Comparative Biochemistry and Physiology, Part A 258 (2021) 110980

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

The role of uptake and degradation in the regulation of peripheral serotonin dynamics in Gulf toadfish, *Opsanus beta*

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ARTICLE INFO

Keywords:
5-hydroxytryptamine
MAO
Monoamine oxidase
Clearance
Metabolism
SERT
Transporter
Excretion
Plasma
Homeostasis

ABSTRACT

The neurotransmitter serotonin (5-hyroxytryptamine, 5-HT) is involved in a variety of peripheral processes. Arguably most notable is its role as a circulating vasoconstrictor in the plasma of vertebrates. Plasma 5-HT is maintained at constant levels under normal conditions through the processes of cellular uptake, degradation, and excretion, known collectively as clearance. However, the degree to which each individual component of clearance contributes to this whole animal response remains poorly understood. The goal of this experiment was to determine the extent to which transporter-mediated uptake and intracellular degradation contribute to 5-HT clearance in the model teleost Gulf toadfish (*Opsanus beta*). Fish that were treated with the 5-HT transport inhibitors fluoxetine, buproprion, and decynium-22 had 1.47-fold higher plasma 5-HT concentrations and a 40% decrease in clearance rate compared to control fish. In contrast, fish treated with the MAO inhibitor clorgyline had a 1.54-fold increase in plasma 5-HT with no change in clearance rate. The results show that transporter-mediated 5-HT uptake plays an important role in controlling circulating 5-HT and whole body 5-HT homeostasis.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an ancient and ubiquitous signaling molecule used in many metabolic pathways. Of particular importance is circulating 5-HT found in the plasma, which has been shown to be a potent vasoconstrictor and, to a lesser extent, can act as a vasodilator (Rapport et al., 1948a; Rapport et al., 1948b; Rapport, 1949; Rapport et al., 1949; Fritsche et al., 1992; Burleson and Milsom, 1995; Sundin et al., 1995; Marcos et al., 2004; Pelster and Schwerte, 2012; Watts et al., 2012; El-Merahbi et al., 2015). For these reasons, plasma 5-HT levels must be tightly regulated. Indeed, both fish and mammals maintain constant plasma 5-HT concentrations of 10^{-9}–10^{-8} M (Maurer-Spurej, 2005). Mammals accomplish this in part by removing excess 5-HT from the extracellular pool and storing it within platelets that are rich with the 5-HT transporter (SERT, SLC6A4), allowing for uptake and removal of 5-HT from the whole blood. In contrast, teleost fish do not have platelets but instead have their evolutionary precursors, thrombocytes, which are believed not to store 5-HT (Fánge, 1992). As a result, most if not all circulating 5-HT in fish is believed to be found in the extracellular pool (Maurer-Spurej, 2005; Caamaño-Tubío et al., 2007; Amador and McDonald, 2018b). As uncontrolled vasoconstriction in response to circulating 5-HT may be problematic to fish, especially during low oxygen (hypoxia) exposure, and SERT function, which could mediate 5-HT uptake from the blood, has been shown to be necessary for the typical cardiovascular response to hypoxia in teleost fish (Panlilio et al., 2016), understanding how blood 5-HT concentrations are controlled may be important for understanding key cardiorespiratory reflexes associated with environmental stress.

To address this, (Amador and McDonald, 2018b) found high SERT mRNA expression and 5-HT uptake in several tissues including the heart and gill of Gulf toadfish (*Opsanus beta*) suggesting that teleost cardiorespiratory organs may remove 5-HT from the extracellular pool similar to mammalian platelets. Despite these tissues showing high SERT mRNA expression, treatment with the SERT inhibitor fluoxetine alone did not reduce uptake (Amador and McDonald, 2018b). Instead, uptake was only reduced when fish were treated with a combination of fluoxetine, buproprion (a norepinephrine [NET] and dopamine transporter [DAT] inhibitor), and decynium-22 (an organic cation and plasma membrane monoamine transporter inhibitor), suggesting that 5-HT enters cells through other promiscuous transporters in addition to SERT (reviewed by (Daws, 2009). While 5-HT uptake at a specific moment in time has been quantified for specific tissues (Amador and McDonald, 2018), a whole animal approach to understanding the role of uptake in 5-HT homeostasis over time has never been attempted.

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https://doi.org/10.1016/j.cbpa.2021.110980
Received 7 April 2021; Received in revised form 11 May 2021; Accepted 12 May 2021
Available online 21 May 2021

In addition to tissue or cellular uptake, 5-HT degradation also contributes to the homeostasis of circulating 5-HT. 5-HT degradation occurs by way of monoamine oxidase (MAO), a mitochondria-bound flavoenzyme which deaminates 5-HT into its metabolite 5-hydroxyindoleacetic acid (5-HIAA) and other compounds (Bortolato et al., 2010). Relatively high 5-HIAA has been measured in the urine of rainbow trout (*Oncorhynchus mykiss*) suggesting that urinary excretion of metabolites plays a major role due to highly efficient renal degradation (Caamaño-Tubío et al., 2007). In mammals, the pulmonary endothelium is the primary location of 5-HT degradation (Wiersma and Roth, 1980; Hart and Block, 1989), and fish gills are thought to have similar capabilities (Olson, 1998); however, the distribution and activity of MAO in teleost tissues varies between species (Hall and Uruena, 1982; Edwards et al., 1986). Distribution and activity of MAO in the Gulf toadfish has never been examined.

Both 5-HT and its metabolites are then excreted from the body *via* the urine, bile and intestinal fluid, or across the gill (Amador and McDonald, 2018; Caamaño-Tubío et al., 2007). 5-HT concentrations are higher in bile than in urine of Gulf toadfish, and 5-HT concentrations in both fluids surpass that of the plasma (Amador & McDonald, submitted), indicating that 5-HT can be actively excreted in the bile and urine at concentrations higher than found in the plasma. A split chamber experiment using [^{3}H]5-HT found that posterior [^{3}H] excretion (combined urinary and biliary excretion) was significantly higher than anterior [^{3}H] excretion in Gulf toadfish, and posterior excretion was reduced when fish were treated with fluoxetine, buproprion, and decynium-22 (Amador & McDonald, 2018). Combined, this suggests that renal and biliary excretion likely account for the majority of unmetabolized 5-HT excretion, with branchial excretion being a relatively minor component, and that posterior excretion of 5-HT is, in part, mediated by SERT and other transporters. 5-HT degradation is known to occur in the intestinal epithelium of mammals (Egashira and Waddell, 1984) and elevated 5-HIAA has been observed in the intestinal mucosa of rainbow trout (Caamaño-Tubío et al., 2007), however, to our knowledge concentrations of 5-HIAA in bile and urine have not been analyzed in teleost fish.

Collectively, uptake, degradation and excretion work together to clear circulating 5-HT from the blood and their combined roles can be quantified by measuring the metabolic clearance rate (MCR) of 5-HT, which is the volume of blood from which 5-HT is entirely cleared through metabolic processes in a given unit of time. The objective of this study was to understand how uptake, degradation, and excretion work together to regulate circulating 5-HT levels in teleosts by measuring the impact of transporter inhibitors and a degradation inhibitor on MCR. We hypothesize that SERT, other transporters, and MAO are playing a major role in 5-HT homeostasis, and that inhibiting transport of 5-HT into tissues or inhibiting intracellular 5-HT degradation will significantly reduce MCR.

2. Materials and methods

2.1. Experimental animals

Gulf toadfish (*Opsanus beta*) were caught as roller trawl bycatch by local shrimpers in Biscayne Bay, FL. Upon arrival, fish were exposed to freshwater for 15 min and returned to seawater. For three consecutive days upon arrival, fish were treated with final concentration 0.1 mg L^{-1} malachite green in 30 mg L^{-1} formalin to treat and prevent infection by ectoparasites. Two and a half weeks later, fish were treated with the same regimen (day one freshwater and malachite, days two and three malachite only). Afterwards, fish were treated every two and half weeks with one day of freshwater and malachite. Fish were housed in a 20-gal aquaria supplied with aerated, flow-through, filtered seawater taken from Biscayne Bay and were fed raw (previously frozen) shrimp weekly until satiation. Experiments were carried out between January 21, 2019 and February 13, 2020 and water temperatures in the lab during that time naturally varied throughout the year, ranging from 18 to 22 °C (average = 20.7 ± 1.3 °C) for fish in the low temperature group and 23 to 24.5 °C (average = 24.6 ± 0.8 °C) for fish in the high temperature group. Thus, fish used in the experiments were acclimated to a range of temperatures. All protocols were carried out with the approval of the University of Miami Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental procedures

Adult toadfish (0.085 ± 0.002 kg; 0.060 kg – 0.122 kg, $n = 50$) were anesthetized in 1 g·L^{-1} tricaine methanesulfonate (MS-222, Western Chemical, Ferndale, WA) buffered with $NaHCO_3$ (pH 8.2) for 10 min and then surgically implanted with a caudal vessel (arterial or venous) catheter (Intramedic PE 50 tubing; Becton Dickinson, Franklin Lakes, NJ) filled with heparinized saline (150 mM NaCl with 50 UI mL^{-1} sodium heparin; Sigma-Aldrich) and heat flare sealed as described previously (Wood et al., 1997). At the same time, fish were fitted with an intraperitoneal catheter (Intramedic PE 160 tubing; Becton Dickinson) filled with peanut oil and sealed with putty as previously described (Morando et al., 2009). Caudal catheters were secured to the fish with a sleeve of larger diameter tubing (Intramedic PE 160 tubing; Becton Dickinson) and sealed in place with Vetbond™ adhesive (3 M Animal Care, St. Paul, MN). Fish were allowed to recover at room temperature (21 °C held constant throughout the year) in individual isolated 1.5 L plastic chambers with aerated, flow-through, 21 °C seawater for 36–48 h before experimentation. After the 36–48 h recovery, water flow to the box was stopped and initial ($t = 0$ h) water (5 mL) and blood samples were taken. A 200 μL pre-sample was taken to remove blood that might have pooled in proximity to the catheter followed by the actual 100 μL blood sample. The blood was spun in a centrifuge (3 min at 14,000 g), the plasma separated from the red blood cell pellet and frozen in liquid N for later analysis. The red blood cell pellet was resuspended in 100 μL saline. The 200 μL pre-sample and the resuspended red blood cells were then re-injected to replace lost blood volume. Syringes were rinsed thoroughly with heparinized saline between each sampling. After the blood sample, the catheter was refilled with heparinized saline and then attached to a MINIPULS® 3 peristaltic pump (Gilson, Middleton, WI) fitted with 0.51 mm manifold pump tubing (Fisher Scientific, Pittsburgh, PA) and PE 50 tubing (Becton Dickinson) with a 23G needle connectors. The pump continuously delivered a solution of 0.32 μCi [^{3}H]5-HT creatine sulfate (40 Ci·$mmol^{-1}$; American Radiolabeled Chemicals, St. Louis, MO) per ml of 150 mM NaCl (Sigma-Aldrich) saline at a rate of 0.3 mL hr^{-1} that was thoroughly vortexed and aliquoted into individual plastic vials for each experimental fish. Subsequent blood samples were taken at $t = 3$, 6, 12, 24, 36, 48, 60, and 72 h with the fish being re-attached to the peristaltic pump after every sample.

At $t = 0$, 12, 24, 36, 48, 60, fish were also injected *via* the intraperitoneal catheter with either peanut oil alone (control; $n = 12$); fluoxetine (F), a SERT inhibitor, combined with buproprion (B), which inhibits dopamine (DAT) and norepinephrine transporters (NET), and decynium-22 (D, as 1,1′ = diethyl-2,2′-cyanine iodide, 97%), which inhibits organic cation (OCT) and plasma monoamine (PMAT) transporters (FBD-treated; $n = 13$); or clorgyline, a MAO inhibitor (MAOI-treated, n = 13). Stock solutions of F (100 μmol ml^{-1}); B (50 μmol ml^{-1}); D (0.6 μmol ml^{-1}); and MAOI (15 μmol ml^{-1}) in 100% ethanol were made, vortexed vigorously, and stored at −20 °C until needed. Working solutions of the drugs were made the night before the start of the experiment, Eppendorf tubes were filled with peanut oil and were overlaid with stock solutions. Tubes were placed open in the fume hood and left to evaporate overnight as described by Amador & McDonald (2018). In the morning, each tube was vortexed vigorously until all precipitate was suspended in peanut oil. Fish were then injected with either 400 μl peanut oil $^{-1}$ 100 g $fish^{-1}$ (control); 36.1 μmol F+ 10.4 μmol B + 22.0 μmol D 400 μl peanut oil $^{-1}$·100 g fish $^{-1}$ (FBD-treated); or 9.2 μmol MAOI·400 μl peanut oil $^{-1}$·100 g fish $^{-1}$ (MAOI –treated) using a 1 mL syringe fitted with an 18G needle.

After 24 h, another water sample was taken, and the fish chamber flushed with 21 °C seawater for 10 min so that nitrogenous wastes and radiolabeled chemicals could flush from the chamber. After 10 min, the flow was turned off again and an initial water sample taken to start the next 24 h flux period. Subsequent water samples were also taken at $t =$ 48 and 72 h to determine excretion of isotope into the water. After the final blood collection at 72 h, fish were euthanized in 3 g·L^{-1} MS-222. Sex and gonadosomatic index were not consistently recorded.

2.3. Analytical techniques and calculations

Plastic scintillation vials containing infusate (25 μl), plasma (25 μl), bile (25 μl), urine (25 μl) or water (5 ml) samples in UniverSol™-ES Liquid Scintillation Cocktail (MP Biomedicals) were analyzed using a LS 6500 liquid scintillation counter (Beckman Coulter, Fullerton, CA). Blood [^{3}H] steady state values for each fish were obtained by fitting plasma [^{3}H] counts at each time point to a Michaelis-Menten curve using PRISM software and using the Vmax value determined by the program (Graphpad v. 8, La Jolla, CA). Metabolic clearance rate (MCR; ml kg^1 h^{-1}) is defined as:

$$MCR = \frac{a}{b}$$

where a is the [^{3}H]-5-HT infusion rate (cpm kg^{-1} h^{-1}), calculated by multiplying the [^{3}H] 5-HT counts per minute (cpm) per ml of infusate by the flow of the peristaltic pump (ml kg^{-1} h^{-1}), and b is the average steady state (cpm ml) and is defined as the average of cpm·ml values taken at or after 36 h. [^{3}H] excretion rate (cpm kg^{-1} h^{-1}) was calculated as:

$$Excretion\ rate = \frac{cd}{ef}$$

where c is the counts in each chamber (final sample – initial sample; cpm·ml^{-1}), d is the volume of the chamber (ml), e is the weight of the fish (kg), and f is the incubation time (24 h). To determine average [^{3}H] excretion rate during steady state, only the [^{3}H] excretion rates measured at 48 h and 72 h were taken into account. Calculations were performed in Excel (Microsoft, Redmond, WA) and copied to Prism (Graphpad) for statistical analysis. Because of the variability in the data, treatment fish were normalized relative to the control from the week of their respective experiment. Control values were compared to the mean of all controls in their respective dataset.

2.4. Statistics

Statistics were preformed using Prism (Graphpad v.8). Data were checked for outliers using ROUT (Q = 1%), and the cleaned data sets were analyzed for normality. Normal data were analyzed using a one-way ANOVA with Tukey's multiple comparisons test (or Mann-Whitney U test) or unpaired Student's t-test. Non-normal data were log transformed and then analyzed with a one-way ANOVA.

3. Results

3.1. Series i: effects of environmental temperature on MCR

MCR values were highly variable within control fish (Fig. 1A). Average MCR for control toadfish acclimated to water temperatures in the holding tanks of 24.6 ± 0.8 °C were 1.7-fold greater than MCR measured for toadfish acclimated to lower water temperatures (20.7 ± 1.3 °C) in the holding tanks (Student's t-test; $p = 0.033$; Fig. 1A). Acclimation temperature did not have a significant effect on plasma 5-HT concentrations, although plasma concentrations tended to be 37.9% lower in fish acclimated to higher temperatures (Mann-Whitney U test, $p = 0.142$; Fig. 1B).

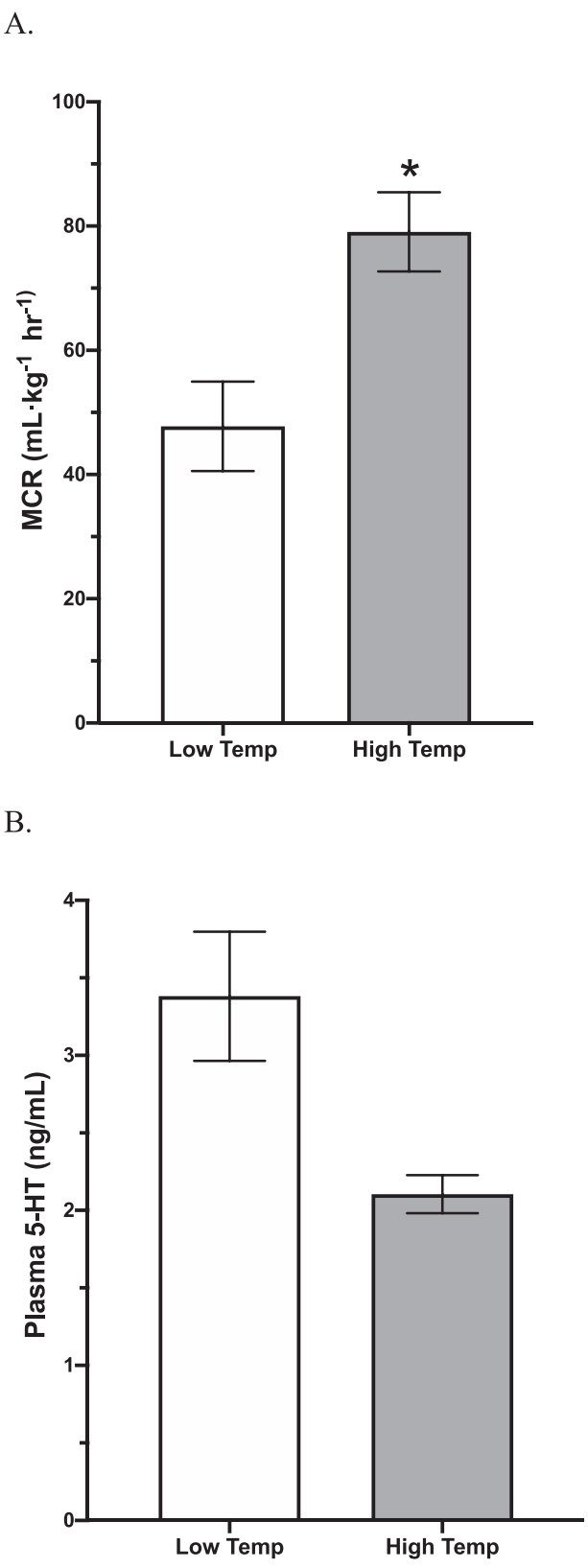

Fig. 1. A: MCR of control fish acclimated to low temperatures (20.7 ± 1.3 °C, n = 12) compared to fish acclimated to high temperature (24.6 ± 0.8 °C, n = 4). B: Average plasma 5-HT concentrations after 24 h of infusion for control fish acclimated to low temperatures compared to fish acclimated to high temperature. Data are means ± 1 SEM; *$p < 0.05$.

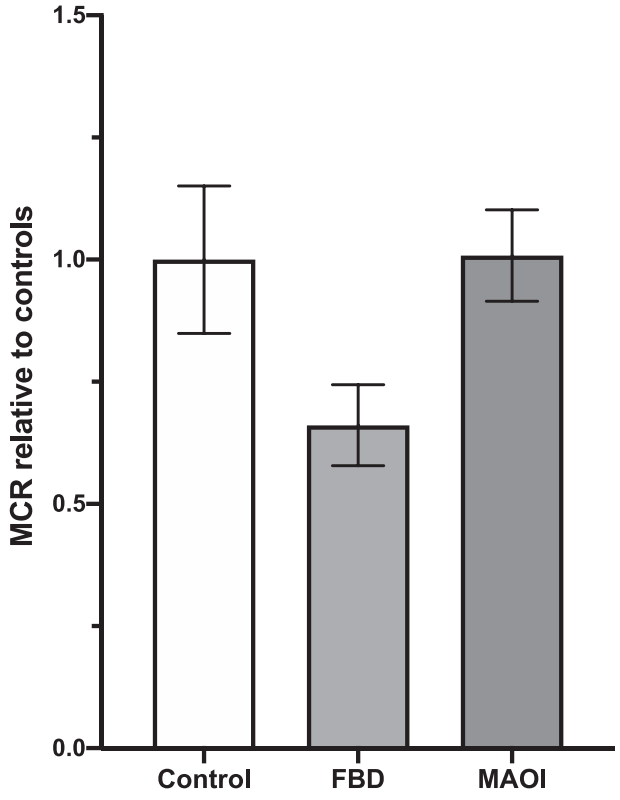

Fig. 2. MCR relative to controls (C, n = 12) in fish in which 5-HT transport is inhibited by fluoxetine, buproprion and decynium-22 (FBD, $n = 13$) or 5-HT degradation is inhibited by clorgyline (MAOI, n = 13). Data are means ± 1 SEM.

3.2. Series ii: effects of transport and degradation inhibitors on MCR

Relative MCR was 40% lower in toadfish when 5-HT transport into cells was inhibited compared to controls, though this was not statistically significant (one-way ANOVA; $p = 0.051$; Fig. 2, Table 1). When 5-HT degradation alone was inhibited, there was no difference compared to controls (Fig. 2, Table 1).

When transport or degradation were inhibited, plasma 5-HT concentrations increased by 48–55% in FBD- or MAOI-treated fish, respectively compared to controls (one-way ANOVA; $p = 0.018$; Fig. 3, Table 1). Drug treatment also had a significant effect on [^{3}H] excretion rate (one-way ANOVA; $p < 0.05$; Fig. 4, Table 1). Analysis of [^{3}H] excretion data showed that FBD-treated fish tended to have a 2.2-fold higher excretion rates relative to controls and had a significant 3.9-fold higher excretion rates compared to MAOI-treated fish (Fig. 4, Table 1). Conversely, fish treated with MAOI tended to have a lower excretion rate relative to controls (Fig. 4, Table 1).

4. Discussion

The objective of this study was to understand how uptake, degradation, and excretion work together to regulate circulating 5-HT levels in teleost fish. Consistent with other hormones, the average MCR of 5-HT in control fish is similar to MCR values measured growth hormone (Sakamoto et al., 1991) and within the observed control range of cortisol MCR reported in seven reported species (Sakamoto et al., 1991; Mommsen et al., 1999). However, 5-HT MCR is 25% lower than reported MCR for prolactin in coho salmon (*Oncorhynchus kitsuch*) (Sakamoto et al., 1991). We do have reason to believe that the MCR for 5-HT was underestimated in the present study (see below).

One interesting finding of the study was that, despite a 36 to 48-h acclimation period and the 72-h experiment during which all fish were held at 21 °C, the MCR in fish from high temperature holding tanks (24.6 ± 0.8 °C) was significantly higher compared to fish previously held at low temperatures (20.7 ± 1.3 °C). When fish acclimated to a higher temperature are then held at a lower temperature, typically the rate of their metabolic processes is lower than those of fish that were always held at the lower temperature and *vice versa*, due to compensatory processes that occur during acclimation (Haschemeyer, 1968). However, the opposite was observed in the present study. It is possible that fish removed from the high temperature 24.6 °C holding tanks were not held long enough at 21 °C for MCR to reach a new stable rate. The 5-HT MCR measured in the present study is a rate that encompasses several metabolic processes that would be hypothesized to be very sensitive to temperature, for example, 5-HT movement through SERT is dependent on Na^+ and Cl^- gradients established by primary active transporters. Furthermore, MAO activity is known to be temperature-dependent in goldfish brain and liver (Hall et al., 1982). Other physiological factors may have influenced the results obtained in this study. Data collection from some low temperature acclimated fish coincided with the Gulf toadfish breeding season, which occurs from late February to April. Cartolano et al. (2019) reported elevated plasma 5-HT in toadfish during breeding months, which coincides with an elevation of GSI (Cartolano et al., 2019). Given that 5-HT is known to play a role in both the physiological and behavioral components of teleost reproduction (Prasad et al., 2015), it would be reasonable to suggest that MCR of 5-HT may be changed during breeding season. Consistent with Cartolano et al. (2019), plasma 5-HT concentrations were slightly elevated in low temperature fish (within breeding season) of the present study compared to high temperature fish (that would have fallen outside of breeding season) of the present study; however, GSI was not measured consistently. The potential relationship between 5-HT homeostasis and breeding physiology is intriguing and should be explored in future studies.

By using [^{3}H]5-HT as a tracer for 5-HT, the MCR for 5-HT, in theory, could be calculated. However, [^{3}H]5-HT is tritiated at both the 1 and 2 positions, therefore, after degradation, one molecule of [^{3}H]5-HT results in the formation of one molecule of [^{3}H]5-HIAA and one molecule of [^{3}H]NH_3. Note that the 1 and 2 positions under IUPAC conventions may also be referred to as the beta and alpha positions, respectively. The inability to differentiate between circulating [^{3}H]5-HT and its [^{3}H] metabolites has major implications for this study, as evident in the lack

Table 1
Average of MCR, plasma 5-HT concentration, and [^{3}H] excretion rate. Values are means ± SEM (n). Values not sharing a letter are significantly different; $p < 0.05$.

	Control	FBD	MAOI
MCR (ml kg^1 h^{-1})	47.75 ± 7.20 (12)	28.86 ± 6.08 (13)	48.98 ± 10.85 (13)
Plasma 5-HT Concentration (ng ml^{-1})	4.66 ± 0.72^a (12)	6.88 ± 1.22^b (12)	7.20 ± 1.02^b (12)
[^{3}H] Excretion rate (cpm kg^1 h^{-1})	2.51 ± 0.67 × 10^{5ab} (11)	4.41 ± 1.07 × 10^{5b} (13)	1.80 ± 0.31 × 10^{5a} (10)

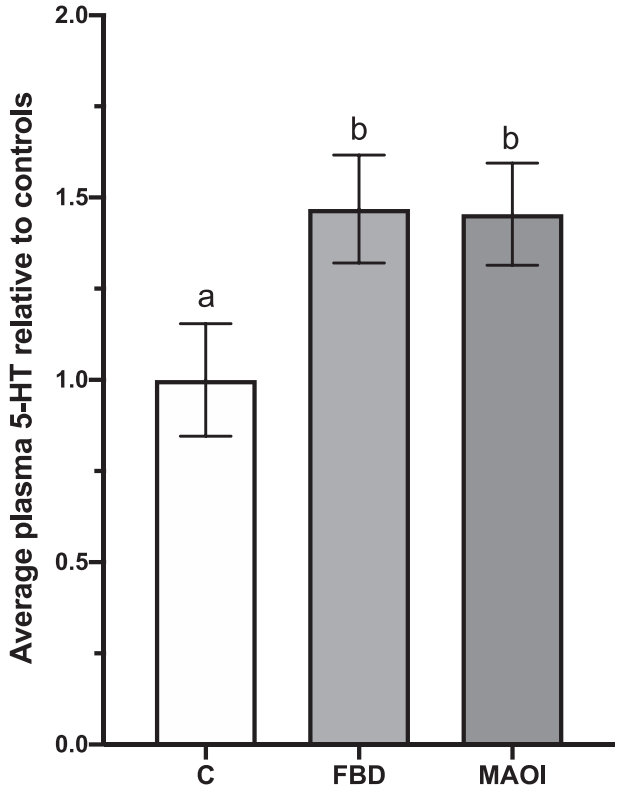

Fig. 3. Plasma 5-HT concentrations for control fish (n = 12) and fish in which 5-HT transport is inhibited by fluoxetine, buproprion and decynium-22 (FBD, n = 12) or 5-HT degradation is inhibited by clorgyline (MAOI, n = 12). Data are means ± 1 SEM. Bars not sharing a letter are significantly different; $p < 0.05$.

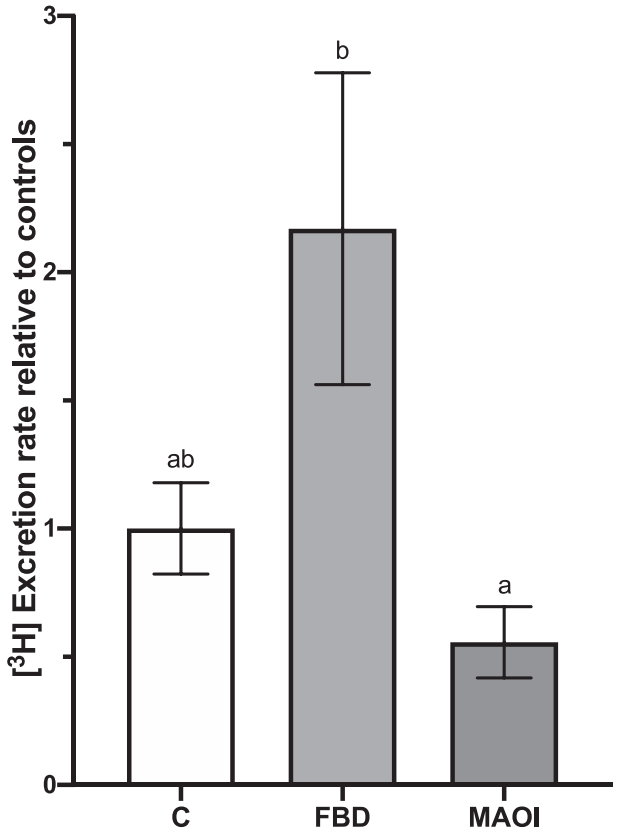

Fig. 4. Average [^{3}H] excretion rate into the water in control fish ($n = 10$) and fish in which 5-HT transport is inhibited by fluoxetine, buproprion and decynium-22 (FBD-treated, n = 13) or 5-HT degradation is inhibited by clorgyline (M-treated, $n = 11$). Data are means ± 1 SEM. Bars not sharing a letter are significantly different; $p < 0.05$.

of an impact on MCR with MAOI treatment despite a significant elevation in plasma 5-HT concentrations (*i.e.*, evidence that there was indeed a reduced clearance of 5-HT from the plasma). This means that clearance rate in control fish is likely underestimated as, after uptake and degradation, [^{3}H] can return to the plasma bound to 5-HIAA and/or NH_3 (Fig. 5A), essentially making it impossible to measure the impact of degradation using this approach. Instead, control MCR is indicating the volume of blood cleared of [^{3}H]5-HT, [^{3}H]5-HIAA and [^{3}H]NH_3, either through uptake or excretion, in an hour.

In contrast, the plasma [^{3}H] counts (and therefore the MCR estimations) in FBD- and MAOI-treated fish more accurately reflect [^{3}H] bound to 5-HT and not metabolites due to their indirect or direct inhibition, respectively, of 5-HT metabolism. Specifically, by inhibiting 5-HT transport into cells, as is the case in FBD-treated fish, the movement of 5-HT into the intracellular pool is reduced or completely inhibited, thereby impacting both uptake and degradation since degradation happens intracellularly (Bortolato et al., 2010) (Fig. 5B). When degradation alone is blocked, as is the case in the MAOI-treated fish, 5-HT may be taken up into cells *via* transporters but will remain unmetabolized. This may explain why there is a decrease in MCR within FBD-treated fish compared to controls (in which the contribution of degradation is impossible to determine), as both uptake and degradation were inhibited, but not a measurable decrease in MAOI-treated fish compared to controls, as only degradation was blocked (making it equivalent to controls in this way). Thus, a better comparison may be between FBD- and MAOI-treated fish rather than compared to controls, given the complications with the control MCR estimations. When compared to each other, FBD-treated fish have 34% lower MCR than MAOI-treated fish (unpaired Student's *t*-test, $p = 0.01$). This shows that transport *via* SERT, DAT, NET, and other transporters is indeed contributing to long-term 5-HT clearance, as the additive effects of uptake and degradation exceed those of degradation alone. It should be noted that FBD treatment, while reducing 5-HT uptake in some tissues by as much at 89%, does not completely abolish 5-HT uptake (Amador and McDonald, 2018a) and it would be reasonable to conclude that MAOI treatment likewise does not completely abolish 5-HT degradation either.

In terms of excretion, 5-HT and 5-HIAA excretion occur through two main pathways - renal excretion *via* urine and hepatic excretion *via* bile. Branchial excretion *via* gills plays a minor role and excretion through the skin cannot be ruled out, as toadfish are scaleless (Amador and McDonald, 2018). NH_3 excretion is predominantly across the gill (Wood et al., 2003). 5-HT and 5-HIAA have both been reported in the urine of rainbow trout (Caamaño-Tubío et al., 2007), and the previously reported presence of 5-HT in the urine of toadfish is noteworthy (Caamaño-Tubío et al., 2007; Amador and McDonald, 2018) as toadfish possess aglomerular kidneys (McDonald and Grosell, 2006) and, as a result, 5-HT or 5-HIAA entry into the urine is likely carrier-mediated. While 5-HT has been previously recorded in teleost bile, 5-HIAA has not. In general, little is known about how 5-HT or 5-HIAA enters the kidney tubule or the bile in fish. In teleosts, there is low SERT mRNA expression within kidney and liver tissue (Amador and McDonald, 2018a) and 5-HT uptake from the blood is not dominated by SERT but instead, low-affinity transport by promiscuous non-SERT transporters may be responsible (Amador and McDonald, 2018). Specifically, 5-HT uptake from the blood is significantly lower in kidney and liver of fish treated only with buproprion & decynium-22 (BD) compared to a combined treatment with fluoxetine (FBD) (Amador and McDonald, 2018). In the same study, divided chamber experiments with [^{3}H]5-HT injected toadfish found that posterior excretion of [^{3}H], which includes 5-HT, 5-HIAA, a small proportion of NH_3, and both renal and hepatic pathways making up approximately 80% of total [^{3}H] excretion, was not reduced in BD-treated fish but was significantly reduced in the

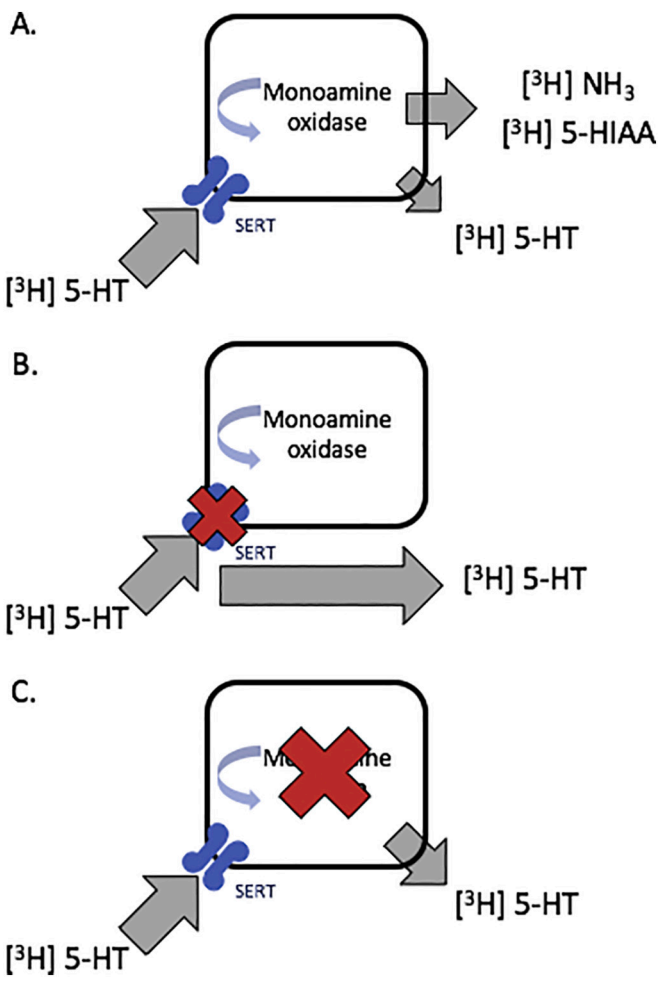

Fig. 5. Schematic depiction of 5-HT uptake and degradation. Under control conditions (A), [^{3}H]5-HT enters a cell through SERT and can be sequestered internally or degraded by MAO. The cell will therefore release some [^{3}H]5-HT, but mostly [^{3}H]5-HIAA and [^{3}H]NH_3 back into the plasma. In FBD-treated fish (B), [^{3}H]5-HT cannot enter the cell through SERT following inhibition. This leads to an elevation of plasma [^{3}H]5-HT and prevents the formation of [^{3}H]5-HIAA and [^{3}H]NH_3 because degradation occurs intracellularly. Therefore, the vast majority of radiolabel in the plasma is bound to 5-HT and not metabolite. In MAOI-treated fish (C), [^{3}H]5-HT can enter the cell *via* SERT and be sequestered, but degradation into [^{3}H]5-HIAA and [^{3}H]NH_3 is prevented due to inhibition of MAO. [^{3}H]5-HT can return to the circulation and plasma 5-HT increases as cells are unable to break down 5-HT into metabolite.

FBD-treated group compared to control. This suggests that 5-HT transport into the urine and/or bile is more dependent on SERT than 5-HT clearance from the blood into these organs. There was no change in the anterior excretion of [^{3}H], which would include larger proportion of NH_3 and makes up about 20% of total excretion, in response to FBD treatment (Amador and McDonald, 2018). These data contradict the findings of the present study, which measured excretion from all routes but found no significant difference between controls and FBD-treated fish. This may be due to methodology, as fish in our study were infused with [^{3}H]5-HT in saline for 72 h as opposed to injected in Amador and McDonald (2018). The fluid loading that occurred likely caused the fish to excrete as much as 40% more urine (McDonald et al., 2003), which may have resulted in a more favorable environment for urinary excretion of 5-HT and its metabolites, especially in FBD-treated fish which had elevated plasma 5-HT concentrations.

5-HT that enters the kidney or liver might also be degraded by MAO found in the kidney and liver tissue itself and then secreted into the urine as 5-HIAA and NH_3. MAO activity has been measured in both fish kidney and liver and varies depending on species. For example, maximum activity (V_{MAX}) was higher in the kidney than liver of rainbow trout (Edwards et al., 1986) but higher in the liver than kidney of goldfish (*Carassius auratus*) (Hall et al., 1982). Alternatively, 5-HIAA may enter kidney and liver from the blood as it is found in significant concentrations in the blood after 5-HT degradation by other organs – for example, rainbow trout gill extract 40% of [^{3}H] 5-HT infused into the arterio-arterial pathway while degrading two-thirds of the remaining 5-HT into metabolite (Gillis et al., 1972; Olson, 1998). Little is known about how 5-HIAA transport occurs in teleosts; however, experiments in mammals and birds have shown the transport of 5-HIAA occurs through organic anion channels in a probenecid-dependent manner (Hakim et al., 1970; Cumming et al., 1992). NH_3 movement across epithelia occurs either passively, with the help of Rhesus (Rh) proteins, or, when in the form of NH_4^+, through transporters that will move K^+ (Wright and Wood, 2009). In the present study, it is not anticipated that 5-HIAA or NH_3 movement into the urine or bile would have been prevented by the drug regime used; however, both FBD-treated and MAOI-treated fish were expected to have had higher 5-HT concentrations and reduced 5-HIAA and NH_3 concentrations within the circulation or within the kidney or liver compared to controls. That both drug treatments worked as intended is supported by the finding that plasma 5-HT levels were significantly elevated in both FBD- and MAOI-treated fish compared to controls. The higher excretion measured in FBD-treated fish compared to MAOI-treated fish may explain why plasma 5-HT levels were not higher in FBD-treated fish compared to MAOI-treated fish, despite FBD-treated fish being unable to take-up or degrade 5-HT (and MAOI-treated fish still having the ability to take up 5-HT). These data suggest that circulating 5-HT concentrations are defended and that excess may be eliminated by uptake and/or excretion. The difference in [^{3}H] excretion between FBD-treated fish and MAOI-treated fish may reflect the proportion of 5-HT that is instead taken up into cells and stored intracellularly in MAOI-treated fish (the component of 5-HT clearance that is inhibited in FBD-treated fish), as both groups should be incapable of degrading 5-HT.

In conclusion, this experiment was able to demonstrate that transporter-mediated uptake contributes to 5-HT clearance in the periphery while failing to capture the contribution of intracellular degradation by MAO. Further investigation into the uptake and degradation of 5-HT will therefore require alternate experimental approaches. Future work characterizing MAO in toadfish will allow for a more comprehensive investigation at 5-HT degradation in various tissues.

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgements

The authors would like to thank Dr. Maria C. Cartolano for surgical assistance.

Grants

This research was funded by the National Science Foundation Grant IOS-1754550 to M.D.M.

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