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Environmental Toxicology and Chemistry, Vol. 35, No. 4, pp. 799–812, 2016
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HUMAN PHARMACEUTICALS IN THE MARINE ENVIRONMENT: FOCUS ON EXPOSURE AND BIOLOGICAL EFFECTS IN ANIMAL SPECIES

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(Submitted 3 March 2015; Returned for Revision 16 April 2015; Accepted 23 June 2015)

Abstract: Marine waters have been poorly investigated for the occurrence of pharmaceutical contamination. Recent data confirm that pharmaceuticals occur widely in marine and coastal environments; therefore, assessment of potential risk to marine species needs further efforts. The present study represents the first extensive review of pharmaceutical contamination in marine environments addressing the effects on the marine biota analyzed at the molecular, cellular, and individual levels. Because pharmaceuticals differ from conventional pollutants, being designed to interact with specific physiological pathways at low doses, the most recent evidence on modes of action and physiological alterations on marine animal species are discussed. Data on spatial distributions of pharmaceuticals in waters and sediments, as well as bioaccumulation rates, are also presented. The present review also seeks to expand knowledge of how the quality of coastal and marine environments could be efficiently monitored to anticipate possible health and environmental risks. *Environ Toxicol Chem* 2016;35:799–812. © 2015 SETAC

Keywords: Pharmaceuticals Marine environment Marine fauna Biomarkers Mode of action

INTRODUCTION

Human and veterinary pharmaceuticals are a large class of chemicals widely discharged into water bodies on a continual basis, mainly from domestic sewage [1,2]. Poorly removed by treatment plants and slowly degraded, they become pseudo-persistent ubiquitous contaminants [3].

In addressing anthropogenic threats to the coastal marine environment, Crain et al. [4] included pollution within the most widespread and pressing threats, together with overexploitation, habitat loss, invasive species, and intrinsic factors, based on species-level assessments. The relative importance of pharmaceuticals among other environmental pollutants cannot be stated at the moment; however, pharmaceuticals can induce profound biological effects [5,6] and thus may act as additional stressors on marine ecosystems already impacted by other factors. Modifications of pH and temperature associated with climate change influence the chemical properties of most pharmaceuticals, which therefore may contribute differently to the global effect [7,8]. Furthermore, the impact of pharmaceuticals on coastal environments is expected to increase with the increasing number of inhabitants in such areas [9,10] and the increasing pressures attributable to human activities such as aquaculture [11], posing relevant implications for marine risk assessment and the determination of potential consequences for ecological and human health that cannot be neglected.

Unlike conventional pollutants, pharmaceuticals are biologically active compounds designed to interact with specific physiological pathways in the target organism. Thus, they represent a class of emerging compounds able to affect specific animal functions (e.g., development, growth, and reproduction) at environmentally relevant concentrations [6].

Although several studies have addressed the occurrence and fate of pharmaceuticals in surface waters [2,12,13], marine waters have long been neglected, under the assumption that dilution would represent a safety factor, per se. Thus, data determining the potential ecosystem and health risks posed by pharmaceuticals are urgently needed. Indeed, there has been a rapid increase in investigations into marine environments, which have shown that pharmaceuticals are present at low but consistent concentrations anywhere they were assessed.

A recent literature review by Gaw et al. [14] focused on the sources, concentrations, and ecotoxicological impacts of the human and veterinary pharmaceuticals most frequently detected in seawater, addressing knowledge gaps and suggesting priorities for future research. After examining pharmaceutical distributions in waters and sediments and their bioaccumulation potential, the present review aims to expand on the review of Gaw et al. [14] by addressing animal responses to pharmaceuticals assessed at different levels of biological complexity, from the molecular to the cellular and organism levels.

We examine effects triggered through conserved targets related to a drug's therapeutic mode of action (MOA), demonstrating that pharmaceuticals are potentially able to alter crucial mechanisms of an organism's homeostasis at concentrations as low as those documented in marine waters, acting in some cases as endocrine-disrupting chemicals. At the same time, physiological, biochemical, or behavioral alterations consistently measurable at environmental concentrations of pharmaceuticals, and potentially applicable as biomarkers for biomonitoring approaches, are highlighted.

OCCURRENCE OF PHARMACEUTICALS IN MARINE ENVIRONMENTS

The available literature shows that the fate and occurrence of pharmaceutical compounds in marine water, sediment, or biota are still largely underestimated, and investigations are mostly limited to spot data from both a spatial and a temporal point of view (Supplemental Data, Tables S1– S3).

This article includes online-only Supplemental Data.

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Published online 25 June 2015 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3131

As shown by the summary of literature data reported in Figures 1 and 2, these compounds have been detected in seawater and sediments of different coastal environments worldwide, with concentrations ranging from 0.01 ng/L to 2.4 μg/L (seawater; Supplemental Data, Table S1) and from 0.1 ng/g dry weight to 820 μg/g wet weight (sediments; Supplemental Data, Table S2). Most of the data collected are from coastal areas with dense populations and intensive industrial and agricultural activities (including animal husbandry and aquaculture), which produce large amounts of domestic, industrial, agricultural, and aquaculture wastes resulting in various anthropogenic impacts. Therefore, they must be regarded as hotspots of pharmaceutical occurrence.

In the following section, we summarize the most recent reports on the occurrence and behavior of pharmaceuticals when released in natural marine environments, with a particular focus on the possible routes of exposure to marine organisms.

Fate and dispersion processes controlling the occurrence of pharmaceuticals in seawater

Despite the general assumption that low pharmaceutical concentrations would result from the relevant dilution rates of freshwaters into seawater bodies, several pharmaceuticals have been detected in seawater at concentrations exceeding the safety threshold for predicted environmental concentrations in surface waters (0.01 μg/L [15]). For example, the analgesic acetaminophen was reported in coastal waters of Costa Rica at concentrations of up to 1952 ng/L, and the lipid regulator gemfibrozil and the nonsteroidal anti-inflammatory drug ketoprofen at levels ranging from 77 ng/L to 758 ng/L and from 185 ng/L to 805 ng/L, respectively [16]. Therefore, physical–chemical processes other than dilution occurring in the marine environment must be considered. Recent studies have highlighted how hydrodynamism of water masses may have a major impact on defining routes of pharmaceutical dispersion in coastal environments. Using the Delft 3D hydrodynamic model, Bayen et al. [17] investigated the dispersion of 40 pharmaceuticals and endocrine-disrupting chemicals in the Singapore coastal marine system. Their results showed that proximate distance to source emissions had little influence on the water concentrations of these contaminants and that those levels greatly depended on coastal mixing patterns and residence times (i.e., levels were highest at sampling sites having the lowest flushing potential). Fenet et al. [18] applied the MARS 3D model to achieve refined predicted concentrations of the antiepileptic drugs carbamazepine and oxcarbazepine and their metabolites in a wastewater treatment plant (WWTP) and in the vicinity of its submarine outfall located in the French Mediterranean coastal area of Montpellier. Modeling of carbamazepine diffusion and advection just above the submarine outfall and in surface seawaters showed the relevant influence of thermocline during summer, with very low diffusion of carbamazepine from the bottom to the surface, thus suggesting a major influence of stratification of water masses. Both studies [17,18] recognize that hydrodynamic models implemented on a regional scale are promising tools that are useful in multidisciplinary approaches to investigate the fate, distribution, and exposure risks of pharmaceuticals in complex coastal systems.

Changes in pH and salt burdens in the transition from freshwater to seawater greatly influence the electrostatic properties of the chemicals, which may have multiple ionizable functional groups with substantially different acid dissociation constant (pK_a) values. The degree of ionization and its

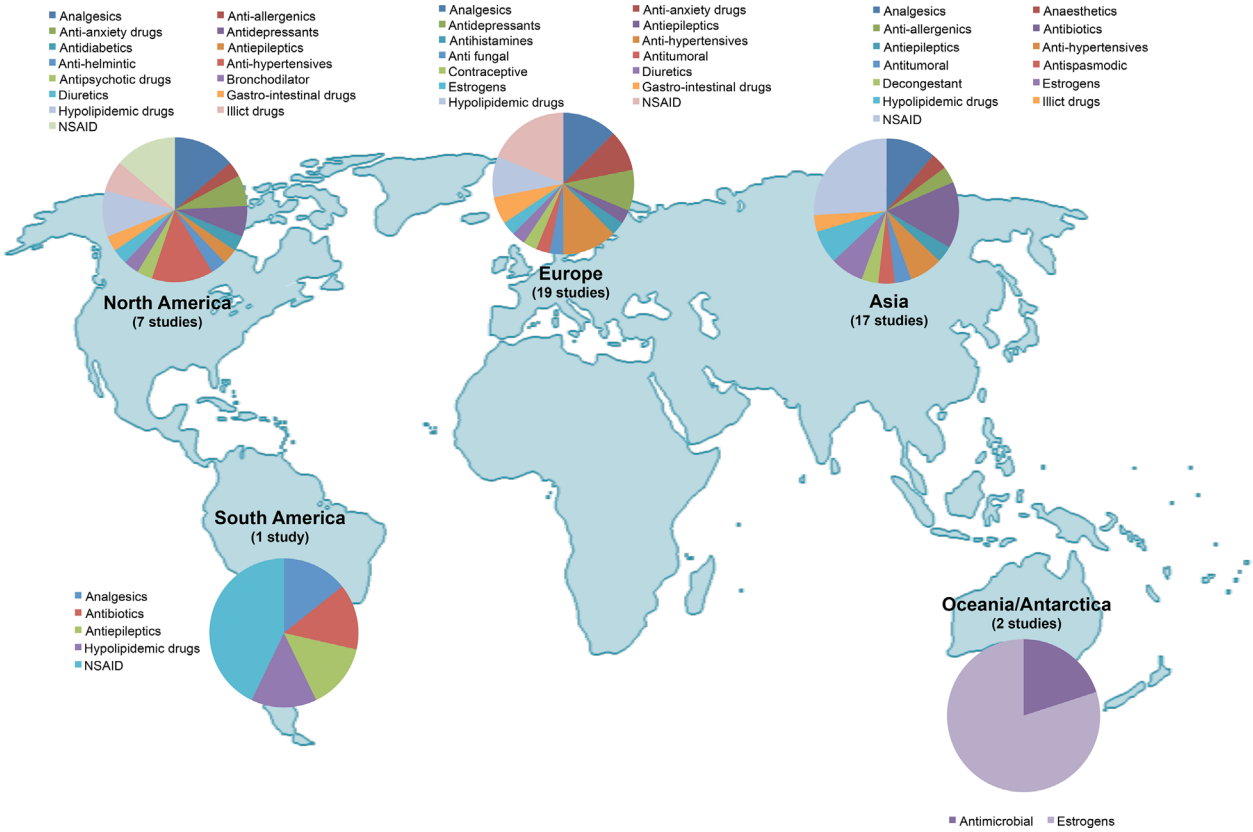


Figure 1. Classes of pharmaceuticals reported in seawater from different locations worldwide. The dataset summarized represents detection of classes of pharmaceuticals reported in detail in Supplemental Data, Table S1. NSAID = nonsteroidal anti-inflammatory drug.

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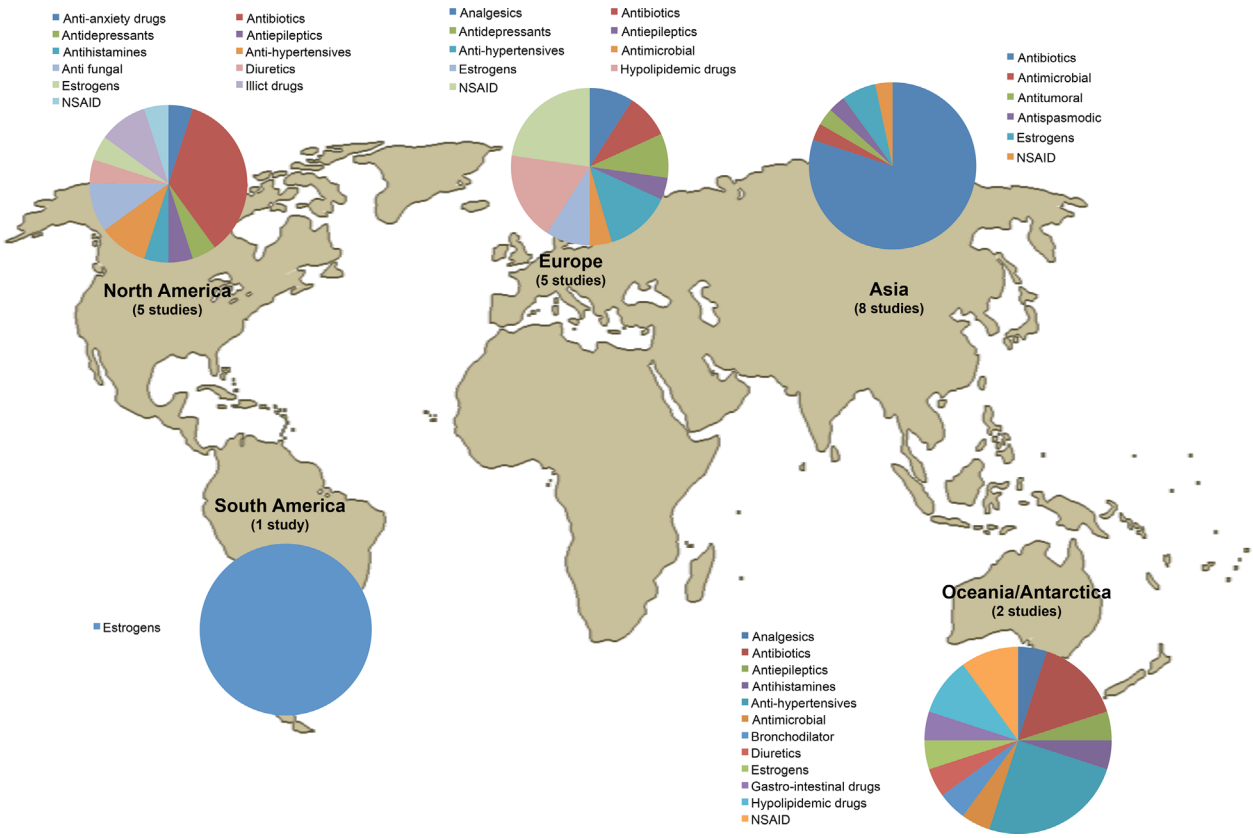


Figure 2. Classes of pharmaceuticals reported in marine sediments from different locations worldwide. The dataset summarized frequencies of detection of classes of pharmaceuticals reported in detail in Supplemental Data, Table S2. NSAID = nonsteroidal anti-inflammatory drug.

properties depend on the pH of the medium, thus influencing their environmental partition. For example, log octanol–water partition coefficient (K_{OW}) values for the β-blocker propranolol and the antidepressant fluoxetine were found to increase linearly with pH [19,20]. Therefore, at the typical pH 8.0 of seawater, the pharmaceuticals are predicted to have enhanced lipophilicity, being more effectively adsorbed onto particulated matter or bioaccumulated by organisms. With a pK_a of 6.6, trimethoprim is not completely ionized in marine surface waters at pH 8.0; the small portion of un-ionized compound remaining in the aqueous phase has a greater affinity for lipophilic matter, leading to a significant bioaccumulation by marine bivalves [21]. Triclosan and carbamazepine exhibit relatively high log octanol–water distribution coefficients (D_{OW}) at pH 8 (5.06 and 3.52, respectively). The D_{OW} accounts for both neutral and ionized compound fractions at a given pH and is a good predictor of the bioaccumulation potential of ionizable compounds [22]. High log D_{OW} values indicate a relatively high potential for accumulation of these compounds in marine organisms because of the anticipated bioconcentration of freely dissolved neutral compounds via lipid–water equilibrium partitioning [17]. Pharmaceuticals including risperidone, simvastatin, sertraline, fluoxetine, paroxetine, bisphenol A, estrogens, and triclosan, which are characterized by a relatively high organic carbon partition coefficient ($K_{OC} > 10^4$), are likely to be more influenced by sorption to suspended solids and subsequent sedimentation/resuspension processes [17]. McEneff et al. [21] showed that measured concentrations of gemfibrozil and mefenamic acid dropped below detectable levels soon after release of WWTP discharges into marine surface waters, possibly because the high salt content of seawater caused these pharmaceuticals to become less soluble and, ultimately, "salt out" of solution [23]. The above evidence shows that transitional environments facing salinity and pH gradients may play a critical role as natural filters for pollutants, including pharmaceuticals. Indeed, Wille et al. [24] and Bayen et al. [17] reported low transfer rates of selected pharmaceuticals from estuaries to the open sea in Belgian and Singapore coastal areas.

Occurrence of pharmaceuticals in marine sediments

Given that pharmaceutical affinities for suspended solids are further enhanced at the typical pH and salinity conditions of seawater, it is likely that marine sediments represent a sink for these compounds [25]. Sediment partitioning of pharmaceuticals in the marine environment has been poorly investigated; however, at least 62 pharmaceuticals have been searched and detected in marine sediments worldwide (Supplemental Data, Table S2). Estrogens are the most widely present, at concentrations up to 130 ng/g (ethinyl estradiol [26]). As expected, the greatest occurrence was reported in coastal areas dominated by sewage treatment plants [27]. Caldwell et al. [28] analyzed vertical profiles of the antimicrobial agent triclosan in dated sediment cores from 4 urbanized estuaries along the East Coast of the United States. Under estuarine conditions, triclosan is likely to be rapidly removed from the water column and to accumulate preferentially in sediments, because it is relatively hydrophobic (log $K_{OW} = 4.8$), and it exhibits a high potential for sorption to organic matter (log $K_{OC} = 4.26$). The reported temporal sediment profiles showed that once sequestered in the sediment, triclosan exhibits long-term stability. Interestingly, its presence in dated sediments has been documented back to its patent date in the 1960s. There is also evidence that the amount of triclosan in sediments is increasing at the estuarine sites analyzed despite improvements in WWTPs. These findings

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show the potential impacts of sediment processes in defining the fate of pharmaceuticals in aquatic systems. Indeed, sediments may act as a secondary pollution source from which pharmaceuticals can be released upon changes in environmental salinity and pH [29], or during tidal changes or storm events.

Occurrence of pharmaceuticals in marine biota

Accumulation of pharmaceuticals in biological tissues is related to a small portion of un-ionized species, with a high affinity for lipophilic matter, remaining in the aqueous phase. However, benthic species, as marine bivalves that are active filter feeders, may bioaccumulate pollutants present in the dissolved phase (through the gills) or adsorbed onto colloids (through the digestive tract) [30–32]. Although the lack of reliable analytical methods limited the investigations on accumulation of pharmaceuticals in marine biota [14], several studies reported that pharmaceutical residues have been detected in wild animal populations worldwide, showing the occurrence and bioavailability of these compounds in natural environments (Supplemental Data, Table S3). Li et al. [33] reported that antibiotics, including quinolones, sulfonamides, and macrolides, were commonly found in wild mollusk species collected from 9 coastal cities along the Chinese Bohai Sea. Quinolones, which were the major compounds reported at concentrations as high as 0.71 μg/kg to 1575.10 μg/kg [33], were strongly adsorbed onto sewage sludge and sediments, with negligible biodegradation [34]. In addition, quinolones were stable in edible animal tissues [35], thus representing a potential health risk for inhabitants consuming high amounts of mollusks in their diet [21].

Antibiotic occurrence in edible marine organisms and human exposure through the diet is particularly high in China, where mariculture production accounts for approximately 80% of the total world amount [36]. Twenty-two of 37 antibiotics were detected in fish, mollusks, shrimp, and crabs collected from Hailing Island (South China [36]). Antibiotics were significantly accumulated in fish muscle and to a lesser extent in mollusks. Only bioaccumulation of erythromycin in adult shrimps was estimated to represent a potential risk to human health [36]. According to a further study carried out in the field (coastal environment of Dalian, China [37]) tetracyclines were the predominant antibiotics in seawater, whereas sulfonamides were the dominant antibiotics in both sediments and aquatic organisms (up to 63.87 μg/kg). The average bioaccumulation factors showed that sulfamethazine, sulfamethiazole, sulfamonomethoxine, and doxycycline were potentially bioaccumulative, whereas sulfadiazine, sulfameter, sulfamethoxypyridazine, and chloramphenicol were bioaccumulative (bioconcentration factor $>$ 5000 L/kg). Overall, antibiotics in the marine environment represent an underrated health risk, and the need for long-term monitoring of both resistance development and transmission to other bacterial species, as well as human risk through the diet, is underlined.

Wild ribbed horse mussels (*Geukensia demissa*) collected from 5 nearshore sites in San Francisco Bay (CA, USA) were analyzed for the presence of 104 residues of pharmaceuticals and personal care products (PPCPs) [38]. Several compounds frequently detected in the Bay surface waters (atenolol, caffeine, gemfibrozil, sulfamethoxazole, meprobamate, valsartan) were not found in mussels. By contrast, the mussels showed high concentrations of nonylphenol, nonylphenol diethoxylate, nonylphenol monoethoxylate, digoxigenin, carbamazepine, triclocarban, and sertraline in their tissues. The different physical–chemical properties of the compounds investigated (e.g., K_{OC}, ionization fraction, pH stability) may account for the observed differences in bioaccumulation, along with different detoxification rates. Moreover, the authors also reported highly variable bioaccumulation factors for the compounds, which may have been influenced by a number of factors. In particular, the extent to which the chemical concentration in the discrete, surface water grab sample collected at each site represented the actual, long-term exposure to the mussels is unknown. In the same study, the illicit drugs amphetamine and cocaine were found in approximately 60% of the samples analyzed, at maximum concentrations in mussel tissues of 40 ng/g wet weight (amphetamine) and 0.3 ng/g wet weight (cocaine) [38]. More recently, Antarctic coastal waters have been assessed for pharmaceutical occurrence and exposure risks to wildlife. Clams (*Laternula elliptica*), sea urchins (*Sterichinus neumayeri*), and Antarctic fish (*Trematomus bernachii*) collected from wild populations in the Erebus Bay showed accumulation of benzophenone-3, estradiol, ethinylestradiol, methylparaben, octylphenol, and propylparaben, related to 2 research base stations (Scott Base and McMurdo) with sewage treatments plants [39]. Interestingly, fish sampled at distances up to 25 km from the research stations' WWTP discharges contained up to 14.1 ng/g, 19.2 ng/g, and 5.0 ng/g dry weight of benzophenone-3, methylparaben, and octylphenol, respectively, indicating that PPCPs from the research stations continuously circulate in the seawaters within Erebus Bay. As already reported for other classes of emerging contaminants [40], the authors postulated that the unique climatic conditions occurring in Antarctica—principally the cold ocean temperatures, the extended periods of darkness, and the presence of sea ice covering coastal sea waters—may reduce microbial degradation processes and photodegradation, and therefore enhance the persistence of pharmaceuticals, allowing for sufficient exposure time to bioaccumulate and likely to enter the trophic chain up to top predatory species [39].

Caged mollusks are increasingly employed to investigate pharmaceutical occurrence in coastal environments, because they allow the measurement of exposure levels as a function of time. In this regard, marine bivalves are excellent sentinels for in situ monitoring of organic micropollutants. Bivalves are sessile organisms living at the sediment–water interface or burrowed into sediments and filtering large volumes of water, suspended materials, and colloids [41]. Caging experiments with blue mussels (*Mytilus edulis*) monitored pharmaceutical occurrence off the Belgian coast over a 6-mo period [42]. Five pharmaceuticals were detected in mussel tissues, including salicylic acid up to 490 ng/g dry weight, whereas lower and fluctuating concentrations of other pharmaceuticals, including carbamazepine, ofloxacin, propranolol, and paracetamol, were found. A recent study by McEneff et al. [21] reported the occurrence of 5 pharmaceuticals (carbamazepine, diclofenac, gemfibrozil, mefenamic acid, trimethoprim) in marine mussels during a 1-yr-long caging experiment along the Irish coastline. All selected pharmaceuticals were continuously detected in surface waters at average concentrations in the mid to high ng/L range, except for gemfibrozil, which was detected sporadically. Three of the 5 compounds were also bioaccumulated by transplanted mussels, with trimethoprim measured at concentrations up to 9.22 ng/g dry weight.

PHARMACEUTICAL INTERACTIONS WITH MARINE FAUNA

For many years, ecotoxicological standard tests led researchers to conclude that no harmful effects were expected

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from pharmaceuticals at the concentrations routinely found in rivers and lakes [12]. Thus, no effects were expected in marine waters, where dilution was higher. However, recent evidence [43] shows that standard tests proposed by current guidelines are not sensitive enough to detect adverse effects provoked by the low environmental concentrations of pharmaceuticals; furthermore, these tests are not sensitive enough to detect the effects of long-term exposures.

Bioluminescence response from *Vibrio fischeri*, and growth inhibition testing with the marine microalga *Isochrysis galbana* showed caffeine, ibuprofen, carbamazepine, and novobiocin toxicity at concentrations well above 1 mg/L, but all compounds were extremely toxic to *Paracentrodus lividus* embryo–larval development at concentrations as low as 0.000001 mg/L to 0.015 mg/L [43]. In previous experiments, Schmidt et al. [44] showed that biomarkers assessed in marine mussels were approximately 3 orders of potency more sensitive to gemfibrozil and diclofenac than toxicity tests. Biomarkers represent physiological, biochemical, or behavioral alterations consistently measurable in living organisms after exposure to contaminants. Thus, it is not unexpected that pharmaceuticals, which are formulated to resist inactivation before exerting their intended therapeutic effects, are able to bioaccumulate and generate stress responses in marine animals. At the same time, animals may possess conserved targets related to the drug's therapeutic MOA. Thus, pharmaceuticals are potentially able to alter crucial mechanisms on the basis of organism homeostasis also at concentrations as low as those documented in marine waters.

Biological effects related to pharmaceutical modes of action

The main inherent feature of pharmaceuticals that makes them peculiar pollutants is that they are specifically designed to be biologically active at low doses and to target specific metabolic, enzymatic, or cell signaling mediators to produce their desired therapeutic effects and reduce the potential for off-target interactions. The evolutionary conservation of molecular targets in a given species potentially increases the possibility that these compounds will be pharmacologically active in organisms unintentionally exposed to the chemical in their natural environment, thus raising the risk of ecotoxicological effects [45,46]. A conceptual model defined MOA approach was developed to address the issue of environmental risk posed by pharmaceuticals [46–48]. The suggested approach assumes that all the relevant information about a pharmaceutical's therapeutic effect and toxicity in mammals must be taken into account and integrated with knowledge of the physiology of the species used as the experimental model. In particular, the degree of evolutionary and functional conservation of drug targets between mammals and the model species should be assessed, together with the physiological pathways potentially affected [45,48]. This background knowledge is then used to make predictions on the MOA and the toxicity of a pharmaceutical in the nontarget species showing a conserved drug target, and to tailor the experimental design of the ecotoxicity tests, for example, by selecting specific endpoints that are relevant to the MOA, or by choosing an appropriate duration of exposure to the drug [48–50].

Recent investigations have confirmed that some specific targets of human pharmaceuticals are conserved during evolution and may interact with drugs in marine animals [51,52]. With an awareness that conservation of therapeutic targets does not necessarily translate to conservation of their function across species, several investigations based on MOA corroborate the proposed biological read-across hypothesis [53]. The read-across hypothesis stipulates that a drug will have an effect on nontarget organisms only if the molecular targets (e.g., receptors, enzymes) have been conserved, resulting in a specific effect when plasma concentrations are similar to human therapeutic concentrations. Although discrepancies are discussed for invertebrates [54], the read-across hypothesis appears readily applicable to fish [53]. If this holds true for different classes of pharmaceuticals, it should be possible to predict the potential environmental impact from information obtained during the drug development process, to identify those pharmaceuticals of major environmental concern, and to develop predictive models for protection of the environment [55].

Studies based on MOAs provide a unique opportunity to develop an understanding of the relationship between molecular/cellular and ecologically relevant endpoints. A conceptual model of the MOA approach applied to the evaluation of fluoxetine effects in some marine organisms is shown in Figure 3. As extensively discussed below, the MOA of fluoxetine targets the serotonin (5-HT) signaling pathway. Because 5-HT is a high-tier physiological controller in aquatic organisms, alterations of the 5-HT pathway by fluoxetine are predicted to have many adverse outcomes on key physiological functions, including reproduction, metabolism, locomotion, and others (Figure 3). It is worth noting that such effects occur in wildlife at concentrations approaching or even below environmental levels (reviewed in Ford and Fong [54]). Therefore, although not directly supporting the definition of guidelines, investigations on MOA should always be included in programs that address pharmaceutical impacts on ecosystems.

The use of an oligo-DNA microarray representing 11 906 transcripts allowed a deeper investigation of the MOA of the anti-inflammatory drug ibuprofen in the digestive gland of *Ruditapes philippinarum* [56]. The functional analysis of differentially transcribed genes suggested that ibuprofen can interfere with various signaling pathways in clams. In particular, ibuprofen altered the expression of several transcripts involved in the arachidonic acid signaling pathway, which is specifically targeted by ibuprofen acting as a cyclo-oxygenase inhibitor [57]. The effects of ibuprofen in clams included overtranscription of phospholipase A2, a key enzyme that catalyzes the hydrolysis of cellular phospholipids to liberate arachidonic acid in one of the first steps of eicosanoid metabolism, which may indicate a potential recruitment of arachidonic acid to increase the substrate availability as a result of cyclo-oxygenase inhibition.

The pharmacological mechanisms by which carbamazepine exerts its therapeutic effects are not entirely clear, although its molecular basis of action has been ascribed to voltage-dependent inhibition of Na^+ and Ca^{2+} channel currents. Carbamazepine induces part of them by interacting with the adenylyl cyclase system and lowering the intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP) in mammalian brain [58,59]. Therefore, the significant reduction in cAMP levels and the activity of the cAMP-dependent protein kinase (PKA) in all tissues of Mediterranean mussels (*Mytilus galloprovincialis*) exposed in vivo to carbamazepine indicated that the specific molecular target of the drug found in mammals is also conserved in mussels [60].

Propranolol is a prototypical β-adrenergic receptor antagonist used in human therapies to counteract cardiovascular pathologies [61]. It is effective on different organs and tissues at low concentrations both in mammals and in lower vertebrates [62]. In addition, propranolol is an effective antagonist

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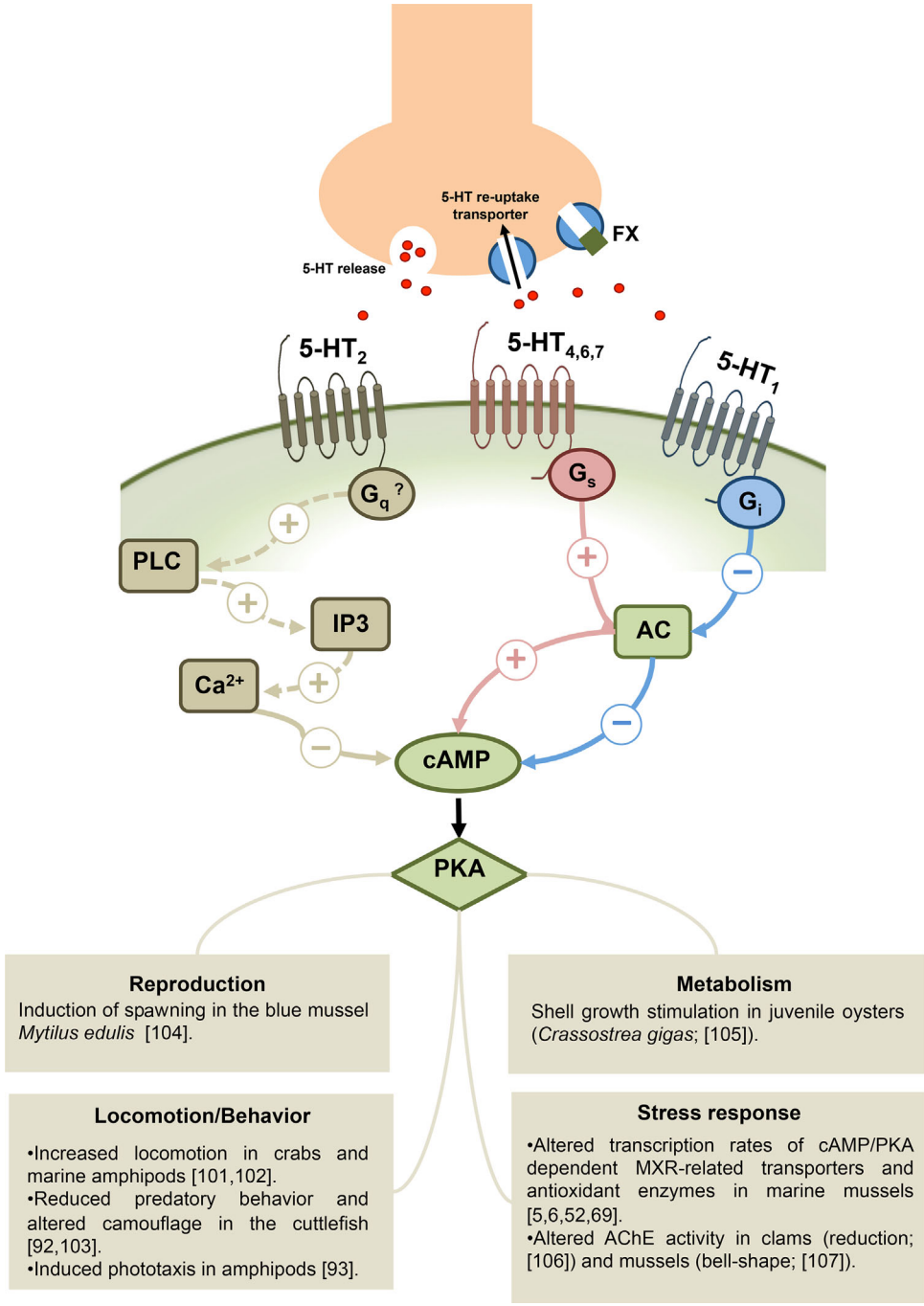


Figure 3. Conceptual model of the mode of action (MOA) approach in marine species considering fluoxetine as a case study. The mode of action of fluoxetine (FX) is to increase serotoninergic neurotransmission by blocking serotonin (5-HT) reuptake at the presynaptic terminal [62]. Physiological functions of 5-HT are mediated by multiple membrane receptors coupled to the stimulation of phospholipase C (PLC) or to the induction/inhibition of adenylyl cyclase (AC) in mammals as well as in bivalves, although pharmacological profiles of invertebrate and mammalian 5-HT receptors are difficult to compare, considering the large phylogenetic distances involved [54,62]. The cyclic adenosine monophosphate (cAMP) pathway has a critical role in invertebrate physiology [62], and high-level physiological effects of FX related to the alterations of the cAMP/cAMP-dependent protein kinase (PKA) system in marine species are reported. AChE = acetylcholinesterase; IP3 = inositol triphosphate; G = guanine nucleotide-dependent proteins.

for type 1 5-HT receptors, and 5-HT affects invertebrate physiology at many levels [63].

The identification of transcripts encoding a putative β-adrenergic receptor (AR) in the oyster *Crassostrea gigas* [64] provided direct evidence on the occurrence of AR-mediated signaling pathways in bivalve tissues (reviewed in Fabbri and Capuzzo [62]). Furthermore, studies on different bivalve species found that β-AR occupancy is coupled to increased cAMP concentrations in tissues [65–68], highlighting a substantial conservation of the β-AR–mediated transduction pathway in bivalves [62]. Accordingly, in vivo exposures of mussels to a range of environmentally realistic concentrations of propranolol resulted in a significant decrease in cAMP levels and PKA activity in digestive glands consistent with propranolol acting as a β-AR blocker [51]. Nevertheless, the increases in cAMP levels and PKA activities in the mantle/gonads of propranolol-exposed mussels observed in same study [51] support an indirect stimulation of propranolol by acting as a 5-

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HT1 receptor antagonist. It is worth noting that transcripts encoding 5-HT1 receptors have been detected in several mussel tissues, including mantle/gonads [5,52,69]. Furthermore, Garnerot et al. [70] showed that serotoninergic fibers are apparently absent or scarce in bivalve digestive gland, whereas a high concentration of these fibers is detected in gonads.

The 5-HT–mediated transduction pathway has also been investigated in mussels and related to the possible biological effects of fluoxetine or further selective serotonin reuptake inhibitors. The therapeutic action of fluoxetine is to increase serotonergic neurotransmission at mammalian synapses by blocking 5-HT reuptake transporters [71]. Thus, fluoxetine mimics the action of a continuous exposure to increased extracellular levels of 5-HT [71]. Consistent with its therapeutic effects and with the expression of putative type 1 5-HT receptors in mussel tissues, mussel in vivo exposure to fluoxetine at environmental concentrations reduced cAMP levels and PKA activity below control values in several tissues, according to the inhibition of adenylyl cyclase activity by a putative increase in 5-HT extracellular levels induced by fluoxetine, and the consequent occupation of 5-HT1 receptors by the agonist [5].

Pharmaceuticals such as fluoxetine and propranolol affecting the same molecular target or targets mediating the same biological process may also interact consistently with their MOAs when released as complex mixtures in natural environments. Specifically, because both fluoxetine and propranolol may affect the 5-HT–mediated pathway indirectly (fluoxetine) by increasing the availability of the agonist molecules or directly (propranolol) by binding to the receptor and preventing its occupation by the agonist, the degree of serotonergic regulation of mussel tissues would play a significant role in determining the net effect of the combined exposure to the pharmaceuticals. As reported by Franzellitti et al. [52], the main effects of mussel coexposure to fluoxetine and propranolol on cAMP-related parameters measured in the digestive gland were ascribed to propranolol, supporting the idea of a prevalent adrenergic control in this tissue over a 5-HT–mediated regulation. In contrast, antagonistic effects of the compounds were observed in the mantle/gonads, consistent with the action of propranolol as a pharmacological antagonist of 5-HT1 receptors, and with a prevalent serotonergic control in this tissue.

The alterations in the cAMP-dependent pathway described above would interfere with neuroendocrinological modulation of important functions in bivalves. As recently reviewed [62], the cAMP-dependent pathway is well known for regulating several functions in mollusks and, among others, the nucleotide modulates the availability of mantle glycogen as fuel for gonad maturation. Furthermore, recent investigations have suggested a role of cAMP in the control of key cytoprotective mechanisms providing an adaptive advantage for marine bivalves to cope with environmental challenges [69,72]. Therefore, conservation of therapeutic effects of pharmaceuticals targeting cell-signaling mediators may impair the ability of organisms to cope with environmental stressors, thus potentially undermining their fitness.

Biomarkers of exposure

In addition to specific effects, further biological alterations more related to the unwanted side effects of pharmaceuticals are documented in exposed marine organisms. The link between exposure and a contaminant, its interaction with cells and organs, and the final adverse outcome at the individual, population, or community level have been established in the framework of adverse outcome pathways [50]. As widely discussed by Hutchinson et al. [73], adverse outcome pathway information could provide a valuable guide for selecting bioassays and biomarkers to be used for effects-based analysis of pharmaceuticals in the marine environment.

The available reports on marine animal exposures mainly rely on laboratory studies under controlled conditions, providing convincing evidence of sublethal effects of pharmaceuticals observed in marine organisms also at environmentally relevant levels (Table 1). Field studies have been neglected, despite their relevance in setting guidelines. This is mainly related to the fact that more knowledge has to be acquired on the possible effects of pharmaceuticals on nontarget organisms, and we are not yet ready to interpret complex effects after in situ exposures and to discriminate the contribution of pharmaceuticals within a combination of stressors. In addition, studies have focused mainly on invertebrates, whereas pharmaceutical effects on marine teleosts have been poorly evaluated.

Lysosome membrane stability. Carbamazepine is used to treat epilepsy and other diseases related to neural hyperexcitability. Humans who are treated at relatively high doses and for prolonged periods are monitored for hepatotoxicity [74]. Marine mussels (*M. galloprovincialis*) exposed to 0.1 μg/L or 10 μg/L carbamazepine for 7 d showed 60% and 80% reductions in hemocyte lysosome membrane stability (LMS), respectively [60], indicating harmful effects of carbamazepine even at the lowest concentration found in coastal habitats. A significant concentration-dependent reduction in LMS also was observed after a 7-d mussel exposure to propranolol in the environmental range of 0.3 ng/L to 30 ng/L [51]. Moreover, fluoxetine reduced LMS by approximately 40% in mussels exposed for 7 d at a concentration as low as 0.03 ng/L, with a dose-dependent effect [5]. Significant reductions in LMS have been observed in the marine mussel *Perna perna* exposed to the antibacterial compound triclosan at 1.2 μg/L and 12 μg/L. The decrease in LMS was time-dependent, from 24 h, to 48 h, and up to 72 h [75]. Reduction in LMS after exposure to pharmaceuticals is not limited to mussels; indeed, it was observed in the clam *R. philippinarum* after a 7-d exposure to 0.1 μg/L to 50 μg/L ibuprofen [76]. In the crab *Carcinus maenas*, caffeine (15 μg/L), ibuprofen (5 μg/L), carbamazepine, and novobiocin (1 μg/L) reduced LMS by 50% compared with control individuals after 28 d of exposure [77,78]. Among other examples, LMS was also greatly reduced in the colonian ascidian *Botryllus schlosseri* after in vitro exposure to ibuprofen (100 μg/L and 1000 μg/L for 60 min) [79].

Lysosomal membrane stability is a sensitive biomarker of cellular health directly correlated with mussel scope for growth and survival [80], thus providing a consistent early warning signal for pharmaceutical harmfulness. Nevertheless, lysosomes may also be potential targets for toxic effects of some pharmaceuticals. As a weakly basic drug with an amino group, fluoxetine was reported to be effectively sequestered by lysosomes of mammalian cells, leading to drug accumulation, increased lysosome volume, and loss of lysosomal functionality [81]. Accordingly, the LMS reduction observed in marine mussels after exposure to fluoxetine by Franzellitti et al. [5] was also accompanied by a significant dose-dependent increase in the lysosomal/cytoplasm ratio and in neutral lipid lysosomal accumulation in digestive glands; these parameters were also correlated with fluoxetine bioaccumulation within the tissue.

Antioxidant responses. Because a major side effect of prolonged or high-dose treatment with pharmaceuticals is the generation of reactive oxygen species (ROS), a large body of

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Table 1. Summary of the most commonly detected biomarker responses to pharmaceuticals in marine animals

Biomarker	Organism	Pharmaceutical	Exposure conditions	Effect	Ref.
Lysosomal membrane stability	Manila clam (*Ruditapes philippinarum*)	Caffeine, carbamazepine, ibuprofen, novobiocin	35-d in vivo exposure at 0.1–50 μg/L	Reduction in hemocytes	[76]
	Mediterranean mussel (*Mytilus galloprovincialis*)	Carbamazepine	7-d in vivo exposure to 0.1 μg/L and 10 μg/L (carbamazepina), 0.03–300 ng/L (fluoxetine), or 0.3–30 000 ng/L (propranolol)	Reduction in hemocytes	[5,51,60]
		Oxytetracycline	4-d in vivo exposure at 1 μg/L and 100 μg/L	Reduction in digestive gland	[7]
	Mussel (*Perna perna*)	Triclosan	24-h, 48-h, and 72-h in vivo exposure to 1.2–12 000 ng/L	Reduction in hemocytes (120–12 000 ng/L)	[75]
	Crab (*Carcinus maenas*)	Caffeine, carbamazepine, ibuprofen, novobiocin	28-d in vivo exposure at 0.1–50 μg/L	Reduction in hemocytes (15–50 μg/L)	[78]
	Colonian ascidian (*Botryllus schlosseri*)	Ibuprofen	60-min in vitro exposure at 100 μg/L and 1000 μg/L	Reduced	[79]
Phase I metabolism	Crab (*C. maenas*)	Carbamazepine	28-d in vivo exposure to 0.1–50 μg/L	Increased EROD and DBF activities in hepatopancreas (10–50 μg/L) and gills (0.1–50 μg/L EROD; 50 μg/L DBF); increased EROD in muscle (10–50 μg/L).	[77]
	Marine polychaete (*Hediste diversicolor*)	Fluoxetine, propranolol, 17-α-ethynylestradiol	17-d in vivo spiking-sediment exposure at 0.01–100 ng/g	Increased EROD activity (fluxetine, propranolol); bell-shaped trend of EROD activity (17-alpha-ethynylestradiol)	[27]
		Ibuprofen	17-d in vivo spiking-sediment exposure at 0.05–500 ng/g	Decreased DBF activity (0.5 ng/g and 50 ng/g)	[27]
	Mediterranean mussel (*M. galloprovincialis*)	Acetaminophen, propranolol	10-d in vivo exposure at 23 μg/L and 403 μg/L	Increased CbE activity in digestive gland	[94]
Lipid peroxidation	Mediterranean mussel (*M. galloprovincialis*)	Acetaminophen	10-d in vivo exposure at 23 μg/L and 403 μg/L	Increased in digestive gland	[94]
		Carbamazepine	7-d in vivo exposure at 0.1 μg/L and 10 μg/L	Increased in gills (0.1 μg/L and 10 μg/L) and mantle/gonads (10 μg/L)	[60]
		Diclofenac, ibuprofen	Time-course (0–15 d) in vivo exposure at 250 ng/L	Reduced in gills, increased (3 d) and reduced (7 d) in digestive gland (diclofenac); bell-shaped trend in digestive gland (ibuprofen)	[82,83]
		Oxytetracycline	4-d in vivo exposure at 1 μg/L and 100 μg/L	Increased in gills	[7]
		Propranolol	10-d in vivo exposure at 11 μg/L and 147 μg/L	Increased in gills (147 μg/L)	[94]
	Crab (*C. maenas*)	Carbamazepine, novobiocin	28-d in vivo exposure at 0.1–50 μg/L	Increase in hepatopancreas, muscle, and gills	[77]
	Marine polychaete (*H. diversicolor*)	Carbamazepine, ibuprofen, propranolol	17-d in vivo spiking-sediment exposure at 0.05–500 ng/g	Increased (500 ng/g carbamazepine; 5 ng/g ibuprofen); bell-shaped trend (propranolol)	[27]
		Fluoxetine, 17-α-ethynylestradiol	17-d in vivo spiking-sediment exposure at 0.01–100 ng/g	Increased (100 ng/g fluoxetine; 0.01 ng/g 17-alpha- ethynylestradiol)	[27]
	European clam (*Ruditapes decussata*)	Carbamazepine	96-h acute test at 0.03–9 μg/L	Reduced levels (9 μg/L)	[85]
	Manila clam (*R. philippinarum*)	Carbamazepine	96-h acute test at 0.03–9 μg/L	Increased levels (9 μg/L)	[85]
Antioxidant responses	Mediterranean mussel (*M. galloprovincialis*)	Acetaminophen	10-d in vivo exposure at 23 μg/L and 403 μg/L	Increased GST activity in digestive gland and gills	[94]
		Carbamazepine	7-d in vivo exposure at 0.1 μg/L and 10 μg/L	Increased CAT activity in digestive gland (0.1 μg/L and 10 μg/L) and mantle/gonads (10 μg/L) Increased GST activity in digestive gland (10 μg/L) and mantle/gonads (0.1 μg/L and 10 μg/L)	[60]
		Diclofenac, ibuprofen	Time-course (0–15 d) in vivo exposure at 250 ng/L	Increase in SOD and GR activities in gills (3-d exposure; diclofenac) Increase of SOD, GST activities (7 d) and CAT activity (3 and 7 d) in difgestive gland (diclofenac); bell-shaped trend of SOD, CAT, GR, and GST activities in digestive gland (ibuprofen)	[82,83]

(*continued*)

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Table 1. (*Continued*)

Biomarker	Organism	Pharmaceutical	Exposure conditions	Effect	Ref.
		Fluoxetine	7-d in vivo exposure at 0.03–300 ng/L	Bell-shaped trend of CAT activity in digestive gland and increase in mantle/gonads (0.3 ng/L and 3–300 ng/L) Increase of GST activity in digestive gland (3–300 ng/L) and in mantle/gonads (0.3 ng/L)	[5]
		Propranolol	7-d in vivo exposure to 0.3–30 000 ng/L	Bell-shaped trend of CAT activity in digestive gland and reduction in mantle/gonads Increase of GST activity in digestive gland and reduction in mantle/gonads	[51]
		Propranolol	10-d in vivo exposure at 11 μg/L and 147 μg/L	Decreased (11 μg/L) and increased (147 μg/L) GST activity in digestive gland; increased GST activity in gills (11 μg/L)	[94]
	Crab (*C. maenas*)	Carbamazepine	28-d in vivo exposure at 0.1–50 μg/L	Increased GST and GPx activities in hepatopancreas (10–50 μg/L), muscle (0.1–50 μg/L), and gills (10–50 μg/L)	[77]
		Fluoxetine	7-d in vivo exposure at 0.5–750 μg/L	Increased GST activity in hepatopancreas (3 μg/L, 120 μg/L, and 750 μg/L) Increased GR activity and total glutathione levels in hepatopancreas (120 μg/L and 750 μg/L)	[101]
		Novobiocin	28-d in vivo exposure to 0.1–50 μg/L	Increased GST and GPx activities (10–50 μg/L) in hepatopancreas; increased GPx activities (10–50 μg/L) in muscle; increased GST (50 μg/L) and GPx (1–50 μg/L) activities in gills	[77]
	Manila clam (*R. philippinarum*)	Acetaminophen	96-h acute test at 0.05–5 mg/L	Decreased (0.05 mg/L) and increased (5 mg/L) GST activity	[86]
		Carbamazepine	96-h acute test at 0.03–9 μg/L	Increased total GSH levels, and GR activity; reduced GSH/GSSG and CAT activity; bell-shaped trend of SOD activity	[85]
	European clam (*R. decussata*)	Acetaminophen	96-h acute test at 0.05–5 mg/L	Decreased GST activity	[86]
		Carbamazepine	96-h acute test at 0.03–9 μg/L	Reduced CAT activity; increased total GSH levels, GSH/GSSG, and GR activity	[85]
	Marine polychaete (*H. diversicolor*)	Carbamazepine, propranolol	17-d in vivo spiking-sediment exposure at 0.05–500 ng/g	Increased GST (0.05 ng/g, 50 ng/g, and 500 ng/g) and GPx (0.05 ng/g) activities (carbamazepine); reduced GST activity (propranolol)	[27]
		Fluoxetine, 17-α-ethynylestradiol	17-d in vivo spiking-sediment exposure at 0.01–100 ng/g	Increased GR activity (1 ng/g, fluoxetine; 0.1 ng/g, 17-alpha-ethynylestradiol)	[27]
Acetylcholinesterase activity	Mediterranean mussel (*M. galloprovincialis*)	Acetaminophen	10-d in vivo exposure at 23 and 403 μg/L	Increased in gills (403 μg/L)	[94]
		Diclofenac	Time-course (0–15 d) in vivo exposure at 250 ng/L	Increased (7 d) in gills	[83]
		Fluoxetine	7-d in vivo exposure at 0.03–300 ng/L	Reduction in gills (0.03–0.3 ng/L and 30–300 ng/L)	[5]
	Crab (*C. maenas*)	Fluoxetine	7-d in vivo exposure at 0.5–750 μg/L	Increase in muscle (120 μg/L and 750 μg/L)	[101]
	Marine polychaete (*H. diversicolor*)	Carbamazepine, ibuprofen	17-d in vivo spiking-sediment exposure at 0.05–500 ng/g	Increased (0.5 ng/g, carbamazepine; 500 ng/g, ibuprofen)	[27]
		Fluoxetine, 17-α-ethynylestradiol	17-d in vivo spiking-sediment exposure at 0.01–100 ng/g	Increased (10–100 ng/g, fluoxetine); bell-shaped trend (17-alpha-ethynylestradiol)	[27]
DNA damage	Crab (*C. maenas*)	Carbamazepine, novobiocin	28-d in vivo exposure at 0.1–50 μg/L	Increased in hepatopancreas (50 μg/L), muscle (50 μg/L), and gills (10–50 μg/L)	[77]
	Colonian ascidian (*B. schlosseri*)	Ibuprofen	60-min in vitro exposure of hemocytes at 100 and 1000 μg/L	Increased	[79]
	Marine polychaete (*H. diversicolor*)	Ibuprofen, propranolol	17-d in vivo spiking-sediment exposure at 0.05–500 ng/g	Increased (5 and 500 ng/g, ibuprofen); decreased (50 ng/g, propranolol)	[27]
		Fluoxetine, 17-α-ethynylestradiol	17-d in vivo spiking-sediment exposure at 0.01–100 ng/g	Decreased	[27]

EROD = ethoxyresorufin O-deethylase activity; DBF = dibenzyl fluorescein dealkylase; CbE = carboxylesterase activity; CAT = catalase activity; GSH = glutathione levels; GST = glutathione-S-transferase activity; GR = glutathione reductase activity; GSH/GSSG = reduced/oxidized glutathione ratio; GPx = gluathione peroxidase activity; SOD = superoxide dismutase activity.

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data is available concerning the activities of antioxidant enzymes in exposed animals. Increases in superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione S-transferase (GST) activities were observed after 3 d and 7 d of Mediterranean mussel exposure to 75 ng/L ibuprofen, which returned to basal levels after 15 d. Similarly, activities of SOD, CAT, and GR were increased by diclofenac after a 3-d mussel exposure, and returned to basal levels at 7 d and 15 d despite the continuous presence of the drug [82,83]. Such a bell-shaped response to pharmaceuticals is shown in most studies on antioxidant enzyme activities. It is hypothesized that a cytoprotective response is elaborated at lower pharmaceutical concentrations, which may be overwhelmed at higher doses, leading to the accumulation of deleterious oxidation products [83].

Treatment of mussel hemocytes with 17β-estradiol significantly enhanced ROS production [72], and increases in CAT activity and mRNA levels were previously shown in digestive glands of mussel injected with low doses of the estrogen, whereas a reduction in CAT activity was observed at higher doses [84]. Antioxidant responses were very different in 2 clam species simultaneously exposed to carbamazepine at a concentration range of 0.03 μg/L to 9 μg/L [85]. Activity of SOD was increased in the clam *Ruditapes decussata* at 0.3 μg/L and 3 μg/L and to a lower extent at 9 μg/L carbamazepine. Activity of CAT was always reduced by the exposure to carbamazepine, whereas GR activity was dose-dependently enhanced at 0.3 μg/L, 3 μg/L, and 9 μg/L carbamazepine. Control levels of SOD activity were very low in exposed clams and were not modified by carbamazepine exposure. Activity of CAT was decreased at 0.3 μg/L and increased at 3 μg/L and 9 μg/L, whereas GR was slightly increased at 9 μg/L carbamazepine. Accordingly, the 2 species also showed differences in detoxification responses after 96 h of exposure to acetaminophen at concentrations much higher than those found in marine waters, namely, 0.05 mg/L, 0.5 mg/L, and 5 mg/L [86]. Indeed, GST activity was reduced at all drug concentrations in *Venerupis decussata* but increased in *Venerupis philippinarum* at 5 mg/L. *Hediste diversicolor* exposed to sediments spiked with carbamazepine, ibuprofen, fluoxetine, ethinylestradiol, or propranolol at environmentally relevant concentrations developed poor responses of antioxidant enzymes [27]. Overall, exposure to a number of environmental pharmaceuticals triggers antioxidant responses. In the majority of studies, the response was rather complex, showing species and tissue specificity (e.g., mussel gills vs digestive glands, [87]; mussel digestive glands vs mantle/gonads [51]) and depending on the physiological state of the animals or concomitant challenges. Therefore, the activities of antioxidant enzymes can give information on the ability of pharmaceuticals to induce oxidative stress in marine animals, although complementary analysis must be performed to consistently diagnose detrimental effects of the chemical compounds.

Oxidative stress products. The products of oxidative stress have also been studied in marine mollusks in terms of lipid peroxidation levels (LPO). *Hediste diversicolor* exposed to a range of carbamazepine, ibuprofen, fluoxetine, ethinylestradiol, or propranolol concentrations (0.01–500 ng/L) showed significantly increased LPO levels at 500 ng/L carbamazepine and fluoxetine; 5 ng/L ibuprofen; 0.01 ng/L ethinylestradiol; and 0.05 ng/L, 0.5 ng/L, and 5 ng/L propranolol [27]. Gills of bivalves appeared to be the organ most sensitive to oxidative effects, and LPO increased linearly with the time of exposure to a mixture of pharmaceuticals (ibuprofen, diclofenac, and fluoxetine) in the ng/L range [87]. Levels of LPO increased significantly in the crab *Carcinus maenas* exposed to 0.1 μg/L to 50 μg/L carbamazepine or novobiocin [77]. No changes in LPO were observed in *V. philippinarum* exposed to acetaminophen, whereas increased levels were shown in *V. decussata* only at the highest concentration tested [86]. Reductions in LPO levels were reported in mussels after fluoxetine exposure (0.3 ng/L and 30 ng/L) [5].

Neurotoxicity. Acetylcholinesterase (AChE) plays an important role in the functioning of the neuromuscular system by preventing continuous muscular contraction in all animals, and AChE activity has been proposed as a biomarker of exposure to neurotoxic compounds in aquatic organisms [41]. Increases in AChE activity were found in *H. diversicolor* exposed to fluoxetine at 10 ng/g and 100 ng/g spiked sediment and to ethinylestradiol at concentrations of 0.01 ng/g, 0.1 ng/g, and 1 ng/g spiked sediment, remaining at basal values at higher concentrations [27]. Activity of AChE was significantly increased in *V. philippinarum* exposed for 3 d to ibuprofen only at concentration as high as 1 mg/L. In contrast, after exposure to propanolol or acetaminophen, AChE activity was reduced in the Mediterranean mussel (*M. galloprovincialis*) [13].

Immune responses. The immune system of *B. schlosseri* was significantly affected after exposure to ibuprofen (100 μg/L and 1000 μg/L for 60 min) [79]. The morphology of phagocytes was modified, because cells withdrew pseudopodia and assumed a spherical shape as a consequence of decreased actin polymerization; phagocytosis was also inhibited [79]. Inhibition of phagocytosis was previously observed in oyster (*C. gigas*) hemocytes after exposure to ibuprofen, in a mixture also including 2 herbicides [88].

Effects at the organism level. The estuarine amphipod *Gammarus locusta* was chronically exposed to the hypercholesterolemic drug simvastatin for 45 d at a 64 ng/L to 8000 ng/L concentration range [89]. In these conditions, simvastatin strongly affected growth, gonad maturation, and reproduction of the amphipod [89]. For estuarine organisms in particular, the osmotic stress may induce additive or synergistic effects with respect to pharmaceutical administration, as demonstrated for growth and development of the common prawn (*Palemon serratus)* larvae exposed to diclofenac and clotrimazole in the low μg/L concentrations [90]. Ibuprofen, diclofenac, and bezafibrate strongly affected the development of *M. galloprovincialis* larvae exposed for 48 h in the range of 0.01 μg/L to 1000 μg/L. In particular, significant effects of diclofenac were seen at concentrations as low as 0.1 μg/L, effects that reached their maximum extent at 1 μg/L; no effect was shown at the highest concentration tested [91].

Behavioral effects were also observed in marine animals after exposure to pharmaceuticals, although in single studies and referring to 1 species. Among these is the reduction of cuttlefish (*Sepia officinalis)* efficiency at striking prey after exposure to fluoxetine at concentration as low as 1 ng/L [92], and the higher preference for light and negative geotaxis in the amphipod *Echinogammarus marinus* exposed to fluoxetine and serotonin at lower concentrations of the range tested (10–100 ng/L [93]). Feeding rate reduction and enhancement were observed in *M. galloprovincialis* after exposure to propranolol and acetaminophen, respectively [94].

Morando et al. [95] observed effects at the systemic level in the gulf toadfish *Opsanus beta* injected with fluoxetine in the range 25 μg/L to 100 μg/L. Concentrations were much higher than those found in the blood of fish exposed to realistic environmental concentrations; however, these experiments highlighted reduction in fish survival and profound

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alteration of osmoregulation because of an increased excretion of urea in the gills, stimulation of intestinal fluid absorption, and lower plasma osmolarity at the lowest doses of injected fluoxetine. Potential effects of low levels of waterborne fluoxetine can be hypothesized.

Future perspectives for biomonitoring of pharmaceuticals. A reduction in pharmaceutical use for human or animal therapy is not an option; and because marine waters represent shared environments, integrated measures need to be defined to deal with the problem of pharmaceutical contamination and to expand our knowledge of how the quality of coastal habitats may be efficiently monitored to anticipate possible health and environmental risks. Current standard ecotoxicity tests do not appear to be sensitive enough to detect adverse effects of pharmaceuticals at environmental levels [43]; thus, new approaches must be developed on the basis of new achievements. The data reported in the present review and in Table 1 point out that LMS evaluated in invertebrate hemocytes displays high sensitivity and consistent reduction after in vitro and in vivo exposure to several pharmaceuticals. Therefore, LMS may be exploited for rapid screening of pharmaceutical exposure. In addition, very recent studies have reported that tests on larval stages could represent a promising approach for marine species given their rapid responses, high sensitivity, and high ecological value [55,96]. In our opinion, the concomitant analysis of multiple MOA relevant endpoints and biomarkers covers different routes of pharmaceutical interactions with marine organisms and may help in developing new biomonitoring strategies for the marine environment.

CONCLUSIONS

Worldwide, the majority of human settlements are located near the coasts, and further increases in costal populations are foreseen [9,10]. Because of their high recreational value, many coastal areas experience significant seasonal peaks in the population, and in some areas the tourists even overshadow the permanent population. These trends suggest the potential for increasing input of human pharmaceuticals into coastal environments and the need for assessment strategies.

Large quantities of pharmaceuticals are consumed daily by humans and partially excreted as parental compounds or metabolites in a biologically active form [13]. They are finally discharged (at different percentages) into coastal waters from WWTP effluents and at higher concentrations in untreated water discharges, which still account for a significant percentage of total discharges [97].

Scientists agree that pharmaceuticals are contaminants of concern in marine environments [14] and thus deserve more attention than that given to date. Appropriate strategies for integrated chemical and biological monitoring at sea have to be set up, with more focus on the particular features of pharmaceuticals. Indeed, the power of these substances has largely been underestimated, and pharmaceuticals have been addressed as conventional contaminants although they are not. Many data have demonstrated that these substances have effects at very low doses; and although nontoxic in the short term, they are detrimental after chronic exposures. Monitoring plans spanning wider regional as well as seasonal dynamic distributions should be improved to fill the gaps previously discussed and to account for the impact of temperature and drought on treatment plants, seasonal pathologies, and tourism. On the whole, the generation of databases on existing pharmaceuticals in marine environments is needed, as suggested by Daughton [98].

Implementation of mitigation actions taken inland [99] and the development of indicators to monitor whether they are effective [100] are also crucial parts of the process, bearing in mind that pharmaceutical concentrations at sea are strictly related to human settlements; thus, threats will increase in parallel with rapidly expanding urbanization along rivers and coastal zones. Similarly, more harmonized and possibly standardized protocols for biological screening need to be developed toward a comprehensive international strategy for assessing the risk posed by pharmaceutical contamination. Unlike the studies that have produced underestimations in the past, chronic exposures and specific MOAs of pharmaceuticals need to be considered as major aspects.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3131.

Data availability—Bibliographic data summarized in Figures 1 and 2 are provided as Supplemental Data in Tables S1, S2, and S3. For all other data, please contact the corresponding author (elena.fabbri@unibo.it).

REFERENCES

1. Daughton CG, Ruhoy IS. 2009. Environmental footprint of pharmaceuticals: The significance of factors beyond direct excretion to sewers. *Environ Toxicol Chem* 28:2495–2521.
2. Lahti M, Brozinski J-M, Segner H, Kronberg L, Oikari A. 2012. Bioavailability of pharmaceuticals in waters close to wastewater treatment plants: Use of fish bile for exposure assessment. *Environ Toxicol Chem* 31:1831–1837.
3. Glassmeyer ST, Kolpin DW, Furlong ET, Focazio MJ. 2008. Environmental presence and persistence of pharmaceuticals: An overview. In Aga DS, ed, *Fate of Pharmaceuticals in the Environment and in Water Treatment Systems*. Taylor & Francis, Boca Raton, FL, USA.
4. Crain CM, Halpern SH, Beck MW, Kappel CV. 2009. Understanding and managing human threats to the coastal marine environment. The year in ecology and conservation biology. *Ann NY Acad Sci* 1162:39–62.
5. Franzellitti S, Buratti S, Capolupo M, Du B, Haddad SP, Chambliss CK, Brooks BW, Fabbri E. 2014. An exploratory investigation of various modes of action and potential adverse outcomes of fluoxetine in marine mussels. *Aquat Toxicol* 151:14–26.
6. Franzellitti S, Buratti S, Du B, Haddad SP, Chambliss CK, Brooks BW, Fabbri E. 2015. A multibiomarker approach to explore interactive effects of propranolol and fluoxetine in marine mussels. *Environ Pollut* 205:60–69.
7. Banni M, Sforzini S, Franzellitti S, Oliveri C, Viarengo A, Fabbri E. 2015. Molecular and cellular effects induced in *Mytilus galloprovincialis* treated with oxytetracycline at different temperatures. *PLoS One* 10:e0128468.
8. Nichols JW, Du B, Berninger JP, Connors KA, Chambliss CK, Erickson RJ, Hoffman AD, Brooks BW. 2015. Observed and modeled effects of pH on bioconcentration of diphenhydramine, a weakly basic pharmaceutical, in fathead minnows. *Environ Toxicol Chem* 34:1425–1435.
9. Li H. 2003 Management of coastal mega-cities—A new challenge in the 21st century. *Mar Policy* 27:333–337.
10. Neumann B, Vafeidis AT, Zimmermann J, Nicholls RJ. 2015. Future coastal population growth and exposure to sea-level rise and coastal flooding—A global assessment. *PLoS One* 10:e0118571.
11. Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K. 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. *Aquaculture* 306:7–23.
12. Fent K, Weston AA, Caminada D. 2006 Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 76:122–159.
13. Santos LH, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC. 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *J Hazard Mater* 175:45–95.

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14. Gaw S, Thomas KV, Hutchinson TH. 2014. Sources, impacts and trends of pharmaceuticals in the marine and coastal environment. *Philos Trans R Soc Lond B* 369:20130572.
15. European Medicines Agency Committee for Medicinal Products for Human Use (CHMP). 2006. European Medicines Agency guideline on the environmental risk assessment of medicinal products for human use. EMEA/CHMP/SWP/4447/00. London, UK.
16. Spongberg AL, Witter JD, Acuña J, Vargas J, Murillo M, Umaña G, Gómez E, Perez G. 2011. Reconnaissance of selected PPCP compounds in Costa Rican surface waters. *Water Res* 45:6709–6717.
17. Bayen S, Zhang H, Desai MM, Ooi SK, Kelly BC. 2013. Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: Influence of hydrodynamics and physical-chemical properties. *Environ Pollut* 182:1–8.
18. Fenet H, Arpin-Pont L, Vanhoutte-Brunier A, Munaron D, Fiandrino A, Martínez Bueno MJ, Boillot C, Casellas C, Mathieu O, Gomez E. 2014. Reducing PEC uncertainty in coastal zones: A case study on carbamazepine, oxcarbazepine and their metabolites. *Environ Int* 68:177–184.
19. Owen SF, Huggett DB, Hutchinson TH, Hetheridge MJ, Kinter LB, Ericson JF, Sumpter JP. 2009. Uptake of propranolol, a cardiovascular pharmaceutical, from water into fish plasma and its effects on growth and organ biometry. *Aquat Toxicol* 93:217–224.
20. Brooks BW, Foran CM, Richards SM, Weston J, Turner PK, Stanley JK, Solomon KR, Slattery M, La Point TW. 2003. Aquatic ecotoxicology of fluoxetine. *Toxicol Lett* 142:169–183.
21. McEneff G, Barron L, Kelleher B, Paull B, Quinn B. 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. *Sci Total Environ* 476–477:317–326.
22. Fu W, Franco A, Trapp S. 2008. Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ Toxicol Chem* 28:1372–1379.
23. Togola A, Budzinski H. 2007. Development of polar organic integrative samplers for analysis of pharmaceuticals in aquatic systems. *Anal Chem* 79:6734–6741.
24. Wille K, Noppe H, Verheyden K, Vanden Bussche J, De Wulf E, Van Caeter P, Janssen CR, De Brabander HF, Vanhaecke L. 2010. Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. *Anal Bioanal Chem* 397:1797–1808.
25. Gilroy EM, Balakrishnan VK, Solomon KR, Sverko E, Sibley PK. 2012. Behaviour of pharmaceuticals in spiked lake sediments—Effects and interactions with benthic invertebrates. *Chemosphere* 86:578–584.
26. Froehner S, Machado KS, Stefan E, Bleninger T, da Rosa EC, de Castro Martins C. 2012. Occurrence of selected estrogens in mangrove sediments. *Mar Pollut Bull* 64:75–79.
27. Maranho LA, Baena-Nogueras RM, Lara-Martín PA, DelValls TA, Martín-Díaz ML. 2014. Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete *Hediste diversicolor* as bioindicator species. *Environ Res* 134:353–365.
28. Caldwell MG, Wilson BA, Zhu J, Wallace GT, King JW, Olsen CR, Burgess RM, Smith JP. 2010. Temporal trends of triclosan contamination in dated sediment cores from four urbanized estuaries: Evidence of preservation and accumulation. *Chemosphere* 78: 347–352.
29. Liang X, Chen B, Nie X, Shi Z, Huang X, Li X. 2013. The distribution and partitioning of common antibiotics in water and sediment of the Pearl River Estuary, South China. *Chemosphere* 92:1410–1416.
30. Gomez E, Bachelot M, Boillot C, Munaron D, Chiron S, Casellas C, Fenet H. 2012. Bioconcentration of two pharmaceuticals (benzodiazepines) and two personal care products (UV filters) in marine mussels (*Mytilus galloprovincialis*) under controlled laboratory conditions. *Environ Sci Pollut Res* 19:2561–2569.
31. Martínez Bueno MJ, Boillot C, Fenet H, Chiron S, Casellas C, Gómez E. 2013. Fast and easy extraction combined with high resolution-mass spectrometry for residue analysis of two anticonvulsants and their transformation products in marine mussels. *J Chromatogr A* 1305: 27–34.
32. Martínez Bueno MJ, Herrera S, Munaron D, Boillot C, Fenet H, Chiron S, Gómez E. 2014. POCIS passive samplers as a monitoring tool for pharmaceutical residues and their transformation products in marine environment. *Environ Sci Pollut Res Int*, in press. DOI: 10.1007/s11356-014-3796-5.
33. Li W, Shi Y, Gao L, Liu J, Cai Y. 2012. Investigation of antibiotics in mollusks from coastal waters in the Bohai Sea of China. *Environ Pollut* 162:56–62.
34. Cruz Moreno-Bondi M, Dolores Marazuela M, Herranz S, Rodriguez E. 2009. An overview of sample preparation procedures for LC-MS multiclass antibiotic determination in environmental and food samples. *Anal Bioanal Chem* 395:921–946.
35. Juan-Garcia A, Font G, Pico Y. 2006. Determination of quinolone residues in chicken and fish by capillary electrophoresis-mass spectrometry. *Electrophoresis* 27:2240–2249.
36. Chen H, Liu S, Xu XR, Liu SS, Zhou GJ, Sun KF, Zhao JL, Ying GG. 2015. Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: Occurrence, bioaccumulation and human dietary exposure. *Mar Pollut Bull* 90:181–187.
37. Na G, Fang X, Cai Y, Ge L, Zong H, Yuan X, Yao Z, Zhang Z. 2013. Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China. *Mar Pollut Bull* 69:233–237.
38. Klosterhaus SL, Grace R, Hamilton MC, Yee D. 2013. Method validation and reconnaissance of pharmaceuticals, personal care products, and alkylphenols in surface waters, sediments, and mussels in an urban estuary. *Environ Int* 54:92–99.
39. Emnet P, Gaw S, Northcott G, Storey B, Graham L. 2015. Personal care products and steroid hormones in the Antarctic coastal environment associated with two Antarctic research stations, McMurdo Station and Scott Base. *Environ Res* 136:331–342.
40. de Wit CA, Alaee M, Muir DCG. 2006. Levels and trends of brominated flame retardants in the Arctic. *Chemosphere* 64:209–233.
41. Viarengo A, Lowe D, Bolognesi F, Fabbri E, Koehler A. 2007. The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comp Biochem Physiol C* 146:281–300.
42. Wille K, Kiebooms JA, Claessens M, Rappé K, Vanden Bussche J, Noppe H, Van Praet N, De Wulf E, Van Caeter P, Janssen CR, De Brabander HF, Vanhaecke L. 2011. Development of analytical strategies using U-HPLC-MS/MS and LC-ToF-MS for the quantification of micropollutants in marine organisms. *Anal Bioanal Chem* 400:1459–1472.
43. Aguirre-Martínez GV, Owuor MA, Garrido-Pérez C, Salamanca MJ, Del Valls TA, Martín-Díaz ML. 2015. Are standard tests sensitive enough to evaluate effects of human pharmaceuticals in aquatic biota? Facing changes in research approaches when performing risk assessment of drugs. *Chemosphere* 120:75–85.
44. Schmidt W, O'Rourke K, Hernan R, Quinn B. 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) and their comparison with standardized toxicity tests. *Mar Pollut Bull* 62:1389–1395.
45. Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ. 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. *Environ Sci Technol* 42:5807–5813.
46. Schmitt H, Boucard T, Garric J, Jensen J, Parrott J, Péry A, Römbke J, Straub J-O, Hutchinson TH, Sánchez-Argüello P, Wennmalm A, Duis K. 2010. Recommendations on the environmental risk assessment of pharmaceuticals: Effect characterization. *Integr Environ Assess Manag* 6:S588–S602.
47. European Centre for Ecotoxicology, Toxicology of Chemicals. 2007. Intelligent testing strategies in ecotoxicology: Mode of action approach for specifically acting chemicals. Technical Report 102. Brussels, Belgium.
48. Christen V, Hickmann S, Rechenberg B, Fent K. 2010. Highly active human pharmaceuticals in aquatic systems: A concept for their identification based on their mode of action. *Aquat Toxicol* 96: 167–181.
49. Ankley GT, Brooks BW, Huggett DB, Sumpter JP. 2007. Repeating history: Pharmaceuticals in the environment. *Environ Sci Technol* 41:8211–8217.
50. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29:730–741.
51. Franzellitti S, Buratti S, Valbonesi P, Capuzzo A, Fabbri E. 2011. The β-blocker propranolol affects cAMP-dependent signaling and induces the stress response in Mediterranean mussels, *Mytilus galloprovincialis*. *Aquat Toxicol* 101:299–308.
52. Franzellitti S, Buratti S, Valbonesi P, Fabbri E, 2013. The mode of action (MOA) approach reveals interactive effects of environmental

15528618, 2016, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/etc.3131 by National Institute Of Biology, Wiley Online Library on [09/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

pharmaceuticals on *Mytilus galloprovincialis*. *Aquat Toxicol* 140–141:249–256.
53. Rand-Weaver M, Margiotta-Casaluci L, Patel A, Panter GH, Owen SF, Sumpter JP. 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. *Environ Sci Technol* 47:11384–11395.
54. Ford AT, Fong PP. 2016. The effects of antidepressants appear to be rapid and at environmentally relevant concentrations. *Environ Toxicol Chem* 35:794–798 (*this issue*).
55. Fabbri E. 2015. Pharmaceuticals in the environment: Expected and unexpected effects on aquatic fauna. *Ann N Y Acad Sci* 1340:20–28.
56. Milan M, Pauletto M, Patarnello T, Bargelloni L, Marin MG, Matozzo V. 2013. Gene transcription and biomarker responses in the clam *Ruditapes philippinarum* after exposure to ibuprofen. *Aquat Toxicol* 126:17–29.
57. Knight J, Taylor GW, Wright P, Clare AS, Rowley AF. 1999. Eicosanoid biosynthesis in an advanced deuterostomate invertebrate, the sea squirt (*Ciona intestinalis*). *Biochim Biophys Acta* 1436:467–478.
58. Chen G, Pan B, Hawver DB, Wright CB, Potter WZ, Manji HK. 1996. Attenuation of cyclic AMP production by carbamazepine. *J Neurochem* 67:2079–2086.
59. Montezinho LP, Mork A, Duarte CB, Penschuck S, Geraldes CF, Castro MM. 2007. Effects of mood stabilizers on the inhibition of adenylate cyclase via dopamine D(2)-like receptors. *Bipolar Disord* 9:290–297.
60. Martin-Diaz L, Franzellitti S, Buratti S, Valbonesi P, Capuzzo A, Fabbri E. 2009. Effects of environmental concentrations of the antiepilectic drug carbamazepine on biomarkers and cAMP-mediated cell signaling in the mussel *Mytilus galloprovincialis*. *Aquat Toxicol* 94:177–185.
61. Weir MR. 2009. Beta-blockers in the treatment of hypertension: Are there clinically relevant differences? *Postgrad Med* 121:90–98.
62. Fabbri E, Capuzzo A, 2010. Cyclic AMP signaling in bivalve molluscs: An overview. *J Exp Zool A* 313:179–200.
63. Tierney AJ. 2001. Structure and function of invertebrate 5-HT receptors: A review. *Comp Biochem Physiol A* 128:791–804.
64. Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, Yang P, Zhang L, Wang X, Qi H, Xiong Z, Que H, Xie Y, Holland PW, Paps J, Zhu Y, Wu F, Chen Y, Wang J, Peng C, Meng J, Yang L, Liu J, Wen B, Zhang N, Huang Z, Zhu Q, Feng Y, Mount A, Hedgecock D, Xu Z, Liu Y, Domazet-Lošo T, Du Y, Sun X, Zhang S, Liu B, Cheng P, Jiang X, Li J, Fan D, Wang W, Fu W, Wang T, Wang B, Zhang J, Peng Z, Li Y, Li N, Wang J, Chen M, He Y, Tan F, Song X, Zheng Q, Huang R, Yang H, Du X, Chen L, Yang M, Gaffney PM, Wang S, Luo L, She Z, Ming Y, Huang W, Zhang S, Huang B, Zhang Y, Qu T, Ni P, Miao G, Wang J, Wang Q, Steinberg CE, Wang H, Li N, Qian L, Zhang G, Li Y, Yang H, Liu X, Wang J, Yin Y, Wang J. 2012. The oyster genome reveals stress adaptation and complexity of shell formation. *Nature* 490: 49–54.
65. Dailianis S, Piperakis SM, Kaloyianni M. 2005. Cadmium effects on ROS production and DNA damage via adrenergic receptors stimulation: Role of Na^+/H^+ exchanger and PKC. *Free Radic Res* 39:1059–1070.
66. Koutsogiannaki S, Evangelinos N, Koliakos G, Kaloyianni M. 2006. Cytotoxic mechanisms of Zn^{2+} and Cd^{2+} involve Na^+/H^+ exchanger (NHE) activation by ROS. *Aquat Toxicol* 78:315–324.
67. Lacoste A, Malham SK, Cueff A, Poulet SA. 2001. Noradrenaline modulates oyster hemocyte phagocytosis via α-adrenergic receptor-cAMP signaling pathway. *Gen Comp Endocrinol* 122:252–259.
68. Shpakov AO, Shipilov VN, Bondareva VM. 2005. Sensitivity of adenylyl cyclase signalling system of the mollusk *Anodonta cygnea* ganglions to serotonin and adrenergic agonists. *Ann N Y Acad Sci* 1040:466–468.
69. Franzellitti S, Fabbri E. 2013. Cyclic-AMP mediated regulation of *ABCB* mRNA expression in mussel haemocytes. *PLoS One* 8:e61634.
70. Garnerot F, Pellerin J, Blaise C, Mathieu M. 2006. Immunohistochemical localization of serotonin (5-hydroxytryptamine) in the gonad and digestive gland of *Mya arenaria* (Mollusca: Bivalvia). *Gen Comp Endocrinol* 149:278–284.
71. Fong PP, Philbert CM, Roberts BJ. 2003. Putative serotonin reuptake inhibitor-induced spawning and parturition in freshwater bivalves is inhibited by mammalian 5-HT2 receptor antagonists. *J Exp Zool A Comp Exp Biol* 298:67–72
72. Koutsogiannaki S, Franzellitti S, Fabbri E, Kaloyianni M. 2014. Oxidative stress parameters induced by exposure to either cadmium or 17β-estradiol on *Mytilus galloprovincialis* hemocytes. The role of signaling molecules. *Aquat Toxicol* 146:186–195.
73. Hutchinson TH, Lyons BP, Thain JE, Law RJ. 2013 Evaluating legacy contaminants and emerging chemicals in marine environments using adverse outcome pathways and biological effects-directed analysis. *Mar Pollut Bull* 74:517–525.
74. Santos NA, Medina WS, Martins NM, Rodrigues MA, Curti C, Santos AC. 2008. Involvement of oxidative stress in the hepatotoxicity induced by aromatic antiepileptic drugs. *Toxicol In Vitro* 22: 1820–1824.
75. Cortez FS, Seabra Pereira CD, Santos AR, Cesar A, Choueri RB, Martini Gde A, Bohrer-Morel MB. 2012. Biological effects of environmentally relevant concentrations of the pharmaceutical triclosan in the marine mussel *Perna perna* (Linnaeus, 1758). *Environ Pollut* 168:145–150.
76. Aguirre-Martínez GV, Buratti S, Fabbri E, DelValls AT, Martín-Díaz ML. 2013. Using lysosomal membrane stability of haemocytes in *Ruditapes philippinarum* as a biomarker of cellular stress to assess contamination by caffeine, ibuprofen, carbamazepine and novobiocin. *J Environ Sci (China)* 25:1408–1418.
77. Aguirre-Martínez GV, Del Valls TA, Martín-Díaz ML. 2013. Early responses measured in the brachyuran crab *Carcinus maenas* exposed to carbamazepine and novobiocin: Application of a 2-tier approach. *Ecotoxicol Environ Saf* 97:47–58.
78. Aguirre-Martínez GV, Buratti S, Fabbri E, Del Valls TA, Martín-Díaz ML. 2013. Stability of lysosomal membrane in *Carcinus maenas* acts as a biomarker of exposure to pharmaceuticals. *Environ Monit Assess* 185:3783–3793.
79. Matozzo V, Franchi N, Ballarin L. 2014. *In vitro* effects of the nonsteroidal anti-inflammatory drug, ibuprofen, on the immune parameters of the colonial ascidian *Botryllus schlosseri*. *Toxicol In Vitro* 28:778–783.
80. Moore MN, Allen JI, McVeigh A, Shaw J. 2006. Lysosomal and autophagic reactions as predictive indicators of environmental impact in aquatic animals. *Autophagy* 2:217–220.
81. Daniel WA, Wójcikowski J. 1997 Contribution of lysosomal trapping to the total tissue uptake of psychotropic drugs. *Pharmacol Toxicol* 80:62–68.
82. Gonzalez-Rey M, Bebianno MJ. 2012. Does non-steroidal anti-inflammatory (NSAID) ibuprofen induce antioxidant stress and endocrine disruption in mussel *Mytilus galloprovincialis*? *Environ Toxicol Pharmacol* 33:361–371.
83. Gonzalez-Rey M, Bebianno MJ. 2014. Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*. *Aquat Toxicol* 148:221–230.
84. Canesi L, Borghi C, Fabbri R, Ciacci C, Lorusso LC, Gallo G, Vergani L. 2007. Effects of 17 beta-estradiol on mussel digestive gland. *Gen Comp Endocrinol* 153:40–46.
85. Almeida A, Calisto V, Esteves VI, Schneider RJ, Soares AM, Figueira E, Freitas R. 2014. Presence of the pharmaceutical drug carbamazepine in coastal systems: Effects on bivalves. *Aquat Toxicol* 156:74–87.
86. Antunes SC, Freitas R, Figueira E, Gonçalves F, Nunes B. 2013. Biochemical effects of acetaminophen in aquatic species: Edible clams *Venerupis decussata* and *Venerupis philippinarum*. *Environ Sci Pollut Res Int* 20:6658–6666
87. Gonzalez-Rey M, Mattos JJ, Piazza CE, Bainy AC, Bebianno MJ. 2014. Effects of active pharmaceutical ingredients mixtures in mussel *Mytilus galloprovincialis*. *Aquat Toxicol* 153:12–26.
88. Luna-Acosta A, Renault T, Thomas-Guyon H, Faury N, Saulnier D, Budzinski H, Le Menach K, Pardon P, Fruitier-Arnaudin I, Bustamante P. 2012. Detection of early effects of a single herbicide (diuron) and a mix of herbicides and pharmaceuticals (diuron, isoproturon, ibuprofen) on immunological parameters of Pacific oyster (*Crassostrea gigas*) spat. *Chemosphere* 87:1335–1340.
89. Neuparth T, Martins C, Santos CB, Costa MH, Martins I, Costa PM, Santos MM. 2012 Hypocholesterolaemic pharmaceutical simvastatin disrupts reproduction and population growth of the amphipod *Gammarus locusta* at the ng/L range. *Aquat Toxicol* 155:337–347.
90. González-Ortegón E, Blasco J, Le Vay L, Giménez L. 2013. A multiple stressor approach to study the toxicity and sub-lethal effects of pharmaceutical compounds on the larval development of a marine invertebrate. *J Hazard Mater* 263:233–238.
91. Fabbri R, Montagna M, Balbi T, Raffo E, Palumbo F, Canesi L. 2014 Adaptation of the bivalve embryotoxicity assay for the high throughput screening of emerging contaminants in *Mytilus galloprovincialis*. *Mar Environ Res* 99:1–8.
92. Di Poi C, Darmaillacq AS, Dickel L, Boulouard M, Bellanger C. 2013. Effects of perinatal exposure to waterborne fluoxetine on memory processing in the cuttlefish *Sepia officinalis*. *Aquat Toxicol* 132–133:84–91.
93. Guler Y, Ford AT. 2010 Anti-depressants make amphipods see the light. *Aquat Toxicol* 99:397–404.

15528618, 2016, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/etc.3131 by National Institute Of Biology, Wiley Online Library on [09/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

94. Solé M, Shaw JP, Frickers PE, Readman JW, Hutchinson TH. 2010. Effects on feeding rate and biomarker responses of marine mussels experimentally exposed to propranolol and acetaminophen. *Anal Bioanal Chem* 396:649–656
95. Morando MB, Medeiros LR, McDonald MD. 2009. Fluoxetine treatment affects nitrogen waste excretion and osmoregulation in a marine teleost fish. *Aquat Toxicol* 95:164–171.
96. Minguez L, Di Poi C, Farcy E, Ballandonne C, Benchouala A, Bojic C, Cossu-Leguille C, Costil K, Serpentini A, Lebel JM, Halm-Lemeille MP. 2014. Comparison of the sensitivity of seven marine and freshwater bioassays as regards antidepressant toxicity assessment. *Ecotoxicology* 23:1744–1754.
97. United Nations-Water. 2015. UN-Water report calls for wastewater focus in post-2015 agenda. *Decade's Weekly*. 2015 February 6. [cited 2015 February 16]. Available from: http://www.un.org/waterforlifedecade/20150206_newsletter.shtml
98. Daughton CG. 2014. The Matthew effect and widely prescribed pharmaceuticals lacking environmental monitoring: Case study of an exposure-assessment vulnerability. *Sci Total Environ* 466: 315–325.
99. Daughton CG, Ruhoy IS. 2013. Lower-dose prescribing: Minimizing "side effects" of pharmaceuticals on society and the environment. *Sci Total Environ* 443:324–337.
100. Liebig M, Floeter C, Hahn T, Koch W, Wenzel A Römbke J. 2014. Risk mitigation measures: An important aspect of the environmental risk assessment of pharmaceuticals. *Toxics* 2:35–49.
101. Mesquita SR, Guilhermino L, Guimarães L. 2011. Biochemical and locomotor responses of *Carcinus maenas* exposed to the serotonin reuptake inhibitor fluoxetine. *Chemosphere* 85:967–976.
102. Bossus MC, Guler YZ, Short SJ, Morrison ER, Ford AT. 2014. Behavioural and transcriptional changes in the amphipod *Echinogammarus marinus* exposed to two antidepressants, fluoxetine and sertraline. *Aquat Toxicol* 151:46–56.
103. Di Poi C, Bidel F, Dickel L, Bellanger C. 2014. Cryptic and biochemical responses of young cuttlefish *Sepia officinalis* exposed to environmentally relevant concentrations of fluoxetine. *Aquat Toxicol*. 151:36–45.
104. National Library of Medicine, Specialized Information Services. 2005. *Hazardous Substances Databank* (HSDB). [cited 2015 February 16]. Available from: http://toxnet.nlm.nih.gov
105. Di Poi C1, Evariste L, Séguin A, Mottier A, Pedelucq J, Lebel JM, Serpentini A, Budzinski H, Costil K. 2014. Sub-chronic exposure to fluoxetine in juvenile oysters (*Crassostrea gigas*): Uptake and biological effects. *Environ Sci Pollut Res Int*, in press. DOI: 10.1007/s11356-014-3702-1.
106. Munari M, Marin MG, Matozzo V. 2014. Effects of the antidepressant fluoxetine on the immune parameters and acetylcholinesterase activity of the clam *Venerupis philippinarum*. *Mar Environ Res* 94: 32–37.
107. Gonzalez-Rey M, Bebianno MJ. 2013. Does selective serotonin reuptake inhibitor (SSRI) fluoxetine affects mussel *Mytilus galloprovincialis*? *Environ Pollut* 173:200–209.

15528618, 2016, 4, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/etc.3131 by National Institute Of Biology, Wiley Online Library on [09/11/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License