Ecotoxicology of pharmaceuticals in coastal and marine organisms

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Abstract

Pharmaceuticals are ubiquitous contaminants in aquatic environments with adverse biological effects linked to exposure, which has led to their classification as emerging pollutants of priority concern. Both parent compounds and metabolites, are continuously released into the aquatic environment via multiple dispersal pathways including treated and untreated wastewater effluents, aquaculture, animal husbandry and pharmaceutical industry, leading to point source acute toxicity and chronic exposure of non-target organisms. Toxicity of pharmaceuticals arises from their design to specifically target biological and metabolic pathways that are in most cases evolutionary conserved. Yet, research focus is overwhelmingly directed towards freshwater systems. Here we overview recent advances in occurrence and ecotoxicology of pharmaceuticals in coastal and marine environments, and critically review sources of major therapeutic classes to transition and coastal marine environments, their pathways and ecotoxicology, highlighting reported adverse effects of exposure at different levels of biological complexity. Overall, laboratory-based studies dominate and antidepressants were the most frequently analysed therapeutic class in coastal and marine species. Regarding endpoints and major taxonomic groups, increased focus on molecular changes and invertebrates was conspicuous. In the end, we outlined key areas and opportunities where future research should be prioritized to underpin effective management options. Ultimately, understanding the effects of pharmaceuticals in the marine environment is key to support effective risk management strategies.

Keywords: marine, coastal and transition environments, pharmaceutical, therapeutic class, exposure, effects, mode of action

1. Introduction

For as long as they have been produced, pharmaceutical compounds have been released in the environment. And albeit they and other personal care products are classified as contaminants of emerging concern, this term does not necessarily imply their occurrence in the environment is recent. It rather alludes to contaminants from multiple sources (domestic, industrial or agricultural) that escaped prior notice and classically were not monitored in spite of their potential to cause adverse effects to the environment; or to compounds for which only recently have environmental concerns been fully raised (Glassmeyer et al. 2007, Sauvé & Desrosiers 2014). In the end, the use of the term emerging contaminants has the intention to highlight the largely unregulated nature of the presence in the environment of substances such as pharmaceutical compounds, but also others such as cosmetics, UV blocker agents (sunscreens) or fragrances (Daughton 2016). Furthermore, the continuous and rapid technological development in highly sensitive analytical instrumentation has enabled the discovery and quantification of numerous compounds and substances in the aquatic environment, and from complex matrices, that previously went undetected (Pérez & Barceló 2007, Sanderson & Thomsen 2009, Klosterhaus et al. 2013).

Pharmaceuticals have come in to particular scrutiny regarding their occurrence and effects on aquatic environments due to a few key features. Firstly, both human and veterinary pharmaceutical compounds are continuously released to the environment worldwide, resulting in their ubiquitous and persistent presence. Moreover, their concentrations in aquatic ecosystems are projected to continue to rise, with mounting environmental concerns, due to an expected increase in both the access and the widespread use of medication by a growing global population (Kuster & Adler 2014). Additionally, unlike several chemical contaminants, pharmaceutical compounds are biologically active and target particular metabolic pathways that in many cases are evolutionary conserved (Gunnarsson et al. 2008, Furuhagen et al. 2014), eliciting effects at very low environmental concentrations (e.g. ng/L) and shown to specifically affect multiple algae and animal functions (e.g. Franzellitti et al. 2013, Aguirre-Martínez et al. 2015, Minguez et al. 2016). However, it is important to notice that the term pharmaceuticals does not refer to a specific or unambiguous class of molecules sharing an *a priori* defined set of chemical, physical or biological similarities, but to a varied group of therapeutic compounds used for human or veterinary treatment encompassing, a wide range of kinetics, metabolism, modes of action (MOA), and ultimately an array of potential underlying effects to the environment (Taylor & Senac 2014).

In this context, over the last couple of decades growing attention has been given to monitoring and evaluating the presence and the ecotoxicology of pharmaceutical compounds in the aquatic environment (Daughton 2016). Yet, in comparison to freshwater systems, where studies on the occurrence and potential effects of pharmaceuticals are manifold, transition and coastal marine environments have been comparatively overlooked or poorly investigated. In part, this is likely due to the assumption that dispersion and dilution processes, including from freshwater sources to estuarine and coastal environments, would be suffice to lessen or cancel any potential effects. Only recently has this trend begun to be reversed, with research gradually focusing towards coastal areas and showcasing that pharmaceuticals are present

throughout transition and marine environments at levels potentially or effectively adverse to different levels of biological complexity (e.g. Fatta-Kassinos et al. 2011, Klosterhaus et al. 2013, Gaw et al. 2014, Aminot et al. 2016, Arpin-Pont et al. 2016, Du et al. 2016, Fabbri & Franzellitti 2016). Moreover, it is important to highlight that presumed impacts on transition and coastal environments are expected to continue to increase allied to population growth and coastal settlement as well from accessory human activities such as aquaculture (Burridge et al. 2010, Gaw et al. 2014, Tornero & Hanke 2016). Overall, the increase in research and literature since 2014 regarding the occurrence, fate and ecotoxicology of pharmaceuticals in coastal and marine environments may, at least in part, be attributed to a review by Gaw et al. (2014), and the call for research prioritization. At that time, Gaw et al. (2014) found 49 studies from the year 2000 onwards reporting concentrations of pharmaceuticals in marine and coastal environments. A number that has since raised considerably, and we were now able to compile information from 124 studies (since the year 2000) focusing on the occurrence and effects of pharmaceuticals in transition and coastal marine environments [Web of Science search in February 2017 with the terms: Marine AND pharmaceutical AND (occurrence OR effect* OR toxicity)].

In the present chapter, we aim to provide a brief overview of the most recent advances in the literature regarding the occurrence and ecotoxicology of pharmaceuticals in coastal and marine environments. We critically assess the state of the art and provide an integrative analysis focusing on the sources of major therapeutic classes of pharmaceuticals to transition and coastal marine environments, their pathways and ecotoxicology to different levels of biological complexity, highlighting reported adverse effects of pharmaceuticals exposure in coastal and marine organisms. In the interest of a focused approach, the scope of the current chapter has been restricted to major therapeutic pharmaceutical compounds, excluding natural and synthesized hormones. Overall, we will prioritize *in situ* evaluations of effects of environmentally relevant concentrations; and aim in the end to highlight knowledge gaps and present-day challenges, and provide an outline of key areas and opportunities where future research should be prioritized to underpin the delineation of effective management options. Ultimately, understanding the effects of pharmaceuticals in the marine environment and unraveling their ecotoxicology, MOA, and bioaccumulation rates, together with research on their occurrence and fate, is key to safeguard potential threats to environmental and human health, and support effective risk management strategies.

2. Sources and occurrence of pharmaceuticals in coastal and marine environments

Tackling what is still a shortage of field data, and developing our understanding on the occurrence of pharmaceutical contaminants in coastal environments, their spatial and temporal patterns, as well as their impacts on the marine biota is paramount. In this section, we outline the major sources of pharmaceutical contaminants to marine environments as well as their contamination pathways, highlighting ranges of concentrations found, and briefly refer the physical and chemical processes that may influence the environmental concentrations of these contaminants in both water and sediments.

111 Sources and pathways of pharmaceutical contaminants to coastal and marine environments are
112 manifold (Kummerer 2009c, Gaw et al. 2014). Yet, estuarine and coastal areas receive a complex mixture
113 of pharmaceutical contaminants that originate from a set of overarching key origins, namely: *i*) human
114 household use; *ii*) hospital use; *iii*) veterinary applications, via aquaculture or from the terrestrial
115 environment, including livestock production or household pets care; *iv*) and industrial and commercial
116 activities linked to the production of pharmaceuticals (Figure 1). All these produce large amounts of waste
117 that via a multitude of entwined pathways result in the presence of pharmaceutical compounds, their
118 metabolites and transformation by-products directly or via diffuse routes in transition and coastal marine
119 environments and organisms. Assumptions that pharmaceuticals in marine environments would be
120 negligible due to hydrodynamics or dilution processes in coastal and marine environments are by large
121 currently refuted. In fact, pharmaceutical contaminants have been detected in marine environments at
122 distances that exceed tens and even hundreds of kilometers from what would be their anticipated sources
123 (e.g. WWTP marine outfalls, coastal areas) (Wille et al. 2010, Zhang et al. 2013, Alygizakis et al. 2016).
124 And even when contaminants are not found in water or sediments, or detected only sporadically,
125 pharmaceuticals are still detected in marine organisms such as bivalves and fish, showcasing their potential
126 for bioconcentration (Wille et al. 2011, Maruya et al. 2012, Klosterhaus et al. 2013).

127 The major route of entry for pharmaceuticals and their by-products in natural aquatic environments
128 are point source wastewater discharges of treated [i.e. outflow of waste water treatment plants (WWTP)
129 and septic tanks] and untreated sewage (Glassmeyer et al. 2007, Fatta-Kassinos et al. 2011). Estuarine and
130 coastal marine environments, particularly those near urban clusters, receive large volumes of these effluent
131 discharges both directly via coastal or offshore underwater outfalls (e.g. Togola & Budzinski 2008,
132 Alygizakis et al. 2016), and indirectly via loadings from streams and rivers where wastewater discharges
133 have taken place (Xu et al. 2013, Cantwell et al. 2016). For instance, the annual loads of pharmaceuticals
134 flushed out to sea from the Yangtze estuary are estimated to surpass 150 metric tons, as a result of the
135 discharge of c. 50 x 10^6 m^3 of sewage (Qi et al. 2014). In another study in South-west France, an assessment
136 of 53 compounds produced an estimated influx of c. 10 kg per day of pharmaceuticals to the Garonne
137 estuary (Aminot et al. 2016). It is important to highlight that even in peak conditions, treatment plants are
138 unable to remove all pharmaceutical contaminants from wastewaters, and that performance efficiency and
139 WWTP removal rates of pharmaceuticals vary significantly from 100 % to <1 %, depending on the type of
140 treatment, operating conditions, chemical loads, and the specific physico-chemical properties of the
141 different pharmaceutical compounds (Kim et al. 2007, Gros et al. 2010, Luo et al. 2014, Silva et al. 2014).
142 As a result, over the years different human and veterinary pharmaceuticals have been found in coastal and
143 marine waters over a wide range of concentrations, from e.g. 0.01 ng/L (e.g. Roxithromycin - antibiotic;
144 Yan et al. 2013) to 6800 ng/L (e.g. Norfloxacin - antibiotic; Zou et al. 2011) and even above 200000 ng/L
145 in areas closely affected by WWTP effluents (e.g. Paracetamol - analgesic; Togola & Budzinski 2008); as
146 well as in sediments (e.g. from 0.01 to c. 17 ng/g dry weight Metoprolol - β-blocker; Cantwell et al. 2016)
147 (see also Gaw et al. 2014, Arpin-Pont et al. 2016, Fabbri & Franzellitti 2016). It is worth highlighting that
148 the contamination and persistence of pharmaceuticals in some transition and coastal environments such as
149 bays, inlets, estuaries and coastal lagoons where water residency and flushing times are reduced, or with
150 periodic connections to the sea, may be of added concern. Many of these coastal areas are favored human

settlement or seasonal holiday hubs, and in addition to direct sewage discharges and other local loadings (e.g. river input, groundwater contamination) there is an increased potential risk hazard associated to the confined nature and the distinctive physico-chemical properties of these systems, where dilution and dispersion of contaminants is likely reduced and changes in sorption kinetics will affect the accumulation of pharmaceuticals over time (Dougherty et al. 2010, Liu et al. 2013, Moreno-González et al. 2015, Aminot et al. 2016).

Other sea based human activities such as shipping, particularly cruise and large passenger ships may have a significant impact in specific coastal and marine areas as a result of wastewater discharges (Alygizakis et al. 2016, Westhof et al. 2016). Over 20 million passengers board cruise ships every year (Cruise Line International Association Industry Outlook), with individual liners that hold passenger and crew numbers above those of small townships regularly visiting highly sought confined or sensitive areas, and though wastewater discharges are regulated (Annex IV of the MARPOL convention on pollution prevention), treatment performance still lacks effective administrative regulation or monitoring, so the potential for contamination is substantial. For instance, Westhof et al. (2016) estimated annual loads of Ibuprofen exceed 3.3 Kg for a ship with 4000 persons on board.

Wastewaters from healthcare and pharmaceutical production facilities are other key sources of pharmaceutical compounds to estuarine and coastal marine environments (Figure 1). By their own nature, hospital activities generate a sizable quantity of contaminated effluents. These are dependent on numerous factors which include, but are not limited to, bed density, number of patients or medical specialties, with several studies characterizing pharmaceutical residues in hospital wastewaters in different regions worldwide (e.g. Santos et al. 2013, Herrmann et al. 2015, Oliveira et al. 2015, Azuma et al. 2016). Compiling information on hospital and healthcare facility effluents, Oliveira et al. (2017) showcased that many pharmaceuticals were present at concentrations below 10 µg/L, though for several of the most common active ingredients values were significantly higher (e.g. Paracetamol 1368 µg/L, Ciprofloxacin 125 µg/L). Overall, though healthcare facilities have been pointed out as key contributors, everyday household discharges are still generally acknowledged as the main contributor of human use pharmaceuticals to the environment (see Kummerer 2009c, Le Corre et al. 2012, Herrmann et al. 2015). In part, this is due to the sheer number of users and the amount of pharmaceutical consumption that takes place in domestic context, with many outpatients also continuing treatment or receiving palliative care outside hospital facilities.

Regarding drug manufacturing, a number of studies have also reported environmental contamination as well as the damaging effects of exposure to effluents from pharmaceutical production sites, with several evidences of high concentrations in effluents, with contamination values reaching tens of mg/L (Fick et al. 2009, Cardoso et al. 2014, Larsson 2014). Remarkably, and for purposes of management and supervision, it is possible to reconstruct exposure pathways and disentangle factory source contamination from human use by evaluating the ratio of pharmaceutical precursor and of its human metabolites (e.g. Prasse et al. 2010). Overall, drug factory discharges, environmental risk and contamination patterns are not specifically linked to use patterns or seasonality, and will mainly affect coastal and marine environments via their localization, or via loading of rivers and streams with subsequent contamination

downstream. Though pharmaceutical industries are mostly located in south east Asia (e.g. Bangladesh, China, India and Pakistan), production sites elsewhere (e.g. Europe, US) are also identified as significant contamination sources (see Cardoso et al. 2014, Larsson 2014, Rehman et al. 2015).

Veterinary applications of pharmaceuticals in both aquaculture and land based animal husbandry or livestock productions are also known contributors of pharmaceuticals to natural environments (Figure 1). In response to the rising demand in seafood products worldwide, aquaculture has been seeing a continued boost in both the number of farms as well as production yield, and this is in part associated to the availability of an array of pharmaceutical compounds that enhance productivity (Sapkota et al. 2008, Tornero & Hanke 2016). The range of veterinary pharmaceuticals accessible to fish farmers include antibiotics, analgesics and antiparasitics, among others, some of them of generic human use, with many compounds applied prophylactically (Cabello 2006, Burridge et al. 2010, Tornero & Hanke 2016). Thus, any sea (coastal or estuarine) based aquaculture activities are direct entry points of pharmaceuticals to the marine environment. By large, pharmaceuticals are incorporated into feed, though other routes such as dilution and immersion in baths are available. In any case, these pharmaceutical compounds, as well as their excreted metabolites and transformation products will fuel environmental contamination and elicit impacts on non-target organisms (see Sapkota et al. 2008, Burridge et al. 2010, Chen et al. 2015). Other key pathways for pharmaceuticals to enter estuarine and coastal environments are wastewater discharges from land based aquaculture activities (Le & Munekage 2004, Zou et al. 2011). Though best practices vary worldwide, pond or tank based aquaculture of multiples species of crustaceans and fish is extensive throughout estuarine and coastal environments, with discharges made directly to these areas. Leakage from ponds is also an acknowledged pathway for veterinary pharmaceuticals to reach estuarine and coastal waters. Over the years the range of concentrations in wastewater discharges or water and sediments surrounding aquaculture activities has been found to vary widely from a few ng/l to 2.5 mg/L (Le & Munekage 2004, Chen et al. 2015, Kim et al. 2017). In some cases, the effect of released pharmaceutical loads may be aggravated by a combination of low flow conditions and the local abundance of juveniles of many species, as aquaculture farms are established in areas (e.g. estuarine habitats, mangroves) renowned to have a key nursery role (Beck et al. 2001) where potential effects on juvenile biota may be subsequently exported to adult populations (Rochette et al. 2010, Vasconcelos et al. 2011, Fonseca et al. 2015).

Discharges of both treated and untreated wastewaters from land based agricultural and livestock productions may ultimately also contribute to the presence of pharmaceuticals in estuaries and coastal environments (e.g. Lim et al. 2013, Awad et al. 2014, Paíga et al. 2016) (Figure 1). Furthermore, fecal excretion of metabolites over pastoral lands can contaminate the groundwater via leaching or run-off, as can the use of waste products as fertilizers (e.g. manure). Overall, the presence of pharmaceuticals in groundwater can originate from many sources, including sewage contamination (e.g. via septic tanks, or sewer leakage), contaminated leachate from landfill (e.g. animal carcasses, pharmaceutical waste products), use of contaminated sludge as fertilizer, as well as by the use of grey waters for irrigation (see review by Sui et al. 2015). The latter can all be relevant sources of pharmaceutical contamination to coastal marine environments, due to discharge and connectivity between groundwater and coastal systems (Dougherty et al. 2010, Sui et al. 2015) (Figure 1). Overall, due to the added risk of contamination to public drinking water, evaluating pathways of groundwater contamination is also paramount (e.g. Fick et al. 2009).

Upon pharmaceutical intake, significant fractions of parent compound are excreted unprocessed as well as in the form of metabolites and transformation by products. Irrespective of their sources, the fate and persistence of pharmaceuticals in coastal environments, as well as their subsequent potential to affect biota or bioconcentrate, are linked to key physico-chemical processes. Primarily, transport, biodegradation, transformation and sequestration (Figure 1). Overall, pathways and routes of exposure rely on both dissolved and particle transport (upon sorption to particulate matter, or sediments), with the fate of individual pharmaceuticals influenced by environmental conditions (e.g. salinity, suspended particulate matter, hydrodynamics, water column mixing, pH, turbidity or light penetration) as well as by their own physical and chemical properties (Glassmeyer et al. 2007). Thus, information collated for degradation or sorption of pharmaceuticals in freshwater environments may not be directly applicable in estuarine and marine contexts (see for instance Fenet et al. 2014, Gaw et al. 2014, Zhao et al. 2015, Fabbri & Franzellitti 2016). Nonetheless, hydrolysis, photolysis, biodegradation and adsorption are recognized as the most likely to alter pharmaceutical compounds. Understanding the complex interactions among sorption kinetics, potential resuspension and transport is crucial. All play a key role in the fate of pharmaceuticals along the interface between estuarine and coastal marine environments, and will determine the persistence of these compounds in the environment, their availability for bioaccumulation and exchange between environments (Kummerer 2009c, Liu et al. 2013). For instance, sorption to colloids can represent a sink for pharmaceuticals, increasing persistence but decreasing bioavailability (pending resuspension), whilst pH and salinity variations can also affect the ionization and solubility of different compounds. In transition areas, changing environmental conditions, as well as major seasonal variations in both environmental conditions and contaminant inputs (e.g. in recreational and holiday areas) may have significant repercussions in the occurrence of pharmaceuticals, bioavailability and transfer to the marine environment (Liu et al. 2013, McEneff et al. 2014, Moreno-González et al. 2015, Zhao et al. 2015).

3. Ecotoxicological effects of pharmaceutical exposure in coastal and marine organisms

Pharmaceuticals are designed to elicit biological effects at low doses, targeting specific metabolic and physiological pathways to achieve the desired therapeutic effects in human and veterinary medicine. In addition to high specificity at low concentrations, the evolutionary conservation of most molecular targets across taxa implies that environmental concentrations of pharmaceuticals have the potential to chronically impact exposed non-target aquatic organisms, with several adverse effects of pharmaceutical exposure reported in organisms at different levels of biological organization (e.g. Huerta et al. 2012).

The majority of ecotoxicological data on pharmaceutical compounds pertains to the freshwater environment (reviews by Crane et al. 2006, Fent et al. 2006), yet scientific contributions on occurrence and effects of pharmaceuticals on coastal and marine biota are increasing. For the current chapter, we found 124 studies focusing on ecotoxicology of pharmaceuticals in coastal and marine biota, as well as on bioaccumulation in wild coastal and marine organisms. Six major therapeutic classes, namely analgesic non-steroid anti-inflammatory drugs (NSAIDs), antidepressants, antibiotics, anticonvulsants,

antihypertensives and lipid regulators, clearly stood out and encompassed c. 91 % of all studies. This is likely due to their frequent detection in the marine environment as well as to their higher sales and consumption. Nonetheless, we cannot exclude a bias towards better-known or more-established compounds and MOA, with researchers favoring the possibility of data comparison with available information.

Concerning major taxonomic groups, mollusks are the most frequent group of organisms in pharmaceutical accumulation and toxicity studies (69 studies), followed by crustaceans and fish (32 and 27 studies, respectively) (Figure 2). Research with marine microorganisms, specifically microalgae and bacteria, were predominantly standard toxicity tests (11 and 6 studies, respectively). Overall, research on the effects of the major therapeutic classes is well distributed among taxonomic groups. Though there is less data available for antihypertensives and lipid regulators on mollusks, in comparison to other therapeutic classes (Figure 2). In terms of study type, most of the data relate to molecular changes (71 studies), which include gene and protein expression as well as other biochemical changes (e.g. biomarkers of oxidative stress and xenobiotics biotransformation) (Figure 2). Molecular endpoints are ubiquitous to all therapeutic classes and are the primary endpoint in analgesic and NSAIDs toxicity studies, whereas behavior endpoints are particularly associated with antidepressant exposures. Effects on development, mortality and reproduction of marine biota are also important endpoints in pharmaceutical toxicity assessments (27, 21 and 17 studies, respectively). 22 studies report the accumulation of pharmaceuticals in finfish, crustaceans and shellfish tissues (Figure 2). This is a noteworthy increase from the 14 studies identified in Gaw et al. (2014). Overall, antibiotics are the main therapeutic class investigated in bioaccumulation studies of marine and coastal organisms, with many studies linked to major aquaculture production. For instance, quinilones, sulfonamides and macrolides were detected in wild mollusk species collected along the coast of the Bohai Sea in China, with highest concentrations ranging from 36 to 1575 µg/kg (Li et al. 2012). Evaluating bioaccumulation and biomagnification of several antibiotic agents in a marine trophic web in Laizhou Bay (China), trimethoprim, nine sulfonamide, five fluoroquinolone and four macrolide antibiotics were all detected in marine invertebrates and fish (Liu et al. 2017). Additionally, sulfonamides and trimethoprim were found to biomagnify along the food web, whilst fluoroquinolones and macrolides were biodiluted. Nonetheless, local seafood consumption was considered unlikely to pose a major human health risk, regarding antibiotic concentrations. Other studies have focused on field monitoring of several pharmaceutical compounds in coastal waters via long term caging experiences with marine bivalves. Wille et al. (2011) detected five pharmaceuticals in caged mussels along the Belgium coast, namely salicylic acid, paracetamol, propranolol, ofloxacin and carbamazepine (highest concentrations ranging from 11 to 490 ng/g dw). McEneff et al. (2014) quantified carbamazepine, mefenamic acid and trimethoprim (peak concentrations of 7.28 to 9.22 ng/g dw) in *Mytilus spp*. following a one year experiment in the Irish coast. Yet, knowledge on fate, biotransformation and bioaccumulation of pharmaceutical compounds in the marine environment is still insufficient, as evidenced by the lack of concordance between field-derived bioaccumulation factors for ribbed horse mussels (*Geukensia demissa*) and model-predicted bioconcentration factors (Klosterhaus et al. 2013), as well as the lack of correlations between accumulation and observed molecular effects of pharmaceuticals (Mezzelani et al. 2016b).

Analgesics and NSAIDs reduce pain and inflammation and are amongst the highest consumed pharmaceuticals worldwide (Fent et al. 2006). Representative compounds include acetaminophen,

diclofenac, ibuprofen, ketoprofen and indomethacin. Biological targets are cyclooxygenases isoforms (Cox1 and Cox2) and these drugs act by non-specific inhibition of the synthesis of various prostaglandins from arachidonic acid (Vane & Botting 1998). Besides being involved in inflammation and pain responses, prostaglandins also play important roles in various physiological functions, including reproduction processes, reducing hypertension, fatty acids metabolism and synthesis of the protective gastric mucosa (Jones 1972). In fish and marine invertebrates, prostaglandins have been related with reproduction, ion regulation and immune responses (Sorbera et al. 2001, Rowley et al. 2005). Accordingly, the marine clam, *Ruditapes philippinarum*, exhibited significant immunological alterations following a 7 days exposure to ibuprofen particularly at the highest concentrations tested (500 and 1000 ug/L), (Matozzo et al. 2012). In the crustacean *Carcinus maenas*, osmoregulatory capacity was impaired with environmentally relevant concentrations of diclofenac over 7 days (10 ng/L and 100 ng/L), although no stress-related effects were observed in these individuals (Eades & Waring 2010). The potential of NSAIDs for endocrine disruption has been suggested in mussels *Mytilus galloprovincialis* exposed to 250 ng/L of ibuprofen or diclofenac for two weeks, with males and females presenting elevated levels of gonad vitellogenin-like proteins (Gonzalez-Rey & Bebianno 2012, 2014). However, a shorter time frame study with diclofenac (1 to 1000 ug/L, 96h) and bivalves *Mytilus spp.* did not show differences in the expression of these vitellogenin-like proteins (Schmidt et al. 2011). Akin to endocrine disruption, variable neurotoxic responses have been reported in mussels in response to analgesics and NSAIDs, namely via tissue specific inhibition and increased activity of acetylcholinesterase (AChE) (e.g. Milan et al. 2013, Mezzelani et al. 2016a, 2016b). Multibiomaker approaches highlighted changes in immunological responses, lipid metabolism and DNA integrity in *M. galloprovincialis* exposed separately to various analgesics and NSAIDs (acetaminophen, diclofenac, ibuprofen, ketoprofen or nimesulide) at 25 µg/L and 0.5 µg/L concentrations for 14 days (Mezzelani et al. 2016a, 2016b). Subsequent gene transcription analysis, via DNA microarrays, corroborated biomarker responses, highlighting the similarities on proposed MOA of NSAIDs between bivalves and vertebrate species (Mezzelani et al. 2016b). Albeit, the lack of a significant change in oxidative stress biomarkers (catalase, glutathione peroxidase and glutathione reductase activities, total glutathione, total oxyradical scavenging capacity) or recovery of the antioxidant system indicate that prooxidant response is not a key target in the pharmacology of these compounds (Gonzalez-Rey & Bebianno 2014, Mezzelani et al. 2016a, 2016b).

To our knowledge, effects of analgesics and NSAIDs in coastal and marine fish species have only been evaluated *in vitro*, and to test their effects on the activities of several enzymes related to xenobiotic and steroid metabolism. Ribalta and Solé (2014) reported that diclofenac significantly interfered in the CYP1A and CYP3A systems of Mediterranean fishes, particularly in the middle slope gadiform *Trachyrincus scabrous*. Ibuprofen exposure (100µM concentration) also inhibited the activity of the CYP3A4 enzyme, benzyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase (BFCOD) in the liver microsomal fraction of *Solea solea*, whilst acetaminophen had no effects on measured enzyme activities (Crespo & Solé 2016).

Antidepressants are neuroactive drugs for the treatment of depression and related psychiatric disorders (e.g. anxiety, obsessive-compulsive disorder, post-traumatic stress disorder). Selective serotonin reuptake inhibitors (SSRIs, such as fluoxetine, sertraline, citalopram) and serotonin and norepinephrine

reuptake inhibitors (SNRIs, such as venlafaxine) are some of the most prescribed antidepressants. Their highly specific MOA is based on the modulation of neurotransmission in the human brain, by targeting and blocking serotonin and norepinephrine reuptake proteins which leads to increased levels of these neurotransmitters in the synaptic cleft (Hiemke & Härtter 2000). Serotonin is also present in lower vertebrates and invertebrates, and as in humans, this biogenic monoamine appears to be involved in various physiological functions and behaviors interacting with reproduction and neuroendocrine processes (e.g. Winberg & Nilsson 1993, Winberg et al. 1997, Fong 1998). A review on the effects of antidepressant exposure on mollusks and crustaceans outlined impacts on metabolism, growth, reproduction, feeding, locomotion and behavior, yet the bulk of information was related to freshwater invertebrates (Fong & Ford 2014). Noteworthy, changes to spawning and larval release in bivalves as well as impaired locomotion and fecundity in snails occurred at environmentally relevant concentrations of antidepressants; although the occurrence of non-monotonic dose response curves were also reported with significant biological effects at lower but not at higher concentrations (Fong & Ford 2014). Considering toxicity to marine invertebrates, altered cognitive capacities (learning and memory retention) and less efficient cryptic behaviors were observed in cuttlefish *Sepia officinalis* following fluoxetine exposure at hatchling stages (1ng/L to 100ng/L) (Di Poi et al. 2013, 2014). Fluoxetine at concentrations ranging from 43 µg/L to 4.34 mg/L, also induced foot detachment from the substrate in five species of marine snails from different habitats (Fong & Molnar 2013). This potentially lethal outcome was also observed in two marine snail species exposed to venlafaxine, albeit different locomotion behaviors at the onset of foot detachment suggest that venlafaxine and fluoxetine have different physiological mechanisms of action (Fong et al. 2015). In another study, long-term exposure to low concentrations of fluoxetine (0.3ng/L to 300 ng/L) diminished algal clearance rates, growth and gonadosomatic index in California mussel *M. californianus* (Peters & Granek 2016). Pharmacological effects of fluoxetine and trait-based sensitivity have also been described for the marine worm *Hediste diversicolor*, based on increased serotonin levels in coelomic fluid and tissues. Effects included weight loss (up to 2% at 500 µg/L), decreased feeding rates (68% at 500 µg/L), and increased oxygen consumption and ammonia excretion (from 10 µg/L), but only limited influence on predator avoidance behaviors (Hird et al. 2016). Regarding sertraline, an early life-stage bioassay with sea urchin embryos found it to be highly toxic given the development of significant abnormalities at ng/L range concentrations (Ribeiro et al. 2015).

As for invertebrates, information on toxicity of antidepressants to fish stem mainly from freshwater species. These include deleterious effects on physiology, reproduction, behavior (e.g. reproductive, predator avoidance, territorial and defensive behaviors) and the potential of SSRIs as endocrine disruption compounds in various fish species (e.g. Mennigen et al. 2010a, 2010b, Schultz et al. 2011, Weinberger & Klaper 2014). Only four studies have evaluated the effects of antidepressants on coastal and marine fish species. Two *in vitro* studies reported species specific responses when assessing the impact of multiple pharmaceuticals on various enzyme activities coastal and deep-sea fishes. In Solé and Sanchez-Hernandez (2015) fluoxetine had no effect on carboxylesterase (CbE). The latter is involved in drugs metabolism and activation in humans and has been associated with pesticide detoxification in fish (Wheelock et al. 2008). In Ribalta and Solé (2014), fluoxetine inhibited cytochrome P450, which are enzymes linked to phase I of xenobiotic metabolism as well as to metabolism of endogenous compounds (e.g. steroids). Moreover, high

concentrations of fluoxetine, administered intraperitoneally to gulf toadfish *Opsanus beta*, affected branchial urea excretion and intestinal osmoregulation and resulted in a severe stress response with high levels of plasma cortisol (Morando et al. 2009). Additionally, fluoxetine (at 300 µg/L) has also been shown to affect marine fish behavior by reducing locomotor activity (EC25 2 µg/L) (Winder et al. 2012).

Beta-adrenergic receptor antagonists or β-blockers are antihypertensive drugs, commonly used to treat high blood pressure, angina, arrhythmias and other cardiac conditions. The MOA of blockers such as propranolol, atenolol and metoprolol consists in their specific binding to adrenoreceptors, competing with β-adrenergic agonists, decreasing resting heart rate, cardiac output and cardiac muscles contractibility, among others (Bourne 1981). A comparative physiology review described the similarity in beta-adrenergic receptors between mammals and fish, highlighting the diversity of physiological processes mediated by these receptors, and proposed biomarkers for β-blockers exposure included cardiovascular dysfunction, with subsequent potential negative effects on fish growth and fecundity (Owen et al. 2007). Only recently have the effects of β-blockers been evaluated in marine fish. Both studies were *in vitro* 100 µM propranolol exposures and described decreased CbE and BFCOD activities in hepatocytes of coastal and deep-sea fishes (Solé & Sanchez-Hernandez 2015, Crespo & Solé 2016).

Sublethal toxicology of propranolol on marine invertebrates include molecular, physiological and behavior changes. Motor activity of amphipod *Gammarus sp* has been shown to decrease even in the presence of predator cues, with respiration rate and feeding rate increasing with propranolol concentrations (100 µ/L to 5mg/L), probably to compensate for higher energy requirements (Wiklund et al. 2011). However, another study documented decreased feeding rate with associated oxidative damage and neurotoxicity in mussels (147 µg/L propranolol) (Solé et al. 2010). Exposure has also been linked with lower scope for growth, byssus strength and byssus abundance, potentially reducing substrate fixation ability in blue mussels, albeit at remarkably high propranolol concentrations (1 to 10 mg/L) (Ericson et al. 2010). A series of complementary experiments with mussels *M. galloprovincialis*, evaluated the MOA, molecular targets and associated endpoints, as well as unspecific effects of exposure to pharmaceuticals interacting with the cAMP-dependent pathway. cAMP cell signaling influences various physiological functions of mussels, namely reproduction, metabolic regulation, and filtering efficiency (Fabbri & Capuzzo 2010). Overall, exposure to environmentally-relevant concentrations of propranolol revealed differences on cAMP-related endpoints, suggesting differential expression of molecular targets in digestive glands, mantle/gonads and gill tissues (Franzellitti et al. 2011). Furthermore, coexposure to fluoxetine and propranolol suggested adrenergic regulation in the digestive gland, whereas serotonergic prevailed in the mantle/gonads of exposed mussels (Franzellitti et al. 2013). A multibiomarker approach revealed altered lysosomal parameters in mussels exposed to low propranolol concentration (0.3 ng/L), but other oxidative stress responses were only observed in the combined fluoxetine and propranolol treatment (Franzellitti et al. 2015). Furthermore, transcriptional and functional regulation of genes (e.g. ABCB) and transporters (e.g. P-glycoprotein) related to the multixenobiotic resistance (MXR) system highlighted the potential of propranolol to impair immunotoxic response in mussels, thus potentially affecting the ability to extrude contaminants and cope with environmental stressors in general (Franzellitti & Fabbri 2013, Franzellitti et al. 2016).

Anticonvulsants, also termed antiseizure or antiepileptics, are neuroactive drugs that interact with the central nervous system to treat epilepsy, bipolar disorder and are increasingly used as mood-stabilizers. Several compounds lead to decreased neuronal activity through different MOA. For example, benzodiazepines (such as diazepam or lorazepam) enhance γ-aminobutyric acid (GABA) neurotransmitter affinity for its receptor increasing chloride channel opening frequency, whilst carbamazepine acts via the blockage of sodium voltage-dependent channels of excitatory neurons inhibiting their sustained firing. Both result in lower cell excitation (Rang et al. 1999). A high degree of evolutionary conservation in GABA receptors (e.g. in fish) whose functions are related with reducing neuronal excitability and muscle tension has been reported (Carr & Chambers 2001). Even if carbamazepine's MOA is not fully understood, molecular targets appear to be conserved in mussels *M. galloprovincialis* following *in vivo* exposure, with reduction of the second messenger cyclic AMP and cAMP-dependent protein kinase (PKA), akin to responses in mammals (Martin-Diaz et al. 2009). Follow-up studies have described transcriptional and functional impairment of the MXR system in this species, highlighting the potential of carbamazepine, and others (i.e. fluoxetine and propranolol), in inducing immunotoxicological effects in marine bivalves at environmental relevant concentrations (Franzellitti et al. 2010, 2014, 2016). Other recent studies have focused on the effects of carbamazepine exposure on biomarker responses in several marine invertebrate species. Biomarkers of cellular health (e.g. lysosomal membrane stability, LMS), xenobiotic metabolism (e.g. EROD, GST), oxidative stress (e.g CAT, SOD, LPO), neurotoxicity (AChE) and genotoxicity (DNAd) have all been induced by varying exposure concentrations of carbamazepine in crab *C. maenas* (Aguirre-Martínez et al. 2013a, 2013c), clams *R. philippinarum* (Aguirre-Martínez et al. 2013b, 2016, Almeida et al. 2014) and *Scrobicularia plana* (Freitas et al. 2015), and in the polychaetes *H. diversicolor* (Pires et al. 2016) and *Diopatra neapolitana* (Freitas et al. 2015). Toxicity of anticonvulsants in coastal and marine fish has seldom been reported. Reduced oxidative stress response, increased swimming lethargy and abnormal posture were observed in the euryhaline fish *Gambusia holbokrii* following acute diazepam exposure (in mg/L range) (Nunes et al. 2008), with acute toxicity LC50 estimated at 12.7 mg/L (Nunes et al. 2005). *In vitro* assays confirmed inhibitory action of carbamazepine on carboxylesterase and BFCOD activity in coastal and deep-sea fish species (Solé & Sanchez-Hernandez 2015, Crespo & Solé 2016).

Lipid regulators or antilipidemic drugs include two major groups of lipid lowering agents: statins (e.g. simvastatin) and fibrates (e.g. bezafibrate, gemfibrozil). Their therapeutic role is to decrease the concentration of cholesterol and triglycerides (fibrates only) in blood plasma. Statins, such as simvastatin and atorvastatin, inhibit the activity of the enzyme HMG-CoA (3-hydroxymethylglutaryl coenzyme A reductase), which is responsible for feedback control of cholesterol synthesis. As a result of decreased intracellular cholesterol concentration, there is an over expression of LDL receptors in hepatocyte membranes which leads to resorption of circulating low-density lipoprotein cholesterol (LDL). Fibrates are peroxisomal proliferators whose MOA is not yet fully described. Their action is mediated through changes in the expression of the genes involved in lipoprotein metabolism. Fibrates bind to nuclear transcription factors of peroxisome proliferator activated receptors (PPARs), which then interacts with various cellular pathways determining hepatic lipid uptake and the metabolism of free fatty acids (Rang et al. 1999).

Antilipidemic toxicity data in marine organisms is limited, nonetheless recent studies have reported a variety of effects on the development and reproduction of invertebrates, whereas in fish responses

have been mainly assessed through molecular and biochemical changes. Chronic exposure to low levels of simvastatin (64 ng/L to 8 µg/L) in the marine amphipod *G. locusta*, ensued severe impacts on growth, gonad maturation and fecundity, the latter at relevant environmental concentrations (Neuparth et al. 2014). In sea urchin *Paracentrotus lividus*, Ribeiro et al. (2015) described delayed embryo development and increased percentage of embryo abnormalities when exposed to simvastatin (5 and 2 mg/L, respectively). Accordingly, another study considering a range of realistic environmental concentrations of simvastatin (0.16 and 1.6 µg/L), reported a decrease in development time and a concomitant increase in body length and growth rate of copepods *Nitokra spinipes* (Dahl et al. 2006). Regarding gemfibrozil, exposure induced vitellin-like proteins (ALP) at 1 mg/L in *Mytilus spp.*, which authors argued reveals the potential for endocrine disruption by this fibrate (Schmidt et al. 2011). Concerning lipid regulators toxicity to fish, gemfibrozil exposure (150 µg/L) upregulated PPAR-related genes transcription in juvenile *Sparus aurata*, albeit no concomitant activation of PPAR pathways was observed (Teles et al. 2016). Activation of immune responses was also suggested following increased mRNA levels of genes linked with pro-inflammatory processes at 15 ug/L gemfibrozil. Increase in cortisol, as evidence of stress related effects from gemfibrozil exposure were also observed, even if only at a concentration of 1.5 mg/L (Teles et al. 2016). Gemfibrozil (injected at 1 mg/kg body weight in *Solea senegalensis*) also induced the activity of CYP-related and phase II (UDPGT) biotransformation enzymes, whilst inhibiting antioxidant defenses (Solé et al. 2014). Furthermore, simvastatin and fenofibrate have been shown to inhibit carboxylesterase activity in various coastal and deep-sea fishes (Solé & Sanchez-Hernandez 2015), with simvastatin exposure also decreasing AChE levels in estuarine *Fundulus heteroclitus*, (1.25 mg/L, and LC50 of 2.68 mg/L) (Key et al. 2009).

Antibiotics are used in both human and veterinary medicine to treat bacterial infections, but may also be used as animal growth promoters. This group encompasses compounds derived from natural products (e.g. secondary metabolites of bacterial origin), semi-synthetic derivatives, or completely synthetic compounds which act through various mechanisms, such as suppression of bacterial cell wall or protein synthesis, and growth (Kummerer 2009a). Penicillins (e.g. penicillin and amoxicillin), macrolides (e.g. erythromycin), quinolones (e.g. ciprofloxacin) and tetracyclins (e.g. tetracycline) are amongst the most common types of antibiotics.

As antibiotics are designed to target microorganisms, their toxicity on bacteria and microalgae is commonly 2 to 3 orders of magnitude above effect levels reported for higher trophic groups (Kummerer 2009a). Accordingly, exposure to clarithromycin and clindamycin induced significant growth inhibition in the marine diatom *Skeletonema marinoi* at very low concentrations (EC50 of 156 and 154 ng/L, respectively) (Minguez et al. 2016). In contrast, Aguirre-Martínez et al. (2015) reported an EC50 of 400 mg/L for inhibition of bacterial luminescence in *Vibrio fischeri*, after 15 min of exposure to the antibiotic novobiocin, and an IC50 of 72.8 mg/L for growth inhibition in the algae *Isochysis galbana* (96h exposure period). This study also reported effects concentrations (in the mg/L range) for other pharmaceuticals, yet novobiocin showed highest toxicity for microorganisms when compared with IC50 values determined for carbamazepine, ibuprofen and caffeine. Growth of marine microalgae (*I. galbana* and *Tetraselmis chui*) was inhibited by three different antibiotics not usually found in the environments (chloramphenicol, florfenicol, and thiamphenicol) with EC50 values ranging from 1.3 to 158 mg/L. Concerning other

phototrophs, one study reported that sulfathiazole exposure, in concentrations commonly used in aquaculture (25 to 50 mg/L), induced growth inhibition on macroalgae *Ulva lactuca* (Leston et al. 2014).

In marine bivalves, exposure to trimethophin (300 to 900 ng/L) and to amoxicillin (100 to 400 µg/L) affected haemocyte parameters in both *R. philippinarum* and *M. galloprovincialis* (Matozzo et al. 2015, Matozzo et al. 2016). Genotoxicity of amoxicillin was also confirmed via increased micronucleus frequency in both species' haemolymph (Matozzo et al. 2016). Similarly, exposure to environmental concentrations of oxytetracycline resulted in decreased lysosomal membrane stability in mussels (Banni et al. 2015). Regarding crustaceans, Han et al. (2016), described several toxicity effects of trimethophin exposure (in the mg/L range) in copepod *Tigriopus japonicas*, including increased ROS levels, upregulation of antioxidant and xenobiotic detoxication-related genes, delayed development time and impaired reproduction. Antibiotic toxicity in marine fish, encompasses thus far, feeding behavior and biomarker responses in juveniles of the common goby *Pomatoschistus microps* exposed to cefalexin (from 1.3 to 10 mg/L) (Fonte et al. 2016). At 20 ºC and over 4 days exposure, predation performance was significantly impaired (> 5 mg/L) and lipid peroxidation levels increased (at 10 mg/L). At 25 ºC cefalexin toxicity increased with a decrease of predation performance at 2.5 mg/L (Fonte et al. 2016).

Antibiotics could also have relevant ecosystem level effects through changes to microbial communities and their functions (e.g. denitrification, organic matter decomposition), compromising ecosystem health (Kummerer 2009a, Caracciolo et al. 2015). Furthermore, constant environmental exposure could promote development of antibiotic resistance (Kummerer 2009b), which is a public health issue if resistance is transferred to human pathogens (Baran et al. 2011).

Despite the limited number of studies, in comparison to freshwater systems, the information currently available on the ecotoxicity of pharmaceuticals to coastal and marine species can already be taken into consideration for management and regulation purposes. The examples highlighted in this chapter clearly demonstrate that multiple pharmaceutical compounds have adverse effects in coastal and marine organisms at environmentally relevant concentrations (e.g. Franzellitti et al. 2016, Minguez et al. 2016). However, current legislation is still mostly based on freshwater toxicity data, albeit the marine environment may be more sensitive to pharmaceutical residues than freshwater (Minguez et al. 2016 - based on a comparative toxicity analyses of 48 pharmaceuticals in both marine and freshwater microalgae and crustacean species). Ultimately, there are still multiple shortcomings in the evaluation of pharmaceutical contamination in coastal and marine environments that we should aim to resolve.

4. Knowledge Gaps, Current Challenges and Futures perspectives

There is still a lack of information regarding concentrations, fate and ecotoxicology of pharmaceuticals in coastal and marine environments (Brausch et al. 2012, Fabbri & Franzellitti 2016). Additionally, there is also a clear disparity of information among regions worldwide which we should tackle. For developing regions, where population increase, higher standards of living and improved access

to pharmaceuticals will likely contribute to increase in environmental contamination this could be a key opportunity to start early monitoring schemes to evaluate environmental accumulation, and develop associated strategies to minimize detrimental impacts both from household use and commercial enterprise (e.g. aquaculture, industry). In developed countries, mitigation plans are necessary as the environmental pressure exerted by pharmaceuticals will continue to rise linked to population ageing and prevalence of chronic diseases. However, up to now most approaches are limited to spatial or temporal isolated data, lacking long-term aims, rather than encompassing large regional and temporal coverage. The latter is particularly important in coastal and transition systems, where variations in loadings are associated to natural fluctuations physical and chemical conditions (e.g. salinity, river flow, temperature, water chemistry) which may imply significant changes to the fate of pharmaceuticals in the environment (Glassmeyer et al. 2007, Zhao et al. 2015).

In the long run, management strategies for contamination by pharmaceuticals should aim to act in advance of ensuing adverse effects, promote the development of a suit of best practices to reduce their occurrence in the environment, and drive the improvement of systems that constrain potential contamination sources, or increase the effectiveness of the removal and degradation of these compounds from the environment. The first line of action to reduce the potential entry of pharmaceuticals in the environmental are WWTP, with continued research on the behavior, degradation and varying removal efficiencies of different WWTP treatments for multiple therapeutic classes still required. Developing novel methodologies that enhance the efficacy of WWTPs tertiary treatment to specifically remove or degrade pharmaceutical compounds is an acknowledged path for reducing the potential impact of pharmaceuticals (Margot et al. 2013, Calisto et al. 2017). In fact, Directive 2013/39 EU (European Parliament, 2013) underlines the importance of finding new ways of tackling water pollution by pharmaceuticals, and unravelling the physico-chemical processes that determine degradation and transformation of pharmaceutical compounds, their metabolites and by-products will further contribute to resolving these issues.

Different pharmaceuticals have been shown to bioaccumulate (Klosterhaus et al. 2013) and even biomagnify (Liu et al. 2017), yet in general there is insufficient information on bioaccumulation and impacts of pharmaceutical residues across the trophic web, namely for top-predators (Gaw et al. 2014). Likewise, given the effects of pharmaceuticals on bacteria and algae (Backhaus et al. 2011, Minguez et al. 2016) and the high degree of homology between chloroplasts and bacteria as well as among other metabolic pathways across multiple phyla (Brain et al. 2008), the lack of research on higher marine phototrophs (e.g. halophytes, plants) is conspicuous.

Compiling information on bioaccumulation, effects, and understanding MOA and adverse outcomes of pharmaceuticals are critical for effective management of pharmaceutical contamination and to safeguard coastal and marine biota. Thousands of different active pharmaceutical ingredients are available for human and veterinary use, which impedes assessing the full spectrum of contaminants in any given monitoring scheme. Furthermore, the consumed amount and toxicity of individual drugs varies greatly, thus it is key to prioritize research directives, monitoring and regulation. Several options have been forwarded over the years (e.g. Schreiber et al. 2011, Caldwell et al. 2014, Rudd et al. 2014), though three main aspects

583 to take into consideration are generally consumption levels, ecotoxicological risk and persistence in the
584 environment. Rather than in isolation, these facets should be evaluated simultaneously, as directing
585 resources to higher risk but low use or persistence pharmaceuticals may not prove good investment of time
586 and resources. Approaches based on MOA take into consideration the evolutionary and functional
587 conservation of molecular targets of pharmaceuticals (e.g. receptors, enzymes), cellular and physiological
588 processes across species, which enables the identification of relevant endpoints and experimental conditions
589 to determine drug toxicity (Christen et al. 2010, Fabbri & Franzellitti 2016). Furthermore, chronic exposure
590 assessments at environmentally significant concentrations are central to evaluate the risk posed by
591 pharmaceutical substances (Fabbri & Franzellitti 2016). Acute testing has several limitations that can
592 compromise resulting environmental regulation. Yet, contamination thresholds are still mostly based on
593 acute standard toxicity tests. Even though, they are less sensitive than other endpoints in non-model species
594 (e.g. Aguirre-Martínez et al. 2015), and neglect potential long-term effects from chronic exposures, which
595 are more representative of the persistent contamination organisms experience in their natural environment
596 (Crane et al. 2006, Fent et al. 2006).

597 Ecotoxicological assessment should strive to fill the gap between sub-cellular endpoints and
598 adverse individual or population level effects. This is a major challenge and requires the development of
599 frameworks that synthesize data at many levels of biological organization. The adverse outcome pathways
600 (AOP) is a good example of this, and several studies have illustrated the potential of AOP for population-
601 modelling and predictive ecotoxicology (Ankley et al. 2010, Franzellitti et al. 2014, Hird et al. 2016).
602 Furthermore, the utility of the AOP approach has been demonstrated for cross species extrapolation and
603 integrating life-history theory (Groh et al. 2015). One of the key issues is ensuring baseline toxicity studies
604 produce robust and accurate quantitative data that can be subsequently integrated in population modelling
605 approaches. Ideally dose-response or concentration-response relationships for both lethal and sub-lethal
606 effects should be defined allowing response curves, effect-thresholds and the probability of effects
607 occurring at different levels of biological organization to be estimated (Kramer et al. 2011).

608 Ultimately, monitoring of prioritized pharmaceuticals, metabolites and by-products in coastal
609 environments should complement risk assessment, and is integral to current European policy. The EU watch
610 list for emerging contaminants under the Water Framework Directive (WFD – Directive 2000/60/EC)
611 currently includes pharmaceutical Diclofenac, and two hormones 17-beta-estradiol (E2), and 17-alpha-
612 ethinylestradiol (EE2); with three additional antibiotics proposed for inclusion (Erythromycin,
613 Clarithromycin and Azithromycin). In addition to consistent water collections and analysis, monitoring
614 strategies can build upon the success of programs such as Mussel Watch (Goldberg & Bertine 2000), which
615 would allow for both bioaccumulation (Wille et al. 2011, McEneff et al. 2014) and monitoring of effects
616 and ecotoxicology a via standardized set of biomarkers (Franzellitti et al. 2015, Mezzelani et al. 2016a).
617 Other prospective monitoring tools for baseline concentration data include the use of passive sampling
618 devices (Martínez Bueno et al. 2016) or the use of unmanned automated sampling devices in ships and
619 marine platforms of opportunity (Brumovsky et al. 2016).

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Figure Legends

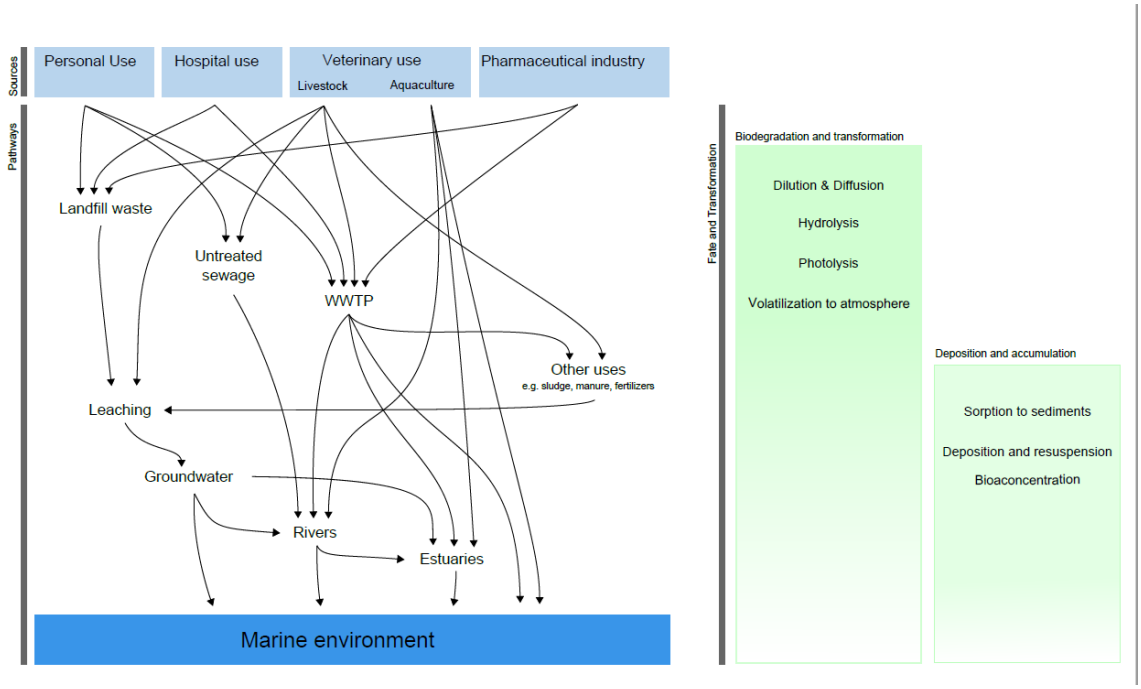

Figure 1 – Major sources and pathways of pharmaceutical contamination into coastal and marine environments. Also shown main fate and transformation processes that affect the presence and concentration of pharmaceutical compounds.

Figure 2 – Tree map representation of studies on the effects of pharmaceutical exposure in coastal and marine organisms per therapeutic class, biological endpoints and major taxonomic groups. Therapeutic classes are antidepressants, analgesics and non-steroid anti-inflammatories (NSAIDs), anticonvulsants, antibiotics, antihypertensives and lipid regulators. Biological endpoints and respective abbreviations are

molecular changes, accumulation (accumul), development (develop), mortality, reproduction (repro) and behavior (behav). Major taxonomic groups and respective abbreviations are fish, tunicates (tun), echinoderms (echi), mollusks (moll), crustaceans (crust), rotifers (rot), annelids (ann), nematods (nem), cnidarians (cni), algae (alg), bacteria (bact). Individual box sizes are proportional to number of entries, and total number of entries per therapeutic class is shown (*n*). Note that a single study may have multiple entrances per therapeutic class (total number of studies 124).