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Electrochemical sensing of hormonal serotonin levels in crayfish

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ABSTRACT

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been implicated as a key biomarker that regulates a range of neurobehavioral functions. 5-HT also plays a prominent role in modulating social behavior across species. Here, we report the successful differentiation of hormonal (circulatory) 5-HT concentrations in socially isolated and communal crayfish samples by a surface-modified carbon fiber microelectrode electrochemical sensor. Monitoring 5-HT concentrations across social conditions provided important insights into mechanisms linking isolation to neurohormones that guide behavior. Utilizing surface coatings and electrochemical etching, our sensor can detect endogenous, nanomolar concentrations of 5-HT *in vitro* from crayfish hemolymph (i.e., blood) collected from communally housed animals and those isolated for one or seven days. Our measurements revealed that crayfish responses to isolation varied by species: *Procambarus* crayfish isolated for seven days exhibited a major increase in 5-HT compared to communally housed or short-term isolated conspecifics, whereas for *Faxonius* crayfish, the hormonal concentrations of 5-HT peaked after one day of isolation but paralleled the levels of communally housed crayfish after one week of isolation. These findings underscore the complex nature of neurochemical pathways in response to social conditions, demonstrate the sensor's utility for studying 5-HT dynamics, and provide a method for monitoring temporal changes in hormonal 5-HT following social isolation.

1. Introduction

Social isolation is increasingly recognized as a major risk factor for poor physical and mental health outcomes across many species (Beller and Wagner, 2018; Cacioppo and Hawkley, 2003; Holt-Lunstad and Steptoe, 2022; Kuriwada, 2016), comparable to smoking, obesity, or high blood pressure. Subjective ("perceived loneliness") and objective social isolation in our society have been connected to suicides (Mumtaz et al., 2018), drug overdose (Christie, 2021; Hosseinbor et al., 2014), and an increased incidence of mood disorders (Calati et al., 2019; Hall-Lande et al., 2007). The negative effects of social isolation are seen across a wide range of animals, from invertebrates (Li et al., 2021; Vora et al., 2022) to humans. The pervasive detrimental effects of social isolation suggest the presence of conversed mechanisms through which isolation disrupts neurophysiology and behavior.

Specific physiological biomarkers, like the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) have been well established as key behavioral modulators. 5-HT is critical to neurological physiology, influencing mood (Jenkins et al., 2016; Margolis et al., 2021), cognition (Schmitt et al., 2006; Smith et al., 2023), and pain perception (Lucki, 1998). Further, 5-HT plays a crucial role in the enteric nervous system (ENS) as a growth factor (Gershon, 2013), modulates immune responses (Grondin and Khan, 2024; Park et al., 2025), and stimulates the vagus nerve for signal transmission (Hwang and Oh, 2025; Sharifa et al., 2023). The role of 5-HT in social behaviors is well established across species (Garofalo et al., 2025; Giannaccini et al., 2013; Maurer-Spurej,

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2005; Zhuang et al., 2018). Among them, crayfish have been a suitable model for examining serotonergic regulation of neurobehavioral function related to social dominance, aggression, predator avoidance, and other behaviors (Fossat et al., 2014; Huber et al., 2001; Panksepp et al., 2003; Tierney et al., 2004). Crayfish are aquatic invertebrates with a reduced nervous system of tractable complexity (Herberholz, 2022). All 5-HT neurons in the crayfish nervous system have been individually identified, and the release mechanism from neurosecretory cells into the bloodstream is well understood (Herberholz, 2022; Real and Czternasty, 1990; Spitzer et al., 2005). Behaviors and identified underlying neural circuitry in crayfish share important similarities with organisms of higher complexity, including much of the relevant neurochemistry, making crayfish a useful model for studying 5-HT and behavioral interactions. Changes in hemolymph 5-HT concentrations can strongly modify crayfish behavior. For example, anxiety-like behavior in crayfish is controlled by 5-HT, and hormonal 5-HT injection can promote it (Fossat et al., 2015), underscoring the need to clarify the link between hormonal 5-HT signaling and behavior.

Despite interest in this topic, the mechanisms linking isolation, 5-HT, and behavior remain elusive. This is, in part, due to the complexity and interactions between social environments and neuromodulatory systems, as well as the lack of tools to quantify biomarker dynamics. Monitoring of neurochemicals, such as 5-HT and dopamine (DA), is commonly achieved with electrochemical methods (Amatatongchai et al., 2019; Bertrand et al., 2008; Introna et al., 2014; Su et al., 2012; Zhan et al., 2024; Zhou et al., 2023), which enable real-time measurements. Cyclic voltammetry (CV) provides molecular selectivity by measuring oxidation at characteristic potentials; the resultant current is proportional to the amount of 5-HT oxidized on the sensor interface.

Nanomaterials, including carbon-based (Ahmad et al., 2025; Chang et al., 2021; Raphey et al., 2019; Wu et al., 2022), and metallic nanoparticle (NP) frameworks (Zhan et al., 2024), have been utilized to improve the detection of 5-HT. Carbon nanotubes (CNTs) enhance 5-HT detection through their graphitic structure, facilitating fast electron transfer at the electrode interface, which improves sensitivity and measurement quality. Sensor selectivity to 5-HT has been demonstrated using polymers such as Nafion (Chang et al., 2021; Tsai et al., 2004), which is a highly stable, negatively charged, ion-exchange membrane that preferentially attracts cationic molecules like 5-HT. Together, these surface modifications improve the sensitivity and selectivity to 5-HT.

Despite surface modifications, long-term sensing of endogenous 5-HT in biological samples remains a challenge in part due to a mismatch between detection sensitivity and endogenous concentrations, limited sample sizes, and a lack of miniaturization of these sensing platforms. Previously, we developed a miniaturized platform for *in vivo* detection of 5-HT and DA in crayfish hemolymph (Han et al., 2024). Utilizing a surface-modified carbon fiber microelectrode (CFME) and miniaturized potentiostat electronics, we were able to investigate 5-HT and DA dynamics in freely-behaving crayfish; however, sensor limitations required prior injection of 5-HT and DA into the crayfish circulatory system before measurements.

The current work reports an improved response from the CFME-based sensor, enabling the detection of endogenous 5-HT *in vitro*. Moreover, successful detection of nanomolar concentrations of 5-HT from *in vitro* hemolymph samples reveals differences between 5-HT concentrations in socially isolated and communally housed crayfish, supporting a clear relationship between social isolation and increased 5-HT concentrations, and establishing a method for uncovering the relationship between social environment and hormonal 5-HT levels.

2. Materials and methods

2.1. Animal protocols, isolation, and sample preparation

Eighty-three sexually mature male (N = 46) and female (N = 37) crayfish from two different genera (Procambarus and Faxonius) were obtained from commercial suppliers and housed separately in large, communal tanks consisting of three or more crayfish. Species in the genus Procambarus (N = 39, with 60 % being males) only included *P. clarkii*, whereas species in the genus Faxonius (N = 44, with 63 % being males) included both F. virilis and F. immunis. Communally housed animals (COMs) were fed twice weekly on Mondays and Thursdays with approximately two shrimp pellets (Formula One Pellets, Ocean Nutrition). COMs (N = 28) were directly obtained from communal tanks before the experiment. Outlined in Fig. 1a, isolated crayfish (ISOs) were placed in 20(L) × 12(W) × 14(H) cm tanks filled with distilled water for one day (N = 23) or seven days (N = 32). 7-Day ISOs were first tested under unfed conditions and subsequently under fed conditions to determine whether feeding had any effect. The fed crayfish (N = 16) were fed two shrimp pellets every day for seven days. The unfed crayfish (N = 18) were not provided food during the period of isolation.

After isolation or communal housing, crayfish were placed on ice for 15 min for anesthesia. 200 μL of hemolymph was then drawn using a 1 mL syringe with a 26 AWG needle. The needle was inserted ½ of its length between the third and fourth walking legs to extract >200 μL of hemolymph from the hemocoel, the hemolymph-containing body cavity, of which 200 μL was micropipetted and mixed with 200 μL of anticoagulant (sodium citrate (0.11 M) and sodium chloride (0.1 M)) in a 1.5 mL microcentrifuge tube. The mixture was then centrifuged for 15 min at 10,000 RPM at 4 °C and refrigerated (−4 °C) before measurements.

2.2. Description of sensor fabrication improvement

The three-electrode sensor for measuring 5-HT is comprised of a carbon fiber microelectrode (CFME) working electrode, Pt counter electrode (CE), and Ag/AgCl reference electrode (RE) (Fig. 1b). The fabrication of the sensor has been previously reported (Han et al., 2023, 2024; Overton et al., 2024). Briefly, the CFME (⌀ = 100 μm, L = 5 mm) was made from T-650 carbon fiber bundles (Solvay, Belgium) and electrically connected to copper wire (30 AWG) using conductive paint (Bare Conductive) inside a glass capillary and secured using biocompatible epoxy (Loctite). The CFME was then modified to improve sensitivity and selectivity by dip-coating in a 0.5 % w/v carbon nanotube/Nafion (Chemours Company) solution for 2 min and electrochemically etched using CV in phosphate buffer solution (1xPBS), scanning from −0.1 to 2.5 V at a scan rate of 100 mV/s.

Previously, *in vivo* measurements utilized an EC/CNT-Nafion CFME (Han et al., 2024). However, to match the electrochemical sensor's detection sensitivity, crayfish were injected with 5-HT. Here, we optimized the surface modification to improve signal-to-noise and sensitivity for *in vitro* 5-HT detection. The CFME was dip-coated in a CNT/Nafion dispersion, then electrochemically etched (CNT-Nafion/EC). While the limit of detection is consistent with the previous fabrication (60 nM (Han et al., 2024)), the signal-to-noise ratio improved 2.8x, enabling successful measurements of endogenous 5-HT *in vitro*.

2.3. CNT-nafion/EC CFME calibration

All sensors used for *in vitro* measurements were normalized to correct for inter-sensor variability, enabling the quantitative comparison of responses across groups and the calculation of absolute concentrations. To normalize sensors, current (Ipa) responses to known, nanomolar (100 nM–1000 nM) concentrations of 5-HT in 1xPBS were measured by CV response with a benchtop potentiostat (VSP-300, Biologic) to determine the signal response to 5-HT in a three-electrode sensor with Pt CE and Ag/AgCl RE. CV was performed from −0.1 to 0.6 V at a scan rate of 100 mV/s. The average Ipa responses (n = 3) were fitted to a linear regression model to generate individual calibration curves, which were subsequently applied to determine absolute concentrations of 5-HT

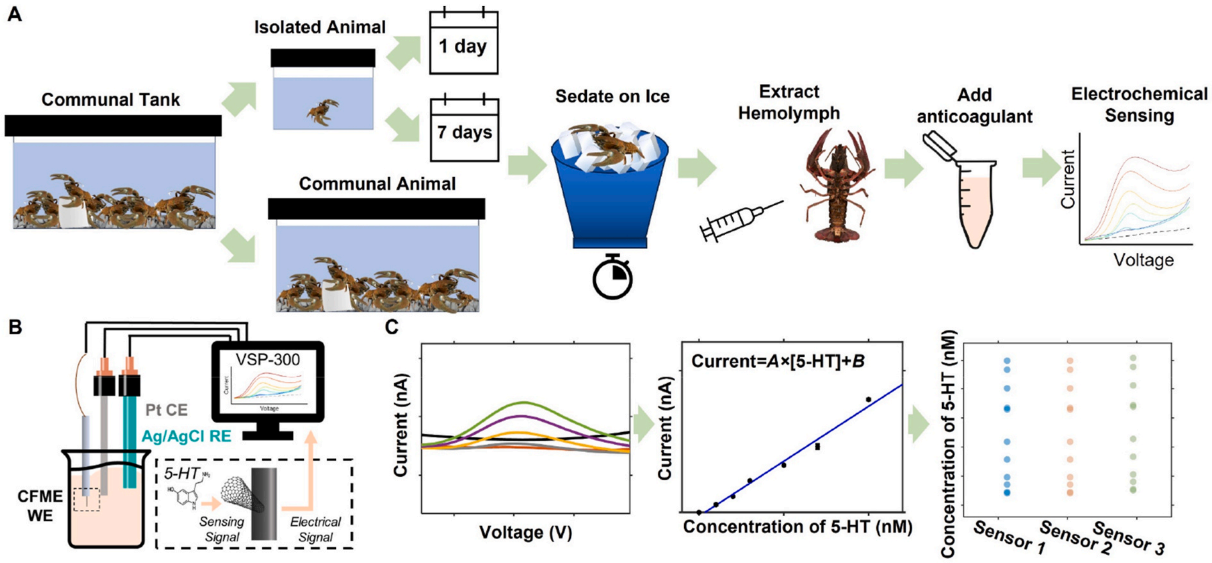

Fig. 1. (A) Overview schematic of the social isolation methodology and subsequent hemolymph sampling and measurements. *P. clarkii* and *F. spp* species crayfish were communally housed except select animals, isolated for one or seven days. Electrochemical measurements to detect 5-HT in extracted hemolymph of crayfish were performed. (B) Illustrative schematic of the electrochemical sensing experiments using a benchtop potentiostat. 5-HT is measured using a CFME modified with CNTs and Nafion dispersion to improve transduction. (C) Representative data and calibration curve illustrating the normalization of raw sensor responses to absolute concentration of 5-HT, enabling cross-sensor comparison.

(Fig. 1c). Calibration data were low-pass filtered and analyzed in using EC-Lab (Biologic).

Calibration performances yield a strong linear relationship between concentrations and Ipa responses. Experimental measurements in 1xPBS with known concentrations of 5-HT were fit to the linear regression model. Calculated concentrations were compared with known concentrations, demonstrating consistent agreement (12.5 % error, average across 10 sensors) between measured and expected values. Reproducibility of measurements was validated using an undiluted hemolymph sample with three sensors, including a 20-min accumulation test, confirming the linear regression model and data normalization, resulting in a coefficient of variation of 8.8 % (Table S1).

2.4. *In vitro electrochemical measurements*

5-HT measurements in crayfish hemolymph were performed on the benchtop using CV. Calibrated regression models were used to convert Ipas from hemolymph samples to concentrations of 5-HT.

In vitro measurements were performed in undiluted crayfish hemolymph (pH = 7.8), while calibration was performed in 1xPBS (pH = 6.8). Comparable baseline current responses indicated similar ionic properties, supporting the validity of calibration across media (Fig. S1), and a one-unit pH difference across media is consistent with insignificant variation in oxidation response. Further, the CNT-Nafion/EC CFME response to 5-HT was assessed in a hemolymph sample and subsequently spiked with known concentrations of 5-HT diluted in 1xPBS. The change in Ipa matched the expected response, supporting the high likelihood that the recorded *in vitro* responses correspond to 5-HT and further validating cross-media calibration (Table S2). Finally, previous results demonstrated the capability to co-detect DA, another neurohormone with a similar oxidation potential (Han et al., 2024). To confirm the improved CNT-Nafion/EC CFME could also co-detect DA, rather than DA interfering with 5-HT measurements, 500 nM 5-HT was spiked with 1 μM DA (Fig. S2). The resultant two-peak waveform verifies that DA did not interfere with 5-HT detection.

The oxidation potential shifts slightly from 0.38 V to 0.29 V between measurements in 1xPBS and hemolymph (Fig. S1). Hemolymph spiked with known concentrations of 5-HT also showed this potential shift, supporting that it is likely due to the presence of sodium citrate in the hemolymph anticoagulant, which is known to chelate with Ag+, shifting the equilibrium potential of the Ag/AgCl RE.

2.5. *Statistical methods*

All statistical analyses were performed using IBM SPSS (IBM Corp.). Ipa data is represented as the mean of measurements from a sample ($n = 3$). Samples where no 5-HT was detected (Ipa = 0 μA) were excluded from statistical analysis. Social groups data are presented as box-and-whisker plots, where the box denotes the interquartile range (IQR; 25th–75th percentile), the central line indicates the median, and the whiskers indicate the minimum and maximum values excluding outliers (>1.5 × IQR) that are shown as individual points. Statistical comparisons among social groups and species were completed using a one-way analysis of variance (ANOVA). Differences between species were analyzed using non-parametric Kruskal-Wallis tests, followed by Mann-Whitney U tests for pair-wise comparison. Single-variable cross-species groups were evaluated using Mann-Whitney U tests. Statistical significance was defined at $p < 0.05$.

3. Results and discussion

3.1. *Modulation of hormonal 5-HT by social isolation*

To investigate how social isolation affects serotonergic mechanisms, we utilized our CNT-Nafion/EC CFME to measure hormonal 5-HT in hemolymph from isolated and communally housed crayfish (Fig. 1a and b). Voltammetry results show a clear oxidation peak at 0.29 V (Fig. 2c and d), demonstrating successful detection of un-spiked, hormonal 5-HT with a CNT-Nafion/EC CFME sensor. Importantly, these findings indicate that hormonal 5-HT levels change in response to social isolation, and these changes are species-dependent. Endogenous 5-HT levels below the sensor's detection limit, indicated by an Ipa of 0 μA, were recorded as 0 nM 5-HT. This does not mean 5-HT is absent in the hemolymph, only that its concentration is below the sensor's detection threshold. In *P. clarkii*, 42 % (N = 17, 7 excluded) from COMs and 28 % (N = 7, 2 excluded) from 1-day ISOs were below the limit. In *F. spp*, 10 % (N = 11, 1 excluded) from COMs were below the limit. ANOVA revealed no significant differences among the species tested ($F = 0.792$, $p =$

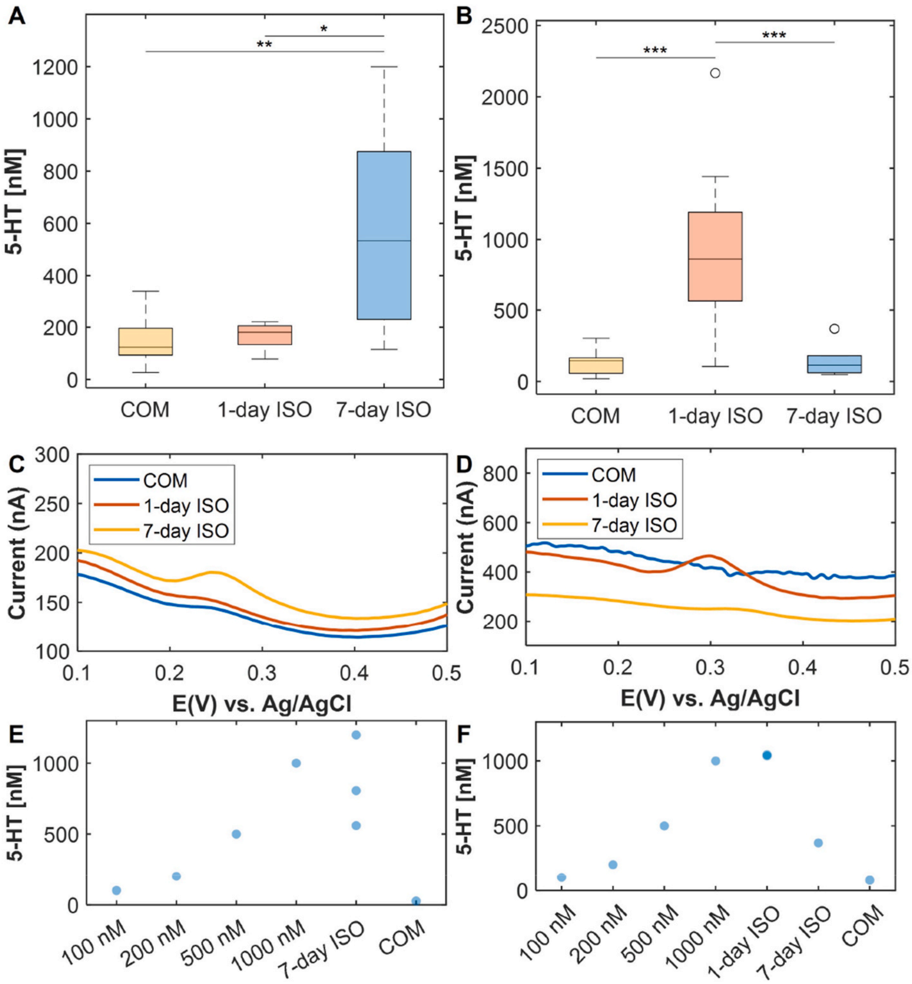

Fig. 2. Effects of social conditions on hormonal 5-HT. Group data are represented as box plots (median, interquartile range, and full data range). Statistical comparisons were performed using one-way ANOVA, with Mann–Whitney U tests used for post hoc pairwise comparisons. Significant differences are indicated by asterisks ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***). (A) *P. clarkii* crayfish across social isolation conditions (COMs N = 10, 1-day ISOs N = 5, 7-day ISOs N = 9). (B) *F. spp* crayfish across social isolation conditions (COMs N = 10, 1-day ISOs N = 16, 7-day ISOs N = 9). (C) CV response to *P. clarkii* after seven days of isolation compared to communally housed. Current peak at 0.3 V corresponds to oxidation of 5-HT. (D) CV response to *F. spp* after one and seven days of isolation compared to communally housed. Scatter plot comparing calibration-derived concentrations (100–1000 nM) to experimentally measured absolute concentrations in *P. clarkii* (E) and *F. spp* (F) across social conditions.

0.378), whereas social isolation had a significant main effect (F = 5.716, p = 0.006). Importantly, there was a significant interaction between species and social isolation (F = 53.957, $p < 0.001$). To assess potential effects of sex and weight on 5-HT concentrations, Mann-Whitney U tests were performed. Weight (p = 0.302) and 5-HT (p = 0.095) were non-significant between males and females, indicating that the differences observed in 5-HT concentrations were related to social isolation. Kruskal-Wallis tests were performed to confirm differences between social conditions in *P. Clarkii* (p = 0.006) and *F. Spp* ($p < 0.001$).

Mann-Whitney tests in *P. clarkii* showed that 7-day ISOs (N = 9) had significantly higher hormonal 5-HT than COM (N = 10) (p = 0.003), while 1-day ISOs (N = 5) had 5-HT concentrations matching COMs (p = 0.297) (Fig. 2a). On the other hand, in *F. spp.*, 1-day ISOs (N = 16) had significantly higher hormonal 5-HT than COMs (N = 10) (p = 0.001), and after seven days of isolation, 5-HT concentrations had decreased to levels matching COMs (p = 0.806, 7-day ISOs N = 9) (Fig. 2b).

P. clarkii crayfish isolated for seven days contained an average of 581.2 nM 5-HT in their hemolymph, a 307.6 % increase in endogenous 5-HT compared to communally housed crayfish of the same species (Fig. 2a–c,e). The difference between the social groups is likely underestimated due to the exclusion of 0 nM samples from the *P. clarkii* communal group. The excluded samples represent very small 5-HT concentrations, which, if detected, would have further reduced the COMs mean. *P. clarkii* 1-day ISOs did not have significantly higher concentrations compared to COMs (p = 0.297, 1-day ISOs vs COM). However, the increase between 1-day ISOs and 7-day ISOs suggests that *P. clarkii* may have a longer onset to changes in hormonal 5-HT when socially isolated. By comparison, *F. spp* 5-HT concentrations in 1-day ISOs were 867.5 nM, a 540.1 % increase in hormonal 5-HT concentrations compared to COMs (with a single 0 nM sample). Interestingly, the concentrations returned to communal baseline values by day seven of isolation, suggesting a possible adaptation in *F. spp* after longer periods of isolation. This differs from *P. clarkii,* which expressed the highest 5-HT levels after seven days of isolation. We hypothesize that these species-specific responses to social isolation are the result of the different social and physiological environments (see below). The dependence on isolation of this species-specific response is confirmed by the lack of significant differences (p = 0.9698) in 5-HT in communally housed crayfish from both genera (Fig. 3a).

P. clarkii are typically found in habitats of standing water. They aggregate in large, dense groups and are considered an aggressive (and invasive) species. *P. clarkii* frequently interact in a robust dominance hierarchy (Graham and Herberholz, 2009; Hemsworth et al., 2007; Herberholz et al., 2016). In contrast, species of the genus *F. spp* (formerly

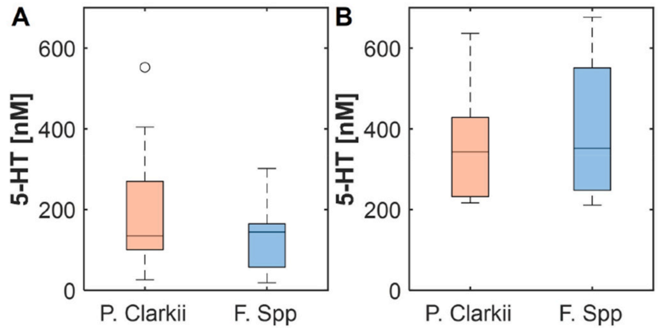

Fig. 3. (A) Boxplots comparing the 5-HT concentrations in communally housed crayfish across species, showing median and interquartile range. Mann–Whitney *U* test shows no significant difference (p = 0.489). (B) Boxplots comparing fed groups for crayfish isolated for seven days in *P. clarkii* (fed N = 6) and *F. spp* (fed N = 8). Mann–Whitney *U* test shows no significant difference across feeding conditions (p = 0.7963).

Orconectes), which live in habitats with flowing water, such as streams and rivers, are more commonly found as single individuals and restrict social contacts to mating or territorial disputes (Berrill and Chenoweth, 1982; Herrmann and Martens, 2024; Larson et al., 2019), and are less aggressive, although this depends on the species (Capelli and Munjal, 1982; Chucholl and Chucholl, 2021). Since *F. spp* crayfish might be more adapted to a solitary lifestyle, removing them from a communally housed environment to an isolated environment may only elicit a brief elevation in hormonal 5-HT due to novelty, which they quickly adapt to, as demonstrated in the quick increase and later decrease of hormonal 5-HT concentrations. However, this remains largely speculative and requires further investigation.

Our results demonstrate that the temporal dynamics of hormonal 5-HT vary largely between crayfish species, and these dynamics are related to the social environment. Varying effects on social interactions in crayfish by 5-HT injections have been reported across species; for example, in *Astacus*, acute hemolymph infusion escalated fighting encounters and altered outcomes (Huber et al., 1997); whereas, in *P. clarkii*, acute 5-HT injections reduced aggression (Tierney et al., 2004). Interestingly, a lower behavioral sensitivity to 5-HT via systemic injections was reported for *F. spp* (i.e., *Orconectes rusticus*) when compared to *P. clarkii*, and acute exposure to fluoxetine, a 5-HT reuptake inhibitor, caused an increase in winning of encounters for male *F. spp* (i.e., *Orconectes virilis*) but not for male *P. clarkii* (Rigo, 2019). 5-HT effects are also modulated by social status, with neurons from isolated individuals responding differently to hormonal 5-HT than those from dominant or subordinate animals (Yeh et al., 1996). Finally, 5-HT also plays a key role in the crayfish stress axis. 5-HT injections into the hemolymph can cause anxiety-like behavior in crayfish (Fossat et al., 2014), and 5-HT stimulates the release of Crustacean Hyperglycemic Hormone, which is converted to glucose, a stress biomarker in crayfish (Prymaczok et al., 2016; Soares et al., 2022). Taken together, it seems possible that the two species in our study responded differently to isolation stress, indicated by their temporal differences in elevated 5-HT levels in the hemolymph.

Past studies across species (e.g., honeybees, flies) using fast-scan cyclic voltammetry (FSCV) have reported concentrations of DA, in the hundreds of nM range, similar to the 5-HT concentrations measured in our COMs (Jarriault et al., 2018; Majdi et al., 2018; Shin and Venton, 2022). Similarly, a previous study in flies using FSCV reported evoked 5-HT concentrations in the hundreds of nM range (Borue et al., 2009). On the other hand, previous crustacean literature using HPLC as the analytical tool have reported lower endogenous concentration of 5-HT than those observed in our study (Livingstone et al., 1981; Tinikul et al., 2008; Weineck et al., 2018). Reversed-phase LC-MS/MS, however, has detected endogenous 5-HT in the nervous tissue of rock crabs at concentrations similar to our study (Cao et al., 2018).

3.2. *Effect of feeding on hormonal 5-HT*

Crayfish isolated for seven days were originally unfed during the isolation period. To explore whether feeding played a role in regulating hormonal 5-HT concentrations, we isolated *P. clarkii* and *F. spp* crayfish and fed them for seven days (one feeding per day). Fed *P. clarkii* (N = 6) and fed *F. spp* (N = 8) revealed no significant difference in 5-HT concentrations between the species (p = 0.852) (Fig. 3b). Interestingly, when comparing 7-day ISOs fed and unfed *P. clarkii,* there was no significant difference (p = 0.219) in hormonal 5-HT concentrations, whereas in *F. spp*, we found a significant difference (p = 0.003) with fed crayfish having higher 5-HT concentrations than the initial unfed 7-day ISOs. We currently can’t explain this difference. It is possible that tryptophan, a 5-HT precursor, which was included at low levels in the shrimp pellets we used as food, was converted into 5-HT. Maybe this effect was only observed in *F. spp* because it was masked by the isolation-induced elevated 5-HT levels in 7-day unfed *P. clarkii*. These results may necessitate the inclusion of additional physiological biomarkers of stress (e.g., glucose (Caldari-Torres et al., 2018; Soares et al., 2022) using a larger sample size.

4. Conclusion

This research reports on the successful detection of hormonal 5-HT concentrations in crayfish by a surface-modified CFME. Changes to the surface-modification method enabled investigation of the relationship between social isolation and 5-HT, where we measured elevations in hormonal 5-HT in the hemolymph of both *P. clarkii* and *F. spp* crayfish following 1-day and 7-day isolation periods. The refined fabrication methodology of the Nafion-CNT/EC CFME detected <100 nM concentrations of 5-HT, and, in the future, we are confident this sensor can be integrated into a miniaturized system (Han et al., 2024) for real-time *in vivo* monitoring of hormonal 5-HT dynamics. Though the small sample size represents a limitation of this study, robust and statistically significant results strongly suggest an underlying effect of social isolation on hormonal 5-HT. These finding provides an exciting avenue for future studies, including those providing higher temporal fidelity on 5-HT concentration changes that modulate neural activities and corresponding behaviors.

CRediT authorship contribution statement

Sydney N. Overton: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Kanishka Balamurugan:** Writing – review & editing, Writing – original draft, Validation, Investigation, Data curation, Conceptualization. **Jens Herberholz:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Reza Ghodssi:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biosx.2025.100732.

Data availability

Data used in this study are publicly available from
Dryad
Electrochemical sensing of hormonal serotonin levels in Crayfish (Original data) Electrochemical sensing of hormonal serotonin levels in Crayfish (Original data)

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