The Biological Effects of Pharmaceuticals in the Marine Environment

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Abstract

Environmental pharmaceuticals represent a threat of emerging concern for marine ecosystems. Widely distributed and bioaccumulated, these contaminants could provoke adverse consequences on aquatic organisms, acting with modes of action like those reported for target species. Compared to a pharmacological use, organisms in field conditions are exposed to complex mixtures of compounds with either similar, different, or even contrasting therapeutical effects. This review summarizes current knowledge on main cellular pathways modulated by the most common classes of environmental pharmaceuticals occurring in marine ecosystems and accumulated by non-target species, including non-steroidal anti-inflammatory drugs (NSAIDs), psychiatric drugs, cardiovascular and lipid regulator agents, steroidal hormones and antibiotics. An intricate network of possible interactions is shown with both synergistic and antagonistic effects on the same cellular targets and metabolic pathways. This complexity reveals the intrinsic limit of the single-chemical approach to predict long-term consequences and future impact of pharmaceuticals at organismal, population and community levels.

1. Introduction on pharmaceuticals in the marine environment

The progress of medical science and continuous formulation of more efficient pharmaceutical drugs has allowed to reduce the impact of several pathologies, determining an increased life expectancy, better prevention of diseases, and general improvement of health quality (Mezzelani et al. 2018a). Consequently, global pharmaceutical market has quickly grown both in terms of total sales and number of synthesized active pharmaceutical ingredients, with a further increase expected in the future due to development and aging of human population (IQVIA, 2019, COM//2019/128 final).

Beside their beneficial role to human society, the huge consumption of pharmaceuticals has become an environmental concern for the widespread occurrence of these compounds in aquatic ecosystems and their potential adverse effects on non-target species. Specifically designed for being biologically reactive at very low concentrations, active ingredients have the potential to interfere on biochemical and physiological processes of non-target species, with virtually unknown long-term effects on marine ecosystems. The urgent need to elucidate such aspects is reflected in international actions, such as the development of guidelines for the Ecopharmacovigilance and regulatory approaches in Europe, USA, Japan and Australia (Jose et al. 2020). The European Strategic Approach to Pharmaceuticals in the Environment encourages efforts to enlighten long-term consequences of these compounds through innovative and multidisciplinary ecosystems-oriented approaches, ensuring that such actions do not limit the access to safe and effective treatments for humans and animals (COM//2019/128 final).

Pharmaceutical residues can enter the aquatic environment by a number of pathways including primarily wastewater treatment plants (WWTPs), but also industrial and hospital discharges, aquaculture facilities, animal farming, runoff of soils and direct discharge of untreated wastewater (Bagnis et al. 2019; Couto et al. 2019, de Oliveira et al. 2020, Mezzelani et al. 2018a). WWTPs are often of limited efficiency toward pharmaceuticals which have biorefractory behaviour and are minimally removed by primary and secondary wastewater treatment processes (Peake et al. 2016).

Tertiary treatments based on finer filtration techniques (sand filter, microfiltration, ultrafiltration, nanofiltration, reverse osmosis) and absorption by activated carbon, represent more advanced technologies with higher removal capacity, but of still limited application due to the elevated costs. Transfer of pharmaceuticals from WWTPs to surface waters has thus been extensively documented preluding the entry of these compounds in the marine environment (Couto et al. 2019, Sathishkumar et al. 2020).

The risk of pharmaceuticals in coastal areas has been ignored for a long time, hypothesizing an illimited dilution capacity of seawater. Contrasting with this misleading view, the presence of pharmaceuticals in marine ecosystems has been demonstrated worldwide, at concentrations ranging from a few ng/L up to hundreds of µg/L (Biel-Maeso et al. 2018, González-Alonso et al. 2017, Kümmerer, 2010, Mezzelani et al. 2018a). From the initial detection of a few anticancer drugs and synthetic steroids in early ’90, more than 160 active principles were measured in 2010, and almost 300 compounds have been actually reported in aquatic environments for various therapeutical classes like antibiotics, non-steroidal anti-inflammatory drugs, antidepressants, antihypertensives and antiepileptics (Aherne et al. 1990, Kümmerer, 2010, Mezzelani et al. 2018a). Despite the environmental persistence of pharmaceuticals is influenced by several variables including physical-chemical characteristic of the molecules, temperature, pH and photolysis by solar irradiation (Couto et al. 2019), the huge consumption and release might confer to these drugs the behaviour of pseudo-persistent pollutants. The urgency to prioritize the environmental sustainability of more than 4000 active principles potentially reaching natural ecosystems, has been identified as a major research need but, at this time, comprehensive monitoring programs for such emerging pollutants remain neither regulated nor mandatory. A basic prerequisite to achieve this goal is to integrate a better knowledge on released loads, with the assessment of bioavailability, uptake and biological effects of pharmaceuticals in non-target organisms.

Common classes of pharmaceuticals occurring in marine ecosystems and accumulated by non-target species include non-steroidal anti-inflammatory drugs (NSAIDs), psychiatric and

88 cardiovascular drugs, steroids and antibiotics. The detection of steroids hormones in sewage effluents
89 in early ’70 provided the first impetus to the study of pharmaceuticals in aquatic environments.
90 Natural and synthetic steroids are largely used in human and veterinary medicine and the amounts of
91 estrogens released by livestock and aquaculture are similar or even higher than the contribution
92 related to human population (Aris et al. 2014, Beardmore et al. 2001, Liu et al. 2015). The 17β-
93 estradiol (E2), 17α-ethynylestradiol (EE2) and Estrone (E1) are the more frequently detected
94 hormones in aquatic environments, and their accumulation has been shown in bivalves and in fish
95 collected from natural environments (Guedes-Alonso et al. 2017). Other active principles measured
96 in tissues of marine organisms include: diethylstilbestrol, estriol, norgestrel, norethisterone, megestrol
97 acetate, progesterone, testosterone, boldenone, nandrolone, cortisone, prednisone and prednisolone.
98 Among 15 steroids detected in molluscs, crabs, shrimps and fish from the southern coast of China,
99 norgestrel and progesterone were the most frequently detected and the occurrence of six synthetic
100 steroids in the feed demonstrated the illegal use of these drugs in local aquaculture farms (Liu et al.
101 2015).

102 Antibiotics have received scientific attention since the’90, given the direct correlation between
103 their growing consumption, occurrence in aquatic ecosystems and rapid expansion of antibiotic
104 resistance in microorganisms. Similarly to steroid hormones, antibiotics in human medicine are
105 coupled with their massive use in livestock and aquaculture. There are actually 5 antibiotics included
106 as priority active principles in the watch-list of the EU Water Framework Directive 2018/840/EU
107 (erythromycin, clarithromycin, azithromycin, amoxicillin and ciprofloxacin) but a recent study
108 suggested that also trimethoprim, sulfamethoxazole, tetracycline, oxytetracycline and ofloxacin are
109 of great environmental concern based on their common detection in surface waters and adverse effects
110 on microbial communities (Kovalakova et al. 2020). Bioaccumulation of antibiotics has been reported
111 in several marine vertebrates and invertebrates. As an example, sulfamethoxazole was dominant in
112 fish sampled from south Africa, in fish and shellfish from the Red Sea and in bivalves from the
113 Mediterranean and South Atlantic coastal areas (Ali et al. 2018, Álvarez-Muñoz et al. 2015, Ojemaye

& Petrik, 2019). Several antibiotics residues were detected in both organisms and fish feeds from the mariculture areas of the Pearl River Delta highlighting again that, despite the use of antibiotics as growth promoters in livestock and aquaculture has been forbidden in EU since 2006, they are still used in other parts of the world (Kovalakova et al. 2020, Xie et al. 2019).

NSAIDs have been shown as common pollutants in marine organisms, from benthic invertebrates up to top predators, with a certain biomagnification behaviour highlighted by the increased concentrations at higher trophic levels (Sathishkumar et al 2020). Diclofenac (DIC) has been detected in mussels from Belgian coasts, Mediterranean, South-East Atlantic Ocean, San Francisco Bay (Capolupo et al. 2017, Cunha et al. 2017, Mezzelani et al. 2020), and in fish species from Spanish and South Africa coasts (Ojemaye & Petrik, 2019). Occasionally, other active principles like acetaminophen, salicylic acid, nimesulide (NIM), naprossen and ibuprofen (IBU) have also been measured in tissues of marine vertebrates and invertebrates (Wolecki et al. 2019).

Possibly surprisingly for some readers, psychiatric drugs are highly represented in marine biota. The antiepileptic carbamazepine (CBZ) was measured in more than 90% of mussels collected from the Tyrrhenian Sea, Adriatic Sea, and Belgian coasts, in oysters from Ebro delta, and in several fish and shellfish species sampled in the Red Sea and Pearl River Delta (Ali et al. 2018, Mezzelani et al. 2020, Xie et al. 2019). The elevated distribution of this antiepileptic drug in marine organisms is partly related to its refractory properties which confer to CBZ an average half-life >200 days in aquatic ecosystems (Bu et al. 2016, Zhu et al. 2019). Marine organisms can also contain residues of antidepressants such as paroxetine (PAR), sertraline (SER), venlafaxine (VEN), citalopram (CIT), lormetazepam (LOR), fluoxetine (FLU) and its metabolites. LOR and PAR were detected in more than 40% of wild mussels from the Mediterranean, while 64% of bivalves collected along the California west coast contained SER; bioaccumulation of VEN, CIT and FLU has been further documented in organisms from the Mediterranean, Red Sea and Atlantic Ocean (Ali et al. 2018, Martínez-Morcillo et al. 2020, Mezzelani et al. 2020).

A few data are also available on bioaccumulation of cardiovascular drugs (CVDs) which include various therapeutic classes, like beta-blockers (atenolol, metprolol, propranolol), angiotensin-converting-enzyme inhibitors, angiotensin II receptor antagonists (valsartan) and calcium channel blockers (diltiazem). Such active principles have been detected in fish and invertebrates from the Portuguese and Spanish coasts, and from the Red Sea (Ali et al. 2018, Álvarez-Muñoz et al. 2015, Moreno-González et al. 2016): considering the massive worldwide consumption of these drugs for the treatment of the first cause of human death (World Health Organization, 2016), the limited number of studies on their environmental occurrence and biological consequences represent an important gap of knowledge.

2. Biological effects of pharmaceuticals on marine organisms

Elucidating biological effects and impacts of pharmaceuticals in marine ecosystems is a faceted and complex challenge. Organisms are typically exposed to complex chemical mixtures where individual compounds can interact through several mechanisms, causing either synergistic or antagonistic feedback, cascade effects which in turn modulate cellular and physiological responsiveness to other stressors, potentially up to population dynamics and ecosystem functioning.

Pioneering studies on deleterious effects and ecological risk of environmental pharmaceuticals were based on the application of acute toxicity tests on bacteria, algae, invertebrates and vertebrates. This approach revealed an apparent lack of risk with EC_{50} values for selected endpoints at concentrations more than one order of magnitude higher than those typically found in marine ecosystems. However, these data more realistically demonstrate that the onset of acute toxicity is highly improbable for such compounds, and that the exclusive use of ecotoxicological bioassays may underestimate potential adverse outcomes in marine organisms exposed to low doses but in long-term, chronic conditions (Brausch & Rand, 2011, Mezzelani et al. 2018a).

Pharmaceuticals exert their biological activity targeting metabolic, enzymatic, or cell-signalling processes through well characterized Mechanisms of Action (MoA): since molecular

targets are often evolutionarily conserved in many aquatic species, specific alterations at molecular and cellular levels may represent sensitive early warning signals for these drugs before the onset of long-term toxicological effects or changes at higher levels of biological organization. Available data on the effects of pharmaceuticals in non-target species are still too fragmented for delineating the overall impact of such active principles on marine organisms.

The aim of the next paragraphs is to summarize the common pathways of action, metabolism and toxicity of the most frequently detected therapeutic classes in marine environment, trying to elucidate parallelisms between target and non-target organisms. Compared to target "patients" who assume specific drugs in response to specific diseases, in field conditions non-target marine organisms are typically exposed to multiple stressors with combinations of several pharmaceuticals and compounds with either similar, completely different, or even contrasting therapeutical effects. In this respect, to provide a more ecologically relevant synthesis of responsiveness to such complexity, individual pathways of various pharmaceutical classes will be combined in a single picture (Figure 1) to highlight the intricate network of metabolic connections which can not be individually disentangled and in turn modulate potential adverse outcomes at biological and environmental levels.

3.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are widely consumed worldwide to treat a variety of symptoms including fever, pain, inflammation, rheumatoid arthritis, muscular and skeletal disorders. Mechanism of action and modulated pathways in target and non-target species are summarized in Figure 1. Irritation or injury conditions provoke increased levels of arachidonic acid (AA), a polyunsaturated fatty acid synthesized from cell membranes by cytosolic phospholipase A2 (PLA_2). The AA is transformed by the cyclo-oxygenase pathway (COX) with the formation of prostaglandins (PGs), an important group of lipid inflammatory mediators, collectively known as eicosanoids. The first COX reaction converts AA to prostaglandin G2 (PGG2), before its reduction to prostaglandin H2 (PGH2), and the final conversion to five biologically active primary PGs:

prostaglandin D2 (PGD2), prostaglandin E2 (PGE2), prostaglandin F2 (PGF2), prostacyclin (PGI2) and thromboxane A2 (TxA2). These eicosanoids differently modulate, either increasing or decreasing, the intracellular levels of cyclic adenosine monophosphate (cAMP) and calcium, exerting their effects on important neurophysiological functions, modulation of inflammation and immune responses, protein metabolism, contraction and relaxation of muscles or blood vessels (Burian, 2007). NSAIDs prevent the conversion of AA to prostaglandins and thromboxanes by inhibiting one or both the isoforms of the COX enzyme (Figure 1): COX-1, constitutively expressed in several tissues and involved in homeostatic, cytoprotective functions, while the inducible COX-2 is mainly responsible for pain perception and inflammation (Bacchi et al. 2012, Ghosh et al. 2015). According to their MoA, available NSAIDs can be classified as "Non-Selective" (i.e. IBU, ketoprofen KET) acting on both COX-1 and COX-2, "Selective" (i.e. celecoxib and rofecoxib) which target only the COX-2, and "Semi-selective" (i.e. DIC, indomethacin, meloxicam) with a higher affinity for COX-2 but also able to inhibit COX-1 pathways. Given the role of prostaglandins in the activation of both innate and adaptive immune responses, their inhibition by NSAIDs leads to the modulation of immune system which, in worst conditions, can result in excessive inflammatory cascades, accumulation of activated macrophages and ulceration.

COX metabolism of AA, prostaglandins (PGs) and other biologically active derivatives of polyunsaturated fatty acids have been detected in several marine invertebrates, such as sponges, corals, and molluscs. These compounds include PGI2, PGE2, PGD2, PGF2, with similar functions as in mammals (Di Costanzo et al. 2019). Evidence that AA metabolism and COX pathways are modulated in aquatic organisms by environmental NSAIDs have been documented in a large number of laboratory studies. Exposure to DIC (1 to 100 µg/L) caused the inhibition of PGE2 synthesis in *M. galloprovincialis*, and changes of COX activity in the digestive gland of the brown mussel *Perna perna* (0.5 µg/L) (reviewed by Sathishkumar et al. 2020). Similarities in MoA of NSAIDs between vertebrates and invertebrates were further confirmed in clams *Ruditapes philippinarum* and *Mytilus spp.*: following exposure to IBU (10-100 µg/L), KET and NIM (0.5-2.5 µg/L) transcriptional profile

revealed the differential expression of genes involved in AA metabolism, including up-regulation of PLA2, down-regulation of Thromboxane A Synthase 1(TBXAS1), Progesterone receptor (PGR1), Prostaglandin E2 receptor EP4 subtype (PTGER4) (Almeida et al. 2020a, Mezzelani et al. 2018b, Milan et al. 2013).

Despite invertebrates lack an adaptive immunity, they possess a potent and complex innate immune system similar to that of vertebrates. Free circulating haemocytes (i.e. granulocytes and hyalinocytes) are responsible for cell-mediated immunity and, when activated, are responsible of phagocytosis, reactive oxygen species production, release of hydrolytic enzymes and antimicrobial peptides. Early activation of immune system was observed in marine species in response to NSAIDs. Transcriptional profiles of marine invertebrates exposed to DIC, IBU, KET and NIM highlighted the modulation of genes regulating lysosomal enzymes, chitinases, Toll-like and NOD-like receptors (TLRs, NLRs), lectins (rhamnose-binding lectin RBL, hepatic lectin HLEC) and scavenger receptors (Almeida et al. 2020a, Mezzelani et al. 2018b, Milan et al. 2013). NLRs are intracellular sensors of pathogen-associated molecular patterns that enter the cell via phagocytosis, interacting with TLRs to regulate the immune response: the activation of immune system in response to environmental NSAIDs (0.05-25µg/L), progressed from transcriptional up to cellular level in *M. galloprovincialis* and *R. philippinarum,* with a significant impairment of haemocytes responsiveness, compromised lysosomal membrane stability, inhibition of phagocytosis activity and granulocytes-hyalinocytes ratio, and an overall reduction of immune surveillance against pathogens (Almeida et al. 2020a).

NSAIDs are also known to be substrates for the cytochrome P450 (CYP) biotransformation pathway (Figure 1). In mammals, CYP2C9 is the most important oxidase primarily responsible for metabolism of several NSAIDs (i.e. celecoxib, IBU, and naproxen), but also cytosolic phase I enzymes (i.e. CYP3A4, CYP2C19, CYP2C8) catalyse oxidative transformations; phase II reactions by UDP-glucuronosyltransferases (i.e. UGT1A1, UGT2B7, UGT1A9 and UGT2B4) produce glucuronides and reactive metabolites which are finally excreted through bile and ABCC2 efflux transporters (Bindu et al. 2020). The role of P450 in NSAIDs metabolism has been considered a key

243 factor in overcoming the adverse effect of these drugs and the elevated DIC toxicity has been
244 associated to its poor metabolization by cytochrome P450. Despite the P450 biotransformation
245 pathway in marine invertebrates still need to be fully elucidated, transcriptional responsiveness of
246 phase I and phase II-related genes was observed in response to environmental levels of NSAIDs
247 (Mezzelani et al. 2018a,b, Milan et al. 2013). Bivalves (*M. galloprovincialis* and *R. philippinarum*)
248 exposed to DIC, IBU, KET and NIM revealed the modulation of drug metabolism genes including,
249 among others, CYP1A, phase II-related genes (GSTA4, SULT1B1, GSTP2) and heat shock proteins
250 (HSP70L, HSP70I) (Figure 1).

251 In addition to COX inhibition and metabolism through CYP450, NSAIDs exert cytotoxic side-
252 effects which mostly derive from their capability to enhance the generation of reactive oxygen
253 species, ROS (Ghosh et al. 2015). Well known mechanisms include a direct targeted toxicity on
254 mitochondria, the potent induction (i.e. by DIC, naprossen, aspirin) of NADPH-oxidases (NOX) and
255 xanthine oxidoreductase (XOR), all typical sources of oxyradicals and lipid peroxidation (Figure 1);
256 NSAIDs contribute to prooxidant mechanisms also through their capability to uncouple inducible
257 nitric oxide synthase (iNOS), resulting in the generation of NO·instead of nitric oxide (NO); further,
258 NSAIDs modulate the ROS-mediated induction of apoptosis through the inhibition of Akt signalling
259 pathways, the downregulation of the transcription factor NF-κB and the alteration of p53 pathway
260 (Figure 1). Additional effects of NSAIDs depend on their high affinity for peroxisome proliferator
261 activated receptors (PPARs), with consequences on lipid metabolism, while their reactivity with
262 peroxidases enzymes is responsible for the inhibition of the acetylcholinesterase activity and the
263 consequent increase of cholinergic transmission (Muraoka & Miura, 2009).

264 In agreement with prooxidant effects described for target species, NSAIDs were shown to
265 modulate NF- kB pathway in both *Mytilus spp.* and clam *R. philippinarum,* increasing the mRNA
266 level of a genuine NF-kB gene (Maria et al. 2016, Milan et al. 2013). NF-kB family is a group of
267 heterodimeric transcription factors found in almost all animal cell types and involved in cellular
268 responses to stress, cytokines, ROS, ultraviolet radiation and trace metals; after its activation in the

cytoplasm, NF-kB is translocated to the nucleus, binds to specific DNA sequences (NF-kB responsive elements) and regulates the transcriptional induction of iNOS, as well as a number of genes mediating inflammatory responses and apoptosis (Regoli & Giuliani, 2014). Also genes involved in the PPAR signalling pathway (i.e. acetyl-coenzyme A acyltransferase 1, phosphoenolpyruvate carboxykinase, and stearoyl-CoA desaturase) were transcriptionally up-regulated in bivalves exposed to IBU, confirming the effects of NSAIDs on lipid and fatty acid metabolism of non-target organisms (Mezzelani et al. 2018b, Milan et al. 2013). Similar evidence was obtained for the effects of these drugs on p53 pathway activated by stimuli including ROS, trace metals, DNA damage and hypoxia. The p53 tumor suppressor protein is a transcription factor which binds to specific DNA regions, the p53 responsive elements (Figure 1) enhancing transcription of stress response genes involved in a variety of anti-proliferative processes, cell cycle checkpoints and arrest, DNA damage and repair, apoptosis (Regoli & Giuliani, 2014). Among these, both mussels and clams exposed to NSAIDs revealed an up-regulation of several related genes including a putative baculoviral IAP repeat-containing 2 (BIRC2), X-linked inhibitor of apoptosis (XIAP), BCL2-associated athanogene 4(BAG4), B-cell CLL/lymphoma 2 (BCL2), suggesting increased anti-apoptotic activity in contrast to effects measured in target species (Mezzelani et al. 2018a,b, Milan et al. 2013).

The effects of NSAIDs in promoting the generation of ROS and the related pro-oxidant mechanisms in marine organisms were confirmed at the cellular, functional level by the significant modulation of antioxidant enzymes (catalase CAT, superoxide dismutase SOD, glutathione reductase GR, glutathione S-transferases GST), the enhancement of peroxidation products like malondialdehyde and lipofuscin, the increment in micronuclei frequency and DNA damages observed in *Mytilus spp.*, *R. philippinarum*, *Gibbula umbillicalis* and *Hediste diversicolor* (Bebianno & Gonzalez-Rey, 2015, Mezzelani et al. 2018a,b, Milan et al. 2013). The decrement of neutral lipids and the inhibition of Acyl-CoA oxidase activity (ACOX) in *Mytilus spp.* further supported COX-mediated effects on AA and lipid metabolisms, while the inhibition of acetylcholinesterase activity

294 in IBU and DIC exposed organisms confirmed effects on cholinergic transmission (Mezzelani et al.
295 2018b, Sathishkumar et al. 2020).

296 Despite clear mechanistic evidence support molecular and cellular effects of NSAIDs in
297 marine organisms, long term consequences on organismal, population, community or ecosystem
298 functioning are still difficult to be predicted. Nonetheless, the effects of DIC in *Mytilus spp.* revealed
299 the disturbance of tyrosine and tryptophan/serotonin pathways allowing the authors to hypothesize
300 adverse consequences for osmotic and reproduction processes (Swiacka et al. 2019). In addition,
301 NSAIDs were shown to modulate genes involved in shell formation and biomineralization of early
302 mussel embryos, such as chitin synthase-CS, carbonic anhydrase-CA and extrapallial protein-EP
303 (Balbi et al. 2018), while in adult mussels these drugs impacted various physiological traits reducing
304 the byssus abundance and strength, along with a significant decrease of the scope for growth; the
305 reduced energy available for growth is considered as an overall index of performance which, if not
306 compensated, might anticipate future adverse consequences at the population level.

307

308 *3.2 Central Nervous System/Psychiatric Drugs*

309 Pharmaceuticals targeting the central nervous system also represent environmental
310 contaminants of emerging concern which include antidepressants acting as Selective Serotonin
311 Reuptake Inhibitors SSRIs (fluoxetine FLU, paroxetine PAR, fluvoxamine FVX, venlafaxine VEN,
312 sertraline SER, citalopram CIT and escitalopram ESC), and the antiepileptic carbamazepine CBZ
313 (Hillhouse & Porter 2015).

314 The SSRIs exert their function at the synaptic cleft by increasing the extracellular level of
315 serotonin (or 5-hydroxytryptamine 5-HT), a small molecule that functions both as a neurotransmitter
316 in the central nervous system and as a hormone in the periphery. Serotonin is synthesized from
317 tryptophan (Figure 1) and stored in synaptic vesicles which prevent its degradation by the enzyme
318 monoamine oxidase (MAO). Following the action potential, vesicles fuse with the presynaptic
319 membrane and release their content into the synaptic cleft where serotonin can bind 5-HT receptors,

modulating the associated signalling pathways. Some of the 5-HT receptors (as 5-HT1A and 5-HT1B) act as inhibitory autoreceptors, indicating whether high levels of 5-HT are present in the synapse, and thus inhibiting the further release of additional serotonin (Nichols & Sanders-Bush 2003). The excess of 5-HT in the synapse is either degraded by MAO or taken back into the presynaptic terminal by active serotonin transporter, 5-HTT (Figure 1). SSRIs are designed to inhibit the serotonin transporter (5-HTT) which determine the accumulation of this neurotransmitter in the synaptic cleft, enhancing the interaction with 5-HT receptors. These receptors are characterized by seven subtypes of transmembrane peptides which modulate different downstream effects and multiple cascades of biochemical reactions (Figure 1); the activation of 5-HT1 and 5-HT5 receptors results in the inhibition of adenylyl cyclase activity (AC), decreased cellular levels of cAMP and of cAMP-dependent protein kinases (PKA); conversely, the other receptors (5-HT4, 6, 7) produce an opposite, excitatory response increasing cellular levels of cAMP and the activation of downstream signalling pathways including MPAK, ERK 1/2 and the CREB cascade resulting in the induction of BDNF gene involved in neuroimmune regulation, fatty acid oxidation and gluconeogenesis (Faure et al. 2006).

Despite simpler than in mammals, serotonergic signalling system is evolutionary conserved and active in marine organisms, including fish and molluscs, where serotonin is involved in many neurophysiological processes, including sensitization and facilitation of withdrawal reflexes, feeding behaviour, locomotion and reproduction. Exposure of *M. galloprovincialis* to environmental concentrations of FLU (0.03-300 ng/L) significantly decreased cAMP levels and PKA activity: these effects were consistent with the inhibition of serotonin reuptake by FLU, its accumulation in post-synaptic cleft, more elevated interaction with 5-HT1 and consequent inhibition of AC activity (Fabbri, 2015, Franzellitti et al. 2014).

Interestingly, the 5-HT1 receptor, coupled to the inhibition of AC, is the unique serotonin receptor pharmacologically demonstrated in invertebrates. Mussels exposed to FLU exhibited a significant upregulation of 5-HT1 mRNA and down-regulation of the ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp), a key component of the multixenobiotic resistance (MXR) mechanism: the

346 modulation of Pgp by SSRIs supports its role in mussels as a general response toward a wide range
347 of chemical stressors (Fabbri, 2015, Franzellitti et al. 2014).

348 The increased serotoninergic neurotransmission in response to SSRIs has been postulated to
349 represent a key process in marine species, influencing many physiological responses (Fabbri, 2015).
350 In this respect, neurotoxic effects of low levels of FLU (0.03-5 µgL) were reported for various
351 bivalves, including *Mytilus.spp* and *R. philippinarum* with a significant inhibition of
352 acetylcholinesterase activity (Figure 1), leading to impairment of numerous cholinergic pathways,
353 hyperstimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission (Cortez et
354 al. 2018, Franzellitti et al. 2014, Gonzalez-Rey & Bebianno, 2013, Munari et al. 2014). In response
355 to FLU and SER the amphipod *Echinogammarus marinus* showed a significant downregulation of
356 *rhodopsin* and *arrestin*, neurological genes related to behaviour and phototransduction cascade, while
357 juvenile oysters, *C. gigas*, chronically exposed to FLU (28-days, 0.1 and 10µg/L), highlighted a
358 transient stimulation of shell growth suggesting a role of serotonin in the regulation of feeding and
359 metabolism in bivalves (Bossus et al. 2014, Gonzalez-Rey & Bebianno, 2013). Additional studies
360 further demonstrated serotonergic effects on locomotion in various marine invertebrates, regulating
361 pedal ciliary activity in marine snails (*Tritonea diomedea*), pedal muscle contractions and swimming
362 in nudibranchs (*Melibe leonine*), gill ciliary activity in mussels and oysters, increased locomotion in
363 crabs (*C. maenas*) and in amphipods (*E. marinus*), inhibited swimming behaviour in early life stages
364 of barnacles (*Amphibalanus Amphitrite*), rotifers (*Brachionus plicatilis*) and mussels (*M.*
365 *galloprovincialis*) (Estévez-Calvar et al. 2017, Fong & Ford, 2014). Low levels of FLU (1-10 ng/L)
366 caused phototactic responses in amphipods (*Gammarus pulex*) and inhibited striking prey efficiency
367 in newborn cuttlefish (*Sepia officinalis*). Similarly, VEN (5 ng/L) acted as a neurodevelopmental
368 toxicant for early life stages of *S. officinalis*, affecting the architecture of the vertical lobe, a key brain
369 structure for cognitive processing: a decrease in norepinephrine and in the relative number of NMDA-
370 like receptor binding sites was observed after 20 days of exposure, supporting a neurological

mechanism of action (Bidel et al. 2016). In the same study, a higher exposure concentration (100 ng/L) impaired the camouflage ability of the cuttlefish, a critical behaviour for its survival.

Beside their effects on serotonergic pathway, SSRIs have also either antioxidant or pro-oxidant activities depending on tissues, dose and presence of a pre-existing oxidative insult (Stefan et al. 2020). In this respect, mechanisms of action include modulation of intracellular ROS formation, antioxidants levels and interactions with key redox signalling pathways, such as the Keap1–Nrf2, NO and NF-κB pathways (Figure 1). Among the antioxidant effects, SSRIs can decrease the ROS levels by suppressing immune cells and secretion of interleukins typically associated to an over-production of oxyradicals; the inhibition of 5-HT reuptake and related metabolism (Kumar & Kumar 2009), as well as the reduced activity of CYP450 (i.e. CYP2D6, CYP1A2, CYP2C9, CYP2C19, CYP3A4) further lower the intracellular ROS release (Figure 1), thus contributing to enhance stability of antioxidant enzymes less subjected to protein carbonylation (Eren et al. 2007, Kumar & Kumar 2009, Rebai et al. 2017). Antioxidant mechanisms of SSRIs have been experimentally demonstrated also in terms of inhibition of NADPH oxidase (NOX) and consequent decrease of superoxide anion, inhibition of iNOS activity and NO levels, modulation of mitochondrial activity and apoptosis induction via the mitochondrial pathway (de Oliveira, 2016). In such less prooxidant cellular conditions, FLU increased cysteine availability, mRNA expression of glutamate cysteine ligase and glutathione synthesis (Eren et al. 2007, Moretti et al, 2012), while chronic exposure to SSRIs has been associated to enhanced levels of antioxidants through the up regulation of Nrf2 and expression of dependent genes (Bouvier et al. 2017) (Figure 1).

In contrast to the above antioxidant effects which typically occur in the presence of an already existing oxidative unbalance, SSRIs can act as prooxidants in non-stressed organisms, further complicating the prediction of their ecological impact in natural, field conditions. Prooxidant effects of FLU have been reported in terms of lipid peroxidation, disruption of cytosolic and mitochondrial membranes, increased mRNA expression of TNF-α, inflammation and apoptosis in hepatic tissues; FLU was shown to activate apoptotic pathway by increasing levels of proapoptotic Bax protein, while

lowering the expression of the antiapoptotic Bcl2 (Djordjevic et al. 2011). Modulation of the NF-kB pathway by SSRIs can further induce both antioxidant and prooxidant effects (Figure 1): the latter derive from the inhibition of Nrf2 and contemporary induction of NADPH oxidase while antioxidant effects are modulated through the increased transcription of several antioxidant genes, and by inhibiting ROS production via TNF-α.

Overall, data on oxidative effects of SSRIs agree with findings measured in several marine species. Induction of glutathione S-transferase activity was measured in *M. galloprovincialis, Perna perna* and *R. philippinarum* in response to FLU exposure (0.03-5µg/L), suggesting the activation of xenobiotic metabolism and antioxidant response: the concomitant impairment of immune parameters, decreased lysosomal stability, increment of MDA and a biphasic variation of antioxidant enzymes (CAT, GR, glutathione peroxidases, SOD) revealed a transient onset of oxidative insult (Franzellitti et al. 2014, Munari et al. 2014). FLU caused a significant down regulation of alkali-labile phosphates (ALP) in mussels gonads (*M. galloprovincialis*) suggesting potential endocrine disruptive effect of SSRIs, given the positive correlation between ALP and vitellogenin-like proteins levels (Franzellitti et al. 2014, Gonzalez-Rey & Bebianno, 2013, Munari et al. 2014). At physiological level, mussels (*M. californianus*) exposed for 107 days to environmental levels of FLU (0.3, 3, 30, and 300 ng/L) revealed adverse effects on algal clearance rates, growth and gonadosomatic index.

Among pharmaceuticals targeting the Central Nervous System, a particular environmental relevance should be given to carbamazepine (CBZ), an anticonvulsant drug used in human medicine to treat epilepsy, neuropathic pain and maniac disorders. In mammals, CBZ was shown to block the voltage-gated sodium channels preventing the generation of action potential and depolarization of cell, thus reducing the frequency of impulses during epileptic crisis (Ambrosio et al. 2002, Siebel et al. 2010); CBZ provoke the same effect also acting as an agonist at the gamma-aminobutyric acid (GABA) receptor with inhibition of glutamate release, and entry of chloride into the cell (Figure 1).

As mentioned above, biological targets of pharmaceuticals are evolutionarily conserved among vertebrates and invertebrates and many studies highlighted a certain similarity for the MoA

of CBZ in non-target marine species (Figure 1). The reduction of ion-channel opening by CBZ is at least partially mediated by a decrease of cAMP levels through direct inhibition of AC and consequent reduction of neuronal excitability (Fabbri, 2015). CBZ has been shown to inhibit AC and PKA activities in *Mytilus spp*., and to down-regulate the expression of ABCB mRNA, potentially lowering the detoxification ability of mussels (Fabbri, 2015). Concerning the effects of CBZ on voltage-, ligand-gated channels and ion transporters (Na + -K+ pump; Na+/Ca2+ exchanger), mussels (*M. galloprovincialis*) revealed a significant down-regulation of sodium-dependent serotonin transporter (SC6A4) at environmental levels of CBZ (1µg/L, 28 days) (Mezzelani et al. 2021). Similarly, the sea bream (*Sparus aurata*) exposed to low levels of this drug (6.95µg/L, 28 days) highlighted the transcriptional modulation of genes involved in transmembrane transport of Ca^{2+} such as calcium binding protein v2-like, calcium calmodulin-dependent serine protein kinase (CASK), and potassium intermediate/small conductance calcium-activated channel (KCNN4), representing an additional proof of similar mechanisms of action of CBZ across species (Hampel et al. 2017).

In mammals, CBZ undergoes to hepatic biotransformation resulting in epoxidation, aromatic hydroxylation, and conjugation reactions (Figure 1). The major drug-metabolizing enzymes involved in CBZ biotransformation include CYP3A4, CYP3A5, CYP2C8, EPHX1 and UGT2B7, with carbamazepine metabolites being excreted through the ABCC2 drug transporter. The cytochrome P450 biotransformation pathway and the enhanced production of ROS are partly responsible the major long-term side effects of CBZ, and similar metabolic pathways have been reported in non-target marine species exposed to low environmental levels of this drug. In *M. galloprovincialis*, CBZ (1 µg/L, 28 days) caused the up-regulation of genes involved in drug metabolism such as cytochrome P450 (CYP4F8, CYP3A2, CYP3A29) and sulfotransferases (SULT1B1), as well as the differential expression of genes involved in cell protection, cell cycle and DNA repair. Among these, a significant upregulation was observed for the stress induced protein Sestrin-3 (SEST3) that has a role in protection against oxidative and genotoxic stress, for genes belonging to GIMAP family (GIMAP4, GIMAP7) with a role in defence, cell differentiation and apoptosis, and for genes coding for

449 Baculoviral IAP repeat-containing protein (BIRC1, BIRC7), which modulate apoptotic processes. It
450 should be also highlighted the significant up-regulation of several genes modulating immune response
451 and inflammation (MYNA, LYZ1, DEFI, MRC1, IRG1, LITAF and PLA2) (Mezzelani et al. 2021).

452 Consistent with molecular evidences, CBZ (0.3-9µg/L) was shown to provoke cellular effects
453 potentially impairing the health status of marine organisms (Figure 1). Onset of oxidative stress,
454 activation of immune responses and neurotoxicity were observed in the bivalves *M. galloprovincialis,*
455 *Venerupis decussata, R. philippinarum, Scrobicularia plana* and in the polychaete *Diopatra*
456 *neapolitana*, revealing the induction of CYP450 3A4, modulation of antioxidant enzymes (CAT,
457 SOD, GR and GST), accumulation of lipid peroxidation products (malondialdehyde, lipofuscin,
458 neutral lipids), decrease of lysosomal membrane stability, inhibition of phagocytosis capacity and
459 increase of AChE activity (Almeida et al. 2020a, Freitas et al. 2016, Hampel et al. 2017, Mezzelani
460 et al. 2021).

461 CBZ was also documented to cause effects possibly leading to adverse consequences at higher
462 levels of biological organization. In early life stages of *M. galloprovincialis*, environmental
463 concentrations of CBZ affected embryo development with appearance of shell malformations in D-
464 veligers 48 h post fertilization even at 0.01µg/L. Transcriptional analyses revealed the down-
465 regulation of genes related to homeostasis of carbonate chemistry at the site of calcification (EP) and
466 in organic matrix synthesis (CS); these genes control calcification rates and morphology of the shell,
467 suggesting a mechanistic explanation for the negative impact of CBZ on regulation of shell
468 biogenesis. The role of CBZ as endocrine disruptor, interfering with synthesis, bioavailability or
469 breakdown of juvenile hormones or ecdysteroids, was highlighted by the significant modulation of
470 androgen and estrogen metabolism (Hampel et al. 2017). At organismal level, this drug altered
471 physiological condition (CI) and gonadosomatic index (GSI) in mussels, leading to hypothesize a
472 potential impairment of reproductive capacity while lower values of CI in chronically exposed
473 organisms were probably related to the reduced feeding capacity (Oliveira et al. 2017). Overall, these
474 responses could trigger a slow-down in the metabolism, resulting in a decrease of the reproductive

475 performance and energy reserves, with long-term adverse consequences on population sustainability
476 (Oliveira et al. 2017).
477
478 *3.3 Cardiovascular drugs and lipid regulating agents*
479 Cardiovascular drugs (CVDs) are largely used in human medicine for treatment of
480 hypertension, myocardial infarction, heart failure and coronary artery disease. CVDs include an array
481 of compounds that directly regulate the function of the heart and blood vessels, like beta-blockers,
482 angiotensin converting-enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs) and
483 calcium channel blockers. These drugs are commonly prescribed with lipid regulating agents (LRs),
484 such as statins and fibrates, which indirectly reduce the risk of severe cardiovascular diseases by
485 treating dyslipidaemias. Compared to other therapeutical classes described so far, ecotoxicological
486 impacts of CVDs and LRs are quite unexplored, especially in marine species, with a few data
487 available mostly for beta-blockers, angiotensin II receptor blockers and lipid regulators.
488 Beta-blockers target β-adrenergic receptors (β-Ars) which, after stimulation from endogenous
489 catecholamines, activate the upregulation of adenylyl cyclase converting ATP to cAMP; the latter is
490 used by cAMP-dependent protein kinase A (PKA) to phosphorylate calcium channels, thus increasing
491 cellular calcium influx available for muscle contraction. By antagonizing β-Ars, beta-blockers (i.e.
492 propranolol PRP, atenolol ATE, metoprolol MET) prevent the binding of norepinephrine and
493 epinephrine, decreasing levels of cyclic AMP and PKA (Figure 1). β-blockers can also induce
494 behavioural side-effects and depressive moods, indicating that they affect some central nervous
495 mechanisms due to their high affinity towards serotonin receptors (Alhayek & Preuss 2021). ACE
496 inhibitors and angiotensin II receptors blockers (ARBs) limit the action of the hormone angiotensin
497 II which has constricting effects on blood vessels, as well as on salt and water retention: while ACE
498 inhibitors reduce the levels of this compound by targeting the enzyme catalysing its formation (renin),
499 ARBs (like Valsartan VAL and Lorsartan LRS) selectively bind to angiotensin type 1 (AT1)
500 receptors. β-blockers are metabolized by P450 (CYP1A2 and CYP2D6) and phase II glucuronidation

501 reactions (Figure 1), while the majority of ARBs are poorly influenced by CYP enzymes and directly
502 undergo to de-ethylation and glucuronidation processes. Among lipid regulators, statins decrease the
503 levels of cholesterol competitively inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase
504 (HMG-CoA reductase), the main enzyme responsible for its biosynthesis, while fibrates lower
505 triglycerides and low-density lipoproteins by stimulating the peroxisomal β-oxidation of fatty acids
506 (Pahan, 2006) (Figure 1).

507 Despite investigations on the effects of CVDs and LRs in non-target organisms are still rather
508 limited (Zhang et al. 2020), adrenoceptors of the β-type were reported in marine species with relevant
509 pharmacological properties similar to those of their mammalian counterparts (Fabbri, 2015). A wide
510 range of environmentally realistic concentrations of PRP (0.3–300 ng/L) significantly decreased
511 cAMP levels and protein kinase A (PKA) activities in digestive gland of *M. galloprovincialis*,
512 consistent with the antagonistic effect of this drug on β-Ars and serotonin receptors: these
513 biochemical observations were paralleled by a similar pattern of mRNA expression for the ABCB1
514 gene, encoding for the membrane transporter Pgp which is also modulated by cAMP and PKA
515 (Fabbri, 2015). β-adrenergic receptors were transcriptionally regulated in zebrafish embryos exposed
516 to propranolol (10 μg/L and above for 96 h) and additional molecular effects of beta-blockers were
517 observed on antioxidant responses (CAT) and apoptosis pathway (p53, PUMA) (Sun et al. 2014).
518 The involvement of oxidative stress in metabolism of beta-blockers by marine organisms was
519 confirmed in mussels exposed to PRP showing decreased lysosomal membrane stability, significant
520 variations of antioxidant enzymes (CAT, GST), induction of carboxylesterase in digestive gland and
521 inhibition of AChE in gills (Fabbri, 2015, Solé et al.2010) (Figure 1).

522 The comparison of pharmaceuticals effects between target and non-target species is difficult
523 when considering angiotensin II receptor blockers (ARBs), given the paucity of investigations
524 performed in marine species. In this respect, LRS (300-3000 ng/L), caused the induction of CYP450
525 like activity, glutathione S-transferases and glutathione peroxidases in mussel *Perna perna* along with
526 the onset of oxidative and cyto-genotoxic effects (Zhang et al. 2020).

A few studies carried out on lipid regulators indicated a significant reduction of triglycerides and an increase of fatty acids in of zebra mussels exposed to low levels of clofibrate (0.2 µg/L, Lazzara et al. 2012), while gemfibrozil (1 µg/L-1000 µg/L) caused oxidative stress conditions with enhanced lipid peroxidation and glutathione transferases (GSTs) (Quinn et al. 2011). Variations of plasma triglycerides and cholesterol levels in fish exposed to gemfibrozil were reflected in changes of representative hepatic genes involved in lipid metabolism, including FABP, APOA1, APOEB, FASN, SREBP2, HMGCR1, PPARA and SREBP1 (Al-Habsi et al. 2016, Skolness et al. 2012).

At physiological level 19-days of PRP exposure (100-1000µg/L) significantly decreased byssus strength and energy available for growth and reproduction in *M. edulis*, suggesting possible consequences at the population level. PRP affected the embryonic development of sea urchin *Paracentrotus lividus* at 5µg/L and led to fecundity decrease, growth inhibition and alterations of heart and metabolic rates in *Daphnia magna*, with reported multi-generational effects (Zhang et al. 2020).

3.4 Steroidal Hormones/estrogens

An increasing body of evidence has demonstrated the high environmental concern for steroidal hormones, provoking deleterious effects in non-target marine species at concentrations one order of magnitude lower than those of other active principles (Almeida et al. 2020b, Aris et al. 2014, Capoluopo et al. 2018, Roark, 2020). The main target of steroids hormones is the endocrine system, responsible for maintaining organisms homeostasis, development, behaviour and reproduction (Aris et al. 2014). Although various hormones have been measured in marine matrices, the majority of studies focussed on estrogens and particularly on 17α-ethinylestradiol (EE2), widely used as a major component in oral contraceptives, and considered the synthetic drug with the highest endocrine disrupting potency (Almeida et al. 2020b).

Physiological estrogens are produced from cholesterol and a detailed discussion on their effects is outside the aim of this paper since excellent reviews are already available both for mammals

553 and marine organisms (Fuentes & Silveyra, 2019, Milla et al. 2011). Very briefly, the classical
554 mechanism of direct genomic signalling is modulated by the nuclear estrogen receptors (ERs) which
555 act as ligand-activated transcription factors (Marino et al. 2006, O'Malley, 2005). Upon binding in
556 the cytoplasm with estrogens (or estrogen-like xenobiotics), a conformational change of ER occurs,
557 inducing receptor dimerization, the complex is then translocated to the nucleus, and its binding to
558 chromatin at ERE sequences, enhances transcription of target genes (Figure 1). Estrogens can also
559 regulate transcription of other genes by indirect genomic signalling when the estrogen receptor
560 complexes do not bind directly to ERE sequences, but rather interact with other transcription factors
561 (TFs) and response elements, thus activating or suppressing target gene expression (Figure 1). Along
562 with the genomic signalling, estrogens are able to exert rapid cellular effects through nongenomic
563 mechanisms. The interaction with membrane bound ER directly activates signalling cascades, such
564 as the Ras/Raf/MAPK cascade, the phosphatidyl inositol 3 kinase (PI3K)/Akt kinase cascade, and the
565 cAMP/ PKA signalling pathways which modulate intracellular levels of Ca^{2+} and NO, as well as the
566 expression of ERE and TF regulated genes (Figure 1).

567 In a variety of tissues, ERs coexist with serotonin receptors, and many studies highlighted
568 effects of estrogens signalling on serotonergic pathway. Both natural and pharmacologically induced
569 changes in estradiol (E2) levels alter the concentration of serotonin, i.e. enhancing the activity of
570 tryptophan hydroxylase (TPH), the rate-limiting step in synthesis of this neurotransmitter from
571 tryptophan (Figure 1). In addition, E2 inhibits the gene expression of the serotonin reuptake
572 transporter (5-HTT) and acts as an antagonist at the 5-HTT, thus promoting the permanence of
573 serotonin in synapses and interstitial spaces (Rybaczyk et al. 2005). The estrogens signalling pathway
574 is also closely related with prostaglandins and arachidonic acid metabolism (Figure 1), and the
575 modulation of PG receptors by sex steroids has been shown as fundamental for key reproductive
576 processes (Blesson et al. 2012).

577 In teleost fishes, three estrogen receptors have been characterized, ERα1, ERβ1 and ERβ2,
578 and agonistic activity of EE2 revealed astonishing similarities in molecular pathways of this

579 signalling system between mammals and fishes (Amenyogbe et al. 2020). One of the most commonly
580 measured effects in fish is the increment of vitellogenin (VTG), an estrogen-inducible yolk precursor
581 protein normally produced only by mature females but detected also in juveniles and males of fish
582 exposed to estrogen-like compounds. The synthesis of VTG is initiated with activation of ERs
583 signalling pathway by estrogens, the enhancement of VTG gene expression, translation and
584 maturation of the protein in the endoplasmic reticulum and Golgi apparatus, before the final
585 packaging into secretory vesicles and release into the circulatory system. Feminization of males is a
586 more severe effect reported in fish exposed to EE2, with development of ovotestes in oviparous
587 species, presence of oocytes in male gonads, impaired spermatogenesis, decrement of sperm motility
588 and sperm counts (Almeida et al. 2020b, Aris et al. 2014, Notch et al. 2007). Additional detrimental
589 effects include an increased frequency of cancers allowing to hypothesize EE2 as a promoter of
590 hepatic tumour formation, by reducing the capability to repair DNA adducts by nucleotide excision
591 repair (NER) processes (Aris et al. 2014, Notch et al. 2007). Behavioural studies provided further
592 support to adverse physiological effects of estrogens with predictable consequences at the population
593 level. Among the several reported evidences, the sand goby *Pomatoschistus minutus* exposed to EE2
594 (41ng/L, 31 days) revealed altered reproductive behaviour of males which normally exhibit specific
595 movements to attract the females' attention and to provide parental care to developing eggs (Saaristo
596 et al. 2010). Exposure to low levels of EE2 (1 ng/L, 10 days) impacted secondary sexual trait
597 expression and mating dynamics of the Gulf pipefish *Syngnathus scovelli*, a species in which males
598 receive and fertilize eggs into a specialized brood pouch where developing embryos are carried for
599 approximately two weeks: the appearance in adult males of female-like secondary sexual traits, while
600 not directly affecting their reproductive capability, induced females to discriminate in mate choice
601 trials, an effect which would reduce male mating opportunities and long-term reproductive success
602 in natural populations (Partridge et al. 2010). Similarly, in the sex-role reversal seahorse
603 *Hippocampus erectus,* environmentally relevant concentrations of EE2 and progesterone (5 ng/L, 50

604 ng/L, 10 ng/L, 100 ng/L, 60 days), significantly inhibited male brood pouch development, impaired
605 the expression of spermatogenesis genes in the testes and caused male feminization (Qin et al. 2020).
606 Knowledge on the effects and mechanisms of action of estrogens is more limited for
607 invertebrates (Almeida et al. 2020b) but the presence of sex-dependent steroids and steroidogenic
608 pathways has been described in different invertebrate groups, particularly in molluscs, where their
609 role has been elucidated in the control of gametogenesis (Janer & Porte, 2007, Porte et al. 2006).
610 Estrogen-like receptors have been characterized in gastropods and cephalopods and corresponding
611 sequences have been identified in bivalves (Canesi et al. 2010). Although phylogenetically clustered
612 with other steroid receptors, molluscan ERs appear to be functionally different, with a constitutive
613 transcriptional activity, not further activated by estrogens. Nonetheless, 17β-estradiol (E2) was shown
614 to activate 'alternative' modes of action in ganglia and immune cells of *Mytilus* spp, i.e. through
615 modulation of Ca^{2+}- and kinase-mediated cascades (Canesi et al. 2004, 2006). Environmentally
616 realistic concentration of EE2 (5 and 50 ng/L) determined significant increase in the expression of
617 VTG and estrogen receptor 2 (ER2) in both female and male mussels (*M. edulis*), along with the
618 decrease in serotonin receptors and COX mRNA levels (Almeida et al. 2020b). In *M.*
619 *galloprovincialis* E2 affected different functional parameters and increased the expression of
620 antioxidant genes in hepatopancreas (Canesi et al. 2010).
621 Available studies on marine invertebrates (polychaete worms, molluscs, and crustaceans)
622 indicate that EE2 can cause developmental delays and female-biased sex ratios (Roark, 2020). In
623 bivalves, changes in the reproductive function and energy metabolism were frequently observed. A
624 lowered percentage of fertilized eggs and of normal larvae were caused in *M. galloprovincialis* by
625 exposure to EE2 (5–500 ng/L, 48 h), probably due to the strong energy depletion on spermatozoa,
626 resulting in a decreased viability when finally exposed to eggs (Almeida et al, 2020b). Gonadal atresia
627 and a delayed gonadal development were caused by EE2 (50 and 500 ng/L, 10 days) in *M. trossulus*,
628 while the rock oysters *Saccostrea glomerata* (at EE2 50 ng/L, 56 days) exhibited VTG induction and
629 intersex in males and females, highlighting the role of estrogens in modulating steroidogenesis and

630 sexual reversion in molluscs (Almeida et al, 2020b). Overall, these data support the hypothesis that,
631 although the invertebrate ERs do not mediate genomic estrogen signalling, conserved nongenomic
632 pathways are likely candidates for similar mechanisms of action.
633
634 *3.5 Antibiotics*
635 A final comment on environmental pharmaceuticals should be related to antibiotics.
636 Compared to other therapeutical classes, these drugs represent an emerging environmental hazard for
637 the development of antibiotic resistant bacteria (ARB) and transfer of antibiotic resistance genes
638 (ARGs), more than for the potential ecotoxicity of such molecules toward non-target species (Välitalo
639 et al. 2017).
640 Antibiotic resistance derives from selective pressure on sensitive bacteria resulting in
641 enrichment of ARGs, which are normally present in microbial communities at background levels.
642 Further exacerbated by environmental factors and co-occurring contaminants, the presence of ARGs
643 confers resistance to antibiotics mostly through transmembrane efflux pumps, enzymatic
644 deactivation, and cellular protection (Carvalho & Santos, 2016, Zheng et al. 2021).
645 In the marine environment, ARGs may be subjected to horizontal gene transfer (HGT)
646 representing a possible risk for organisms and humans: in this respect, the World Health Organization
647 recognized the occurrence of ARB and ARGs as one of the most important public health concerns of
648 this century (Ben et al. 2019, Kovalakova et al. 2020). ARGs can be transferred by direct contact with
649 seawater or indirectly through food webs and seafood consumption. Shaping typology and resistance
650 of gut bacteria, the transfer of ARGs can have adverse effects on important functions like digestive
651 processes, immune responses and vulnerability to infectious diseases (Zheng et al. 2021).
652 The environmental impact of antibiotics and ARGs has been shown particularly on microbial
653 communities, affecting richness and diversity of primary producers and decomposers, which are
654 essential for the microbial ecosystem functioning (Zheng et al. 2021). Cyanobacteria and ammonium
655 oxidizing bacteria appeared sensitive to antibiotics such as amoxicillin, ampicillin, ciprofloxacin and

clarithromycin, with EC50 values similar to realistic seawater concentrations (reviewed by Välitalo et al.2017). Reported effects spanned from protein synthesis and inhibition or interference with DNA replication, to the modulation of photosynthesis-mediated calcification and enhancement of cyanobacteria biofilms formation; the latter results highlight how antibiotic residues in marine environment may also influence the biofilm-associated ecological functions of cyanobacteria, i.e., promoting precipitation of carbonate and the increment of atmospheric carbon dioxide concentration (Kovalakova et al. 2020, Välitalo et al.2017). At a lower extent compared to cyanobacteria, also green algae exhibit a certain sensitivity toward antibiotics: several species, such as *Pseudokirchneriella subcapitata, Desmodesmus subspicatus, Chlorella vulgaris, Scenedesmus vacuolatus,* and *Tetraselmis suecica*, were affected by the macrolides clarithromycin and erythromycin with EC50 values below 1 mg/L: mechanisms of toxicity could be related to the inhibition of pathways involved in chloroplast and photosynthetic metabolism, leading to final impairment of cell growth (Välitalo et al. 2017). Long-term exposure to environmental levels of sulfamethoxazole and norfloxacin inhibited the green microalgae *Chlorella sp.*, while stimulated the growth of *Prorocentrum lima,* indicating a role of antibiotics in the bloom of red tides (Niu et al. 2019). The bactericide and bacteriostatic effects of these compounds can also cause disappearance of some microbial subpopulations with consequent effects on their ecological functions like modulation of biogeochemical cycles, changes in nitrogen transformation, methanogenesis, sulfate reduction, nutrient cycling, and organic matter degradation (Kokalokova et al. 2020): the inhibition of denitrification by sulfonamides was shown to stimulate the release of nitrous oxide (N_2O) with consequent enhancement of eutrophication processes and greenhouse effects (Mezzelani et al. 2018a).

Beside the indirect effects modulated by ARGs, knowledge is actually limited on direct, chronic toxicity of environmental antibiotics in non-target species. In this respect, the clam, *R. philippinarum* exposed to realistic concentrations of trimethoprim, highlighted the alteration of haemocytes parameters, with a significant decrement in lysosomal membrane stability, while oxidative stress responses were only slightly affected (Binelli et al. 2009, Matozzo et al. 2015).

682 Similarly, low doses of sulfamethoxazole affected the metabolomic profile in *M. galloprovincialis*,
683 with alterations in amino acids levels (aspartate, phenylalanine, valine, and tryptophan) pinpointing
684 disturbances in osmotic regulation and energy metabolism (Serra-Compte et al. 2019). Mechanisms
685 of action of antibiotics in non-target species are still to be clarified, and further effort is thus needed
686 to fill this gap of knowledge (Kovalakova et al. 2020, Välitalo et al. 2017).
687
688 **5. Final Thoughts**
689 Environmental consequences of pharmaceutical residues have recently emerged as a major
690 research area in marine science. Fragmented information is still available for non-target species, with
691 prevalence of studies focussing on NSAIDs and psychiatric drugs compared to cardiovascular,
692 synthetic steroidal hormones and antibiotics. Well-documented effects of single classes of
693 pharmaceuticals, dosed at low and environmentally realistic concentrations, evidenced marked
694 similarities in modes of action between target and non-target species, showing the same cellular
695 pathways involved in metabolism or onset of adverse consequences.
696 However, a key feature to consider when assessing the impact of pharmaceuticals on marine
697 species is the typology and conditions of exposure. Target organisms typically assume specific
698 compounds intended to alleviate a particular disturbance at defined posology and time of treatment:
699 conversely, in field conditions non-target species are exposed, potentially for the entire duration of
700 their life cycle, to low doses of several classes of co-occurring drugs that, at the same time, will
701 modulate a variety of pathways and metabolic processes. This review highlighted the complexity of
702 such interactions, revealing either synergistic or antagonistic effects on the same cellular targets.
703 Noteworthy, the intricate network of mechanisms regulating organism responsiveness to
704 pharmaceuticals might be further challenged by the simultaneous presence of other typologies of
705 chemical pollutants (trace metals, polycyclic or halogenated hydrocarbons, microplastics, biotoxins,
706 etc.) or environmental stressors (such as ocean acidification and temperature increase) which target
707 the same cellular pathways.

The reviewed results need to be interpreted with a certain caution, given the high variability in reported biological endpoints, doses and mode of exposures, typologies of investigated drugs, life stage and characteristics of non-target species. Nonetheless, the intrinsic limit of a single-chemical approach appears evident, and predictions obtained evaluating potential risk of individual classes of pharmaceuticals, might not necessarily be confirmed in natural, environmental conditions.

Future research and field monitoring studies need to consider more comprehensive strategies for assessing the impact of multiple stressors, particularly considering that the continuous development of new drugs makes risk assessment of pharmaceuticals not affordable on a chemical characterization alone. Due to the lack of acute effects, it is imperative to move toward evaluation of chronic effects for which, however, the contribution of various stressors is more difficult to disentangle. Despite convincing mechanistic hypotheses can explain molecular or cellular effects of pharmaceuticals, only a few studies documented a progression of adverse consequences on physiological performances as a function of dose and time of exposure. In this respect, links with organismal, population, community or ecosystem functioning are still hard to be forecasted, but not less important for this difficulty.

Disclosure Statement

The authors, Marica Mezzelani and Francesco Regoli, declare that they have no known competing financial interests or personal relationships with other people or organizations that could have inappropriately influenced the work reported in this paper.

Literature cited

Aherne GW, Hardcastle A, Nield AH. 1990. Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples. *J. Pharm. Pharmacol*. 42, 741-42.

732 Al-Habsi AA, Massarsky A, Moon TW. 2016. Exposure to gemfibrozil and atorvastatin affects
733 cholesterol metabolism and steroid production in zebrafish (Danio rerio). *Comp. Biochem. Physiol.*
734 *B Biochem. Mol. Biol.* 199, 87–96.
735 Alhayek S, Preuss CV. 2021. Beta 1 Receptors. Stat Pearls Publishing, LLC. PMID: 30422499
736 Ali AM, Rønning HT, Arif WMA, Kallenborn R, Al-Lihaibi, SS. 2017. Occurrence of
737 pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of
738 the Red Sea. *Chemosphere* 175, 505–13.

739 Almeida A, Solé M, Soares A, Freitas R. 2020a. Anti-inflammatory drugs in the marine environment:
740 bioconcentration, metabolism and sub-lethal effects in marine bivalves. *Environ. Pollut.* 263, 114442.
741 Almeida Â, Silva M G, Soares A, Freitas R. 2020b. Concentrations levels and effects of 17alpha-
742 Ethinylestradiol in freshwater and marine waters and bivalves: A review. *Environ. Res.*185, 109316.
743 Amenyogbe E, Chen G, Wang Z, Lu X, Lin M, Lin AY.2020. A Review on Sex Steroid Hormone
744 Estrogen Receptors in Mammals and Fish. *Int J Endocrinol*. 5386193.
745 Alvarez-Muñoz D, Rodríguez-Mozaz S, Maulvault AL, Tediosi A, Fernández- Tejedor M, et al.
746 2015a. Occurrence of pharmaceuticals and endocrine disrupting compounds in macroalgaes,
747 bivalves, and fish from coastal areas in Europe. *Environ. Res.* 143, 56–64.

748 Ambrosio AF, Soares-Da-Silva P, Carvalho CM, Carvalho AP. 2002. Mechanisms of action of
749 carbamazepine and its derivatives, oxcarbazepine, BIA 2-093, and BIA 2- 024. *Neurochem Res.* 27,
750 121-130.
751 Aris AZ, Shamsuddin AS, Praveena SM. 2014. Occurrence of 17α -ethynylestradiol (EE2) in the
752 environment and effect on exposed biota: a review. *Environ. Int.* 69, 104–19.
753 Bacchi S, Palumbo P, Sponta A, Coppolino MF. 2012. Clinical pharmacology of non-steroidal anti-
754 inflammatory drugs: a review. *Antiinflamm Antiallergy Agents Med Chem* 11: 52–64.

755 Bagnis S, Fitszsimons, MA, Snape J, Tappin A, Comber S. 2019. Impact of the wastewater-mixing
756 zone on attenuation of pharmaceuticals in natural waters: implications for an impact zone inclusive
757 environmental risk assessment. *Sci. Total Environ.* 658, 42–50.
758 Balbi T, Montagna M, Fabbri R, Carbone C, Franzellitti S, Fabbri E, Canesi L. 2018. Diclofenac
759 affects early embryo development in the marine bivalve Mytilus galloprovincialis. *Sci. Total Environ*.
760 642, 601-09
761 Beardmore JA, Mair GC, Lewis RI. 2001. Monosex male production in finfish as exemplified by
762 tilapia: applications, problems, and prospects. *Aquaculture.* 197(1–4):283–301.
763 Bebianno MJ, Gonzalez-Rey M. 2015. Ecotoxicological Risk of Personal Care Products and
764 Pharmaceuticals in Claude Amiard-Triquet, Jean-Claude Amiard and Catherine Mouneyrac, Aquatic
765 Ecotoxicology, Advancing Tools for Dealing with Emerging Risks, vol. 16. Academic Press Ch, pp.
766 383-416.
767 Ben YJ, Fu CX, Hu M, Liu L, Wong, MH, Zheng C.M. 2019. Human health risk assessment of
768 antibiotic resistance associated with antibiotic residues in the environment: a review. *Environ. Res.*
769 169, 483-93.
770 Bidel F, Di Poi C, Budzinski H, Pardon P, Callewaert W et al. 2016. The antidepressant venlafaxine
771 may act as a neurodevelopmental toxicant in cuttlefish (Sepia officinalis). *Neurotoxicology* 55, 142-
772 53.
773 Biel-Maeso M, Baena-Nogueras RM, Corada-Fernández C, Lara-Martín PA. 2018. Occurrence,
774 distribution and environmental risk of pharmaceutically active compounds (PhACs) in coastal and
775 ocean waters from the Gulf of Cadiz (SW Spain). *Sci. Total Environ.* 612, 649-59.
776 Bindu S; Mazumder S; Bandyopadhyay U. 2020. Non-steroidal anti-inflammatory drugs (NSAIDs)
777 and organ damage: A current perspective. *Biochem. Pharmacol.*180, 114147.
778 Binelli A, Parolini M, Cogni D, Pedriali A, Provini A. 2009. A multi-biomarker assessment of the
779 impact of the antibacterial trimethoprim on the non-target organism zebra mussel (Dreissena
780 polymorpha). *Comp. Biochem. Physiol*., C 150, 329-36.

781 Blesson CS, Büttner E, Masironi B, Sahlin L. 2012. Prostaglandin receptors EP and FP are regulated
782 by estradiol and progesterone in the uterus of ovariectomized rats. *Reprod. Biol. Endocrinol.: RB&E*,
783 10, 3.
784 Bossus MC, Guler YZ, Short SJ, Morrison ER, Ford AT. 2014. Behavioural and transcriptional
785 changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and
786 sertraline. *Aquat. Toxicol.*151, 46–56.
787 Bouvier E, Brouillard F, Molet J, Claverie D, Cabungcal JH et al. 2017 Nrf2-dependent persistent
788 oxidative stress results in stress-induced vulnerability to depression. *Mol Psychiatry* 22, 1701–13.
789 Brausch JM, Rand GM. 2011. A review of personal care products in the aquatic environment:
790 environmental concentrations and toxicity. *Chemosphere* 82, 1518-32.
791 Bu Q, Shi X, Yu G, Huang J, Wang B. 2016. Assessing the persistence of pharmaceuticals in the
792 aquatic environment: challenges and needs. *Emerg. Contam.* 2 (3), 145–147.
793 Burian M. 2007. Chapter NSAIDs, Mode of Action, Encyclopedia of Pain, ISBN: 978-3-540-43957-
794 8.
795 Canesi L, Borghi C, Ciacci C, Fabbri R, Vergani L, Gallo G. 2007. Bisphenol-A alters gene
796 expression and functional parameters in molluscan hepatopancreas. *Mol. Cellul. Endocrinology,*
797 276(1-2), 36–44.
798 Canesi L, Ciacci C, Lorusso LC, Betti M, Guarnieri T, Tavolari S, Gallo G. 2006. Immunomodulation
799 by 17β-estradiol in bivalve hemocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 291, R664-73.
800 Canesi L, Lorusso LC, Ciacci C, Betti M, Gallo G. 2004. Environmental estrogens can affect the
801 function of mussel hemocytes through rapid modulation of kinase pathways. *Gen. Comp. Endocrinol.*
802 138, 58-69.
803 Capolupo M, Diaz-Garduno B, Martin-Diaz ML. 2018. The impact of propranolol, 17α-
804 ethinylestradiol, and gemfibrozil on early life stages of marine organisms: effects and risk assessment.
805 *Environ. Sci. Pollut. Res*. 25, 3196-220.

806 Capolupo M, Franzellitti S, Kiwan A, Valbonesi P, Dinelli E, et al. 2017. A comprehensive evaluation
807 of the environmental quality of a coastal lagoon (Ravenna, Italy): integrating chemical and
808 physiological analyses in mussels as a biomonitoring strategy. *Sci. Total Environ.* 598, 146-159.

809 Carvalho IT, Santos L. 2016. Antibiotics in the aquatic environments: a review of the European
810 scenario. *Environ. Int.* 94, 736-57.

811 Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of
812 substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC
813 of the European Parliament and of the Council and repealing Commission Implementing Decision
814 (EU) 2015/495 (notified under document C, 2018, p. 3362.

815 Communication from the Commission to the European Parliament, the Council and the European
816 Economic and Social Committee. European Union Strategic Approach to Pharmaceuticals in the
817 Environment COM/2019/128 final.

818 Cortez FS, Souza L, Guimarães LL, Almeida JE, Pusceddu, FH, et al. 2018. Ecotoxicological effects
819 of losartan on the brown mussel Perna perna and its occurrence in seawater from Santos Bay (Brazil).
820 *Sci. Total Environ*. 637-638, 1363-71.

821 Couto CF, Lange LC, Amaral MCS. 2019. Occurrence, fate and removal of pharmaceutically active
822 compounds (PhACs) in water and wastewater treatment plants—a review. *J. Water Process Eng.* 32,
823 100927.

824 Cunha SC, Pena A, Fernandes JO. 2017. Mussels as bioindicators of diclofenac contamination in
825 coastal environments. *Environ. Pollut*. 225, 354-60.

826 de Oliveira MR, Frihling BEF, Velasques J, Filho FJCM, Cavalheri PS, Migliolo L. 2020.
827 Pharmaceuticals residues and xenobiotics contaminants: occurrence, analytical techniques and
828 sustainable alternatives for wastewater treatment *Sci. Total Environ.* 10, 135568

829 de Oliveira MR. 2016. Fluoxetine and the mitochondria: a review of the toxicological aspects. Toxicol
830 Lett 258:185-91.

831 Di Costanzo F, Di Dato V, Ianora A, Romano G. 2019. Prostaglandins in Marine Organisms: A
832 Review. *Mar Drugs* 17 (7), 428.
833 Djordjevic J. Djordjevic A, Adzic M, Elaković I, Matić G, Radojcic MB 2011. Fluoxetine afects
834 antioxidant system and promotes apoptotic signaling in Wistar rat liver. *Eur J Pharmacol* 659:61–
835 66.
836 Eren I, Naziroğlu M, Demirdaş A. 2007. Protective effects of lamotrigine, aripiprazole and
837 escitalopram on depression-induced oxidative stress in rat brain. *Neurochem Res* 32:1188-95.
838 Estévez-Calvar N, Canesi L, Montagna M, Faimali M, Piazza V, Garaventa F. 2017. Adverse effects
839 of the SSRI antidepressant sertraline on early life stages of marine invertebrates. *Mar. Environ. Res.*
840 128, 88–97.
841 Fabbri E. 2015. Pharmaceuticals in the environment: expected and unexpected effects on aquatic
842 fauna. *Ann. N. Y. Acad. Sci.* 1340, 20-28.
843 Faure C, Ouissame MF, Nasser H. 2006. Long-term adaptive changes induced by serotonergic
844 antidepressant drugs *Expert Rev Neurother*. 6, 235-45.
845 Fong PP, Ford AT. 2014. The biological effects of antidepressants on the molluscs and crustaceans:
846 a review. *Aquat. Toxicol.* 151, 4-13.
847 Franzellitti S, Buratti S, Capolupo M, Du B, Haddad SP. et al. 2014. An exploratory investigation of
848 various modes of action and potential adverse outcomes of fluoxetine in marine mussels. *Aquat.*
849 *Toxicol.* 151, 14-26.
850 Freitas R, Almeida A, Calisto V, Velez C, Moreira A et al. 2016. The impacts of pharmaceutical
851 drugs under ocean acidification: new data on single and combined long-term effects of carbamazepine
852 on Scrobicularia plana. *Sci. Total Environ.* 541, 977-85.
853 Fuentes N, Silveyra P. 2019. Estrogen receptor signaling mechanisms. *Adv Protein Chem Struct Biol.*
854 116:135-170.
855 Ghosh R, Alajbegovic A, Gomes AV. 2015. NSAIDs and Cardiovascular Diseases: Role of Reactive
856 Oxygen Species. Oxidative Medicine & Cellular Longevity.25, 536962.

857 Gonzalez-Alonso S, Merino LM, Esteban S, Lopez de Alda M, Barcelo D et al. 2017. Occurrence of
858 pharmaceutical, recreational and psychotropic drug residues in surface water on the northern
859 Antarctic Peninsula region. *Environ. Pollut.* 229, 241-54.
860 Gonzalez-Rey M, Bebianno MJ, 2013. Does selective serotonin reuptake inhibitor (SSRI) fluoxetine
861 affects mussel Mytilus galloprovincialis? *Environ. Pollut.* 173, 200-09.
862 Guedes-Alonso R, Sosa-Ferrera Z, Santana-Rodríguez JJ. 2017. Determination of steroid hormones
863 in fish tissues by microwave-assisted extraction coupled to ultra-high performance liquid
864 chromatography tandem mass spectrometry. *Food Chem* 237:1012-20
865 Hampel M, Blasco J, Babbucci M, Ferraresso S, Bargelloni L, Milan M. 2017. Transcriptome analysis
866 of the brain of the sea bream (Sparus aurata) after exposure to human pharmaceuticals at realistic
867 environmental concentrations. *Mar. Environ. Res.* 129, 36-45.
868 Hillhouse TM, Porter JH. 2015. A brief history of the development of antidepressant drugs: from
869 monoamines to glutamate. *Exp. Clin. Psychopharmacol* 23, 1-21.
870 IQVIA Institute for Human Data Science. 2019. The Global Use of Medicine in 2019 and Outlook to
871 2023 Forecasts and Areas to Watch.
872 Janer G, Porte C. 2007. Sex steroids and potential mechanisms of non-genomic endocrine disruption
873 in invertebrates. *Ecotoxicology* 16, 145-160.
874 Jose J, Sandra Pinto J, Kotian B, Mathew Thomas A, Narayana Charyulu R. 2020. Comparison of the
875 regulatory outline of ecopharmacovigilance of pharmaceuticals in Europe, USA, Japan and Australia.
876 *Sci. Total Environ.* 709, 134815.
877 Kajiwara M, Kuraku S, Kurokawa T, Kato K, Toda S. et al. 2006. Tissue preferential expression of
878 estrogen receptor gene in the marine snail, Thais clavigera. *Gen. Comp. Endocrinol.* 148, 315-26.
879 Kovalakova P, Cizmas L, Donald TJM, Marsalek B, Feng M, Sharma VK. 2020. Occurrence and
880 toxicity of antibiotics in the aquatic environment: a review. *Chemosphere* 251, 126351.

881 Kumar P, Kumar A. 2009. Possible role of sertraline against 3-nitropropionic acid induced behavioral,
882 oxidative stress and mitochondrial dysfunctions in rat brain. *Prog Neuro-Psychopharmacol Biol*
883 *Psychiatry* 33, 100-08.
884 Kümmerer K. 2010. Pharmaceuticals in the Environment. *Annu. Rev. Environ. Resour.* 35, 57-75.
885 Lazzara R, Fernandes D, Faria M, López JF, Tauler R, Porte C. 2012. Changes in lipid content and
886 fatty acid composition along the reproductive cycle of the freshwater mussel Dreissena polymorpha:
887 its modulation by clofibrate exposure. *Sci. Total Environ.* 432, 195-201.
888 Liu J, Lu G, Xie Z, Zhang Z, Li S, Yan Z. 2015. Occurrence, bioaccumulation and risk assessment
889 of lipophilic pharmaceutically active compounds in the downstream rivers of sewage treatment plants.
890 *Sci. Total Environ.* 511, 54-62.
891 Maria VL, Amorim MJB, Bebianno MJ, Dondero F. 2016. Transcriptomic effects of the non-steroidal
892 anti-inflammatory drug Ibuprofen in the marine bivalve Mytilus galloprovincialis Lam. *Mar.*
893 *Environ. Res.* 119, 31-39.
894 Marino M, Galluzzo P, Ascenzi P. 2006. Estrogen signaling multiple pathways to impact gene
895 transcription. *Curr. Genomics,* 7(8), 497-508
896 Martínez-Morcillo S, Rodríguez-Gil JL, Fernández-Rubio J, Rodríguez-Mozaz S, María Prado
897 Míguez-Santiyán MP et al. 2020. Presence of pharmaceutical compounds, levels of biochemical
898 biomarkers in seafood tissues and risk assessment for human health: results from a case study in
899 North- Western Spain. *Int. J. Hyg Environ. Health* 223, 10-21.
900 Matozzo V, De Notaris C, Finos L, Filippini R, Piovan A. 2015. Environmentally realistic
901 concentrations of the antibiotic Trimethoprim affect haemocyte parameters but not antioxidant
902 enzyme activities in the clam Ruditapes philippinarum. *Environ. Pollut.* 206, 567-74.
903 Mezzelani M, Nardi A, Bernardini I, Milan M, Peruzza L. et al. 2021. Environmental
904 pharmaceuticals and climate change: The case study of carbamazepine in M. galloprovincialis
905 under ocean acidification scenario. *Environ. Intern.* 146, 106269.

906 Mezzelani M, Fattorini D, Gorbi S, Nigro M, Regoli F. 2020. Human pharmaceuticals in marine
907 mussels: evidence of sneaky hazard along Italian coasts. *Mar. Environ. Res.*162, 105137.
908 Mezzelani M, Gorbi S, Regoli F. 2018a. Pharmaceuticals in the aquatic environments: evidence of
909 emerged threat and future challenges for marine organisms. *Mar. Environ. Res.* 140, 41-60.
910 Mezzelani M, Gorbi S, Fattorini D, d'Errico G, Consolandi G. et al. 2018b. Long-term exposure of
911 Mytilus galloprovincialis to Diclofenac, Ibuprofen and Ketoprofen: insights into bioavailability,
912 biomarkers and transcriptomic changes. *Chemosphere* 198, 238-48.
913 Milan M, Pauletto M, Patarnello T, Bargelloni L, Marin MG, Matozzo V. 2013. Gene transcription
914 and biomarker responses in the clam *Ruditapes philippinarum* after exposure to ibuprofen. *Aquat.*
915 *Toxicol.* 126, 17-29.
916 Milla S, Depiereux S, Kestemont P. 2011. The effects of estrogenic and androgenic endocrine
917 disruptors on the immune system of fish: a review. *Ecotoxicology* 20, 305-19.
918 Moreno-González R, Rodríguez-Mozaz S, Huerta B, Barceló D, León VM. 2016. Do pharmaceuticals
919 bioaccumulate in marine molluscs and fish from a coastal lagoon? *Environ. Res.* 146, 282-98.
920 Moretti M, Colla A, de Oliveira Balen G, dos Santos DB, Budni J. et al. 2012. Ascorbic acid
921 treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage
922 induced by chronic unpredictable stress. *Journal of Psychiatric Res.* 46 (3), 331-40.
923 Munari M, Marin MG, Matozzo V. 2014. Effects of the antidepressant fluoxetine on the immune
924 parameters and acetylcholinesterase activity of the clam Venerupis philippinarum. *Mar. Environ. Res.*
925 94, 32-37.
926 Muraoka S, Miura T, Inactivation of cholinesterase induced by non-steroidal anti-inflammatory drugs
927 with horseradish peroxidase: implication for Alzheimer's disease. 2009. *Life Sciences.* 84, 272-77.
928 Nichols CD, Sanders-Bush E. 2003. Serotonin. Encyclopedia of the Neurological Sciences, Academic
929 Press, 245-248, ISBN 9780122268700.

930 Niu Z, Xu W, Na J, Lv Z, Zhang Y. 2019. How long-term exposure of environmentally relevant
931 antibiotics may stimulate the growth of Prorocentrum lima: A probable positive factor for red tides.
932 *Environ. Pollut.* 255, 113149.
933 Notch EG, Miniutti DM, Mayer GD 2007. 17α-Ethinylestradiol decreases expression of multiple
934 hepatic nucleotide excision repair genes in zebrafish (Danio rerio). *Aquat Toxicol.* 84:301-9.
935 O'Malley BW. 2005. A life-long search for the molecular pathways of steroid hormone action. *Mol*
936 *Endocrinol.* 19(6), 1402-11.
937 Ojemaye CY, Petrik L. 2019. Occurrences, levels and risk assessment studies of emerging pollutants
938 (pharmaceuticals, perfluoroalkyl and endocrine disrupting compounds) in fish samples from Kalk
939 Bay harbour, South Africa. Environ Pollut 252, 562-72
940 Oliveira P, Almeida Â, Calisto V, Esteves VI, Schneider RJ et al. 2017. Physiological and
941 biochemical alterations induced in the mussel Mytilus galloprovincialis after short and long-term
942 exposure to carbamazepine. *Water Res.* 117, 102-14.
943 Pahan K. 2006. Lipid-lowering drugs. *Cell. Mol. Life Sci.* 63, 1165-78.
944 Partridge C, Boettcher A, Jones AG. 2010. Short-term exposure to a synthetic estrogen disrupts
945 mating dynamics in a pipefish. *Horm Behav.* 58,800-7.
946 Peake BM, Braund R, Tong AYC, Tremblay LA. 2016. The Life-cycle of Pharmaceuticals in the
947 Environment. Woodhead Publishing Series in Biomedicine Number 51.
948 Porte C, Janer G, Lorusso LC, Ortiz-Zarragoitia M, Cajaraville MP, Fossi MC, Canesi L. 2006.
949 Endocrine disruptors in marine organisms: approaches and perspectives. *Comp. Biochem. Physiol.*
950 143C, 303-15.
951 Qin G, Zhang Y, Zhang B, Zhang Y, Liu Y, Lin Q. 2020. Environmental estrogens and progestins
952 disturb testis and brood pouch development with modifying transcriptomes in male-pregnancy lined
953 seahorse Hippocampus erectus. *Sci Total Environ.* 715,136840.

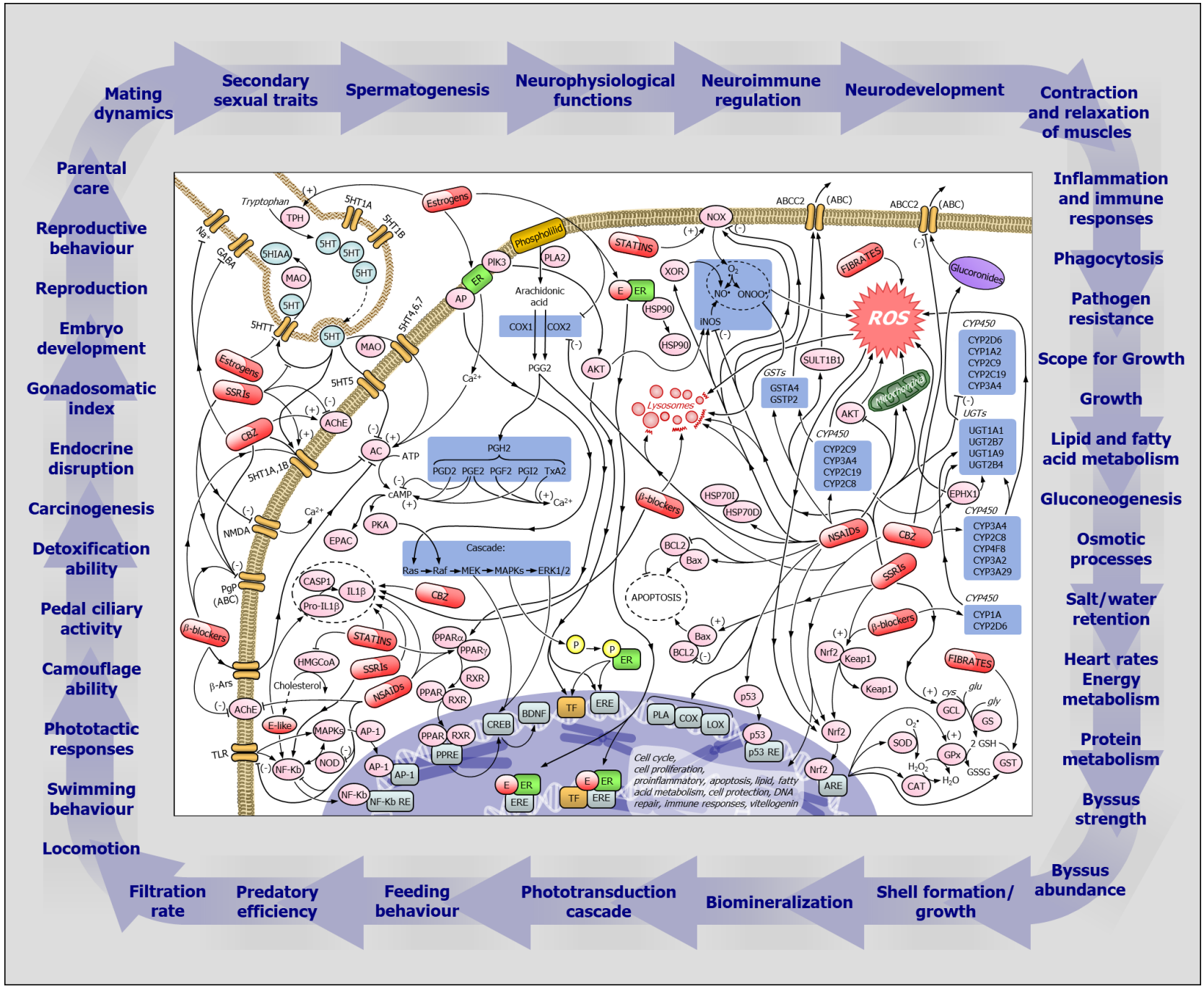
954 Quinn B, Schmidt W, O'Rourke K, Hernan R. 2011. Effects of the pharmaceuticals gemfibrozil and
955 diclofenac on biomarker expression in the zebra mussel (Dreissena polymorpha) and their comparison
956 with standardised toxicity tests. *Chemosphere* 84, 657-63.
957 Rebai R, Jasmin L, Boudah A. 2017. The antidepressant effect of melatonin and fuoxetine in diabetic
958 rats is associated with a reduction of the oxidative stress in the prefrontal and hippocampal cortices.
959 *Brain Res Bull* 134:142-50.
960 Regoli F, Giuliani ME, 2014. Oxidative pathways of chemical toxicity and oxidative stress
961 biomarkers in marine organisms. *Mar. Environ. Res.* 93, 106-17.
962 Roark, A. 2020. Endocrine Disruptors and Marine Systems. *Encyclopedia of the World's Biomes.*
963 Rybaczyk LA, Bashaw MJ, Pathak DR, Moody SM, Gilders RM, Holzschu DL. 2005. An overlooked
964 connection: serotonergic mediation of estrogen-related physiology and pathology. *BMC women's*
965 *health*, 5, 12.
966 Saaristo M, Craft JA, Lehtonen KK, Lindström K. 2010. An endocrine disrupting chemical changes
967 courtship and parental care in the sand goby. *Aquat Toxicol* 97, 285-92.
968 Sathishkumar P, Meena RAA, Palanisami T, Ashokkumar V, Palvannan T, Gu FL. 2020. Occurrence,
969 interactive effects and ecological risk of diclofenac in environmental compartments and biota – a
970 review. *Sci. Total Environ.* 698, 134057
971 Serra-Compte A, Álvarez-Muñoz D, Solé M, Cáceres N, Barceló D, Rodríguez-Mozaz S. 2019.
972 Comprehensive study of sulfamethoxazole effects in marine mussels: Bioconcentration, enzymatic
973 activities and metabolomics. *Environ. Res.* 173, 12-22.
974 Siebel AM, Rico EP, Capiotti KM, Piato AL, Cusinato CT et al. 2010. In vitro effects of antiepileptic
975 drugs on acetylcholinesterase and ectonucleo-tidase activities in zebrafish *(Danio rerio)* brain.
976 *Toxicol. In Vitro* 24, 1279-84.
977 Skolness SY, Durhan EJ, Jensen KM, Kahl MD, Makynen EA et al. 2012. Effects of gemfibrozil on
978 lipidmetabolism, steroidogenesis, and reproduction in the fathead minnow (Pimephales promelas).
979 *Environ. Toxicol. Chem.* 31, 2615-24.

980 Solè M, Shaw JP, Frickers PE, Weadman JW, Hutchinson TH. 2010. Effects on feeding rate and
981 biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen.
982 *Anal. Bioanal. Chem.* 396 (2), 649-56.
983 Sun L, Xin L, Peng Z, Jin R, Jin Y, Qian H, Fu Z. 2014. Toxicity and enantiospecific differences of
984 two β-blockers, propranolol and metoprolol, in the embryos and larvae of zebrafish (Danio rerio).
985 *Environ. Toxicol.* 29, 1367-78.
986 Świacka K, Maculewicz J, Smolarz K, Szaniawska A, Caban M. 2019. Mytilidae as model organisms
987 in the marine ecotoxicology of pharmaceuticals - a review *Environ. Pollut.* 254, 113082.
988 Thornton JW, Need E, Crews D. 2003. Resurrecting the ancestral steroid receptor: ancient origin of
989 estrogen signaling. *Science* 301, 1714-17.
990 Välitalo P, Kruglova A, Mikola A, Vahala R. 2017. Toxicological impacts of antibiotics on aquatic
991 micro-organisms: a mini-review. *Int. J. Hyg Environ. Health* 220, 558-69.
992 Wolecki D, Caban M, Pazdro K, Mulkiewicz E, Stepnowski P, Kumirska J. 2019. Simultaneous
993 determination of non-steroidal anti-inflammatory drugs and natural estrogens in the mussels Mytilus
994 edulis trossulus. *Talanta* 200, 316-23.
995 World Health Organization, 2016. Anatomical Therapeutic Chemical (ATC) Classification. World
996 Health Organization, Geneva.
997 Xie H, Hao H, Xu N, Liang X, Gao D. et al. 2019. Pharmaceuticals and personal care products in
998 water, sediments, aquatic organisms, and fish feeds in the Pearl River Delta: occurrence, distribution,
999 potential sources, and health risk assessment. *Sci. Total Environ.* 659, 230-39.
1000 Zhang K, Zhao Y, Fent K. 2020. Cardiovascular drugs and lipid regulating agents in surface waters
1001 at global scale: Occurrence, ecotoxicity and risk assessment. *Sci. Total Environ.* 729, 138770.
1002 Zheng D, Yin G, Liu M, Chen C, Jiang Y, Hou L, Zheng Y. 2021. A systematic review of antibiotics
1003 and antibiotic resistance genes in estuarine and coastal environments. *Sci. Total Environ.* 777, 146009
1004 Zhu S, Dong B, Wu Y, Buc L, Zhou S. 2019. Degradation of carbamazepine by vacuum-UV oxidation
1005 process: kinetics modeling and energy efficiency. *J. Hazard. Mater* 368, 178-85.

CAPTION OF FIGURE 1

Figure 1: Pathways of action, metabolism and toxicity of the most frequently detected therapeutic classes in marine environment: non-steroidal anti-inflammatory drugs (NSAIDs), psychiatric drugs (Selective Serotonin Reuptake Inhibitors SSRIs and carbamazepine CBZ), cardiovascular drugs (β-blockers), lipid regulators (statins and fibrates), steroidal hormones (estrogens). Acronyms arranged in alphabetical order: ABCC2: ATP Binding Cassette Subfamily C Member 2; ABC: ATP-binding cassette transporters; AC: adenylyl cyclase; AChE: acetylcholinesterase; AKT: protein kinase B; AP: activator protein; AP-1: activator protein 1; ARE: antioxidant responsive element; ATP: adenosine triphosphate; BAX: Bcl-2-associated X protein; BCL2: B-cell lymphoma 2 protein; BDNF: brain-derived neurotrophic factor; cAMP: cyclic adenosine monophosphate; CASP1: caspase 1 CAT: catalase; CBZ: carbamazepine; COX: cyclooxygenase; COX1: cyclooxygenase 1; COX2: cyclooxygenase 2; CREB: cAMP response element-binding protein; CYP450: cytochrome P450 enzymes *Cys:* cysteine; E: estrogen; E-like: estrogen like compounds; EPAC: exchange protein activated by cyclic AMP; EPHX1: epoxide Hydrolase 1; ER: estrogen receptor; ERE: estrogen response element; ERK1/2: extracellular signal-regulated kinases 1 and 2; GABA: γ amino-butyric acid receptor; GCL: glutamate-cysteine ligase; *Glu:* glutamate; *Gly:* glycine; GPx: glutathione peroxidases; GS: glutathione synthetase; GSH: reduced glutathione; GSSG: oxidized glutathione; GST: glutathione S-transferase; HMGCoA: β-Hydroxy β-methylglutaryl-CoA; HSP70 I/D: heat shock protein 70 I and 70D; HSP90: heat shock protein 90; IL1β: interleukin 1 beta; iNOS: inducible nitric oxide synthetase; Keap1: Kelch-like ECH-associated protein 1; LOX: leukotrienes; MAO: monoamine oxidase; MAPKs: mitogen-activated protein kinases; NF-kB RE: nuclear factor-kB responsive element; NF-kB: nuclear factor-kB; NMDA: N-methyl-D-aspartate receptor; NOD: nucleotide-binding oligomerization domain proteins; NOX: NAPH oxidases; Nrf 2: nuclear factor (erythroid-derived 2)-like 2; NSAIDs: non-steroidal anti-inflammatory drugs; P: phosphate; p53 RE: protein 53 responsive element; p53: protein 53; PGD2: prostaglandin D2; PGE2: prostaglandin E2; PGF2: prostaglandin F2; PGG2: prostaglandin G2; PGH2: prostaglandin H2; PGI2: prostaglandin I2; PgP: P-glycoprotein; PKA: protein kinase A; PLA: phospholipase; PLA2: phospholipase A2; PlK3: Polo like kinase 3; PPAR: peroxisomal proliferator activated receptor; PPARα: peroxisomal proliferator activated receptor α; PPAR β: peroxisomal proliferator activated receptor β; PPRE: peroxisomal proliferator responsive element; Pro- IL1β: Pro-inflammatory interleukin 1 beta; Ras/Raf/MEK: Ras-signalling cascade; RXR: retinoid X receptor; SOD: superoxide dismutase; SSRIs: selective serotonin re-uptake inhibitors; SULT1B1: sulfotransferase Family 1B Member 1; TF: transcription factor; TLR: Toll like receptors; TPH: tryptophan hydroxylase; *Trp:* tryptophan; TxA2: thromboxane A2; UGTs: UDP-glucuronosyltransferases; XOR: xanthine oxido-reductase; β-

Ars: β-Adrenergic receptors; 5HIAA: 5-Hydroxyindoleacetic acid; 5HT 1,4,5,6,7: 5-hydroxytryptamine receptors; 5HT: 5-hydroxytryptamine (Serotonin); 5HT1A: 5-hydroxytryptamine 1A receptor; 5HT1B: 5-hydroxytryptamine 1B receptor; 5HTT: 5-hydroxytryptamine transporter.


Mating dynamics
Secondary sexual traits
Spermatogenesis
Neurophysiological functions
Neuroimmune regulation
Neurodevelopment
Contraction and relaxation of muscles
Parental care
Reproductive behaviour
Reproduction
Embryo development
Gonadosomatic index
Endocrine disruption
Carcinogenesis
Detoxification ability
Pedal ciliary activity
Camouflage ability
Phototactic responses
Swimming behaviour
Locomotion
Inflammation and immune responses
Phagocytosis
Pathogen resistance
Scope for Growth
Growth
Lipid and fatty acid metabolism
Gluconeogenesis
Osmotic processes
Salt/water retention
Heart rates Energy metabolism
Protein metabolism
Byssus strength
Byssus abundance
Filtration rate
Predatory efficiency
Feeding behaviour
Phototransduction cascade
Biomineralization
Shell formation/ growth
ROS
Cell cycle, cell proliferation, proinflammatory, apoptosis, lipid, fatty acid metabolism, cell protection, DNA repair, immune responses, vitellogenin