Chapter 2

Pharmacology and Molecular Identity of Serotonin Receptor in Bivalve Mollusks

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Abstract

It is now known that 5-HT regulates several neurobehavioral systems such as mood, appetite, sleep, learning, and memory. It also plays critical roles in the physiological functions of peripheral organs involved in stress, growth, and reproduction in the animal kingdom. 5-HT content has seen to be higher in the nervous system of bivalves than those of other examined invertebrates and vertebrates. Thus, bivalves have been considered as an excellent model to investigate 5-HT functions in neurological and peripheral systems. The present study reviews knowledge on 5-HT signaling mediated through 5-HT receptor and its physiological contribution to regulate reproduction in bivalves. Two G-protein-coupled 5-HT_1-like receptors have been cloned in bivalve species. However, binding affinities of the 5-HT agonists and antagonists to the isolated plasma membrane proteins and their effects on spawning in bivalves suggest the presence of a single or mixed 5-HT_1-, 5-HT_2-, and 5-HT_3-like receptors. It has suggested that the 5-HT-like receptors in bivalves are distinct from those of mammalian 5-HT receptors due to pharmacological properties. The present review pays a special attention to future research perspectives to better understand 5-HT regulation of reproduction in bivalves, which can provide us with satisfactory knowledge to elucidate reproductive disorders associated with dysfunctions of the neurotransmitter system.

Keywords: gonad, nervous system, oocyte, serotonin biosynthesis, serotonin metabolism and reuptake, serotonin receptor, sperm

1. Introduction

5-hydroxytryptamine called serotonin (5-HT) is a transmitter substance of the nervous system in animal kingdom. 5-HT has also been identified in bivalves from the period of its first discovery and earlier studies on these animals have led to convince the neurobiologist that it acts as a neurotransmitter.

A brief bibliography of discovery for 5-HT receptor and its physiological functions is provided in **Table 1**. Gaddum and Picarelli [6] were the first who demonstrated that 5-HT acts through a receptor-mediated pathway. Further studies have then directed toward pharmacological characterization of the 5-HT receptors in the nervous system and peripheral organs using radiolabelled ligands [7, 8] until the first molecular identity of the 5-HT receptor [9]. In 1960–1980s, 5-HT neurons have localized in the nervous system and peripheral organs (including gonad) of bivalves. Then, Sugamori et al. [10] and Tanabe et al. [11] cloned the 5-HT receptors in the nervous system and reproductive system of pond snail (*Lymnaea stagnalis*) and Yesso scallop (*Patinopecten yessoensis*), respectively. Taken together, bivalves and mammals become model organisms to investigate receptor-mediated mechanism of 5-HT physiological function because of small size, a simple nervous system and a high content of 5-HT in the nervous system.

Year	Scientists	Contribution to discovery of identification, localization, and characterization of 5-HT	References
1957	Gaddum and Picarelli	Suggestion of two types of 5-HT receptors (5-HT_M and 5-HT_D) in the guinea-pig ileum	[6]
1978	Fillion et al.	Identification of 5-HT receptors in the bovine brain using radiolabelled ligands: [^{3}H]-5-hydroxytryptamine and [^{3}H]-lysergic acid diethylamide	[7]
1979	Peroutka and Snyder	Evidence for the presence of two distinct 5-HT (5-HT_1 and 5-HT_2) in the rat brain derived from their selective recognition by radiolabelled ligands	[8]
1982	Matsutani and Nomura	Serotonin stimulates spawning in Yesso scallop (Bivalvia, Mollusca)	[18]
1984	Hirai and Koide	5-HT stimulates oocyte maturation in surf clam	[27]
1985	Osanai	5-HT regulation of the oocyte signaling required to undergo germinal vesicle breakdown	[28]
1988	Fargin et al.	Molecular identity of 5-HT_{1A} receptor	[9]
1991	Bandivdekar and Koide	Pharmacological identification of serotonin receptor in surf clam	[29]
1993	Sugamori and Van Tol	Molecular identity of 5-HT receptor in pond snail (Gastropoda, Mollusca)	[10]
2010	Tanabe and Osada	Molecular identity of 5-HT receptor in Yesso scallop	[11]

Species: pond snail, *Lymnaea stagnalis*; surf clam, *Spisula solidissima*; Yesso scallop, *Patinopecten yessoensis*.

Table 1. Bibliography of 5-hydroxytryptamine (serotonin, 5-HT) receptor: from discovery to physiological characterization.

Serotonin regulates various neurobehavioral systems (such as mood, appetite, sleep, learning, and memory). However, studies have revealed that it also plays critical roles in physiological functions of peripheral organs such as stress and growth [1–3]. One of the major system that 5-HT contributes to its regulation is reproduction. In both mammals and bivalves, it has observed that 5-HT regulates reproductive endocrine system, oocyte maturation, and sperm motility [12–23].

Although 5-HT biosynthesis and its receptor structure have been reviewed in bivalves [24–26], however, there is a gap of review on physiological signaling of 5-HT in these animals. The present study reviews the biology of 5-HT in bivalves, particularly, its contribution to reproduction. Particular attention has then paid to pharmacological characteristics of the 5-HT receptor and 5-HT-stimulated spawning through a receptor-mediated mechanism. This study provides future perspectives that await investigation to better understand 5-HT network and signaling in bivalve reproduction.

2. Molecular identity and pharmacological characteristics of the 5-HT receptors

Since the time Gaddum and Picarelli [6] suggested the presence of two kinds of tryptamine receptor, further studies have been conducted to identify and localize the 5-HT receptors to elucidate serotonergic signaling in biological systems. Fargin et al. [9] were the first who reported that the protein product of an orphan receptor (G21) encoding a G-protein-coupled receptor (GPCR) transiently expressed in monkey kidney cells possesses all the typical ligand-binding characteristics of the 5-HT_{1A} receptor. Molecular identity of 5-HT receptors has revealed that there are, so far, a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptors which are classified into seven groups. Except of the 5-HT_3 receptor that is a ligand-gated ion channel [35, 36], the 5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 belong to GPCR superfamily [4, 5, 37–41]. In invertebrates, pharmacological properties of the 5-HT receptors do not allow us to classify them in mammalian categories, although some signal transduction characteristics are similar [26].

2.1. Pharmacological characteristics of 5-HT receptors in bivalves

In bivalves, primary studies have used pharmacological 5-HT agonists and antagonists to investigate their binding affinities onto isolated membrane proteins of the oocytes and sperm using radiolabelled [^{3}H]5-HT [29, 42–45]. The results showed that only 5-HT and its analogs are capable of inhibiting [^{3}H]5-HT-specific binding to the isolated plasma membrane proteins of the oocytes in surf clam, whereas other monoamines (such as acetylcholine, haloperidol, carbachol, pyrilamine, and so on) are without effects [43, 44].

In surf clam, 1 μM ICS 205930, 5-HT, 5-CT, mianserin, methysergide, 8-OH-DPAT, 2-methyl-5-HT, BMY 7378, α-methyl-5-HT, ketanserin, quipazine, and PBG inhibit [^{3}H]5-HT binding to the isolated proteins of the oocyte plasma membrane by 49, 46, 40, 40, 37, 35, 33, 28, 26, 25, 22, and 11%, respectively [29]. The authors suggested that 5-HT receptors in the oocyte of

surf clam possess sites that interact with the 5-HT_1 and 5-HT_3 receptor analogs, because of the binding affinity of the 5-HT_1 receptor (5-CT, mianserin, methysergide, and 8-OH-DPAT) and the 5-HT_3 receptor (ICS 205930 and 2-methyl-5-HT) analogs. However, current pharmacological characterization of 5-HT receptor analogs reveals that 5-CT is a non-selective agonist, and mianserin and methysergide are particularly selective antagonists of the 5-HT_2 receptor (**Table 2**). These may suggest that the 5-HT_2 receptor also exist on the membrane of the oocytes in surf clam, in addition to the 5-HT_1 and 5-HT_3 receptors [29, 46].

Krantic et al. [43, 44] studied dose-dependent effects of the 5-HT analogs and observed that 5-HT, 8-OH-DPAT, metoclopramide, MDL 72222, mianserin, ICS 205930, ritanserin, imipramine, propranolol, and TFMPP inhibit specific [^{3}H]5-HT binding to the isolated membrane

Receptor	Agonists	Reference	Antagonist	Reference
5-HT_1	8-OH-DPAT (5-HT_{1A})	[47]	Propranolol (5-HT_{1B})	[49, 50]
	TFMPP (5-$HT_{1A, 1B, 1D}$)	[48]	NAN-190 (5-HT_{1A})	
			BMY 7378	[61, 62]
5-HT_2	TFMPP (5-$HT_{2A, 2C}$)	[49]	Ketanserin (5-HT_{2A})	[50]
	mCPP (5-$HT_{2B, 2C}$)	[50]	Spiperone (5-HT_{2A})	[50]
	PBG	[51]	1-NP (5-$HT_{2A, 2B, 2C}$)	[63, 64]
			Cyproheptadine (5-$HT_{2A, 2B}$)	
			Mianserin (5-$HT_{2A, 2B, 2C}$)	[50]
			Ritanserin (5-$HT_{2A, 2B, 2C}$)	[65]
			Methysergide (5-$HT_{2B, 2C}$)	[66]
5HT_3	1-m-c-b (mCPBG)	[52]	Metoclopramide	[67, 68]
	2-methyl-5-HT	[53]	ICS 205-930 (Tropisetron)	[53, 69–71]
	Quipazine	[54]	LY-278584	[72, 73]
			MDL-72222 (Bemesetron)	[69, 74]
			Ondansetron	
Non-selective	α-Methyl-5-HT (5-$HT_{1, 2}$)	[55]	Methiothepin (5-$HT_{1A, 1B, 1D, 5A}$)	[75]
	5-CT (5-$HT_{1A, 1B, 1D, 5A, 7}$)	[56–60]		

α-methyl-5-HT, α-methyl-5-hydroxytryptamine; 1-m-c-b, 1-methyl-chlorophenyl biguanide; 2-methyl-5-HT, 2-methyl-5-hydroxytryptamine; 1-NP, 1-(1-naphthyl)piperazine; 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, 7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol; mCPP, *meta*-chlorophenylpiperazine; MDL-72222 (Bemesetron) PBG, 1-phenylbiguanide; and TFMPP, 3-trifluoromethylphenylpiperazine.

8-OH-DPAT also acts as a 5-HT_7 receptor agonist [76] and possesses serotonin reuptake blocking property [77]. TFMPP binds to SERT and evokes 5-HT release [78]. *mCPP acts as 5-HT reuptake inhibitor/releasing agent* [79]. Unlike mCPP, TFMPP has insignificant affinity for the 5-HT_3 receptor [80]. BMY-7378 is a weak partial 5-HTlA agonist compared to 8-OH-DPAT that is a full 5-HT1A agonist [81, 82] and is a selective antagonist of α_{1D}-adrenoceptors [83]. PBG and mCPBG have dopamine releasing properties [84]. Methysergide also acts as a 5-$HT_{1A, 1B, 1D}$ receptors' partial agonist. 5-HT and methysergide appear not to compete for the same site, whereas ketanserin and methysergide do appear to compete for the same site [56, 66, 85]. Quipazine also acts via 5-HT_2 receptor as an agonist [86, 87] or antagonist of 5-HT_3 receptor [88, 89]. Metoclopramide acts as antagonist of dopamine D_2 receptors [90] and as a 5-HT_4 receptor agonist [91].

Table 2. Pharmacological agonists and antagonists of the 5-hydroxytryptamine (serotonin, 5-HT) receptors.

proteins of the oocytes in surf clam by 100, 67, 63, 61, 57, 57, 55, 49, 47, and 12% with IC_{50} of 0.52, 0.05, 0.06, 0.13, 0.45, 3.05, 0.42, 4.2, 1.32, and >100 μM, respectively. Hence, these results showing affinities of the 5-HT analogs to the 5-HT_1, 5-HT_2, and 5-HT_3 receptors in the oocyte of surf clam; however, the receptor possesses distinct 5-HT binding sites from 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors in mammals and *Drosophila*. For instance, the 5-HT_{1A} receptor is more sensitive to 8-OH-DPAT than 5-HT, insensitive to ritanserin, and relatively sensitive to TFMPP in mammals. 8-OH-DPAT is a weak agonist on the *Drosophila* 5-HT receptors. Ritanserin, but not TFMPP, inhibits [^{3}H]5-HT binding to the isolated membrane protein of the oocyte in surf clam, although isolated 5-HT receptor is highly sensitive to 8-OH-DPAT more than that of 5-HT. The 5-HT receptor in the oocyte of surf clam does not possess pharmacological 5-HT_2 receptor characteristics in mammals, as it is not equally sensitive to TFMPP and 8-OH-DPAT. The pharmacological characteristics of the isolated 5-HT receptor also differ from the 5-HT_3 receptor. In mammals, the 5-HT_3 receptor is at least 100-fold more sensitive to 8-OH-DPAT than to metoclopramide; however, 8-OH-DPAT and metoclopramide are equipotent in inhibition of [^{3}H]5-HT binding to the 5-HT receptor in the surf clam. Based on these different responses of the isolated membrane protein of the surf clam oocytes to the 5-HT analogs, the authors suggested the presence of a novel 5-HT receptor in the plasma membrane of the surf clam oocytes.

In Yesso scallop, Osada et al. [45] observed that [^{3}H]5-HT binding to the oocyte plasma membrane is inhibited to 93, 83, 70, 44, 41, and 36% in the presence of 100 μM metoclopramide, 8-OH-DPAT, 5-HT, ritanserin, α-methyl-5-HT, and methiothepin, respectively. In the Pacific oyster, [^{3}H]5-HT binding to the oocyte plasma membrane is inhibited to 96, 83, 58, 49, 21, and 16% in the presence of 100 μM metoclopramide, 8-OH-DPAT, 5-HT, α-methyl-5-HT, ritanserin, and methiothepin respectively [45]. Ritanserin-, α-methyl-5-HT-, and methiothepin-inhibited [^{3}H]5-HT binding to the 5-HT receptor isolated from the oocyte of Yesso scallop suggest that mixed 5-HT_1 and 5-HT_2 receptors function in this species. However, the authors suggested that a single 5-HT_1 receptor functions in the Pacific oyster as methiothepin acts mainly as a 5-HT_1 antagonist (**Table 2**). In addition, this study shows that metoclopramide does not influence [^{3}H]5-HT binding to 5-HT receptor isolated from the oocyte of Yesso scallop and the Pacific oyster and 8-OH-DPAT is also a weak agonist, suggesting that 5-HT signaling is not mediated by 5-HT_3 receptor and is distinct from mammalian 5-HT_{1A} receptors in these species.

Pharmacological characteristics of the 5-HT receptor in sperm have only studied in surf clam [42]. The results have shown that 1 μM ICS 205930, 2-methyl-5-HT, 8-OH-DPAT, BMY 7378, 5-HT, 5-CT, mianserin, methysergide, α-methyl-5-HT, PBG, and ketanserin inhibit 45, 43, 37, 32, 31, 31, 30, 26, 13, 4, and 1% of [^{3}H]5-HT binding to the sperm plasma membrane, respectively. Considering current pharmacological characterization of 5-HT receptors, analogs of 5-HT_3, 5-HT_1, and 5-HT_2 receptors are more potent to compete with 5-HT to inhibit [^{3}H]5-HT binding to the sperm plasma membrane.

2.2. Molecular identity and cellular localization of 5-HT receptors in bivalves

In mollusks, the 5-HT_{Lym} and 5-HT_{2Lym} are first identified in the central nervous system of the pond snail (*L. stagnalis*). They display some pharmacological characteristics of the 5-HT_1 and 5-HT_2 receptors in mammals, and thus are currently considered as the 5-HT_1-like receptor and the 5-HT_2-like receptor, respectively [10, 92]. The Ap5-HT_{B1} and Ap5-HT_{B2} [93], 5-HT_{1AP}[94],

and 5-HT_{2AP} [95] are identified in California sea slug (*Aplysia californica*). The Ap5-HT_{B1} and Ap5-HT_{B2} (79.5% homologous to each other) are expressed in the reproductive system and the nervous system, respectively; however, they are not classified into any 5-HT receptor subtypes in mammals due to differences in their amino acid sequences [93]. The 5-HT_{1AP} is distributed in most organs, including the nervous system, kidney, gills, and heart, and its amino acid sequence and pharmacological profiles suggest that it is a 5-HT_1 receptor subfamily [94]. The 5-HT_{2AP} shares 68 and 34% of its amino acid sequence identity with the 5-HT_{Lym} and 5-HT_1 receptor in mammals, its pharmacological characteristics is very similar to those of the 5-HT_{Lym} receptor, and it is only expressed in the nervous system [95].

In bivalves, the 5-HT receptors are cloned in the ovary of the Yesso Scallop [11], and Pearl oyster, *Pinctada fucata* [96] (**Figure 1**). Molecular identity of the 5-HT receptor is also predicted for the Pacific oyster (5-HT_{cg}) [97]. In the Yesso scallop, an 1818 bp cDNA encodes a putative 5-HT_{py} receptor that includes a 232-bp 5′-untranslated region (UTR), a 1362-bp open reading frame (ORF) encoding a putative protein of 454 amino acids, and a 224-bp 3′-UTR. In the Pearl oyster, a 2541 bp cDNA encodes a putative 5-HT_{pf} receptor that includes a 296-bp 5′-UTR, a 1416-bp ORF encoding a putative protein of 471 amino acids, and an 829-bp 3′-UTR. The 5-HT_{pf} is calculated to have a molecular weight of 53.55 kDa. The hydrophobicity analysis of the deduced amino acid sequence revealed seven putative transmembrane domains, which are highly conserved between 5-HT_{py}, 5-HT_{pf}, and other 5-HT_1 receptors coupled with $G_{i/o}$. The 5-HT_{py} contains two potential sites for N-linked glycosylation in the extracellular N-terminal region and the third intracellular domain. The 5-HT_{pf} receptor contains five potential sites for N-linked glycosylation in the extracellular N-terminal region. There are 12 and 8 sites for phosphorylation by protein kinase A or C in the Yesso scallop and Pearl oyster, respectively, among which 7 sites are located in the third cytoplasmic loop. A relatively long third cytoplasmic loop and a short fourth inner terminal domain (C-terminal tail) are present in the 5-HT_{py} and 5-HT_{pf} sequence.

An amino acid sequence alignment of 5-HT receptor homologs from different species reveals that a relatively high level of amino acid sequence identity exists between 5-HT_{py} and 5-HT_{pf} (52%) and between 5-HT_{py} and 5-HT_{cg} (48%). The amino acid sequence identity is between 5-HT_{pf} and 5-HT_{cg} (71%). There are conserved amino acid regions when the 5-HT_{py} and 5-HT_{pf} are aligned to 5-HT_1 subtypes in human (**Figure 1**). The 5-HT_{py} amino acid sequence is 40, 40, 37, 38, and 38% identical to the human 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptor, respectively. The 5-HT_{pf} amino acid sequence is 42, 39, 39, 40, and 40% identical to the human 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptor, respectively. The 5-HT_{cg} was not considered in alignment analysis as it is a predicted sequence. The amino acid sequence identity is higher within the transmembrane domains, compared to those of the intracellular and extracellular region. However, lower amino acid sequence identity exists between the 5-HT receptors in bivalves with the other 5-HT receptors (5-HT_2, 5-HT_5, 5-HT_6, and 5-HT_7) in vertebrates. The phylogenetic analysis of the 5-HT receptors in invertebrates suggests that the 5-HT receptors of bivalves resemble the 5-HT receptors in the California sea slug (*A. californica*), pond snail (*L. stagnalis*), and air-breathing snail (*Planorbella trivolvis*), which are known to be as 5-HT_1-like receptor (**Figure 2**). These known 5-HT receptors are differentiated into a major branch, compared to the other known invertebrate 5-HT receptors. Four 5-HT receptors

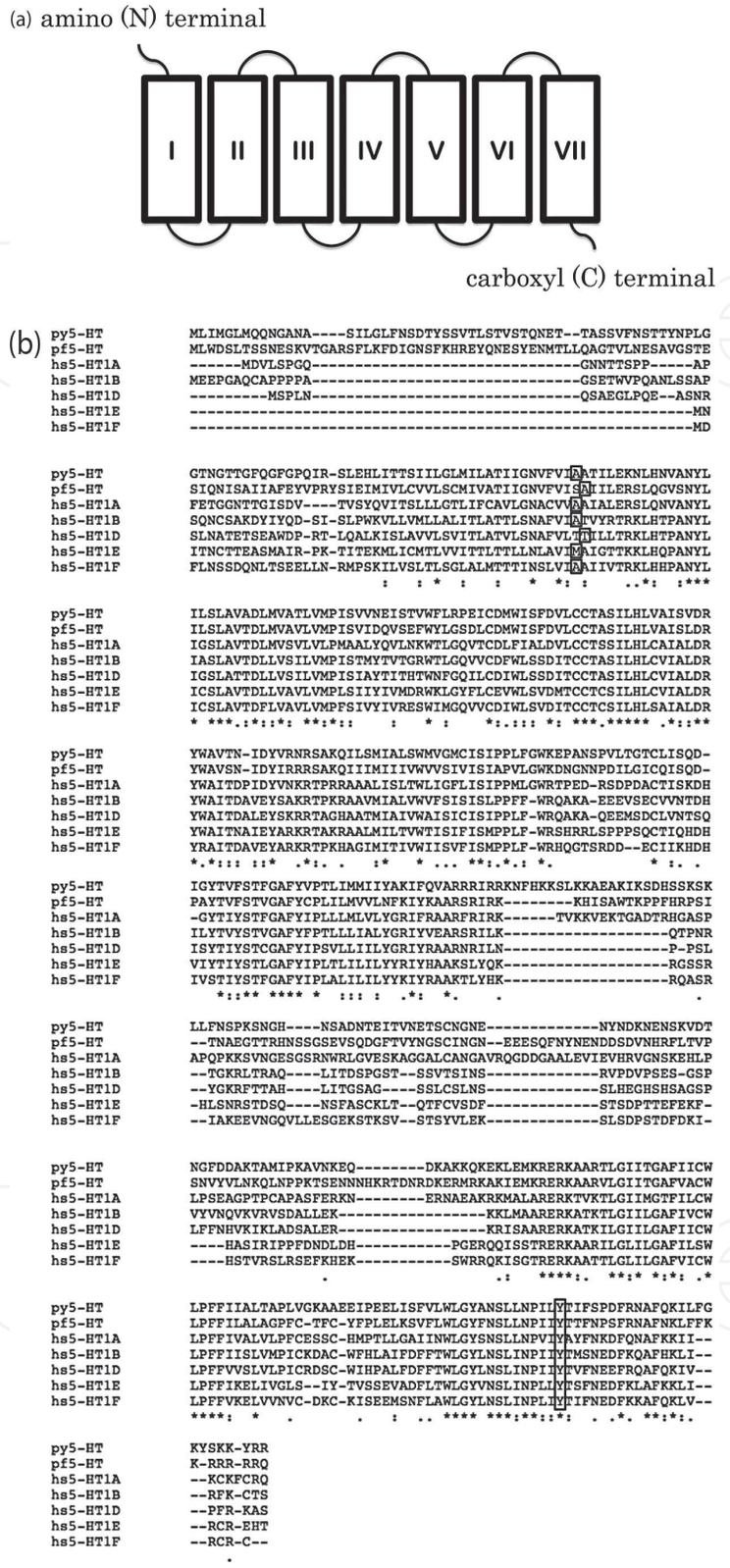

Figure 1. A schematic representation of the G-protein-coupled 5-hydroxytryptamine (serotonin, 5-HT) receptor showing seven transmembrane domains (A). (B) Multiple alignment of deduced amino acid sequence of 5-HT receptors of the Yesso scallop (*Patinopecten yessoensis*, py5-HT) and pearl oyster (*Pinctada fucata*, pf5-HT) with the 5-$HT_{1A\text{-}F}$ receptors in human. The marked amino acids indicate seven transmembrane regions. Sequences are aligned with MUSCLE configured for highest accuracy (www.phylogeny.fr).

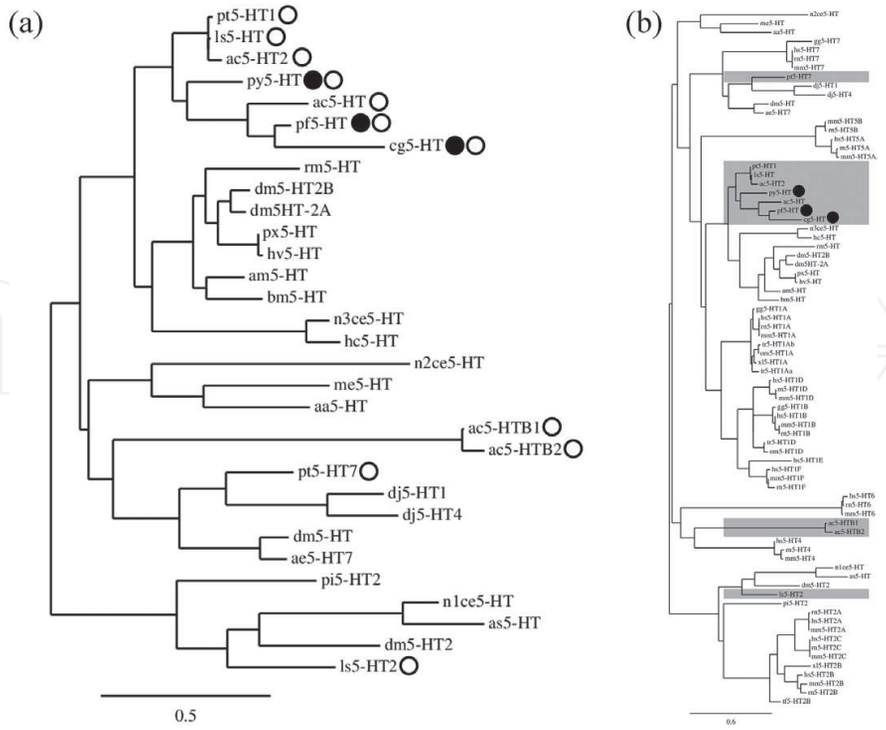

Figure 2. Phylogenetic analysis of the 5-hydroxytryptamine (serotonin, 5-HT) receptor known from invertebrates (A) and from invertebrates and vertebrates (B). Filled circles indicate bivalve species. Open circles or dark background indicate mollusk species. Note that the 5-HT_3 receptors are excluded in this analysis, as they are ligand-gated ion channel. Phylogeny trees are constructed using the maximum likelihood method implemented in the PhyML program. The amino acid sequences of the 5-HT receptors are aligned with MUSCLE configured for highest accuracy (MUSCLE with default settings). After alignment, ambiguous regions (i.e. containing gaps and/or poorly aligned) are removed (www.phylogeny.fr). Accession numbers of applied 5-HT receptors are as follows: **invertebrates** dm5-HT (AAA28305, 5HT-dro), dm5-HT2 (CAA57429, 5-HT2-dro), dm5HT-2A (CAA77570, 5HT-dro2A), dm5-HT2B (CAA77571, 5HT-dro2B), ae5-HT7 (AAG49292), am5-HT (NP-001164579), px5-HT (BAD72868), rm5-HT (AAQ89933), bm5-HT (CAA64862), hv5-HT (CAA64863), dj5-HT1 (BAA22404), dj5-HT4 (BAA22403), 1ce5-HT (AAC15827), 2ce5-HT (NP-491954), 3ce5-HT (NP-497452), as5-HT (AAC78396), hc5-HT (AAO45883), ac5-HTB1 (Q16950, Ap5HTB1), ac5-HTB2 (Q16951, Ap5HTB2), ac5-HT2 (AAM46088, Ap5-HT2), ac5-HT (AAC28786, Ap5-HT), ls5-HT2 (AAC16969, Lym5-HT2), ls5-HT (AAA29290, Lym5-HT), pt5-HT1 (AAQ95277), pt5-HT7 (AAQ84306), py5-HT (BAE72141), pf5-HT (AIW04132), cg5-HT (EKC38511), pi5-HT2 (AAS57919, 5-HT type 2), me5-HT (AAS05316), aa5-HT (BAA12013), and **vertebrates** tr5-HT1Aa (CAA65175, 5-HT1Aalpha), tr5-HT1Ab (CAA65176, 5-HT1Abeta), om5-HT1A (AAP83427), xl5-HT1A (CAA69208), gg5-HT1A (NP-001163999), rn5-HT1A (NP-036717), mm5-HT1A (NP-032334), hs5-HT1A (NP-000515), gg5-HT1B (NP-001166252), rn5-HT1B (NP-071561), mm5-HT1B (NP-034612), hs5-HT1B (AAH69065), tr5-HT1D (CAA58745), om5-HT1D (AAP83428), rn5-HT1D (NP-036984), mm5-HT1D (NP-032335), hs5-HT1D (NP-000855), hs5-HT1E (NP-000856), rn5-HT1F (NP-068629), mm5-HT1F (NP-032336), hs5-HT1F (NP-000857), rn5-HT2A (NP-058950), mm5-HT2A (NP-766400), hs5-HT2A (NP-000612), tf5-HT2B (CAC85912), xl5-HT2B (CAD71264), rn5-HT2B (NP-058946), mm5-HT2B (NP-032337), hs5-HT2B (NP-000858), rn5-HT2C (NP-036897), mm5-HT2C (NP-032338), hs5-HT2C (NP-000859), rn5-HT4 (NP-036985), mm5-HT4 (CAA70775), hs5-HT4 (CAC22248), rn5-HT5A (NP-037280), mm5-HT5A (NP-032340), hs5-HT5A (NP-076917), rn5-HT5B (NP-077371), mm5-HT5B (NP-034613), rn5-HT6 (NP-077341), mm5-HT6 (NP-067333), hs5-HT6 (NP-000862), gg5-HT7 (NP-001165240), rn5-HT7 (NP-075227), mm5-HT7 (NP-032341), hs5-HT7 (NP-000863). First letters of the genus and species are used to construct the phylogenetic analysis; fruit fly (*Drosophila melanogaster*, dm); mosquito (*Aedes aegypti*, ae); honey bee (*Apis mellifera*, am); butterfly (*Papilio xuthus*, px); tick (*Rhipicephalus microplus*, rm); silkworm (*Bombyx mori*, bm); moth (*Heliothis virescens*, hv); planarian flatworm (*Dugesia japonica*, dj); nematode roundworm (*Caenorhabditis elegans*, ce); nematode roundworm (*Ascaris suum*, as); nematode (*Haemonchus contortus*, hc); California sea slug (*Aplysia californica*, ac); pond snail (*Lymnaea stagnalis*, ls); air-breathing snail (*Planorbella trivolvis*, pt); scallop (*Mizuhopecten yessoensis*, py); Pearl oyster (*Pinctada fucata*, pf); Pacific oyster (*Crassostrea gigas*, cg); lobster (*Panulirus interruptus*, pi); shrimp (*Metapenaeus ensis*, me); barnacle (*Amphibalanus amphitrite*, aa); pufferfish (*Takifugu rubripes*, tr); pufferfish (*Tetraodon fluviatilis*, tf); Tilapia (*Oreochromis mossambicus*, om); frog (*Xenopus laevis*, xl); chicken (*Gallus gallus*, gg); rat (*Rattus norvegicus*, rn); mouse (*Mus musculus*, mm); and human (*Homo sapiens*, hs).

of mollusks (5-HT_2 in pond snail, 5-HT_7 in the air-breathing snail, 5-HT_{B1} and 5-HT_{B2} in the California sea slug) are differentiated into different branch. Except of two latter case which display difficulties to be classified in terms of 5-HT receptors in vertebrates [26], the 5-HT_2 in pond snail and the 5-HT_7 in the air-breathing snail are considered as the 5-HT_2-like and the 5-HT_7-like receptors, respectively [92, 98].

The 5-HT_{py} and 5-HT_{pf} are expressed in most of the organs, including the ovary, testis, mantle, adductor muscle, gill, the nervous system (cerebral-pedal ganglia and VG), digestive gland, or kidney [11, 96]. *In situ* hybridization has shown that the 5-HT_{py} mRNA is localized in the oocytes and epithelium of the gonoducts in the ovary and in the spermatids and epithelium of the gonoduct in the testis [11]. It has histologically observed that, at spawning, mature oocyte and sperm are collected and evacuated from the acini into the surrounding aquatic environment via gonoducts in the great scallop [99]. Real-time PCR analyses of the 5-HT_{pf} mRNA transcription reveals that the order of decreasing is as follows: mature ovary > mature testis, VG, and digestive gland > mantle, gills, and adductor muscle. In addition, the testicular and ovary 5-HT_{pf} mRNA transcription does not differ among resting, developmental, and mature stages, however, increases in the ovary at spawning stage [96].

3. Receptor-mediated 5-HT stimulation of spawning in bivalves

Matsutani and Nomura [18] observed that injection of homogenates of CG, PG, or VG into the gonad of Yesso scallop induces spawning in 100% of males; however, they are without effects on females. In another experiment, they observed that 5-HT induces spawning in 100% of males and 73.3–80% of females. No other neurotransmitters, including adrenaline, noradrenaline (NA), and ϒ-aminobutyric acid, induced spawning [100–103]. Acetylcholine and dopamine (DA) induce spawning in males (40%), however they are without effects on females. Similarly, further studies have shown that neurotransmitters except of 5-HT are not potent to induce spawning in the surf clam [40], Zebra mussel [104], and Peruvian scallop [33, 105]. It is worth to note that DA at high dose (2×10^{-3} M) is capable of inducing spawning in males of Peruvian scallop [105] and in both males and females of Lion's paw scallop (*Nodipecten nodosus*) and Nucleus scallop (*Argopecten nucleus*) [106]. Omitting these exceptions, it has been accepted that 5-HT is the most potent neurotransmitter that induce spawning in bivalves at physiological concentration (**Table 3**). Other studies also show that injection of 0.4 mM 2–20 $\times 10^{-4}$ M 5-HT induces spawning in bivalve species, including the Atlantic deep-sea scallop, butter clam (*Saxidomus gigantea*), Gaper clam (*Tresus capax*), Manila clam (*Ruditapes philippinarum*), Pacific geoduck (*Panopea generosa*), Pacific littleneck clam (*Protothaca staminea*), Pacific oyster, Pacific razor clam (*Siliqua patula*), Pink scallop (*Chlamys rubida*), Rock scallop (*Hinnites multirugosus*), Weathervane scallop (*Patinopecten caurinus*), and Yesso scallop [107, 108]. It has also observed that 10^{-4} to 10^{-6} M 5-HT stimulates the release of the oocytes from the ovary tissues and sperm from the testicular tissues following a 90-min incubation, *in vitro* [109–112]. These are in agreement with identification of 5-HT and localization of nerve fibers transferring 5-HT from nervous system to gonad, which are observed around acini or gamete collective tubules. Both males and females response to exogenous 5-HT in a dose-dependent

manner. However, it seems that females usually require higher amount of 5-HT than that of a male to release the oocytes. The observed sex-specificity might be related to inter-sex differences in the concentration of 5-HT, which are shown to be higher in males than in females [32, 34]. Moreover, studies show that 5-HT fully stimulates spawning in ripe individuals.

As 5-HT fibers are localized in the gonad of bivalves, these observations pioneered further research to elucidate mechanism through which 5-HT induces spawning. In Zebra mussel, methiothepin, a non-selective 5-HT_1 receptor antagonist (**Table 2**), decreases 5-HT-induced spawning when it is added into the aquarium 5 min after addition of 5-HT. However, it is without effects on 5-HT-induced spawning when it is added into the aquarium 10 min after addition [120]. A 2 h pre-treatment of the Zebra mussel with 10^{-4} M methiothepin decreases parturition from 65 to 8% and from 82 to 1% in the individuals treated with 10^{-4} and 10^{-3} M 5-HT, respectively. These suggest that 5-HT-induced spawning requires a certain period of time and that 5-HT-induced spawning is irreversible.

To better understand which type of 5-HT receptor is involved in 5-HT-induced spawning, further experiments have conducted using 5-HT receptor analogs. It has observed that 10^{-4} M 8-OH-DPAT, 5-HT, and TFMPP induce 80, 70, and 56% spawning in Zebra mussel; however,

Species	Notes	Spawning of female (%)		Spawning of male (%)		References
		Control	5-HT (mM)	Control	5-HT (mM)	
Yesso scallop *Patinopecten yessoensis*	T: 6.7–10.5M: Injection to gonadD: 0.4 ml of 5-HT solution C: FSW	011.1	2: 73.3, 800.2: 1000.02: 200.002: 0	0	2: 1000.2: 800.02: 1000.002: 800.0002: 400.00002: 0	[18]
Yesso scallop *Patinopecten yessoensis*	T: 17–19M: Injection to gonadD: 0.4 ml of 0.1 mM 5-HT C: ASW	12.5	T_e: 87.5T_e: 91.7T_e: 100	–	100	[30]
American oyster *Crassostrea virginica*	T: 25M: Injection to gonadD: 0.4 ml of 2 mM 5-HT C: FSW	0	0	0	100	[2][113]
Bay scallop *Argopecten irradians*	T: 20–21M: Injection to gonadD: 0.4 ml of 2 mM 5-HT C: FSW	33.3	3.5	66.7	96.6	[2][113]
Hard clam *Mercenaria mercenaria*	T: 28–29M: Injection to muscleD: 0.4 ml of 2 mM 5-HT C: FSW	0	15.3	0	84.7	[2][113]
Hard clam *Mercenaria mercenaria*	T: 20M: Injection to muscleD: 0.4 ml of 5-HT solution C: FSW	20: 02: 00.2: 00.02: 0	20: 02: 1.10.2: 12.20.02: 2.2	20: 02: 00.2: 00.02: 0	20:23.32: 40.00.2: 36.60.02: 14.4	[114]

Species	Notes	Spawning of female (%)		Spawning of male (%)		References
Ocean quahog *Arctica islandica*	T: 15–16M: Injection to muscleD: 0.4 ml of 2 mM 5-HTC: FSW	0	T_{e1}: 16.7T_{e2}: 22.2T_{e3}: 23.1T_{e4}: 9.1	0	T_{e1}: 83.3T_{e2}: 77.8T_{e3}: 76.9T_{e4}: 90.9	[3][115]
Ocean quahog *Arctica islandica*	T: 15–16M: Injection to muscleD: 0.4 ml of 2 mM 5-HTC: FSW	0	21.1	0	79.0	[2][113]
Ribbed mussel *Geukensia demissa*	T: 28M: Injection to muscleD: 0.4 ml of 2 mM 5-HTC: FSW	0	11.1	100	88.9	[2][113]
Surf clam *Spisula solidissima*	T: 19M: Injection to gonadD: 0.4 ml of 2 mM 5-HTC: FSW	100	33.3	0	66.7	[2][113]
Surf clam *Spisula solidissima*	T: NDM: Injection to gonadD: 0.5 ml of 5-HT solutionC: ASW	0	2: 1000.2: 66.70.02: 66.70.002: 250.0002: 0	0	2: 1000.2: 85.70.02: 400.002: 00.0002: 25	[19]
Japanese baking scallop *Pecten albicans*	T: 12–16M: Injection to gonadD: 0.5–1 ml of 5-HT solutionC: FSW			0	2.5: 900.25: 87.50.025: 93.8	[4][116]
Giant clam *Tridacna gigas*	T: 27.8–30.5M: Injection to gonadD: 1–7 ml of 2 mM 5-HTC: FSW	0	2.6	0	66.7	[1][117]
Southern giant clam *Tridacna derasa*	T: 27.8–30.5M: Injection to gonadD: 1.5–4.5 ml of 2 mM 5-HTC: FSW	0	4.3	0	47.8	[1][117]
Maxima clam *Tridacna maxima*	T: 27.8–30.5M: Injection to gonadD: 0.5–2 ml of 2 mM 5-HTC: FSW	0	18.8	0	93.8	[1][117]
Crocus clam *Tridacna crocea*	T: 27.8–30.5M: Injection to gonadD: 0.5–1 ml of 2 mM 5-HTC: FSW	0	0	0	73.3	[1][117]
Scaly clam *Tridacna squamosal*	T: 27.8–30.5M: Injection to gonadD: 1.5–3 ml of 2 mM 5-HTC: FSW	0	0	0	67	[1][117]

Species	Notes	Spawning of female (%)		Spawning of male (%)		References
Bear paw clam *Hippopus hippopus*	T: 27.8–30.5M: Injection to gonadD: 1–5 ml of 2 mM 5-HT C: FSW	0	52.5	0	100	[1][117]
Zigzag scallop *Pecten ziczac*	T: 20M: Injection to muscle and gonadD: 0.4 ml of 2 mM 5-HT C: FSW	Feb.: 0Mar.: 0Apr.: 0	Feb.: 0Mar.: 0Apr.: 0	Feb.: 0Mar.: 0Apr.: 0	Feb.: 55Mar.: 100Apr.: 90	[118]
Doughboy scallop *Mimachlamys asperrima*	T: 15M: Injection to gonadD: 0.05 ml of 5-HT solutionC: Saline solution (Instant Ocean, Sarrebourg, France)	0	0.001: 00.01: 1000.1: 1001: 10010: 100	20	0.001: 200.01: 600.1: 601: 10010:100	[119]
Zebra mussel *Dreissena polymorpha*	T: 12M: 5-HT has added into aquarium, *in vivo*	0	1: 1000.1: 48.7	0	1: 1000.1: 65.4	[120]
Fingernail clam *Musculium transversum*	T: 23M: 5-HT has added into aquarium, *in vivo*	0	1 M: 1000.1: 560.01:0			[5][121]
Peruvian scallop *Argopecten purpuratus*	T: NDM: Injection to gonadD: 0.4 ml of 0.02–2 mM 5-HT C: FSW	0	0–20	0	100	[105]
Japanese clam *Mactra chinensis*	T: NDM: Injection to footD: 0.4 ml of 0.001–2 mM 5-HT C: FSW	0	2: 1001: 1000.1: 93.30.05: 1000.02: 1000.01: 26.70.001: 0	0	2: 1001: 1000.1: 93.30.05: 1000.02: 1000.01: 26.70.001: 0	[122]
Catarina scallop *Argopecten ventricosus*	T: 23M: Injection to gonadD: 0.025–2.5 mM 5-HTC: ND	0	0	0	100	[123]
Manila clam *Ruditapes philippinarum*	T: NDM: Injection to footD: 0.2 ml of 5-HT solution C: FSW	0	8.8	0	10: 801: 600.1: 86.70.01: 1000.001: 500.0001: 0	[124]
Nucleus scallop *Argopecten nucleus*	T: 22M: Injection to gonadD: 0.2 ml of 1 mM 5-HT solution C: FSW	40	67	20	90	[106]

Species	Notes	Spawning of female (%)		Spawning of male (%)		References
Lion's paw scallop *Nodipecten nodosus*	T: 22M: Injection to gonadD: 0.2 ml of 1 mM 5-HT solution C: FSW	6	48	24	93	[106]
Atlantic deep-sea scallop *Placopecten magellanicus*	T: 5 and 10M: Injection to gonadD: 0.4 ml of 2 mM 5-HT C: FSW			0	100	[125]

Abbreviation: ASW, artificial seawater; C, injection to control; D, dose; FSW, filtered seawater; M, method; ND, not determined; T, temperature (°C), T_e, experimental trial.

[1]Values for control are 0% as no individual injected with filtered seawater exhibited spawning behavior [117].

[2]Numbers of female and male injected with 5-HT are not determined. Values show percentage of spawned females and males from total number of individuals that spawned following injection of 5-HT. Total percentage of spawning are 27.1% (Ocean quahog), 82.9% (Bay scallop), 70% (American oyster), 45.0% (Ribbed mussel), 41.6% (Hard clam), and 60.0% (Surf clam). In the control group of Bay scallop, Ribbed mussel, and Surf clam, 8.6, 5.0, and 2.2% spawned, respectively. Individual in the control group of American oyster, Hard clam, and Ocean quahog did not spawn.

[3]Numbers of female and male injected with 5-HT are not determined. Values show percentage of spawned females and males from total number of individuals that spawned following injection of 5-HT. Total percentage of spawning are 17.1, 22.5, 37.1, and 35.5% in individual spawning trail 1 (T_{e1}), individual spawning trial 2 (T_{e1}), mass spawning trial 1 (T_{e3}), and mass spawning trial 2 (T_{e4}), respectively. Individual spawning represents spawning of a specimen placed in a glass dish (1 l FSW). Mass spawning represents placing of all individuals in troughs (140 l FSW). Individual in any control group did not spawn.

[4]Induction of spawning in the male phase of hermaphrodite scallop.

[5]Animals are exposed, and the percentage of parturition is evaluated based on the number of the release of juveniles.

Table 3. 5-hydroxytryptamine (serotonin, 5-HT) stimulates spawning in various species of bivalve mollusks.

2-methyl-5-HT and α-methyl-5-HT are without effects (4.1 and 0%) [104]. None of these 5-HT receptor agonists induce spawning at 10^{-5} M. A 2 h pre-treatment of Zebra mussel with 10^{-4} M cyproheptadine and mianserin results in 50 and 30% inhibition of 10^{-3} M 5-HT-induced spawning, respectively, whereas propranolol, 1-NP, NAN-190, and ketanserin are without effects. In addition, cyproheptadine is the only effective analog that totally inhibits 10^{-4} M 5-HT-induced spawning. A 2 h pre-treatment of Zebra mussel with 10^{-4} M cyproheptadine or mianserin totally suppress spawning at 10^{-4} or 10^{-3} M 8-OH-DPAT-induced Zebra mussel. In addition, 10^{-4} and 10^{-3} M 8-OH-DPAT-induced spawning are inhibited by 30 and 60% in the presence of 10^{-4} M NAN-190, respectively. These results may suggest that 5-HT_1 receptor agonists are potent to induce spawning. Antagonists of 5-HT_2 receptor are strongly potent to interfere with spawning induced by 5-HT_1 receptor agonist; however, they are capable of partially inhibiting 5-HT-induced spawning. The latter note, itself, represents interaction between 5-HT binding sites [104] or suggests the presence of more than one type 5-HT receptor to regulate 5-HT-induced spawning.

In Japanese clam [122], 1, 10, 20, 50, 100, and 1000 μM α-methyl-5-HT injected into the foot induces spawning in 0, 25, 31, 63, 75, and 100% of specimens, respectively, compared to 0% in control and 100% in ≥20 μM 5-HT. In addition, Japanese clams injected with 10, 100, and

1000 μM 8-OH-DPAT into the foot spawns 15, 33, and 100%, respectively. In this species, neither TFMPP nor mCPBG induces spawning in Japanese clam. Injection of mianserin into the foot of Japanese clam decreases spawning to 25 and 0% at 100 and ≥500 μM, respectively. The mianserin-inhibited spawning can be partially overcome by the second injection of 20 μM 5-HT, resulting in 60 and 50% spawning at 100 and 500 μM, respectively. Based on the rank order of potency of the 5-HT agonists, the authors suggested that a mixed 5-HT_1/5-HT_2 receptor mediates 5-HT-induced spawning in this species. However, spawning of the individual pre-treated with mianserin may also suggest that 5-HT binding sites to induce spawning are different from those of mianserin. On the other hand, there might be more than one 5-HT receptor in the Japanese clam; however, 5-HT signaling seems to be mediated via a 5-HT_1 receptor.

4. Conclusion and future research perspectives

A few studies exist that investigate the characteristics of 5-HT binding site in the plasma membrane of the oocyte and sperm. Pharmacological profiles of binding sites in competition experiments suggest the presence of a single or mixed 5-HT_1, 5-HT_2, and 5-HT_3 receptors in bivalves. The phylogenetic analysis of 5-HT receptor suggests that classification of the bivalve 5-HT receptors based on available mammalian 5-HT receptor classification is not successful. It might be due to sensitivity and insensitivity of 5-HT binding sites to 5-HT analogs. On the other hand, the 5-HT receptor(s) in bivalves is distinct from those of other organisms. However, molecular identity of 5-HT receptor shows that the 5-HT receptor in bivalve seems to be a homolog of 5-HT_1 receptors in mammals.

Tissue distribution of the 5-HT receptor has shown that it is widely expressed in various organs, although its mRNA transcription is relatively high in the ovary and testis. This suggests multifunctional characteristics of 5-HT in bivalves. In addition, transcription of the 5-HT receptor undergoes seasonal variation. Studying 5-HT content and expression of 5-HT receptor in the nervous system and the gonad of bivalves will help us to better understand 5-HT signaling in reproduction.

To better understand receptor-mediated 5-HT signaling, it requires to produce genetic models of bivalves that do not express 5-HT receptor(s). Another valuable biological tool is to use bivalves that show natural alternations in 5-HT biosynthesis or natural disruption of reproduction. Bivalves host some parasites that particularly infect the reproductive system. For instance, Garnerot et al. [31] observed histopathological changes in the gonad of soft-shell clam infected with a trematode *Prosorhynchus squamatus*. In infected individual, the follicles and genital follicles are not surrounded by 5-HT-IR fibers around, and 5-HT staining is clearly visible inside the parasite. Another example is protozoan *Marteilioides chungmuensis* that become mature in the oocyte of the pacific oyster [126]. The parasites affect the reproductive follicles causing irregular enlargement of the infected gonadal tissues [127]. Although infected female oysters produced oocytes continuously and spawned repeatedly, however the parasites cause nutritional wasting and mortality, and affect the reproductive output of

infected female oyster [127, 128]. Ngo et al. [129] also reported that *M. chungmuensis* delays spawning and cause damages to ripe oocytes. These biological examples of parasite-infected bivalves can provide us with model organisms to study 5-HT regulation of gonadal development and gamete maturation.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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