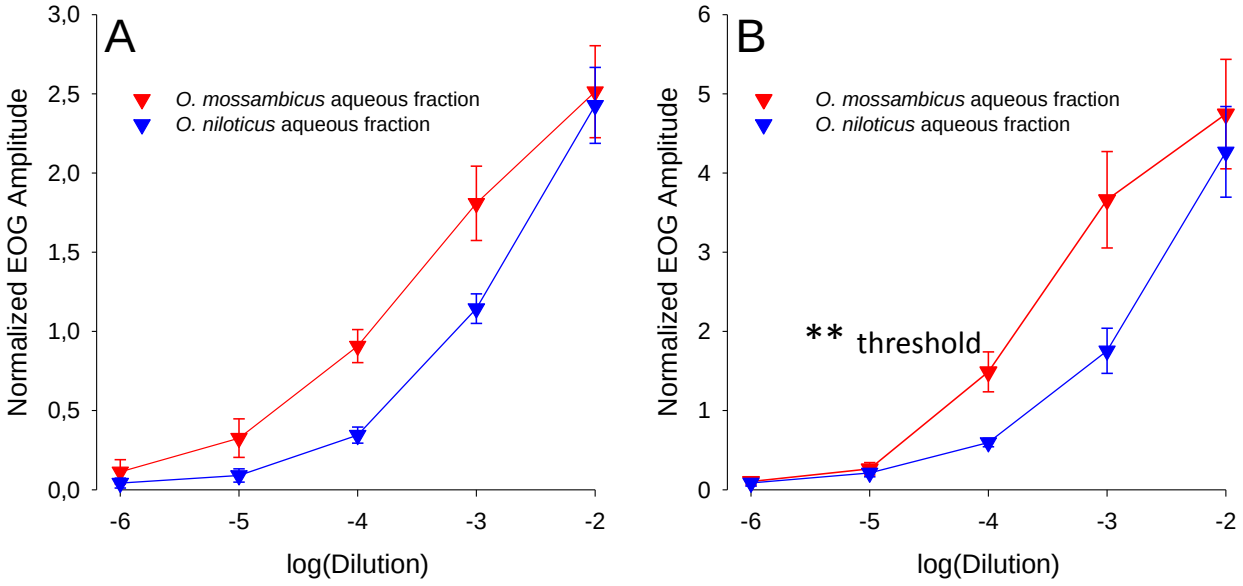

Figure 3.

Figure 4

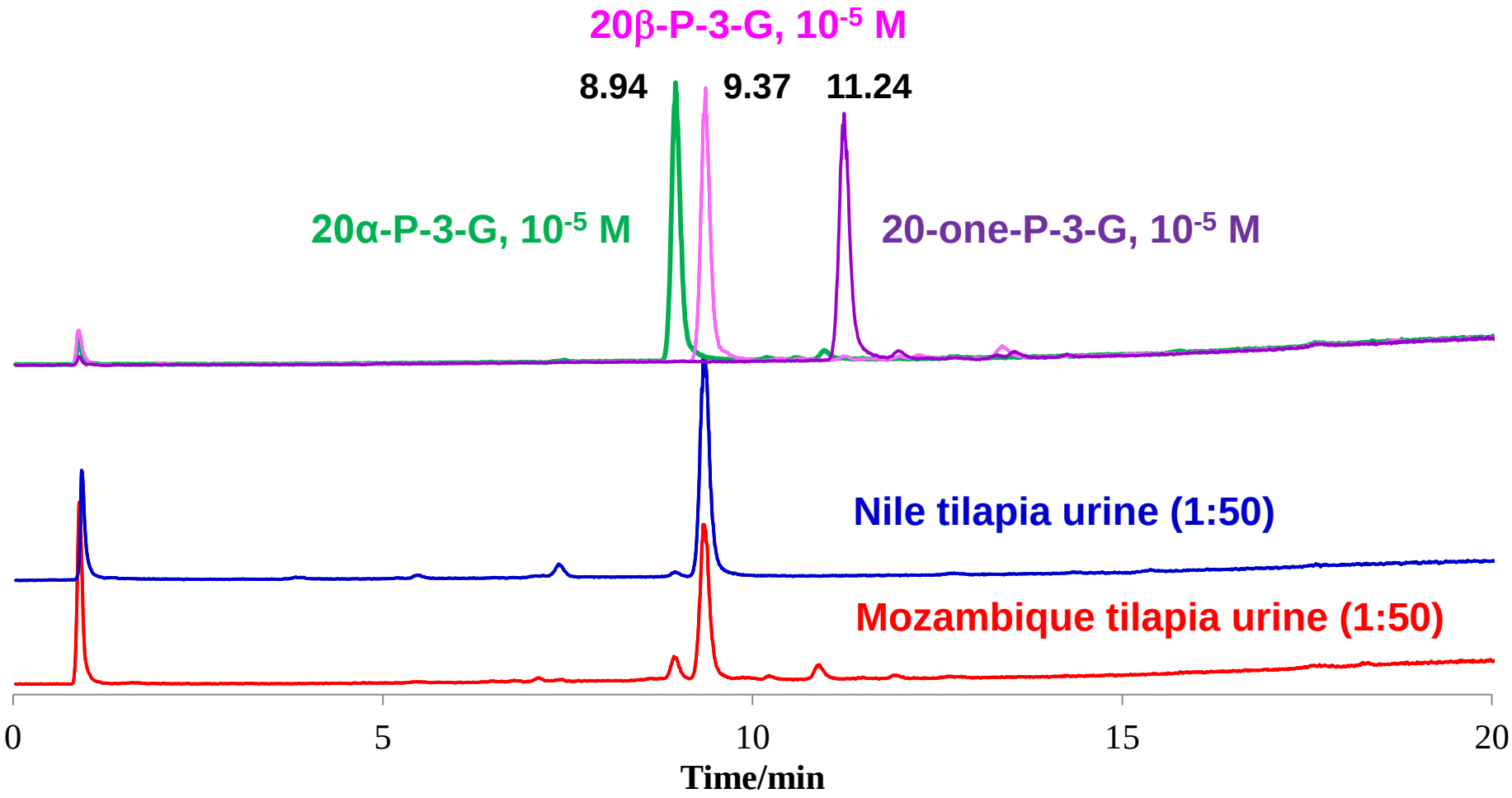
20β-P-3-G, 10-5 M
8.94
9.37
11.24
20α-P-3-G, 10-5 M
20-one-P-3-G, 10-5 M
Nile tilapia urine (1:50)
Mozambique tilapia urine (1:50)
0
5
10
15
20
Time/min

Figure 5

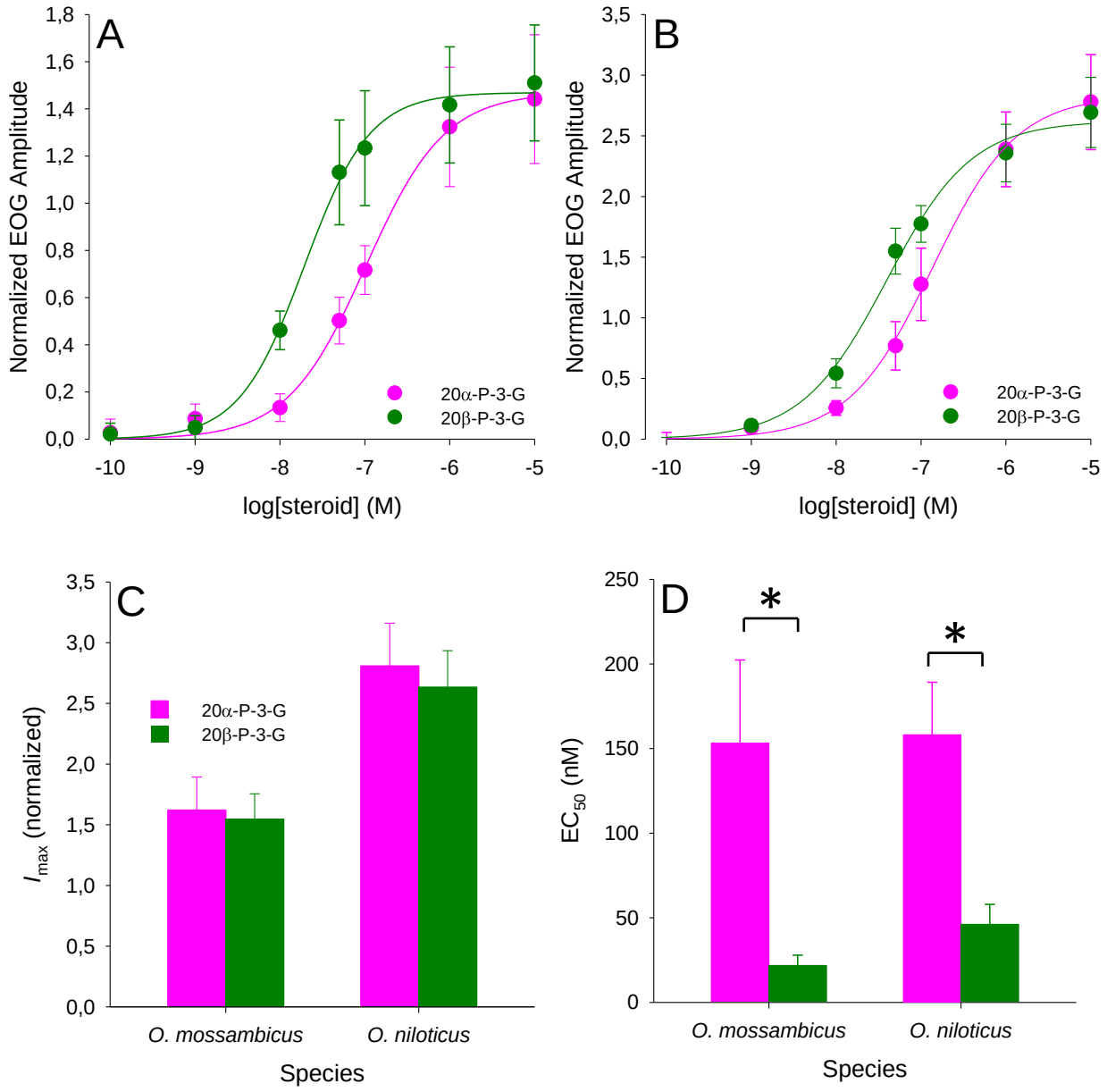

Figure 5.

Figure 6

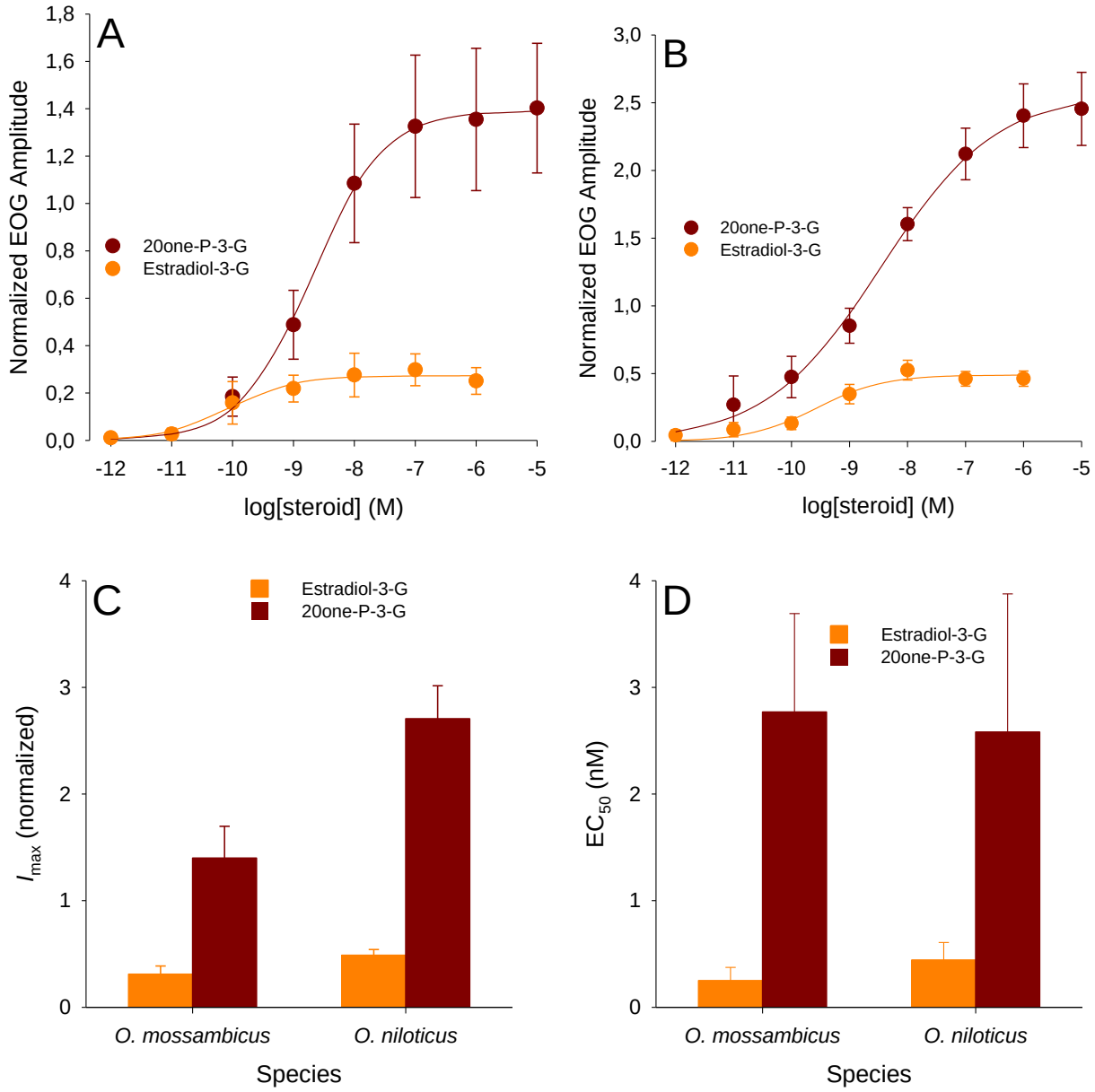

Figure 6.