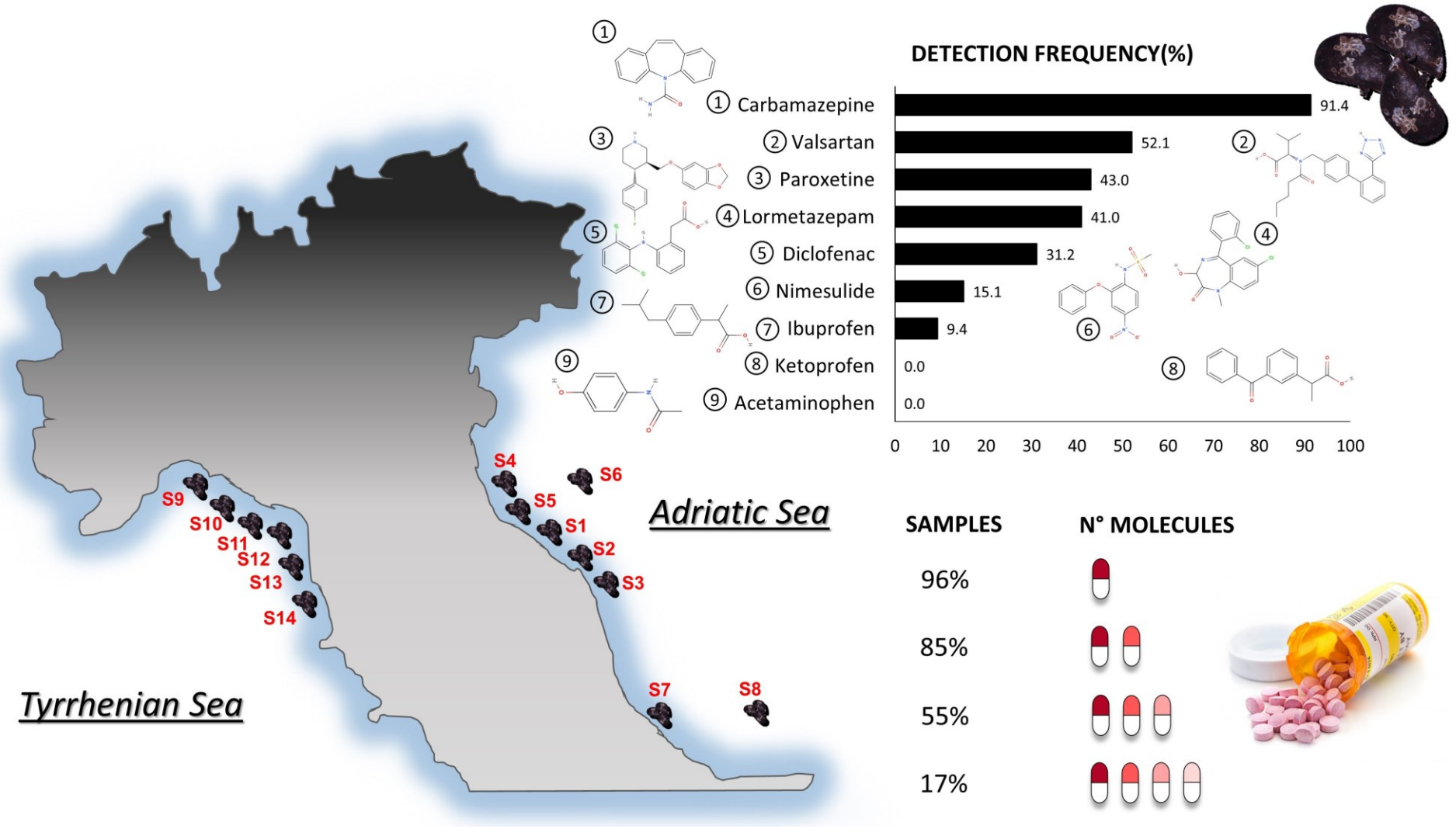
Human pharmaceuticals in marine mussels: Evidence of sneaky environmental hazard along Italian coasts

HIGHLIGHTS

- Pharmaceuticals are ubiquitously present in tissues of Mediterranean mussels
- Organisms contained drugs of several therapeutic classes
- Carbamazepine was measured in more than 90% of analysed samples
- 91% of analysed mussels contained at least 1 drug, 55% at least 3
- Seasonality had a limited influence on pharmaceuticals bioaccumulation


DETECTION FREQUENCY(%)
① Carbamazepine 91.4
② Valsartan 52.1
③ Paroxetine 43.0
④ Lormetazepam 41.0
⑤ Diclofenac 31.2
⑥ Nimesulide 15.1
⑦ Ibuprofen 9.4
⑧ Ketoprofen 0.0
⑨ Acetaminophen 0.0
0 10 20 30 40 50 60 70 80 90 100
Adriatic Sea
Tyrrhenian Sea
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
SAMPLES
N° MOLECULES
96%
85%
55%
17%

Human pharmaceuticals in marine mussels: evidence of sneaky environmental hazard along Italian coasts

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Abstract

Despite the increasing interest for pharmaceuticals in the marine environment, their accumulation in wild organisms and consequent environmental hazards are still poorly known. The Mediterranean Sea is highly challenged by the density of coastal populations, large consumption of pharmaceuticals and their often limited removal by Wastewater Treatment Plants (WWTPs). In this respect, the present study aims to provide the first large-scale survey on the distribution of such contaminants of emerging concern in native mussels, *Mytilus galloprovincialis* from Italian coasts. Organisms were collected from 14 sites representative of relatively unpolluted marine waters along the Adriatic and Tyrrhenian Sea and analysed for 9 common pharmaceuticals including Non-Steroidal Anti-Inflammatory Drugs (NSAIDs: Diclofenac DIC, Ibuprofen IBU, Ketoprofen KET and Nimesulide NIM), the analgesic Acetaminophen AMP, the antiepileptic Carbamazepine CBZ, the antihypertensive Valsartan VAL, the anxiolytic Lormetazepam LOR and the antidepressant Paroxetine PAR. Results indicated the widespread occurrence of the majority of pharmaceuticals in mussel tissues: CBZ was measured in >90% of analysed samples, followed by VAL (>50%), PAR (>40%), and DIC (>30%), while only AMP and KET were never detected. Heterogeneous tissue concentrations ranged from a few units up to hundreds of ng/g (d.w.), while seasonal and interannual variability, investigated over 4 years, did not highlight any clear temporal trend. Limited differences obtained between the Adriatic and Tyrrhenian Sea, as well as coastal versus off-shore sampling sites, suggest that analysed levels of pharmaceuticals in mussels tissues should be considered as baseline concentrations for organisms collected in unpolluted areas of the Mediterranean. This study provided the first unambiguous evidence of the widespread occurrence of pharmaceuticals in marine mussels from Italian coasts, giving novel insights on the potential ecotoxicological hazard from such compounds in marine species.

Keywords

Pharmaceuticals, Contaminants of Emerging Concern, Bioaccumulation, Mediterranean Sea, Environmental Hazard

1. Introduction

The remarkable progress in human and veterinary pharmacology certainly represented one of the greatest benefits of the modern society also contributing, however, to the increased occurrence of pharmaceutical compounds in aquatic environments (Boxall et al. 2012; Erzinger et al. 2013; Gonzalez-Rey et al. 2015; Burket et al. 2019). Such contaminants of emerging concern have been detected in seawater and sediments worldwide, with variable levels ranging within ng/L-µg/L and ng/g respectively (Mezzelani et al. 2018a). The most frequently detected compounds are represented by active principles belonging to steroid hormones, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), psychiatric and cardiovascular drugs (Moreno-González et al. 2015; Ebele et al. 2017; Desbiolles et al. 2018; Mezzelani et al. 2018a,b; Miller et al. 2018) . Laboratory experiments suggested the capability of bioindicator species to accumulate various classes of pharmaceuticals and the onset of deleterious outcomes from molecular up to physiological levels. Depending on the type and doses, measured alterations included the activation of immune system, modulation of lipid and oxidative metabolism, onset of genotoxic effects, impairment of endocrine system with adverse effects in organisms homeostasis, development, behaviour and reproduction (Almeida et al., 2020; Fabbri and Franzellitti, 2016; Mezzelani et al., 2018a,b; Moreno-González et al. 2015). Although laboratory studies documented ecotoxicological effects of pharmaceuticals in non-target species, the field distribution of these compounds in wild marine organisms has been less studied. As contaminants of emerging concern, pharmaceuticals are not included in any environmental regulation and neither routinely monitored: among over 4000 substances classified as pharmaceuticals, only seven (two estrogens, 17-α-ethinylestradiol and 17-β-estradiol, and five antibiotics such as erythromycin, clarithromycin, azithromycin, amoxicillin and ciprofloxacin) are included in a dynamic watch-list of the European Union Water Framework Directive (2018/840/EU), based on

their potential adverse effects for aquatic ecosystem (Miller et al. 2018). Recently, the European Commission (EC) acknowledged the importance of environmental pharmaceuticals, and on March 2019 a Communication was adopted outlining a set of actions toward the multifaceted challenges of those residues in natural ecosystems. The "Strategic Approach to Pharmaceuticals in the Environment" (COM/2019/128 final) emphasizes the need of gathering monitoring data as an important prerequisite to develop an appropriate risk assessment of such emerging pollutants.

Pharmaceutically active compounds are typically designed to cross biological membranes and various compounds belonging to the most common therapeutic classes (i.e. antibiotics, psychiatric, anti-inflammatory, cardiovascular drugs, etc.) have been detected in aquatic biota. The majority of studies focussed on riverine and lacustrine fauna, and fish species have been preferentially investigated in comparison to molluscs and other invertebrates (Liu et al. 2015; Burket et al. 2019; Miller et al. 2019). This is a crucial aspect since concentrations of antibiotics and antidepressants in bivalves can be one order of magnitude higher than those measured in fish from the same sampling area (Du et al. 2014).

Only a few studies were carried out on species living in coastal areas (Huerta et al., 2012; Martinez-Bueno et al., 2014; Maruya et al., 2014; Alvarez-Muñoz et al., 2015a,b; Serra-Compte et al., 2017; Martinez-Morcillo et al., 2020). A variable accumulation of eighteen compounds was reported in fish and molluscs from the Mar Menor Lagoon (Moreno-González et al. 2016) and pharmaceuticals were also detected in *Mytilus* spp. from Adriatic Sea, Belgian and Portuguese Atlantic coasts (Wille et al. 2011; Mezzelani et al. 2016a; Cunha et al. 2017). The Mediterranean Sea is the largest enclosed sea, highly challenged by elevated anthropogenic pressures, with reported impacts from human activities proportionally stronger than in any other sea (Coll et al. 2012; Suaria et al. 2016). The density of population inhabiting coastal areas, the large consumption of pharmaceuticals for both human and veterinary medicine, and the often inadequate presence or typology of wastewater treatment plants (WWTPs) make the Mediterranean an ideal basin for investigating the occurrence and distribution of pharmaceutical compounds in marine organisms. The

common Mediterranean mussel, *Mytilus galloprovincialis*, is one of the most widely used sentinel organisms with a marked capability to bioaccumulate xenobiotics and to exhibit sensitive molecular and cellular responses to such stressors (Canesi et al, 2007; Beyer et al. 2017; Oliveira et al. 2017; Regoli et al. 2014, 2019; Swiacka et al., 2019). In addition, considering the extensive mussels farms along the coasts, assessment of pharmaceuticals residues in these organisms is relevant also in terms of potential human exposure through food consumption, (Wille et al., 2011; Alvarez-Muñoz et al., 2015a,b; Serra-Compte et al., 2017; Martinez-Morcillo et al., 2020).

The aims of this study were (*i*) to investigate how widespread is the occurrence of pharmaceuticals in tissues of wild mussels along the Adriatic and Tyrrhenian Sea (Figure 1), (*ii*) to highlight seasonal and interannual fluctuations and (*iii*) to evaluate differences between coastal and off-shore locations. In this respect, concentrations of model pharmaceuticals were measured in wild mussels collected from a total of 14 sites along the Adriatic and Tyrrhenian Sea. In 3 sites of the Central Adriatic, samplings were carried out over 4 years to better characterize baseline levels and the presence of seasonal or inter-annual patterns of variation. The selected compounds included 9 among the most commonly used active principles in Italy, representative of different therapeutic classes such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with Diclofenac (DIC), Ibuprofen (IBU), Ketoprofen (KET) and Nimesulide (NIM), analgesics with Acetaminophen (AMP), and psychiatric drugs with the antiepileptic carbamazepine (CBZ), the hypnotic-sedative lormetazepam (LOR), the antidepressant paroxetine (PAR); the antihypertensives valsartan (VAL).

Considering the paucity of data on bioaccumulation of pharmaceutical compounds in marine organisms, this study represents the first large and temporal scale survey for the Mediterranean. Obtained results were expected to provide novel insights on the potential risk of such emerging environmental contaminants, aimed to effectively implement knowledge-based reduction and prevention measures.

2. Materials and Methods

2.1 Sampling activities

Sampling activities were designed to fulfil the three objectives of the present study: spatial, temporal and coastal vs off-shore evaluations. The collection of common mussels is not subjected to ethical review permissions according to both European and Italian normative (Directive 2010/63/UE, Italian Legislative Decree n. 26, 4/03/2014) and monitoring guidelines widely recommend this species as an appropriate bioindicator for assessing bioavailability of environmental pollutants and their possible transfer to humans through food consumption (Beyer et al. 2017).

Individuals of the Mediterranean mussels, *M. galloprovincialis* of homogeneous dimensions (5.0 ± 1.0 cm shell length) were seasonally collected, from 2014 to 2017, in three different sites of the central Adriatic coast namely Senigallia (S1), Torrette (S2) and Portonovo (S3), in the district of Marche region (Figure 1 and Table SM1 for additional information). Selected sites are representative of relatively unpolluted coastal areas, where local authorities regularly monitor the presence of traditional pollutants to detect environmental disturbance. Those sites are typical summer touristic destinations which since several years, are awarded with the Blue Flag certification by the Foundation for Environmental Education, assigned to beaches, marinas, and sustainable boating tourism operators as an indication of their high environmental and quality standards. Mussels are farmed for human consumption in S1 and S3; those from site S3, widely characterized for the seasonal fluctuations of trace elements and biological responses to stressful conditions (Bocchetti and Regoli, 2006; Fattorini et al., 2008; Pisanelli et al., 2009), represent a well-known reference population to use in environmental studies. For each sampling site and period, mussels were collected and the whole tissues were removed from 50 specimens, pooled in 10 samples (each containing 5 organisms), and stored at -20 °C until the chemical analyses on pharmaceuticals concentrations.

Additional 11 sampling sites were selected along the Adriatic and Tyrrhenian coasts, to increase the information on a larger geographical scale (Figure 1 and Table SM1 for additional

149 information). Adriatic coastal sites included a natural park (S4), the entrance of Pesaro canal harbour
150 (S5), and a mussel farm (S7), while off-shore locations were a 10 m depth artificial reef for fish
151 repopulation at about 7 nautical miles (NM) from the coast (S6), and a gas platform at 10 NM offshore
152 (S8): mussels were collected on July 2016 (S7-S8) and on May, July and September 2017 (S4-S6).
153 The Tyrrhenian sites, located along the coasts of Liguria and Tuscany regions, were representatives
154 of a mussel farm (S9), natural parks influenced by rivers runoffs (S10-S11), non-industrial harbour
155 (S12), touristic areas with recreational beaches (S13-S14). Mussels were collected on December 2016
156 (S9, S10, S11, S12), September 2017 (S12), March and August 2017 (S13, S14). For each sampling
157 site and period, the whole tissues were removed from 25 mussels, pooled in 5 samples (each
158 containing 5 organisms), and stored at -20 °C until the chemical analyses.

159159

160 *2.2 Chemical analyses*

161 Levels of AMP, CBZ, DIC, IBU, KET, LOR, NIM, PAR and VAL in mussels tissues were
162 determined on 5 replicates, each constituted by whole tissues of 5 specimens. Measurement of tissue
163 levels of CBZ, PAR, LOR and VAL could be performed in mussels collected after July 2015 when
164 analytical protocols were developed and validated for such compounds. Sample preparation and
165 analytical determination followed a modification of previously validated methods which allow the
166 sensitive detection of investigated molecules through a High Performance Liquid Chromatography
167 (HPLC) approach with fluorimetric and diode array detection (Mezzelani et al. 2016a,b; Mezzelani
168 et al. 2018b). Such analytical methods are detailed in Supplementary Materials (SM) with a complete
169 description of homogenization and extraction buffers used for various compounds, solid phase
170 extraction (SPE) procedures, conditions for chromatographic separations and peaks detection, limits
171 of detection and quantification of individual molecules (SM Tables SM2, SM3, SM4).

172 Concentrations of various pharmaceuticals were quantified by comparison with signals of
173 pure standard solutions. Due to the lack of appropriate Certified Standard Reference Materials

(SRMs), recovery for each compound was estimated on samples of control mussels (n=5) spiked with various concentrations of investigated molecules, as described in detail in the SM. The optimal working range corresponded to the analytical limit of measurement which guarantees an acceptable variability (CV<20%) on 10 replicates and a good linearity ($R^2 \geq 0.99$), assuring at least 95% of recovery. The intensive validation of the method carried out for the control of precision, accuracy, variability and analytical recovery did not require the further use of internal standards, preventing the intrinsic difficulty of identifying suitable surrogate substances for HPLC-DAD analytical methods. The water content in mussel tissues was determined by the measurement of wet weight and dry weight ratio in 5 replicates (each constituted by 5 organisms), for each sampling period and site; final concentrations of detected pharmaceuticals were normalized and expressed as ng/g dry weight (d.w.).

Considering the analytical conditions and the preparation procedures described in SM, the minimum measurable amounts in mussels tissues (Limit of Quantification, LOQ) were 1 ng/g dry weight (d.w.) for AMP, 1.37 ng/g d.w. for DIC, 8 ng/g d.w. for IBU, 0.86 ng/g d.w. for KET, 2.10 ng/g d.w. for NIM, 1.03 ng/g d.w. for CBZ, 0.43 ng/g d.w. for LOR, 0.95 ng/g d.w. for PAR and 0.49 ng/g d.w. for VAL, as described in detail in the SM and reported in the Table SM2.

189189

2.3 Statistical analyses

Differences between tissue concentrations of pharmaceuticals were tested for S1, S2 and S3 by two-way analysis of variance (ANOVA) considering the factors “month”, “year” and the interaction of the two factors (IBM SPSS Statistics). Values <LOQ were computed using half of the LOQ, data were normalized, and the homogeneity of variance was checked by the Levene’s test at the 95% of confidence interval. Given the high variability measured in tissues concentrations during months and years, for each site the criteria adopted prior to test ANOVA were *(i)* tissue concentrations > LOQ in at least 3 different months, *(ii)* tissue concentrations > LOQ in at least 3 different years. When statistically significant differences were obtained from the two-way ANOVA analysis, the

199 Student-Newman-Keuls *post-hoc* comparison (SNK) was applied to discriminate between means of
200 values, at the 95% of confidence interval. In addition, one-way analyses of variance (ANOVA) was
201 applied to test differences of pharmaceuticals concentrations between mussels collected from all the
202 Adriatic and Tyrrhenian sites (S1-S14).

203 A non-metric Multi-Dimensional Scaling (nMDS) was performed as multivariate ordination
204 analysis in which variables describing a multidimensional space are scaled on a two-dimension plot
205 based on their similarity, thus maximizing the distance among points (Clarke and Gorley, 2001). The
206 nMDS function from the Vegan R-package (Oksanen et al. 2011) was applied on bioaccumulation
207 levels of DIC, IBU, NIM, CBZ, PAR, LOR and VAL measured in mussels sampled from S1-S2-S3
208 in 2015-2016-2017.

209

210 **3. Results**

211 Tissue concentrations of individual pharmaceuticals, the sum of Non-Steroidal Anti-
212 inflammatory Drugs, ∑NSAIDs (DIC, IBU, NIM), and the sum of Psychiatric drugs ∑PSY (CBZ,
213 PAR, LOR) measured in different months and years in mussels from S1, S2 and S3 are reported in
214 Table 1. Results of analysis of variance revealed significant differences related to "month" and "year"
215 (Supplementary Material Table 6, SM6). Among NSAIDs, DIC was the most frequently detected
216 compound with concentrations ranging from below 1.40 to more than 170 ng/g (dry weight of the
217 tissues, d.w.). Significant temporal variations were observed only in mussels from S1 with higher
218 DIC levels in July 2015, May and October 2016, May 2017 (p<0.001, Table 1). The elevated
219 variability of results obtained in S2 and S3 prevented to reach a statistical significance despite the
220 higher values were always measured between late spring and early autumn, particularly in the
221 recreational beach (S3) during the touristic season. In all the sites, DIC was typically below detection
222 limit in autumn and winter months (September, November, February, Table 1). IBU and NIM were
223 occasionally measured with spotted peaks of concentrations ranging from less than 8 to more than
224 140 ng/g (d.w.), and from <2 to more than 80 ng/g (d.w.), respectively (Table 1): such fluctuations

225 did not appear to be related to any seasonal cycle. Levels of AMP and KET were always below the
226 limit of quantification (LOQ), of 1 ng/g (d.w.) and 0.86 ng/g (d.w.) respectively (data not shown).
227 The ∑NSAIDs was given as the sum of tissue levels of DIC, IBU and NIM, expressed as nmol/g to
228 normalize different molecular weights of these compounds. Although the general trend of ∑NSAIDs
229 was mostly influenced by DIC, overall it highlighted that in almost all sampling periods mussels
230 contained at least one residue of this pharmaceutical class.

231 CBZ was detected in more than 95% of analysed samples, being generally the most abundant
232 among measured pharmaceuticals with mean values ranging from 35 up to 280 ng/g (d.w.) (Table 1).
233 The significant differences observed as a function of "month" and "year" (Table SM6) did not
234 highlight a clear seasonality of CBZ concentrations, although lower average values were detected in
235 Summer (July and August of various years, Table 1). Concentrations of PAR in mussels were
236 detectable in all the sites for most of the periods, typically ranging between 2 and 10 ng/g (d.w.), and
237 with significantly higher values in S1 in July 2015 and 2016 (up to 30 ng/g d.w.), and in S3 in July
238 2017 (up to 50 ng/g, Table 1). Tissue levels of LOR were characterized by an elevated variability,
239 both in terms of occurrence in different sites/periods, and of concentrations ranging from a few up to
240 300 ng/g (d.w.) (Table 1).

241 The sum of concentrations measured for CBZ, PAR and LOR (∑PSY) confirmed the
242 variability already reported for individual compounds, further highlighting the presence of psychiatric
243 drugs in mussels tissues in all the sites and sampling periods (Table 1). Finally, the antihypertensive
244 VAL was detectable in the majority of analysed samples with tissue levels from less than 0.5 to
245 approximately 7 ng/g (d.w.) (Table 1).

246 With the only exception of AMP and KET always below the detection limit, the Non Metric
247 Multidimensional Scaling analyses (nMDS) was carried out on all pharmaceutical compounds
248 measured in different periods (2015-2017) and sites (S1-S3): a rather moderate separation was shown
249 among variables, supporting previous evidences of a limited influence of seasonality on
250 pharmaceuticals concentrations in mussels tissues (Figure 2). Based on this evidence, average tissues

concentrations of individual pharmaceuticals, ∑NSAIDs (DIC, IBU, NIM), and ∑PSY (CBZ, PAR and LOR) were calculated for mussels collected from all the Adriatic and Tyrrhenian locations, and the comparison between sites was thus irrespective of sampling month (Figure 3). The obtained results revealed the widespread bioaccumulation of all the investigated pharmaceuticals in mussels from all the sites (Figure 3). Despite some geographical differences, the concentrations were not significantly different (one-way ANOVA) between mussels from Adriatic and Tyrrhenian locations (S1-S8 and S9-S14, respectively): the overall range of measured concentrations was comparable to that characterized for S1-S3 on a seasonal/interannual basis, and no evident differences occurred from coastal toward off-shore sites, S6-S8 (one-way ANOVA).

The relative frequency of pharmaceuticals detection in mussels collected from all sampling sites and periods is summarized in Figure 4A. CBZ occurred in more than 90% of analysed organisms, VAL in 52%, followed by PAR and LOR, 43 and 41% respectively. Among NSAIDs, DIC was measured in 31% of samples, while KET and AMP were always below the LOQ (Fig 4). Overall, the 96% of samples contained at least 1 active principle, the 85% at least 2, and 55% at least three of the analysed pharmaceutically active compounds (Fig.4B).

4. Discussion

4.1. Occurrence of pharmaceuticals in mussels tissues

The large consumption of pharmaceuticals is posing an important global issue and despite scientific research has been active for 20 years, the environmental relevance of this problem is still unclear and not adequately considered at political and regulatory levels. In this respect, scientific data should support political authorities in developing reliable normative guidelines, appropriate strategies for environmental risk assessment, knowledge-based reduction and prevention measures. Pharmaceuticals are characterized by a complex environmental fate modulated by their excretion rate after human or veterinary usage, unproper domestic disposal, limited removal by WWTPs,

276 particularly under specific environmental conditions which influence the consequent transport into
277 and across aquatic bodies. Research progress contributed to extensively document the presence of
278 pharmaceuticals in wastewaters, rivers, lakes, coastal water and sediments, but such data do not
279 provide specific information on biological hazards to organisms and ecosystems (Miller et al. 2019).
280 Studies to clarify the bioaccumulation of pharmaceuticals in field conditions are recommended by
281 international Authorities (i.e. European Commission, COM/2019/128 final), although this approach
282 is often hampered by the complexity to identify molecules to prioritize, and limited availability of
283 analytical protocols. The improvement of extraction methods and of advanced analytical techniques,
284 such as gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-
285 tandem mass spectrometry (LC MS/MS), has recently increased the possibility to detect the
286 distribution of pharmaceuticals, enabling the simultaneous determination of more than 20 compounds
287 even in complex, environmental samples (Klosterhaus et al. 2013; Martínez Bueno et al. 2013; Miller
288 et al. 2019). These approaches are fundamental especially when it is necessary to ensure elevated and
289 certified analytical sensitivity, as for residues in food matrices. However, due to the elevated costs,
290 the presence and application of such sophisticated analytical methodologies is still limited in many
291 environmental laboratories involved in routinely biomonitoring activities on marine species (Dodder
292 et al. 2014; Mc Eneff et al. 2014; Álvarez-Muñoz et al. 2015; Moreno-González et al. 2016 Cunha et
293 al. 2017). In this respect, considering the urgent need to fill the gap of knowledge on pharmaceuticals
294 in the aquatic ecosystem (COM/2019/128 final), the possibility to apply a commonly used analytical
295 approach, despite more time-consuming, can represent an important alternative to increase available
296 data on the presence of such compounds in marine organisms and to raise public awareness. In the
297 present study we used specific HPLC analyses with diode array and fluorescence detection which
298 represent an update and implementation of previously reported methods (Mezzelani et al., 2016a,b,
299 2018a). Presented protocols, optimized for the measurement of representative classes of drugs, have
300 been extensively tested in terms of extraction, purification, chromatographic separation and detection,

301 to maximize recovery and to guarantee an environmentally appropriate sensitivity (see
302 Supplementary Material).

303 Based on our knowledge, the present study represents the largest temporal- and spatial-scale
304 investigation on the occurrence of pharmaceuticals in Mediterranean mussels, covering 9
305 pharmaceuticals of 6 therapeutic classes, 14 sites, two basins and 4 years, contributing to provide an
306 important volume of data on the occurrence of these emerging substances in the marine ecosystems
307 and on the bioaccumulation capacity of benthic species.

308 Besides specific peculiarities, all the sampling areas sustain several goods and ecosystem
309 services spanning from leisure and recreational activities (i.e. touristic beaches) to food production
310 (mussels farms): none of selected sites was close to specific sources like WWTPs discharges.

311 The pharmaceuticals investigated in the present study were selected as model compounds
312 within the most consumed therapeutic classes in Italy, which include analgesics, anti-inflammatories,
313 antidepressants, anxiolytics, anticonvulsants and anti-hypertensives (OsMed, 2017). The obtained
314 results clearly revealed the ubiquitous occurrence of pharmaceuticals in mussels collected in different
315 sites and periods of unpolluted coastal areas of Italy. Considering NSAIDs, DIC was detected in more
316 than 30% of samples, showing a wide interval of concentrations, ranging from 1.21 up to 280 ng/g.
317 Lower values (0.5-4.6 ng/g) were previously detected in *M. galloprovincialis* transplanted in a North
318 West Adriatic Coastal Lagoon (Pialassa Piomboni) and *Mytilus* spp. from the Portuguese and Irish
319 coasts (McEneff et al., 2014; Capolupo et al., 2017; Cunha et al. 2017). In fish species DIC was
320 measured in in golden grey mullets (*Liza aurata*) and flounders *P. flesus* from the Mar Menor lagoon,
321 Tagus and Scheldt estuaries, (Alvarez-Muñoz et al., 2015a; Moreno-Gonzalez et al. 2016). Notably,
322 despite diclofenac is one of the most frequently detected pharmaceuticals in water column or
323 sediments and deeply studied for the deleterious effects to wildlife (Oaks et al. 2004; Bonnefille et
324 al. 2018; Mezzelani et al. 2018a), it has been recently removed from the last update of the European
325 Watch List (2018/840/EU).

Limited information is available on occurrence of NIM and IBU in marine organisms, confirming the importance to increase availability of data on bioaccumulation of environmental pharmaceuticals. Our data showed the presence of these compounds in 15.1 and 9.3 % of samples with values between a few up to several tenths of ng/g. Levels of IBU below the LOQ were observed in *Geukensia demissa* from the San Francisco Bay (Klosterhaus et al. 2013), while lacustrine fish species exhibited concentrations up to 62 ng/g (Xie et al. 2017), comparable to mean values measured in mussels from the present study.

AMP and KET were the only pharmaceuticals always below their LOQ. Similar results were obtained for KET in *M. edulis* from the Baltic Sea (Wolecki et al. 2019), while AMP was detected in *M. edulis* caged along the Belgian coastline (up to 115 ng/g, Wille et al.2011), in the same species from the Gulf of Gdansk (up to 80 ng/g, Caban et al. 2016), and in the freshwater *C. fluminea* sampled close to a WWTP effluent (up to 347 ng/g, Burket et al. 2019). Recent laboratory experiments with *M. galloprovincialis* highlighted the lack of accumulation for both KET and AMP, but the contemporary onset of molecular, biochemical and cellular alterations; these results allowed to hypothesize the potential biotransformation capability by Mediterranean mussels (Mezzelani et al. 2016a,b; 2018b), thus suggesting species-specific differences in bioaccumulation and metabolism of these pharmaceuticals.

Among the investigated drugs, CBZ was by far characterized by the highest frequency of detection in Mediterranean mussels, being measured in more than 90% of samples. The widespread distribution of this antiepileptic drug in aquatic environment is documented and related to its refractory properties, such as resistance to conventional water treatments (coagulation, flocculation, sand filtration, chlorination), biotreatments and photodegradation (Zhu et al. 2019). The half-life of CBZ in water column requires between 4.5 and 25 sunny summer days for degradation through direct photolysis (Calisto et al. 2011), while exposure to sunlight in winter or at higher latitudes (50°N) determines a persistence in aquatic environments greater than 100 days (Andreozzi et al. 2003; Oliveira et al. 2017); laboratory and field investigations defined an average half-life of CBZ >200

352 days, with a maximum of 1200 days in a Swedish lake (Bu et al. 2016; Zou et al. 2015). Tissue
353 concentrations measured in *M. galloprovincialis* from the Adriatic and Tyrrhenian sites (30-300 ng/g)
354 are at least one order of magnitude higher than those reported for mussels farmed in Southeastern
355 France (up to 3.5 ng/g d.w., Martínez Bueno et al. 2013), in caged *M. edulis* deployed for 6- months
356 along the Belgian coast (up to 11 ng/g, Wille et al. 2011), in *G. demissa* from San Francisco Bay (2.4
357 ng/g, Klosterhaus et al. 2013), in *Cassostrea gigas* from Ebro delta (2.1 ± 0.04 ng/g (d.w.), Álvarez-
358 Muñoz et al. 2015). The high concentrations and frequency of CBZ in Mediterranean mussels
359 highlight an elevated pressure and concern for this antiepileptic drug, the toxicological effects of
360 which have been reported for non-target marine organisms (Freitas et al. 2016; Oliveira et al. 2017;
361 Mezzelani et al. 2018b).

362 Our study also documented for the first time an elevated occurrence of PAR and LOR, both
363 detected in more than 40% of analysed mussels with tissue levels typically below 10 ng/g (d.w.) and
364 frequent peaks up to 50 ng/g (d.w.). Also for these compounds, no information is actually available
365 on potential accumulation in marine species: levels of PAR were <LOQ in *M. galloprovincialis* from
366 the Portuguese coast, while no data were reported for LOR (Moreno-Gonzalez et al. 2016). PAR is a
367 typical antidepressant belonging to the Selective serotonin reuptake inhibitors (SSRIs), while LOR is
368 a Benzodiazepine (BZP) prescribed for its anxiolytic, hypnotic and tranquilizer properties. Within
369 such therapeutical classes, PAR and LOR are the most prescribed active principles in Italy (OsMed,
370 2017); consumption of LOR increased by more than 10 folds from 1983 to 2017, representing the
371 only BZP with this atypical trend, and virtually replacing the usage of the other similar active
372 principles (Faccini et al. 2012).

373 More than 50% of analysed organisms also showed the presence of VAL, an angiotensin II
374 receptor antagonist, usually prescribed as anti-hypertensive. Despite frequently detected in water
375 column (Gros et al. 2012; Klosterhaus et al. 2013; Moreno-González et al. 2015; Pereira et al. 2015;
376 Alygizakis et al. 2016; Mezzelani et al. 2018a; Mijangos et al., 2018), only one study documented its
377 accumulation in the freshwater annelid *Erpobdella octoculata* with levels up to 2.3 ng/g (Grabicova

et al. 2015), rather comparable to those measured in Adriatic and Tyrrhenian mussels: no data, however, is actually available on its potential adverse effects on marine organisms.

Similarly, to LOR and PAR, also VAL is the main active principle consumed in Italy within its therapeutical class. These compounds are scarcely detected in marine and freshwater environments worldwide (Salgado et al. 2011; De Solla et al. 2016; Mole and Brooks, 2019). Their widespread distribution in mussels from Italian coasts, may thus suggest that national prescribing and sale trends directly affect the fate of such compounds in environment and biota, reinforcing the concept that the life-cycle of pharmaceuticals does not end with their usage (Peake et al. 2016).

386386

4.2. Seasonal and geographical differences of pharmaceuticals in mussels tissues

The seasonal and interannual analyses carried out for almost 4 years in mussels from 3 Adriatic sites (S1-S3) did not highlight any clear temporal trend in pharmaceuticals bioaccumulation, in spite of the significant differences among months and years (Two-Way ANOVA, Table SM6). Compared to concentration of other chemical pollutants which are influenced by reproductive cycle of organisms (Fattorini et al., 2008), seasonal variations of pharmaceuticals in marine species were not observed in tissues of different wild species sampled from Mar Menor Lagoon in spring and autumn (Moreno-González et al. 2016). Nonetheless, in our study lower tissue levels of CBZ detected in summer periods (July and August) would agree with a greater photolytic degradation of this drug at higher seawater temperature and solar irradiation, (Andreozzi et al. 2003). At the same time, some peaks observed for other pharmaceuticals (NIM, LOR, PAR) during the summer months (July and August), suggest the impact of a greater anthropogenic pressure in touristic areas. However, the elevated variability of results also confirms the complexity of factors modulating the release, persistence and bioavailability of such molecules in the marine environment (Cui et al. 2016). Among these, extreme hydrological events, rivers runoff, flow rates and efficiency of WWTPs influence the irregular inputs of pharmaceutically active compounds in coastal areas, while environmental factors such as seawater temperature, pH and solar irradiation can affect stability and intrinsic chemical-

404 physical properties of active principles (Ebele et al. 2017; Välitalo et al. 2017). The unpredictable
405 interactions among so many variables can determine a constant but heterogeneous distribution of
406 pharmaceuticals in coastal organisms.

407 The comparison of mussels from all the sites revealed a similar pattern of pharmaceuticals
408 accumulation, without significant dissimilarities in various geographical areas. Despite the different
409 oceanographic and hydrographic characteristics, Adriatic and Tyrrhenian basins are both affected by
410 freshwater inputs from the Po river and Arno river, respectively (Giovanardi et al. 2018). The Po
411 River is the main Italian watercourse, flows in Northern Italy through some of the most anthropized
412 Italian regions. Its surface extends for over 71.000 Km^2, crossing 3.200 municipalities with around
413 16 million inhbitants and receiving civil, industrial and agricultural effluents. The Arno river has a
414 lower extension (9.000 Km^2) affecting 164 municipalities with more than 2 million inhabitants and it
415 receives mostly civil and industrial effluents. All the sites investigated in the present study reflected
416 common typologies of coastal sites, sustaining different ecosystemic services including protected
417 areas, recreational and touristic beaches, mussel farms, small marinas and non-industrial harbours:
418 the common characteristic of the sampled locations was the lack of specific sources for
419 pharmaceuticals release. To our knowledge no information on concentrations in water column are
420 available for the selected compounds in sampling sites and obtained results can be considered as
421 baseline levels of pharmaceuticals accumulation in Mediterranean mussels; this hypothesis is further
422 supported by similar levels of drugs measured in mussels from coastal sites and those collected at 5-
423 10 NM offshore.

424 The ubiquitous occurrence of pharmaceuticals in tissues of *M. galloprovincialis* confirmed
425 the capability of these non-target organisms to accumulate such compounds, clearly reflecting an
426 environmental side effect of massive consumption of medicines, their limited removal from
427 wastewater treatment plants and consequent release into the aquatic environments (Hernando et al.
428 2006; Ankley et al. 2007; Kay et al. 2017). Such results highlight the importance to improve
429 pharmaceuticals characterization in marine species on a global scale, applying all available and

reliable analytical procedures. Worthy to note is the constant co-exposure of *M. galloprovincialis* to multiple pharmaceuticals, evidenced by more than 55% of samples containing simultaneously at least 3 of analysed compounds: this result highlight the urgent need to consider pharmaceutical contaminants as mixtures potentially exacerbating the consequences on aquatic species through the onset of additive or synergistic effects (Giuliani et al., 2013; Ding et al. 2016; Freitas et al. 2016; Godoy et al. 2019; Trombini et al. 2019). Although concentrations measured in this study are far from human therapeutic doses of administration, and estimation of possible hazard for human consumption was outside the scope of this work, nonetheless it should be noted that accumulation of similar levels of DIC, IBU and NIM in mussels, was shown to modulate the appearance of subtle biological effects spanning from changes of transcriptional profile to immune responses, genotoxicity, alterations of lipid metabolism, oxidative and neurotoxic effects (Mezzelani et al. 2016a,b; Mezzelani et al. 2018b).

5. Conclusions

This study demonstrated the ubiquitous presence of pharmaceuticals in mussels tissues from the Mediterranean, suggesting a baseline occurrence of these contaminants of emerging concern. The large temporal- and spatial-scale analysis of multiple compounds demonstrated the contemporary accumulation of pharmaceuticals belonging to different therapeutic classes. 91% of analysed mussels contained at least 1 of the investigated drugs, and 55% at least 3; carbamazepine was the most frequent, being detected in more than 90% of analysed samples. Considering the limited number of pharmaceuticals selected in this study on the total of prescribed compounds, the concern on such environmental contaminants should be raised, promoting more frequent monitoring campaigns, even with the use of simple but reliable analytical approaches. Overall results support the need to prioritize pharmaceutical compounds for an appropriate environmental risk assessment in aquatic ecosystems, actually recognised as a priority from scientists, European Commission (COM/2019/128 final), the United Nations Agenda 2030, the G7/G20 and World Health Organisation. Such a challenging issue requires a multidisciplinary approach to integrate different competences and perspectives: scientists

need to better elucidate environmental fate and mechanisms of action of pharmaceuticals in non-target organisms, contributing to raise public awareness and to promote adequate behaviours. At the same time, medical doctors might consider the environmental fate and hazard of different active principles when prescribing drugs, thus stimulating also pharmaceutical industries toward a sustainable innovation combining therapeutical efficacy with environmental impact of new molecules. Finally, reliable normative guidelines should be recognized as technological opportunities for designing new wastewater treatment processes and plants, to improve removal efficiency and decrease the input of pharmaceuticals in aquatic bodies.

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Table 1. Concentrations of pharmaceuticals in the whole tissues of *M. galloprovincialis* collected in Senigallia (S1), Torrette (S2) and Portonovo (S3) from 2014 to 2017. Data are given as mean values ± standard deviations (n=5). NSAIDs: Non Steroidal Anti-Inflammatory Drugs; PSY: psychiatric drugs; AH: antihypertensive drug; DIC: diclofenac; IBU: ibuprofen; NIM: nimesulide; ∑ NSAIDs: Total NSAIDs (sum of DIC, IBU and NIM); CBZ: carbamazepine; PAR: paroxetine; LOR: Lormetazepam; ∑PSY: Total PSY (sum of CBZ, PAR and LOR); VAL: valsartan; n.a. not analyzed. Values are expressed as ng/g dw for all the individual drugs while ∑NSAIDs and ∑PSY are expressed as nmol/g to normalize different molecular weights of different compounds. *p* values are reported and letters indicate significant differences between groups of means (whithin each site and for each molecule) (Newman Keuls post hoc); n.s. not statistically significant; n.t. not tested.

Site	Year	Season	Month	NSAIDs										PSY										AH	
				DIC ng g^{-1} (d.w.)		IBU ng g^{-1} (d.w.)		NIM ng g^{-1} (d.w.)		*∑ NSAIDs nmol g^{-1} (d.w.)*				CBZ ng g^{-1} (d.w.)		PAR ng g-1 (d.w.)		LOR ng g-1 (d.w.)		*∑ PSY nmol g^{-1} (d.w.)*				VAL ng g-1 (d.w.)	
S1	2014	Summer	Jul	<1.4		<8.0		2.3 ± 2.2		0.01 ± 0.02	*(a)*			n.a.		n.a.		n.a.		*n.a.*				n.a.	
			Aug	1.4 ± 0.8	(a)	<8.0		2.2 ± 1.0		*0.03 ± 0.00*	*(ab)*			n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Autumn	Sep	27.8 ± 46.9	(a)	<8.0		<2.0		0.09 ± 0.16	*(ab)*			n.a.		n.a.		n.a.		*n.a.*				n.a.	
	2015	Spring	Apr	29.6 ± 10.2	(b)	143.7 ± 242.0		<2.0		*0.12 ± 0.03*	*(ab)*			n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Summer	Jul	103.8 ± 27.9	(b)	<8.0		<2.0		*0.35 ± 0.09*	*(ab)*			45.5 ± 14.7	(b)	16.7 ± 8.8	(b)	6.4 ± 8.5		*0.26 ± 0.07*	*(b)*			1.2 ± 2.0	(a)
			Aug	<1.4		<8.0		<2.0		*<0.0044*				151.1 ± 27.9	(c)	9.1 ± 4.5	(ab)	<0.4		*0.66 ± 0.11*	(c)			3.2 ± 1.2	(b)
		Autumn	Nov	<1.4		<8.0		<2.0		*<0.0044*				279.6 ± 67.6	(d)	4.5 ± 2.5	(ab)	<0.4		*1.20 ± 0.29*	*(d)*			6.0 ± 0.9	(b)
	2016	Winter	Feb	<1.4		<8.0		80.6 ± 74.9		*0.28 ± 0.24*	*(ab)*			228.2 ± 101.3	(cd)	5.9 ± 5.5	(ab)	<0.4		*0.98 ± 0.43*	*(cd)*			5.7 ± 0.6	(b)
		Spring	May	99.1 ± 24.0	(b)	<8.0		<2.0		*0.33 ± 0.08*	*(b)*			270.5 ± 107.4	(d)	1.6 ± 2.6	(a)	<0.4		*1.15 ± 0.45*	*(d)*			<0.5	
		Summer	Jul	2.5 ± 2.5	(a)	<8.0		<2.0		*0.02 ± 0.02*	*(a)*			32.7 ± 9.0	(ab)	30.0 ± 17.0	(b)	<0.4		*0.22 ± 0.08*	*(b)*			0.9 ± 0.8	(a)
		Autumn	Oct	58.4 ± 55.5	(b)	14.9 ± 15.9		2.1 ± 2.1		*0.26 ± 0.26*	*(ab)*			140.7 ± 23.9	(c)	4.5 ± 9.0	(a)	439.5 ± 77.0		2.19 ± 0.70	*(e)*			6.7 ± 1.1	(b)
	2017	Spring	May	109.3 ± 50.7	(b)	<8.0		<2.0		*0.37 ± 0.16*	*(b)*			139.4 ± 57.8	(c)	5.3 ± 1.1	(ab)	<0.4		*0.61 ± 0.24*	*(c)*			1.5 ± 1.4	(a)
		Summer	Jul	<1.4		<8.0		<2.0		*<0.0044*				21.9 ± 6.5	(a)	3.4 ± 5.4	(ab)	2.4 ± 3.1		*0.11 ± 0.03*	*(a)*			0.5 ± 0.4	(a)
	Sig.			*p < 0.001*		*(n.t.)*		*(n.t.)*		*p < 0.001*				*p < 0.001*		*p < 0.01*		*(n.t.)*		*p < 0.001*				p < 0.001	
S2	2014	Summer	Jul	<1.4		<8.0	(n.t.)	56.0		0.2				n.a.		n.a.		n.a.		*n.a.*				n.a.	
			Aug	1.7 ± 1.2		13.6 ± 10.6		5.8 ± 6.8		*0.09 ± 0.04*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Autumn	Sep	<1.4		<8.0		<2.0		*<0.0044*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
	2015	Spring	Apr	27.2 ± 16.9		<8.0		<2.0		*0.11 ± 0.05*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Summer	Jul	20.6 ± 27.3		<8.0		<2.0		0.07 ± 0.10				62.9 ± 7.7	(b)	2.7 ± 2.1		1.4 ± 1.6		*0.28 ± 0.03*	*(bc)*			1.3 ± 0.8	(b)
			Aug	<1.4		<8.0		61.0 ± 134.0		*0.22 ± 0.43*				34.6 ± 25.8	(a)	7.0 ± 5.7		18.9 ± 25.1		*0.22 ± 0.15*	*(bc)*			<0.5	
		Autumn	Nov	<1.4		<8.0		<2.0		*<0.0044*				299.7 ± 46.2	(b)	1.1 ± 1.1		<0.4		*1.27 ± 0.20*	*(c)*			5.4 ± 0.4	(c)
	2016	Winter	Feb	<1.4		<8.0		<2.0		*<0.0044*				181.8 ± 41.3	(b)	4.1 ± 3.6		<0.4		*0.78 ± 0.17*	*(c)*			<0.5	
		Spring	May	36.4 ± 35.1		<8.0		3.7 ± 4.0		0.14 ± 0.11				<1.0		<1.0		104.8 ± 105.9		*0.31 ± 0.32*	*(b)*			1.3 ± 1.1	(b)
		Summer	Jul	14.0 ± 29.9		28.4 ± 19.7		<2.0		*0.19 ± 0.07*				<1.0		4.0 ± 5.3		<0.4		*0.01 ± 0.01*	*(a)*			1.8 ± 1.5	(b)
		Autumn	Oct	12.7 ± 26.9		9.4 ± 12.1		<2.0		*0.09 ± 0.09*				201.3 ± 35.1	(b)	4.0 ± 7.8		42.8 ± 24.7		*0.99 ± 0.16*	*(c)*			3.4 ± 0.8	(c)
		Winter	Dec	<1.4		27.9 ± 32.9		7.1 ± 10.8		*0.16 ± 0.16*				181.8 ± 84.9	(b)	2.1 ± 3.7		235.2 ± 218.4		*1.48 ± 0.81*	*(c)*			<0.5	
	2017	Spring	May	6.5 ± 12.9		<8.0		<2.0		*0.04 ± 0.04*				216.8 ± 78.9	(b)	6.2 ± 4.0		2.2 ± 4.3		*0.94 ± 0.34*	*(c)*			<0.5	
		Summer	Jul	84.3 ± 82.1		<8.0		<2.0		*0.29 ± 0.26*				52.3 ± 31.4	(a)	1.0 ± 1.3		<0.4		*0.22 ± 0.13*	*(bc)*			0.4 ± 0.3	(a)
	Sig.			(n.s.)		*(n.t.)*		(n.t.)		(n.s.)				*p < 0.001*		(n.s.)		(n.s.)		*p < 0.001*				p < 0.001	
S3	2014	Summer	Jul	<1.4		<8.0	(n.t.)	6.7 ± 9.9	(n.t.)	*0.04 ± 0.03*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
			Aug	16.1 ± 14.7		9.4 ± 0.6		4.2 ± 2.5		*0.11 ± 0.06*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Autumn	Sep	<1.4		<8.0		6.1 ± 4.4		*0.04 ± 0.01*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
	2015	Spring	Apr	5.4 ± 8.1		<8.0		3.8 ± 2.6		*0.05 ± 0.03*				n.a.		n.a.		n.a.		*n.a.*				n.a.	
		Summer	Jul	171.1 ± 233.6		<8.0		<2.0		*0.56 ± 0.73*				76.1 ± 30.1		11.2 ± 7.8	(a)	2.2 ± 4.4		*0.36 ± 0.14*	*(ab)*			1.8 ± 1.6	(ab)
			Aug	52.1 ± 37.5		<8.0		<2.0		*0.19 ± 0.12*				74.4 ± 24.0	(ab)	2.3 ± 2.5	(a)	<0.40		*0.32 ± 0.10*	*(ab)*			<0.5	
		Autumn	Nov	<1.4		<8.0		13.6 ± 28.1		*0.07 ± 0.09*				144.7 ± 30.5	(b)	5.0 ± 1.7	(a)	<0.40		*0.63 ± 0.13*	*(b)*			<0.5	
	2016	Winter	Feb	<1.4		<8.0		<2.00		*<0.0044*				240.2 ± 39.1	(b)	4.7 ± 4.2	(a)	<0.40		*1.03 ± 0.17*	*(b)*			4.0 ± 2.2	(ab)
		Spring	May	53.6 ± 31.4		<8.0		4.6 ± 7.9		*0.20 ± 0.11*				<1.0		6.7 ± 14.0	(a)	288.0 ± 130.7		*0.88 ± 0.37*	*(b)*			1.8 ± 0.7	(ab)
		Summer	Jul	31.9 ± 32.9		<8.0		3.4 ± 3.8		*0.13 ± 0.10*				51.2 ± 46.4	(a)	<1.0		62.1 ± 138.4		*0.40 ± 0.53*	*(b)*			3.6 ± 0.6	(b)
		Autumn	Oct	34.8 ± 48.2		<8.0		<2.0		*0.13 ± 0.15*				181.6 ± 108.5	(ab)	<1.0		207.6 ± 140.4		*1.39 ± 0.58*	*(b)*			4.6 ± 1.4	(b)
		Winter	Dec	10.5 ± 22.0		40.9 ± 50.9		<2.0		*0.23 ± 0.23*				147.8 ± 35.3	(b)	18.4 ± 21.2	(a)	137.5 ± 157.3		*1.09 ± 0.38*	*(b)*			0.5 ± 0.2	(a)
	2017	Spring	May	16.8 ± 36.1		<8.0		<2.0		*0.08 ± 0.11*				128.7 ± 55.0	(b)	1.6 ± 1.6	(a)	<0.40		*0.55 ± 0.23*	*(b)*			<0.5	
		Summer	Jul	82.0 ± 27.2		<8.0		<2.0		*0.28 ± 0.09*				35.2 ± 14.2	(ab)	49.9 ± 22.2	(b)	<0.40		*0.28 ± 0.09*	*(ab)*			<0.5	
	Sig.			*n.s.*		*(n.t.)*		(n.t.)		(n.s.)				*p < 0.001*		*p < 0.001*		(n.t.)		*p < 0.001*				*p < 0.001*	

Legend of the Figures:

Figure 1. Map of sampling locations along the Tyrrhenian and Adriatic Sea.

Figure 2. nMDS analyses on bioaccumulation of DIC, IBU, NIM, CBZ, PAR, LOR and VAL, in *M. galloprovincialis* from Senigallia S1, Torrette S2, and Portonovo S3, sampled in different months and years. Different shapes indicate each site: "square" for S1; "triangle" for S2 and "circle" for S3, while different fillings indicate seasons: "white" for Autumn; "black" for Winter; "dotted" for Spring and "gray" for Summer AMP and KET were always below detection limit and not elaborated.

Figure 3. Concentrations of Diclofenac, Ibuprofen, Nimesulide, Carbamazepine, Paroxetine, Lormetazepam, Total NSAIDs (sum of DIC, IBU and NIM), Total PSY (sum of CBZ, PAR and LOR) and Valsartan in the whole tissues of *M. galloprovincialis* collected in different sites along the Adriatic (S1-S8) and Tyrrhenian Sea (S9-S14). For sites sampled in more than one period, results are given as mean values ± standard deviation of means concentrations measured in each sampling time; in all the other cases, results are given as mean values ± standard deviation of replicates.

Figure 4. A) Detection frequency (% of analysed samples) of different pharmaceuticals in tissues of *M. galloprovincialis* from all sampling sites and periods B) Percentage of samples containing at least 1, 2, 3 or 4 pharmaceuticals.

Figure 1.

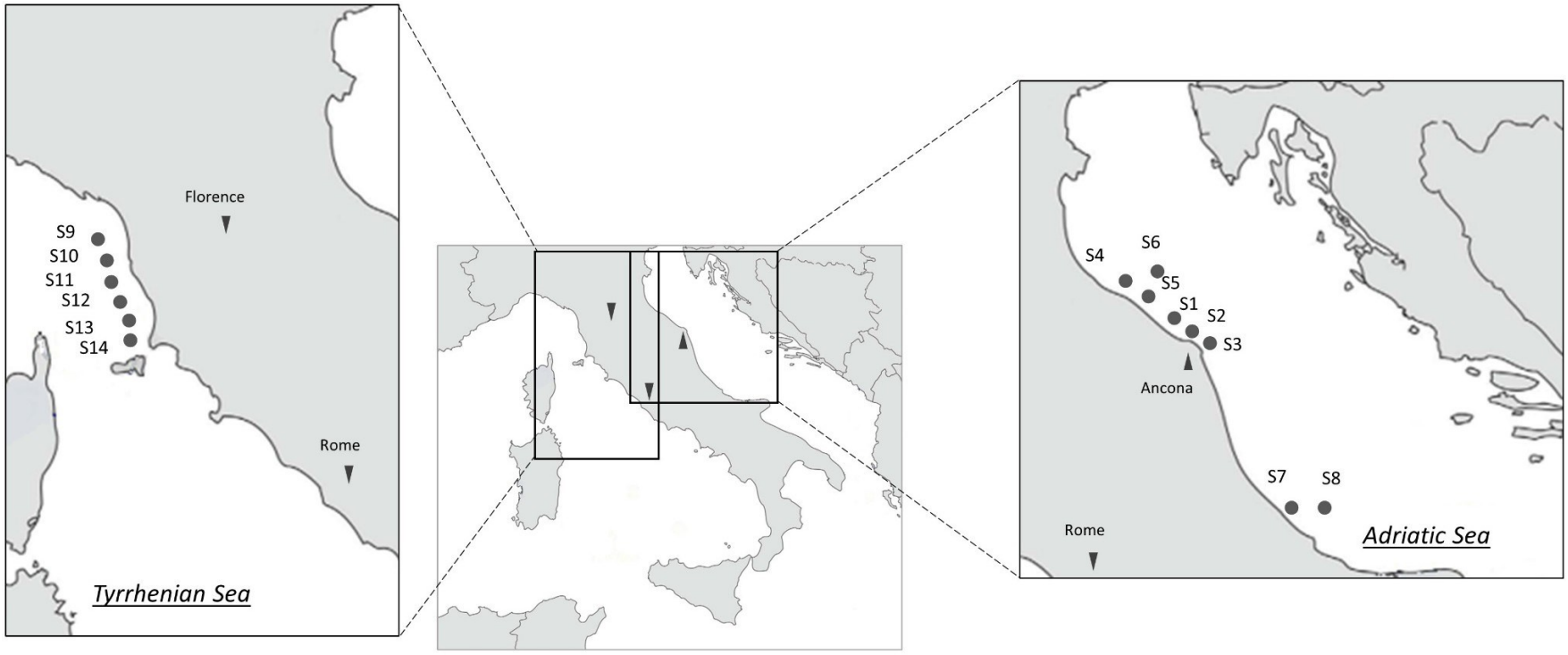
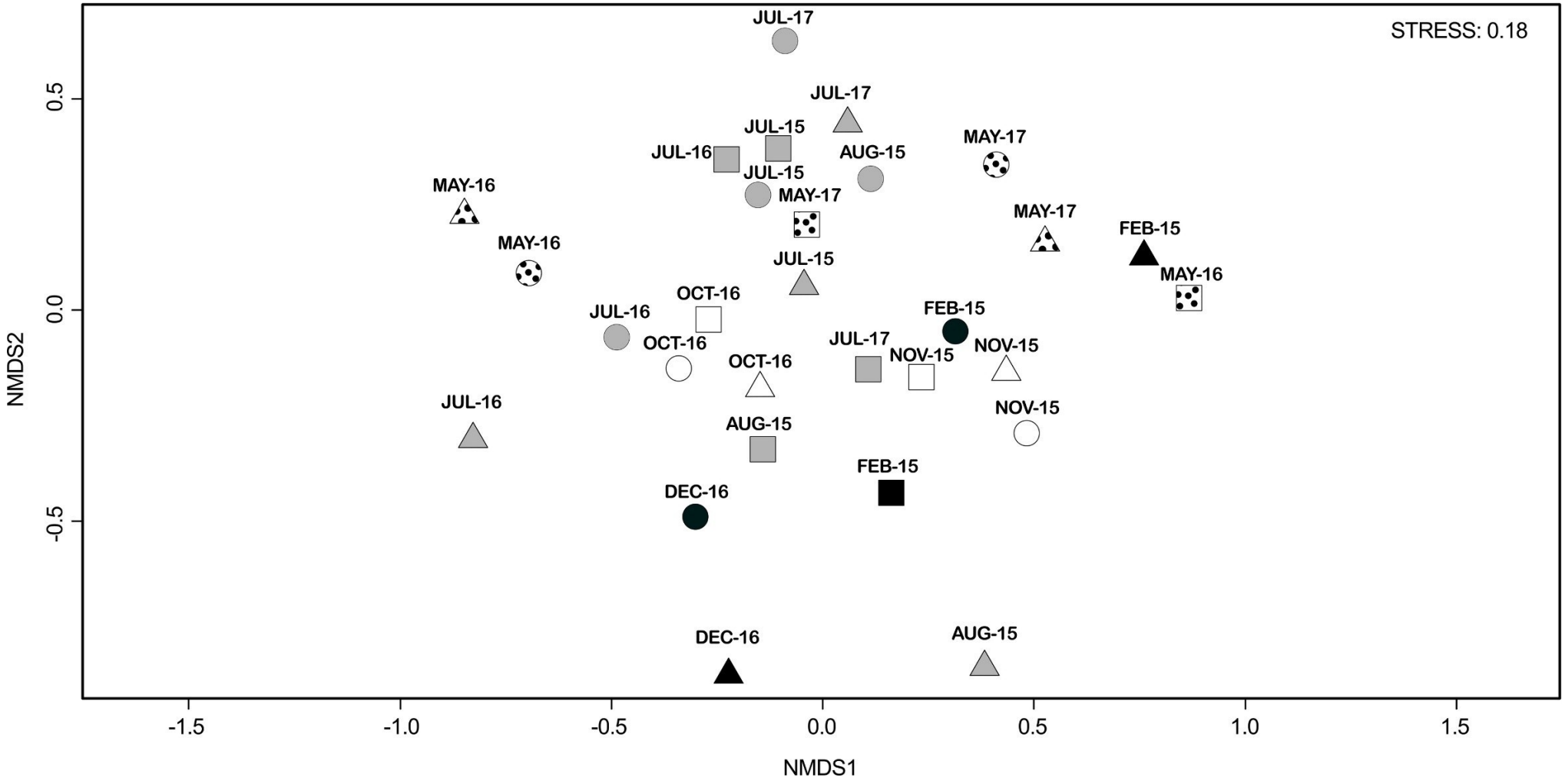

Florence
S9
S10
S11
S12
S13
S14
Rome
Tyrrhenian Sea
S4
S6
S5
S1
S2
S3
Ancona
S7
S8
Rome
Adriatic Sea

Figure 2.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NMDS2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
0.5
0.0
-0.5
JUL-17
JUL-17
JUL-15
JUL-16
AUG-15
MAY-17
JUL-15
MAY-16
MAY-17
MAY-17
FEB-15
MAY-16
MAY-16
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MAY-16
OCT-16
JUL-16
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OCT-16
NOV-15
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OCT-16
JUL-16
NOV-15
AUG-15
FEB-15
DEC-16
DEC-16
AUG-15

Figure 3.

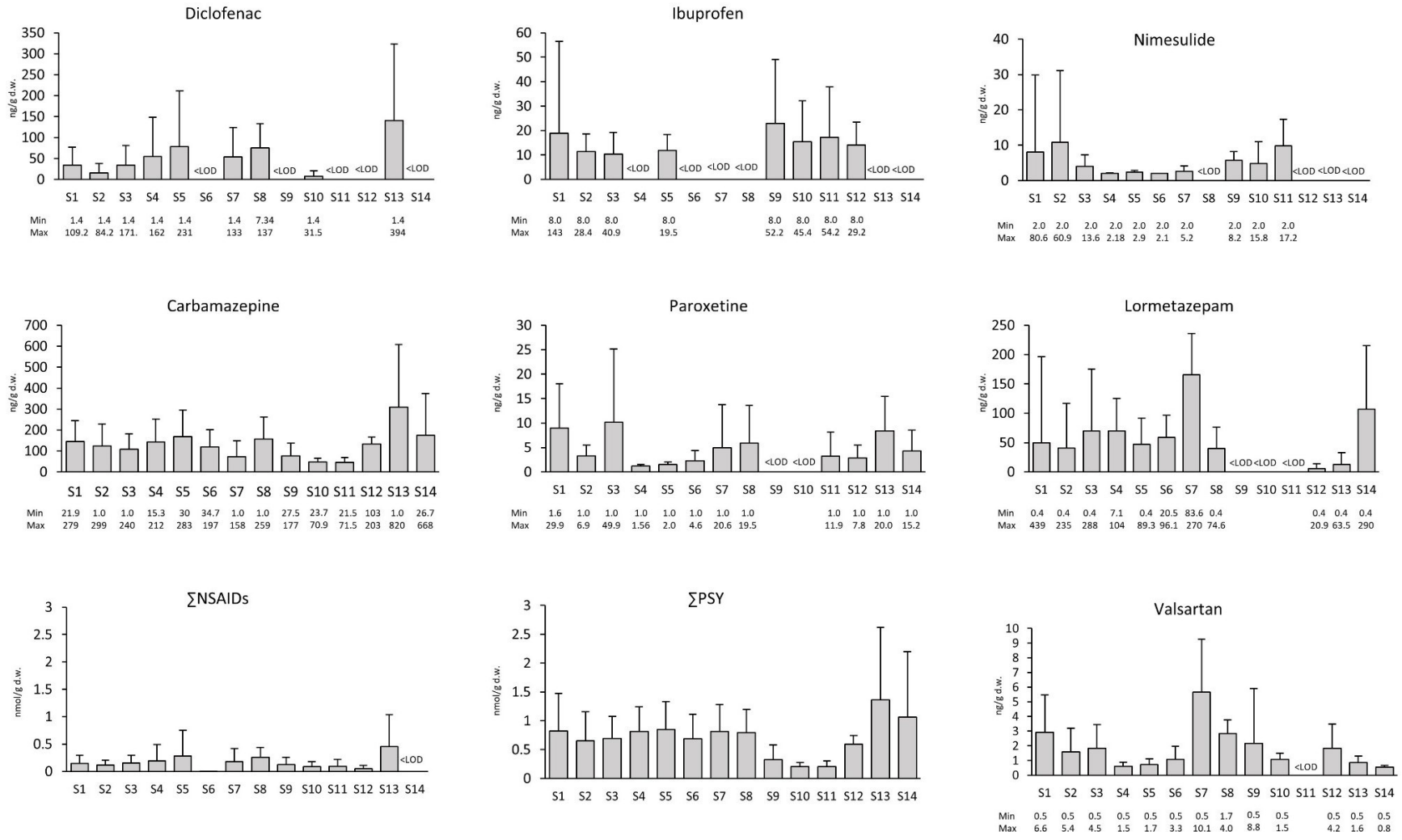
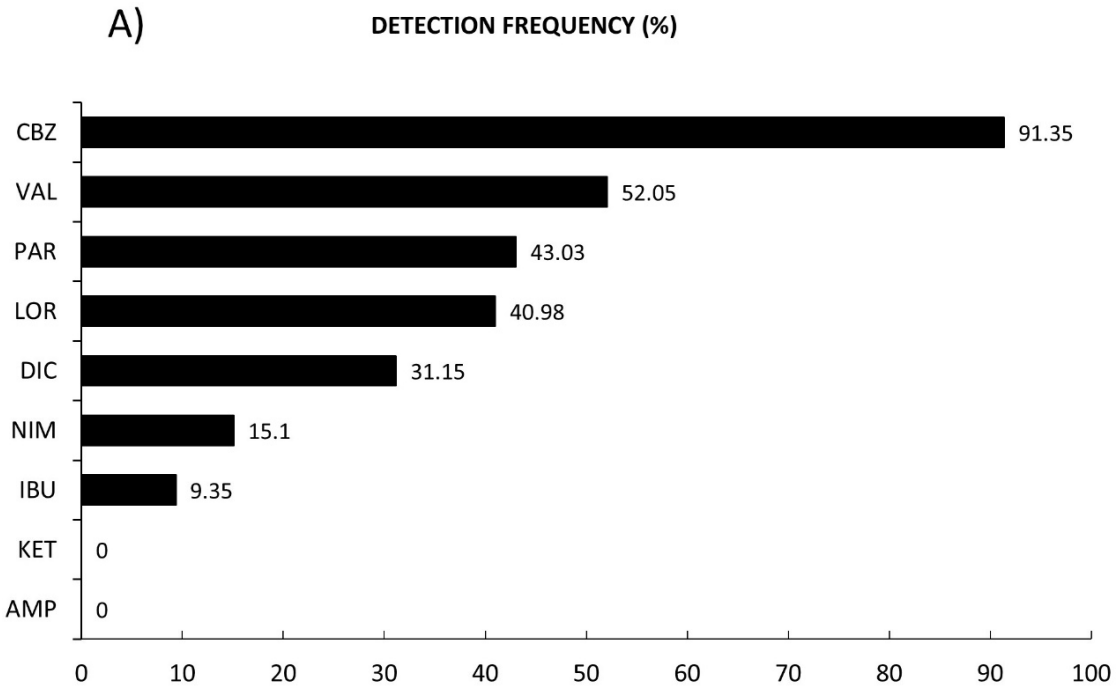

Diclofenac
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 1.4 1.4 1.4 1.4 1.4 1.4 7.34 1.4 1.4
Max 109.2 84.2 171. 162 231 133 137 31.5 394
Ibuprofen
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 8.0 8.0 8.0 8.0 8.0 8.0 8.0
Max 143 28.4 40.9 19.5 52.2 45.4 54.2 29.2
Nimesulide
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 2.0 2.0 2.0 2.0 2.0 2.0 2.0 2.0 2.0 2.0
Max 80.6 60.9 13.6 2.18 2.9 2.1 5.2 8.2 15.8 17.2
Carbamazepine
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Min 21.9 1.0 1.0 15.3 30 34.7 1.0 1.0 27.5 23.7 21.5 103 1.0 26.7
Max 279 299 240 212 283 197 158 259 177 70.9 71.5 203 820 668
Paroxetine
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 1.6 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0
Max 29.9 6.9 49.9 1.56 2.0 4.6 20.6 19.5 11.9 7.8 20.0 15.2
Lormetazepam
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 0.4 0.4 0.4 7.1 0.4 20.5 83.6 0.4 0.4 0.4 0.4
Max 439 235 288 104 89.3 96.1 270 74.6 20.9 63.5 290
∑NSAIDs
nmol/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
∑PSY
nmol/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Valsartan
ng/g d.w.
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
<LOD
Min 0.5 0.5 0.5 0.5 0.5 0.5 0.5 1.7 0.5 0.5 0.5 0.5 0.5
Max 6.6 5.4 4.5 1.5 1.7 3.3 10.1 4.0 8.8 1.5 4.2 1.6 0.8

Figure 4.


A)
DETECTION FREQUENCY (%)
CBZ
VAL
PAR
LOR
DIC
NIM
IBU
KET
AMP
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52.05
43.03
40.98
31.15
15.1
9.35
0
0
0
10
20
30
40
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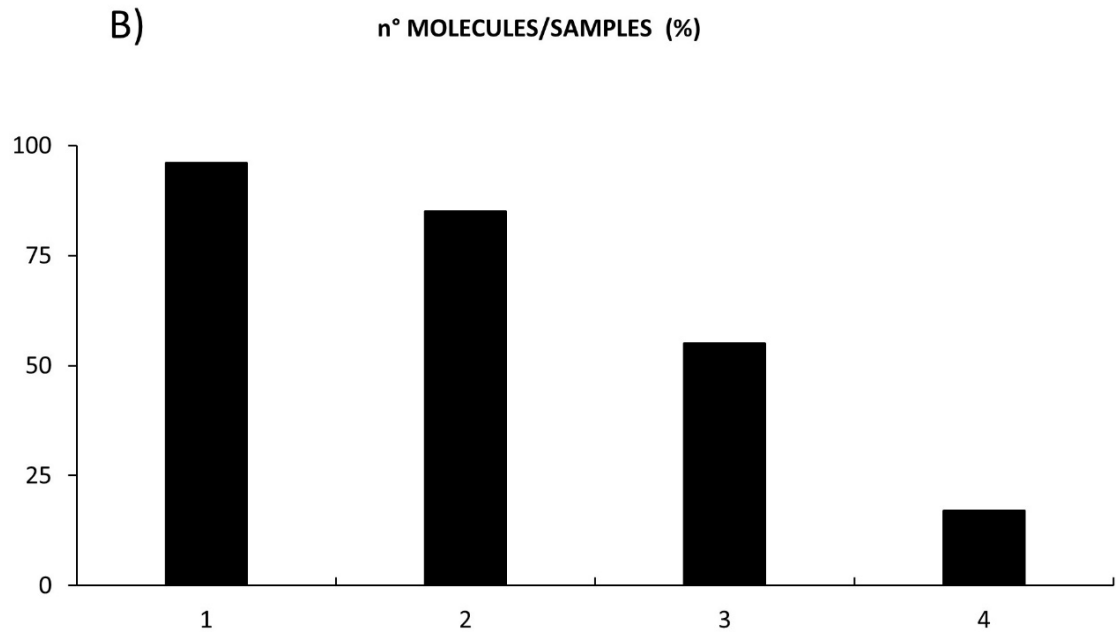
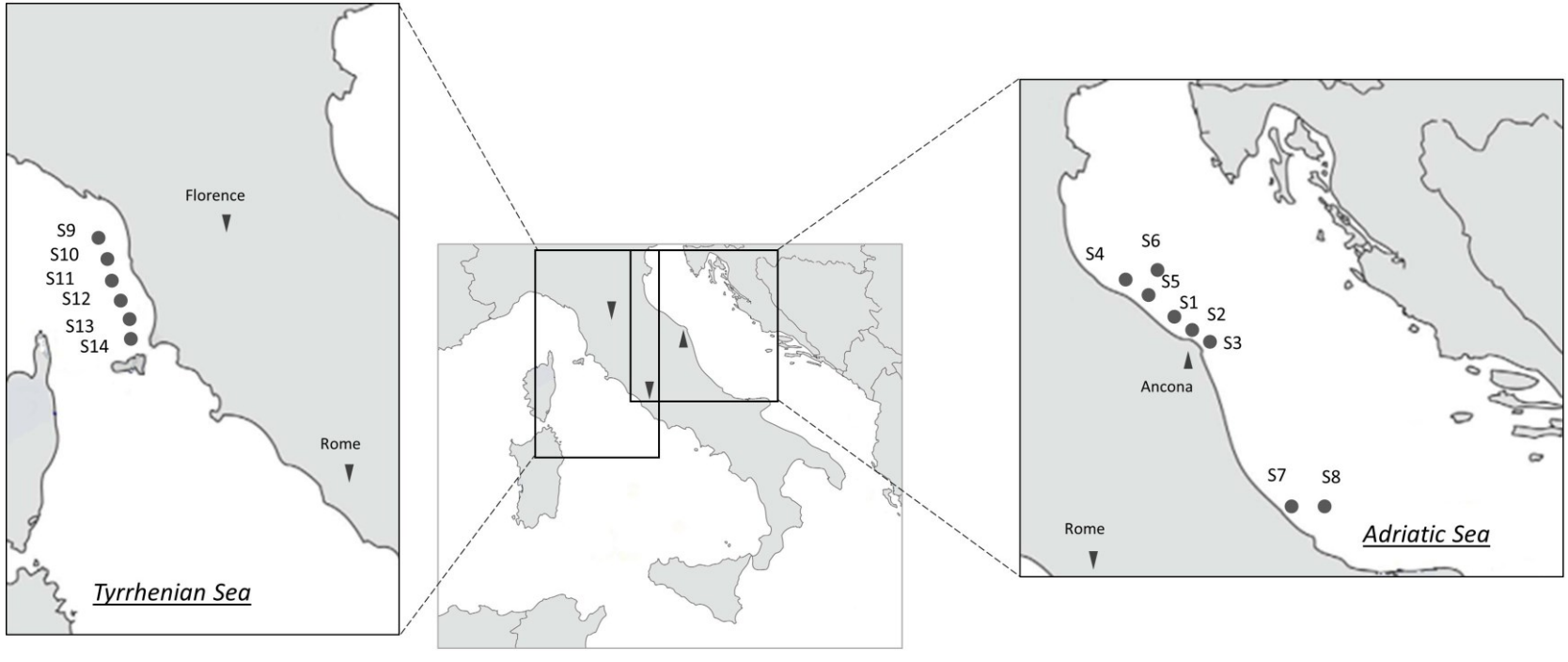
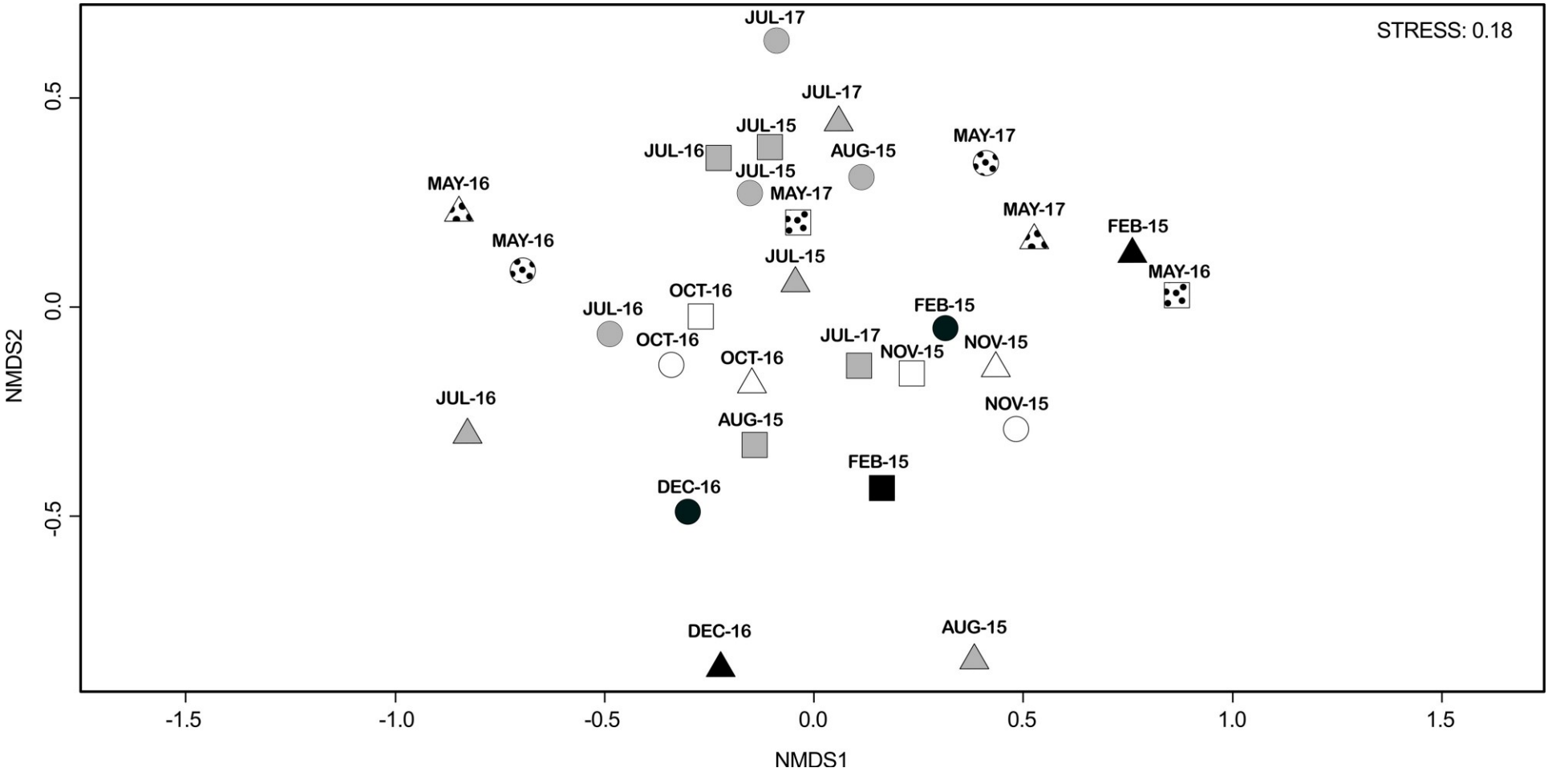

B)
n° MOLECULES/SAMPLES (%)
100
75
50
25
0
1
2
3
4


Florence
S9
S10
S11
S12
S13
S14
Rome
Tyrrhenian Sea
S6
S4
S5
S1
S2
S3
Ancona
S7
S8
Rome
Adriatic Sea


STRESS: 0.18
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JUL-15
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MAY-17
JUL-15
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FEB-15
MAY-16
JUL-15
MAY-16
OCT-16
JUL-16
FEB-15
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JUL-17
NOV-15
NOV-15
OCT-16
JUL-16
NOV-15
AUG-15
FEB-15
DEC-16
DEC-16
AUG-15
NMDS1
NMDS2
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0.0
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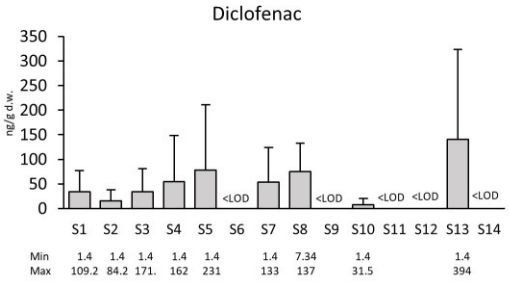
Diclofenac
ng/g d.w.
350
300
250
200
150
100
50
0
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S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
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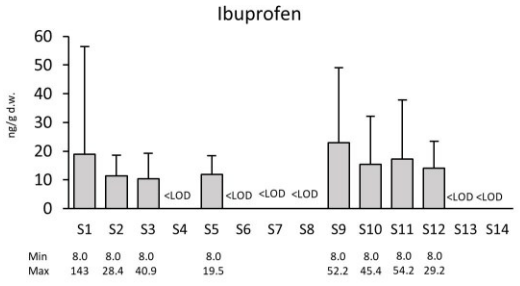
Max 109.2 84.2 171. 162 231 133 137 31.5 394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ng/g d.w.
60
50
40
30
20
10
0
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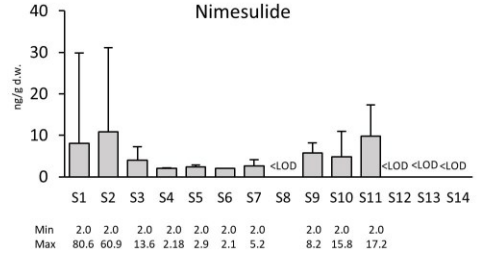
Max 143 28.4 40.9 19.5 52.2 45.4 54.2 29.2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ng/g d.w.
40
30
20
10
0
<LOD
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
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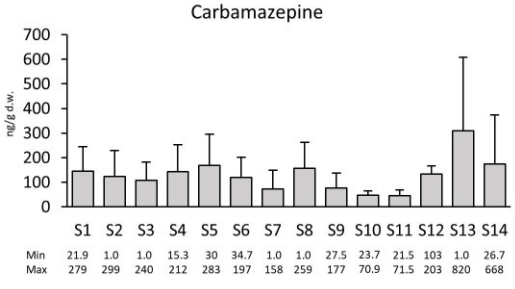
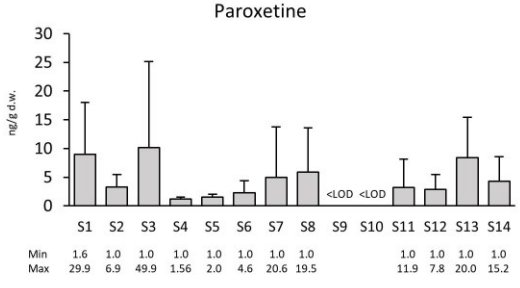
Max 80.6 60.9 13.6 2.18 2.9 2.1 5.2 8.2 15.8 17.2


Carbamazepine
ng/g d.w.
700
600
500
400
300
200
100
0
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Min 21.9 1.0 1.0 15.3 30 34.7 1.0 1.0 27.5 23.7 21.5 103 1.0 26.7
Max 279 299 240 212 283 197 158 259 177 70.9 71.5 203 820 668


Paroxetine
ng/g d.w.
30
25
20
15
10
5
0
<LOD
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Min 1.6 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0
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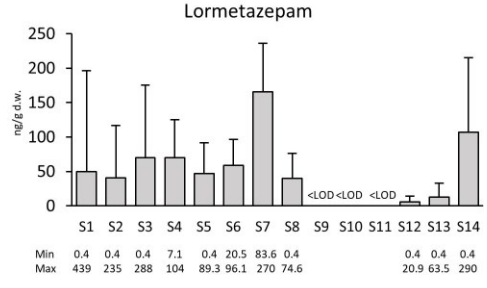
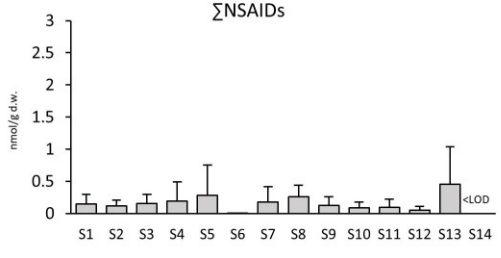

Lormetazepam
ng/g d.w.
250
200
150
100
50
0
<LOD
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Min 0.4 0.4 0.4 7.1 0.4 20.5 83.6 0.4 0.4 0.4 0.4
Max 439 235 288 104 89.3 96.1 270 74.6 20.9 63.5 290


∑NSAIDs
nmol/g d.w.
3
2.5
2
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1
0.5
0
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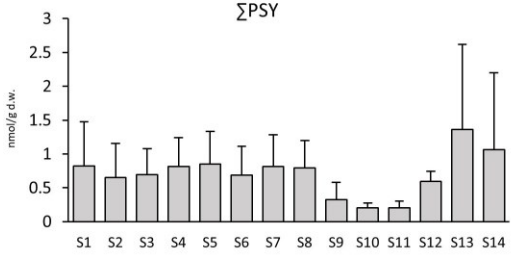
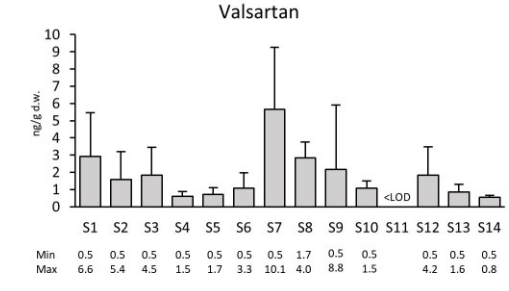
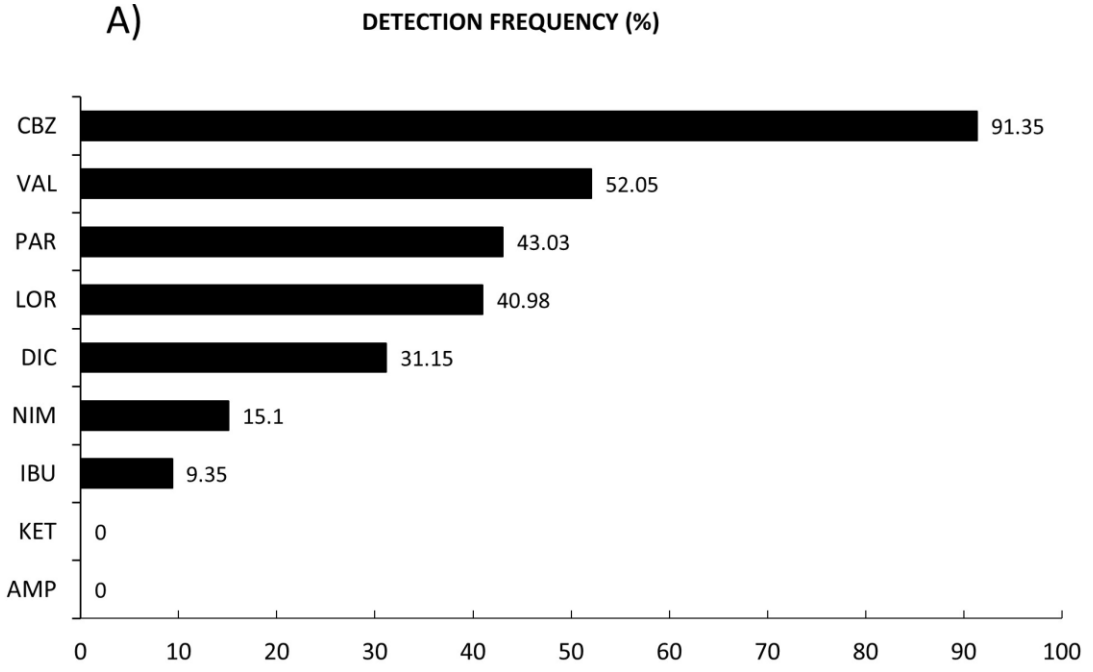
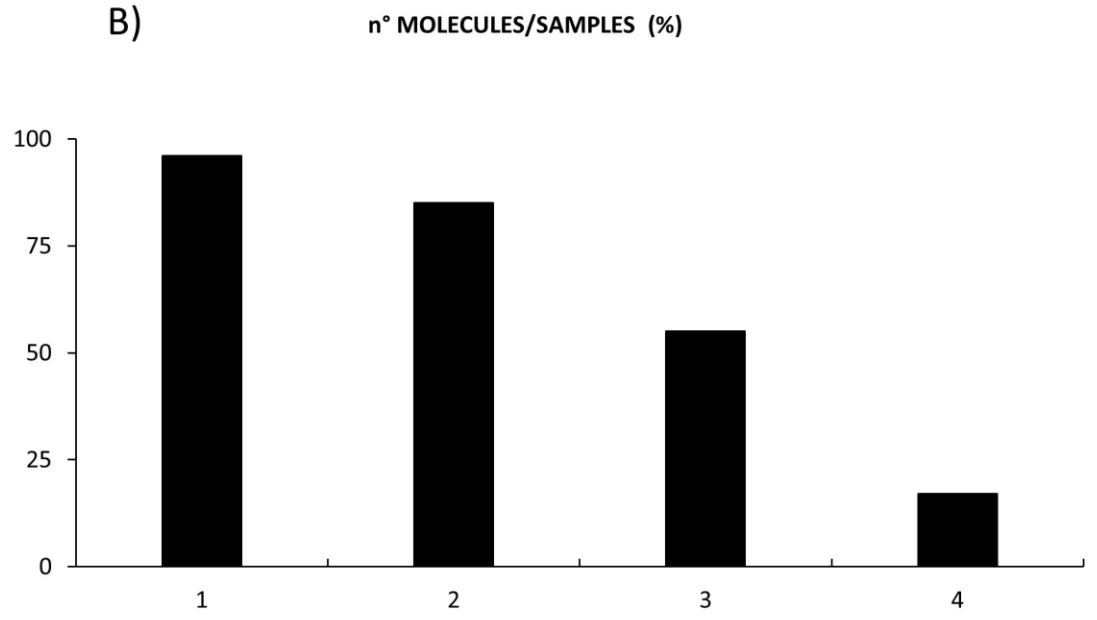
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14


∑PSY
nmol/g d.w.
3
2.5
2
1.5
1
0.5
0
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14


Valsartan
ng/g d.w.
10
9
8
7
6
5
4
3
2
1
0
<LOD
S1 S2 S3 S4 S5 S6 S7 S8 S9 S10 S11 S12 S13 S14
Min 0.5 0.5 0.5 0.5 0.5 0.5 0.5 1.7 0.5 0.5 0.5 0.5 0.5
Max 6.6 5.4 4.5 1.5 1.7 3.3 10.1 4.0 8.8 1.5 4.2 1.6 0.8


A)
DETECTION FREQUENCY (%)
CBZ
VAL
PAR
LOR
DIC
NIM
IBU
KET
AMP
91.35
52.05
43.03
40.98
31.15
15.1
9.35
0
0
0
10
20
30
40
50
60
70
80
90
100


B)
n° MOLECULES/SAMPLES (%)
100
75
50
25
0
1
2
3
4

Declaration of interests

☒ The authors (Marica Mezzelani, Daniele Fattorini, Stefania Gorbi, Marco Nigro, Francesco Regoli) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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

Prof. Francesco Regoli, on behalf of all the authors.

CRediT author statement

Marica Mezzelani: she participated to conceptualization of the study; she was responsible of data curation, formal analysis and original draft writing. **Daniele Fattorini:** he was responsible for analytical methods development, he participated to interpretation and data curation, formal analysis, review & editing. **Stefania Gorbi:** she participated to the conceptualization of the study, participated to the review of obtained results and editing of the manuscript. **Marco Nigro:** he participated to conceptualization of the study, discussion of results, support in sampling activities. **Francesco Regoli:** he participated to the conceptualization of the study and general supervision, funding acquisition, discussion of results and of manuscript structure, review and editing.

SUPPLEMENTARY MATERIALS (SM)

Human pharmaceuticals in marine mussels: evidence of sneaky environmental hazard along Italian coasts

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Materials and Methods

Sampling sites and periods

All the sampling locations and relative periods are indicated in Table SM1, which include the GPS coordinates and the main characteristics of the areas.

Analytical methods for measurements of pharmaceuticals in mussels tissues.

Sample preparation and analytical measurements represent an update of previously reported methods (Mezzelani et al., 2016a, b; Mezzelani et al., 2018b). Protocols developed for the extraction, purification, separation and detection of AMP, DIC, IBU, KET and NIM were modified to include CBZ, LOR, PAR and VAL among pharmaceuticals detected in mussels tissues and the main applied HPLC conditions are summarized in the Table SM2.

HPLC grade methanol, acetonitrile and analytical standards (purity of ≥97%) were purchased from Sigma Aldrich (Milan, Italy) for the following PhACs: Acetaminophen AMP (CAS 103-90-2), Carbamazepine CBZ (CAS 298-46-4) Diclofenac DIC (CAS 15307-86-5), Ibuprofen IBU (CAS 15687-27-1), Ketoprofen KET (CAS 22071-15-4) Lormetazepam LOR (CAS 848-75-9) Nimesulide NIM (CAS 51803-78-2), Paroxetine PAR (CAS 110429-35-1) and Valsartan VAL (CAS 137862-53-4). Stock solutions (1 mg mL^{-1}) were prepared every 5 days in methanol acidified at 0.1% with acetic acid in amber vials and stored at +4 °C in the dark to reduce possible degradation. Working solutions were prepared daily in methanol acidified at 0.1% with acetic acid.

Different homogenization and extraction buffers were used for various compounds: acetic acid 0.1%, pH = 3.26 (buffer 1) for CBZ, DIC, IBU, KET LOR, NIM, PAR and VAL, while ammonium phosphate 10 mM, pH = 4.0 with citric acid 100 mM (buffer 2) for AMP. About 3 g of wet tissues were homogenized in 5mL of buffer at room temperature for 20 minutes using a dispersing, stirring, homogenizing and grinding system (IKA ULTRA® TURRAX® Tube Drive). After centrifugation at 4500 ×g for 30 minutes, samples were purified by Solid Phase Extraction (SPE) with reversed-phase tubes (Discovery DSC-18, 1g x 6 mL, Supelco, Bellefonte, Pennsylvania, USA). SPE tubes were conditioned with 6 mL of methanol, followed by 18 mL of ultra-pure water. Samples were diluted (1:1) with ultra-pure water and loaded onto the SPE cartridges; after washing with 12 mL of potassium bicarbonate $KHCO_3$ and 6 mL of ultra-pure water, analytes were eluted and recovered using 2 mL of a solution constituted by methanol acidified at 0.1% with acetic acid (HPLC, gradient grade, Carlo Erba). The final conditions for the purification of the extracts with SPE techniques, including the type (C18) and mass (1g) of resin, the volume applied and recovered, are

the result of numerous tests conducted during the validation of the method, to guarantee the best extraction and purification yields. The C18 cartridges are widely used for the purification of environmental matrices. Obtained samples were filtered using Phenex^{TM}-RC membrane (Regenerated Cellulose/Polypropylene 0.45 µm, 15mm syringe filters, Phenomenex, US) and then centrifuged again at 12000 ×g for 20 minutes.

Analytical detection of extracted PhACs was performed by High Performance Liquid Chromatography, with fluorimetric and diode array detectors DAD (Agilent Infinity 1260 series). Chromatographic separations of CBZ, DIC, IBU, KET, LOR, NIM, PAR and VAL were performed on a Kinetex column (C18,5 µm, 150 mm length, 4.6 mmID, Phenomenex, US), equipped with a security guard column (C18, 5 µm, 4 mm length, 2.0 mmID, Phenomenex, US). For CBZ, DIC, KET and VAL a mobile phase composed by ultra-pure water (26%), acetonitrile (42%) and Buffer 1 (32%) was used under isocratic condition. Analyses of IBU, LOR, NIM and PAR were performed using ultra-pure water, acetonitrile, Buffer 1 gradient (from 35%:30%:35% to 0%:65%:35% linearly for 23 minutes). To guarantee the optimal resin cleaning condition, after each analyses a gradient was applied to linearly increase the organic phase content in the column (87.5%: 12.5% to 10%: 90%).
During the validation phase mussel tissues (n=10) were homogenized, divided in two subsamples, one considered as Blank, while the other was spiked with various concentrations of standard solution of each tested compound. Spiked mussel samples were in contact with pharmaceuticals for 10 hours, before being analysed by HPLC. Both samples were injected in the HPLC to compare the spectra of Blank with those from the spiked sample: the chromatography was corrected to obtain the spectra in the cleaner field (i.e. area without interferences of other signals). Once obtained the best preliminary conditions, 20 samples (each constituted by 5 mussels), were tested to observe the variability of the background. When necessary, additional chromatography corrections have been tested to obtain the best conditions. After this, the optimal wavelengths were selected for each molecule (assuring at least 85% of the maximum absorbance) and the best qualifiers (with 50-75% of maximum absorbance) were identified. All wavelengths (main and qualifiers), have been repeatedly tested trough the analyses of samples (mussels homogenates) spiked with known concentrations of standards, to verify linearity, dispersion of the points with respect to the calibration curve (n =10). These checks were further used to determine LOD/LOQ (Table SM3)

Following these procedures, DAD was used for monitoring the spectra from 190 nm to 350 nm, and the signal for CBZ, DIC, KET and LOR was obtained at 286 nm, 276 nm, 250 nm e 232 nm, respectively. NIM was detected using DAD from 190 nm to 410 nm and monitoring at 298 nm. Analytical detection of IBU, PAR and VAL was obtained by fluorimetric detector with excitation/emission wavelengths at 230/294 nm for IBU, 296/338 nm for PAR and 205/380 for VAL.

With such analytical conditions Limits of Detection (LOD) for individual pharmaceuticals were 0.60 ng for AMP, 0.82 ng for DIC, 4.80 ng IBU, 0.51 ng for KET, 1.26 ng for NIM, 0.62 ng for CBZ, 0.26 ng for LOR, 0.57 ng for PAR and 0.29 ng for VAL.

Separation of AMP was carried out by an Agilent Eclipse Plus column (C18, 3.5 μm, 100 mm length, 4.6 mmID) with a security guard column (C18, 5 μm, 4 mm length, 2.0 mmID, Phenomenex, US), and a mobile phase composed by Buffer 2 (87.5%) and methanol (12.5%) under isocratic condition; a fast, post-run gradient was applied to remove any unresolved compound retained by the analytical column (from 87.5%:12.5% to 10%:90%). Detection was obtained using diode array in the range 190-350 nm, monitoring the signal at 248 nm and checking for quality control and assurance with additional qualifying signals. Concentrations of various PhACs were quantified by comparison with signals of pure standard solutions. Due to the lack of appropriate Certified Standard Reference Materials (SRMs), recovery for each compound was estimated on samples of control mussels (n=10) spiked with various concentrations of investigated molecules According to EU standards 2002/657/EC, retention time of all analytes are comparable with those obtained from the calibration standard in spiked mussels, within a margin of ± 2,5%.

The parameters related to the signals detection are reported in the Table SM3, including the retention time (RT), the wavelength of both main signals and qualifiers (2 for each compounds). The relative LOD, LOQ, minimum (MIN), median and maximum (MAX) values are also included. Reproducibility, recovery and RSD for each compound were determined adding aliquots of know concentrations of pure standard in mussels samples: For each compound, 5 levels of concentrations were tested on 5 samples (each constituted by 5 organisms) and the obtained data are included in the Table SM4.

The following identification criteria were applied for the detection of each analytes: 1) retention time (RT); for each compound the RT is verified using the chromatogram relating to the main signal and in those of at least two signals used as qualifiers. The RTs were previously determined through the separation of pure standard solutions with known concentration and selected in a time window equal to ±2.5% of the RT itself. The verification of the RT was daily checked, corresponding to each individual analytical session, after fresh mobile phase solutions are prepared. 2) at least two qualifying signals were used for the verification and determination of each of the components. The qualifiers were chosen from the signals that ensure a value at least of 50% of the main signal, except for one of the two AMP qualifiers: the noise of background lead to select a signal in a cleaner chromatographic region, with a value <50%. For the same reason (influence of background) one of selected qualifiers for LOR, is characterized by a value higher than the main signal. The relative relationship between the main signal and that of the qualifiers was determined after calibrations

carried out with solutions of pure standards at a known concentration (at least 10 calibration points, each characterized by at least 5 replicates); the calibrations were periodically checked at each analytical session. The peaks for each analyte were accepted if both the selected qualifiers guarantee a signal equivalent to the ratio determined after calibration ±20% of percentage variation; 3) each peak was accepted if the main signal and those of the qualifiers are sufficiently distinguishable from the background, with a peak separated for at least half of the height from any interfering signals; in any case, only peaks with a signal / noise ratio of at least 10/1 are accepted. 4) for the compounds that are determined by fluorescence as the main signal, i.e. IBU, PAR, VAL, in addition to the qualifying signals, cross check of signals obtained by DAD detector was checked; 5) for each compound, the UV/VIS absorption spectrum obtained by the DAD was compared with that of appropriate standard solutions and with that of mussel samples added with known aliquots of pure standards. 6) for compounds detected with DAD, the peak purity was evaluated through the specific software provided by Agilent Technologies (ChemStation Edition-OpenLAB CDS, Rev.C.01.03[37]), comparing the spectra of standard and sample solutions.

The water content in mussels tissues was determined by the measurement of wet weight and dry weight ratio in 5 replicates (each constituted by 5 organisms), for each sampling period and site as reported in Table SM5; final concentrations of detected pharmaceuticals were normalized and expressed as ng/g dry weight (d.w.). Considering these analytical conditions and the described preparation procedures, the minimum measurable amounts (Limit of Quantification, LOQ) in mussels tissues were 1 ng/g dry weight (d.w.) for AMP, 1.37 ng/g d.w. for DIC, 8 ng/g d.w. for IBU, 0.86 ng/g d.w. for KET, 2.10 ng/g d.w. for NIM, 1.03 ng/g d.w. for CBZ, 0.43 ng/g d.w. for LOR, 0.95 ng/g d.w. for PAR and 0.49 ng/g d.w. for VAL (Table SM3). All those values always ensure an appropriate analytical accuracy.

Table SM1. General characteristics of sampling sites in both the Adriatic and Tyrrhenian Sea.

Site	Location	Area	Latitude	Longitude	Characteristic	Year	Sampling period
S1	Senigallia	Adriatic Sea	43° 43' 14.93'' N	13° 13' 33.30'' E	Public beach	2014	Summer, Autumn
						2015	Spring, Summer, Autumn, Winter
						2016	Spring, Summer, Autumn, Winter
						2017	Spring, Summer
S2	Torrette	Adriatic Sea	43° 36' 42.28'' N	13° 27' 13.31'' E	Public beach	2014	Summer, Autumn
						2015	Spring, Summer, Autumn, Winter
						2016	Spring, Summer, Autumn, Winter
						2017	Spring, Summer
S3	Portonovo	Adriatic Sea	43° 34' 51.72'' N	13° 34' 22.91'' E	Public beach	2014	Summer, Autumn
						2015	Spring, Summer, Autumn, Winter
						2016	Spring, Summer, Autumn, Winter
						2017	Spring, Summer
S4	Fiorenzuola	Adriatic Sea	43° 57' 08.00'' N	12° 49' 51.00'' E	Natural park	2017	Spring, Summer, Autumn
S5	Baia Flaminia	Adriatic Sea	43° 55' 25.60'' N	12° 53' 58.73'' E	Canal harbour	2017	Spring, Summer, Autumn
S6	Tripodi	Adriatic Sea	43° 58' 29.00'' N	12° 54' 51.10'' E	Off-shore (7 NM)	2017	Spring, Summer, Autumn
S7	Vasto	Adriatic Sea	42° 03' 00.00'' N	14° 55' 12.00'' E	Mussel farm	2016	Summer
S8	Off-shore platform	Adriatic Sea	42° 12' 11.16'' N	14° 58' 14.88'' E	Off-shore (10 NM)	2016	Summer
S9	La Spezia	Tyrrhenian Sea	44° 01' 55.36'' N	09° 52' 38.82'' E	Mussel farm	2016	Winter
S10	Gombo	Tyrrhenian Sea	43° 43' 29.19'' N	10° 14' 39.91'' E	Natural Park	2016	Winter
S11	Fiume Morto	Tyrrhenian Sea	43° 44' 43.62'' N	10° 12' 18.06'' E	Natural Park	2016	Winter
S12	Livorno	Tyrrhenian Sea	43° 32' 24.39'' N	10° 17' 51.97'' E	Harbour	2016	Winter
						2017	Autumn
S13	Forte dei Marmi	Tyrrhenian Sea	43° 57' 22.07'' N	10° 09' 01.33'' E	Public beach	2017	Spring, Summer
S14	Lido di Camaiore	Tyrrhenian Sea	43° 53' 48.53'' N	10° 12' 58.05'' E	Public beach	2017	Spring, Summer

Table SM2: Main chromatographic conditions.

Method	Coditions	Mobile phase	%	Detection	N° Channels	Duration (min)
Method 1	Isocratic	Ultra-pure water	26	DAD / FLD	6 + 3	20
		Acetonitrile	42			
		Buffer 1*	32			
Method 2	Gradient	Ultra-pure water	35 to 0	DAD / FLD	6 + 3	27
		Acetonitrile	30 to 65			
		Buffer 1*	35 to 35			
Method 3	Isocratic	Methanol	12.5	DAD	3	6
		Buffer 2**	87.5			

Table SM3: Detection parameters, including the retention times (RTs ± RDS), wavelength of both Main and Qualifier (2) signals, relative responsiveness of qualifier (±RDS), LOD and LOQ (±RDS and CV%), minimum, median and maximum obtained values in the present application.

Compound		ACETAMINOPHEN	CARBAMAZEPINE	DICLOFENAC	IBUPROFEN	KETOPROFEN	LORMETAZEPAM	NIMESULIDE	PAROXETINE	VALSARTAN
ID		AMP	CBZ	DIC	IBU	KET	LOR	NIM	PAR	VAL
Method*		M3	M1	M1	M2	M1	M2	M2	M2	M1
Retention time	(min)	4.28 ± 0.10	2.70 ± 0.06	12.70 ± 0.32	18.50 ± 0.45	5.30 ± 0.12	12.10 ± 0.30	16.10 ± 0.40	5.32 ± 0.12	6.70 ± 0.16
Main signal	(nm)	DAD: 248	DAD: 286	DAD: 276	FLD: Ec 296, Em 338	DAD: 250	DAD: 232	DAD: 298	FLD: Ec 296, Em 338	FLD: Ec 250, Em 380
Qualifier 1	(nm)	268	294	266	Ec: 296, Em: 326	266	236	274	Ec: 296, Em: 326	Ec: 250, Em: 360
Qualifier 1	Relative sig. (%)	37 ± 24%	88 ± 2.4%	86 ± 27%	54 ± 9.7%	85 ± 3.8%	93 ± 16%	64 ± 4.4%	73 ± 31%	75 ± 6.8%
Qualifier 2	(nm)	228	276	294	Ec: 296, Em: 350	276	200	348	Ec: 296, Em: 350	Ec: 250, Em: 410
Qualifier 2	Relative sig. (%)	80 ± 2.4%	90 ± 8.8%	71 ± 9.8%	65 ± 2.2%	53 ± 2.7%	115 ± 31%	72 ± 4.4%	90 ± 25%	64 ± 7.5%
LOD (N=10)	(ng)	0.60 ± 0.12	0.62 ± 0.12	0.82 ± 0.16	4.80 ± 0.96	0.51 ± 0.10	0.26 ± 0.05	1.26 ± 0.25	0.57 ± 0.11	0.29 ± 0.05
LOD (N=10)	(ng/mL)	0.30 ± 0.06	0.31 ± 0.06	0.41 ± 0.08	2.40 ± 0.48	0.25 ± 0.05	0.13 ± 0.02	0.63 ± 0.12	0.28 ± 0.05	0.14 ± 0.02
LOQ (N=10)	(ng/g d.w.)	1.00 ± 0.20	1.03 ± 0.20	1.37 ± 0.27	8.00 ± 1.60	0.86 ± 0.17	0.43 ± 0.08	2.10 ± 0.42	0.95 ± 0.19	0.49 ± 0.09
LOQ (N=10)	CV%	20.0%	19.4%	19.7%	20.0%	19.8%	18.6%	20.0%	20.0%	18.4%
MIN value	(ng/g d.w.)	< 1	< 1.03	< 1.37	< 8	< 0.86	< 0.43	< 2.1	< 0.95	< 0.49
MEDIAN value	(ng/g d.w.)	< 1	111	< 1.37	< 8	< 0.86	< 0.43	< 2.1	< 0.95	< 0.49
MAX value	(ng/g d.w.)	< 1	821	443	105	< 0.86	895	301	81	10

**See the Table SM2 for method specifications*

Table SM4: Reproducibility and recovery of results based on 5 concentrations levels (for each level, N=5).

			ACETAMINOPHEN	CARBAMAZEPINE	DICLOFENAC	IBUPROFEN	KETOPROFEN	LORMETAZEPAM	NIMESULIDE	PAROXETINE	VALSARTAN
Concentration Level 1	Nominal	(ng/g d.w.)	5.28	4.92	6.60	31.44	4.48	2.44	8.44	4.32	2.09
	Measured	(ng/g d.w.)	5.01 ± 0.13	4.68 ± 0.10	6.32 ± 0.07	29.88 ± 0.56	4.28 ± 0.11	2.34 ± 0.03	8.03 ± 0.21	4.17 ± 0.10	1.99 ± 0.04
	Measured	CV%	2.64%	2.32%	1.25%	1.89%	2.66%	1.69%	2.62%	2.51%	2.46%
	Recovery	%	95.0 ± 2.5%	95.2 ± 2.2%	95.8 ± 1.1%	95.0 ± 1.7%	95.5 ± 2.5%	95.9 ± 1.6%	95.1 ± 2.4%	96.5 ± 2.4%	95.2 ± 2.3%
Concentration Level 2	Nominal	(ng/g d.w.)	6.60	6.15	8.25	39.30	5.60	3.05	10.55	5.40	2.62
	Measured	(ng/g d.w.)	6.32 ± 0.09	5.85 ± 0.09	7.88 ± 0.10	37.50 ± 0.76	5.34 ± 0.11	2.92 ± 0.05	10.07 ± 0.19	5.15 ± 0.10	2.50 ± 0.04
	Measured	CV%	1.54%	1.71%	1.30%	2.05%	2.06%	2.04%	1.94%	2.02%	1.98%
	Recovery	%	95.9 ± 1.4%	95.1 ± 1.6%	95.5 ± 1.2%	95.4 ± 1.9%	95.4 ± 1.9%	96.0 ± 1.9%	95.4 ± 1.8%	95.4 ± 1.9%	95.6 ± 1.8%
Concentration Level 3	Nominal	(ng/g d.w.)	7.92	7.38	9.90	47.16	6.72	3.66	12.66	6.48	3.14
	Measured	(ng/g d.w.)	7.60 ± 0.13	7.02 ± 0.11	9.54 ± 0.19	45.36 ± 0.66	6.45 ± 0.12	3.49 ± 0.06	12.06 ± 0.19	6.18 ± 0.08	3.00 ± 0.04
	Measured	CV%	1.78%	1.61%	2.04%	1.46%	1.99%	1.91%	1.63%	1.45%	1.58%
	Recovery	%	96.0 ± 1.7%	95.1 ± 1.5%	96.4 ± 1.9%	96.1 ± 1.4%	95.9 ± 1.9%	95.4 ± 1.8%	95.3 ± 1.5%	95.3 ± 1.3%	95.8 ± 1.5%
Concentration Level 4	Nominal	(ng/g d.w.)	9.24	8.61	11.55	55.02	7.84	4.27	14.77	7.56	3.66
	Measured	(ng/g d.w.)	8.88 ± 0.16	8.26 ± 0.14	11.12 ± 0.18	53.03 ± 0.98	7.61 ± 0.06	4.14 ± 0.05	14.13 ± 0.19	7.23 ± 0.11	3.48 ± 0.04
	Measured	CV%	1.84%	1.73%	1.70%	1.86%	0.91%	1.29%	1.36%	1.59%	1.33%
	Recovery	%	96.1 ± 1.7%	95.9 ± 1.6%	96.3 ± 1.6%	96.3 ± 1.7%	97.1 ± 0.8%	97.0 ± 1.2%	95.6 ± 1.2%	95.7 ± 1.5%	95.2 ± 1.2%
Concentration Level 5	Nominal	(ng/g d.w.)	10.56	9.84	13.20	62.88	8.96	4.88	16.88	8.64	4.18
	Measured	(ng/g d.w.)	10.16 ± 0.18	9.45 ± 0.14	12.72 ± 0.18	60.52 ± 0.96	8.59 ± 0.12	4.68 ± 0.08	16.09 ± 0.25	8.29 ± 0.11	4.00 ± 0.05
	Measured	CV%	1.81%	1.52%	1.43%	1.59%	1.46%	1.86%	1.60%	1.43%	1.34%
	Recovery	%	96.2 ± 1.7%	96.0 ± 1.4%	96.3 ± 1.3%	96.2 ± 1.5%	95.9 ± 1.3%	96.0 ± 1.7%	95.3 ± 1.5%	95.9 ± 1.3%	95.6 ± 1.2%
	R^2		0.9942	0.9951	0.9956	0.9950	0.9947	0.9943	0.9950	0.9950	0.9955

Table SM5: Average fresh and dry weight of mussel tissues measured at various locations in different sampling periods.

Site	Period	Year	Average Fresh weight (g)	Average Dry weight (g)	Average Ratio	CV%
S1	Summer	2014	3.10 ± 0.03	0.64 ± 0.07	4.57 ± 0.57	11.7%
	Autumn	2014	3.00 ± 0.26	0.63 ± 0.05	5.09 ± 0.48	10.0%
	Spring	2015	3.05 ± 0.22	0.71 ± 0.05	4.35 ± 0.60	14.2%
	Summer	2015	3.20 ± 0.06	0.62 ± 0.04	5.16 ± 0.55	11.4%
	Autumn	2015	3.10 ± 0.15	0.66 ± 0.08	4.71 ± 0.58	12.1%
	Winter	2015	3.15 ± 0.12	0.58 ± 0.02	4.49 ± 0.43	10.0%
	Spring	2016	3.28 ± 0.05	0.68 ± 0.07	4.82 ± 0.50	12.4%
	Summer	2016	3.09 ± 0.09	0.70 ± 0.01	4.41 ± 0.59	12.9%
	Autumn	2016	3.20 ± 0.13	0.65 ± 0.01	4.92 ± 0.75	15.0%
	Winter	2016	3.40 ± 0.11	0.70 ± 0.03	4.86 ± 0.83	17.3%
	Spring	2017	3.60 ± 0.05	0.73 ± 0.07	4.93 ± 0.77	15.8%
	Summer	2017	3.24 ± 0.18	0.63 ± 0.02	5.14 ± 0.69	13.0%
	Autumn	2017	3.08 ± 0.21	0.70 ± 0.05	4.40 ± 0.56	12.8%
	Winter	2017	3.21 ± 0.25	0.62 ± 0.05	5.18 ± 0.78	15.1%
S2	Summer	2014	3.09 ± 0.13	0.65 ± 0.02	4.75 ± 0.74	15.9%
	Autumn	2014	3.28 ± 0.08	0.70 ± 0.05	4.68 ± 0.77	16.2%
	Spring	2015	2.90 ± 0.13	0.59 ± 0.06	4.91 ± 0.62	12.8%
	Summer	2015	3.10 ± 0.05	0.61 ± 0.03	5.08 ± 0.75	14.7%
	Autumn	2015	3.02 ± 0.23	0.64 ± 0.06	4.72 ± 0.50	10.8%
	Winter	2015	3.20 ± 0.14	0.66 ± 0.02	4.84 ± 0.62	12.6%
	Spring	2016	3.12 ± 0.07	0.70 ± 0.01	4.45 ± 0.54	12.6%
	Summer	2016	3.40 ± 0.18	0.69 ± 0.08	4.92 ± 0.94	18.9%
	Autumn	2016	3.17 ± 0.10	0.71 ± 0.09	4.46 ± 0.76	17.2%
	Winter	2016	3.09 ± 0.12	0.63 ± 0.05	4.90 ± 0.56	11.9%
	Spring	2017	3.3 ± 0.09	0.66 ± 0.03	5.02 ± 0.65	12.8%
	Summer	2017	3.18 ± 0.05	0.61 ± 0.04	5.21 ± 0.73	13.9%
	Autumn	2017	3.24 ± 0.16	0.76 ± 0.06	4.26 ± 0.64	14.7%
	Winter	2017	3.14 ± 0.23	0.66 ± 0.07	4.75 ± 0.80	17.2%
S3	Summer	2014	3.18 ± 0.07	0.70 ± 0.02	4.54 ± 0.68	14.6%
	Autumn	2014	3.24 ± 0.03	0.63 ± 0.02	5.14 ± 0.52	10.2%
	Spring	2015	3.05 ± 0.08	0.69 ± 0.06	4.42 ± 0.58	12.9%
	Summer	2015	3.12 ± 0.03	0.60 ± 0.02	5.22 ± 0.63	11.7%
	Autumn	2015	2.5 ± 0.11	0.48 ± 0.01	5.20 ± 0.73	13.7%
	Winter	2015	3.52 ± 0.05	0.70 ± 0.05	5.02 ± 0.67	12.9%
	Spring	2016	3.27 ± 0.06	0.64 ± 0.07	5.10 ± 0.52	10.0%
	Summer	2016	3.43 ± 0.11	0.71 ± 0.03	4.83 ± 0.63	12.6%
	Autumn	2016	3.21 ± 0.09	0.68 ± 0.04	4.72 ± 0.52	10.8%
	Winter	2016	3.08 ± 0.17	0.60 ± 0.08	5.13 ± 0.79	15.2%
	Spring	2017	3.14 ± 0.04	0.65 ± 0.03	4.83 ± 0.55	11.2%
	Summer	2017	3.22 ± 0.16	0.71 ± 0.08	4.53 ± 0.81	17.5%
	Autumn	2017	3.14 ± 0.09	0.68 ± 0.06	4.98 ± 0.68	13.0%
	Winter	2017	3.22 ± 0.05	0.67 ± 0.06	4.80 ± 0.62	12.7%
S4	Spring	2017	3.13 ± 0.10	0.60 ± 0.03	5.21 ± 0.73	13.8%
	Summer	2017	3.36 ± 0.12	0.65 ± 0.07	5.17 ± 0.69	13.0%
	Autumn	2017	3.06 ± 0.08	0.72 ± 0.06	4.25 ± 0.52	12.5%
S5	Spring	2017	3.41 ± 0.14	0.80 ± 0.05	4.26 ± 0.69	16.0%
	Summer	2017	3.25 ± 0.05	0.74 ± 0.06	4.39 ± 0.53	11.9%
	Autumn	2017	3.11 ± 0.12	0.60 ± 0.04	5.18 ± 0.73	14.2%
S6	Spring	2017	3.03 ± 0.08	0.71 ± 0.03	4.26 ± 0.65	15.4%
	Summer	2017	3.51 ± 0.15	0.82 ± 0.08	4.28 ± 0.48	11.5%
	Autumn	2017	3.29 ± 0.06	0.75 ± 0.07	4.38 ± 0.66	14.6%
S7	Summer	2016	3.34 ± 0.18	0.79 ± 0.03	4.23 ± 0.78	18.2%
S8	Summer	2016	3.09 ± 0.09	0.68 ± 0.01	4.54 ± 0.76	13.2%
S9	Winter	2016	3.22 ± 0.14	0.75 ± 0.06	4.30 ± 0.71	16.2%
S10	Winter	2016	3.15 ± 0.17	0.67 ± 0.04	4.68 ± 0.78	16.5%
S11	Winter	2016	3.42 ± 0.09	0.70 ± 0.07	4.89 ± 0.74	14.8%
S12	Winter	2016	3.07 ± 0.13	0.64 ± 0.05	4.80 ± 0.48	10.3%
	Autumn	2017	2.89 ± 0.08	0.78 ± 0.06	4.30 ± 0.69	16.6%
S13	Spring	2017	3.35 ± 0.14	0.76 ± 0.08	4.41 ± 0.78	17.3%
	Summer	2017	3.29 ± 0.18	0.66 ± 0.03	4.95 ± 0.64	12.5%
S14	Spring	2017	3.41 ± 0.04	0.62 ± 0.01	5.53 ± 0.67	12.0%
	Summer	2017	3.03 ± 0.16	0.58 ± 0.07	5.26 ± 0.94	18.2%

Table SM6: Results of two-way analysis of variance for concentrations of pharmaceuticals in the whole tissues of M. galloprovincialis collected in Senigallia (S1), Torrette (S2) and Portonovo (S3) from 2014 to 2017. DIC: diclofenac, ∑ NSAIDs: Total Non-Steroidal Anti-Inflammatory Drugs (sum of DIC, IBU and NIM), CBZ: carbamazepine, PAR: paroxetine, LOR: lormetazepam, ∑PSY: Total Psychiatric Drugs (sum of CBZ, PAR and LOR), VAL: valsartan, dF, degree of freedom; F, F test; P, probability level; n.s. not significant, n.t. not tested.

VARIABLE	SITE	MONTH			YEAR			MONTHxYEAR		
		dF	F	p	dF	F	p	dF	F	p
Dic	S1	7	30.46983	p < 0.001	3	15.6858	p < 0.001	2	16.71058	p < 0.001
	S2	8	2.176708	p < 0.05	3	0.178904	n.s.	n.t.	n.t.	n.t.
	S3	8	3.794528	p < 0.01	3	3.445155	p < 0.05	2	3.976906	p < 0.05
∑NSAIDs	S1	7	7.316792	p < 0.001	3	2.86113	p < 0.001	2	7.059261	p < 0.01
	S2	8	4.409396	p < 0.001	3	4.196254	p < 0.05	2	0.728584	n.s.
	S3	8	3.026138	p < 0.01	3	2.421515	p < 0.01	2	2.363707	n.s.
Cbz	S1	5	49.47053	p < 0.001	2	10.30637	p < 0.001	1	0.861587	n.s.
	S2	6	271.3531	p < 0.001	2	578.3507	p < 0.001	1	21.24534	p < 0.001
	S3	6	11.12197	p < 0.001	2	18.74909	p < 0.001	1	15.01065	p < 0.001
Lor	S2	6	4.121298	p < 0.01	2	1.73088	n.s.	n.t.	n.t.	n.t.
Par	S1	5	1.746727	n.s.	2	1.64296	n.s.	n.t.	n.t.	n.t.
	S2	6	1.573198	n.s.	2	1.490586	n.s.	n.t.	n.t.	n.t.
	S3	6	4.076	p < 0.01	2	7.888001	p < 0.01	1	16.77622	p < 0.001
∑PSY	S1	5	46.09712	p < 0.001	2	11.81652	p < 0.001	1	5.89E-05	n.s.
	S2	6	18.48101	p < 0.001	2	26.41206	p < 0.001	1	0.480851	n.s.
	S3	6	5.119507	p < 0.001	2	1.886574	n.s.	n.t.	n.t.	n.t.
Val	S1	5	19.12311	p < 0.001	2	0.754014	n.s.	n.t.	n.t.	n.t.
	S2	6	31.22737	p < 0.001	2	19.27081	p < 0.001	1	0.31066	n.s.
	S3	6	7.626437	p < 0.001	2	24.26529	p < 0.001	1	0.718008	n.s.

Note: Ibuprufen and Nimesulide are not reported since the two-way ANOVA could not be performed given the high variability of obtained results.

Table SM7. Complete analytical data-set obtained in the present study. Concentrations of pharmaceuticals in the whole tissues of M. galloprovincialis collected in Senigallia (S1), Torrette (S2) and Portonovo (S3) from 2014 to 2017. Data are given as mean values ± standard deviations (n=5). NSAIDs: Non Steroidal Anti-Inflammatory Drugs; PSY: psychiatric drugs; AH: antihypertensive drug; DIC: diclofenac; IBU: ibuprofen; NIM: nimesulide; ∑ NSAIDs: Total NSAIDs (sum of DIC, IBU and NIM); CBZ: carbamazepine; PAR: paroxetine; LOR: Lormetazepam; ∑PSY: Total PSY (sum of CBZ, PAR and LOR); VAL: valsartan; n.a. not analyzed. Values are expressed as ng/g dw for all the individual drugs while ∑NSAIDs and ∑PSY are expressed as nmol/g to normalize different molecular weights of different compounds.

Site	Year	Season	Month	NSAIDs				PSY				AH
				DIC	IBU	NIM	∑ NSAIDs	CBZ	PAR	LOR	∑ PSY	VAL
				ng g-1 (d.w.)	ng g-1 (d.w.)	ng g-1 (d.w.)	nmol g-1 (d.w.)	ng g-1 (d.w.)	ng g-1 (d.w.)	ng g-1 (d.w.)	nmol g-1 (d.w.)	ng g-1 (d.w.)
S1	2014	Summer	Jul	<1.4	<8.0	2.3 ± 2.2	0.01 ± 0.02	n.a.	n.a.	n.a.	n.a.	n.a.
			Aug	1.4 ± 0.8	<8.0	2.2 ± 1.0	0.03 ± 0.00	n.a.	n.a.	n.a.	n.a.	n.a.
		Autumn	Sep	27.8 ± 46.9	<8.0	<2.00	0.09 ± 0.16	n.a.	n.a.	n.a.	n.a.	n.a.
	2015	Spring	Apr	29.6 ± 10.2	143.7 ± 242	<2.00	0.12 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.
		Summer	Jul	103.8 ± 27.9	<8.0	<2.00	0.35 ± 0.09	45.5 ± 14.72	16.66 ± 8.8	6.426 ± 8.5	0.26 ± 0.1	1.239 ± 2.0
			Aug	<1.4	<8.0	<2.00	<0.004	151.1 ± 27.87	9.089 ± 4.5	<0.40	0.66 ± 0.1	3.195 ± 1.2
		Autumn	Nov	<1.4	<8.0	<2.00	<0.004	279.6 ± 67.57	4.477 ± 2.5	<0.40	1.2 ± 0.3	5.965 ± 0.9
	2016	Winter	Feb	<1.4	<8.0	80.6 ± 74.9	0.28 ± 0.24	228.2 ± 101.3	5.855 ± 5.5	<0.40	0.982 ± 0.4	5.682 ± 0.6
		Spring	May	99.1 ± 24.0	<8.0	<2.00	0.33 ± 0.08	270.5 ± 107.4	1.645 ± 2.6	<0.40	1.15 ± 0.5	<0.5
		Summer	Jul	2.5 ± 2.5	<8.0	<2.00	0.02 ± 0.02	32.73 ± 8.966	29.99 ± 17.0	<0.40	0.219 ± 0.1	0.938 ± 0.8
		Autumn	Oct	58.4 ± 55.5	14.95 ± 15.91	2.1 ± 2.1	0.26 ± 0.26	140.7 ± 23.94	4.501 ± 9.0	439.5 ± 77.0	2.19 ± 0.7	6.681 ± 1.1
	2017	Spring	May	109.3 ± 50.7	<8.0	<2.00	0.37 ± 0.16	139.4 ± 57.8	5.329 ± 1.1	<0.40	0.605 ± 0.2	1.479 ± 1.4
		Summer	Jul	<1.4	<8.0	<2.00	<0.004	21.9 ± 6.455	3.363 ± 5.4	2.38 ± 3.1	0.109 ± 0.0	0.521 ± 0.4
S2	2014	Summer	Jul	<1.4	<8.0	56.0	0.20	n.a.	n.a.	n.a.	n.a.	n.a.
			Aug	1.7 ± 1.2	13.57 ± 10.6	5.8 ± 6.8	0.09 ± 0.04	n.a.	n.a.	n.a.	n.a.	n.a.
		Autumn	Sep	<1.4	<8.0	<2.00	<0.004	n.a.	n.a.	n.a.	n.a.	n.a.
	2015	Spring	Apr	27.2 ± 16.9	<8.0	<2.00	0.11 ± 0.05	n.a.	n.a.	n.a.	n.a.	n.a.
		Summer	Jul	20.6 ± 27.3	<8.0	<2.00	0.07 ± 0.10	62.9 ± 7.709	2.667 ± 2.1	1.373 ± 1.6	0.277 ± 0.0	1.256 ± 0.8
			Aug	<1.4	<8.0	61.0 ± 134.0	0.22 ± 0.43	34.6 ± 25.79	6.976 ± 5.7	18.94 ± 25.1	0.222 ± 0.2	<0.5
		Autumn	Nov	<1.4	<8.0	<2.00	<0.004	299.7 ± 46.23	1.122 ± 1.1	<0.40	1.272 ± 0.2	5.431 ± 0.4
	2016	Winter	Feb	<1.4	<8.0	<2.00	<0.004	181.8 ± 41.26	4.142 ± 3.6	<0.40	0.781 ± 0.2	<0.5
		Spring	May	36.4 ± 35.1	<8.0	3.7 ± 4.0	0.14 ± 0.11	<1.0	<1.00	104.8 ± 105.9	0.314 ± 0.3	1.318 ± 1.1
		Summer	Jul	14.0 ± 29.9	28.42 ± 19.7	<2.00	0.19 ± 0.07	<1.0	4.002 ± 5.3	<0.40	0.011 ± 0.0	1.802 ± 1.5
		Autumn	Oct	12.7 ± 26.9	9.424 ± 12.13	<2.00	0.09 ± 0.09	201.3 ± 35.15	3.976 ± 7.8	42.81 ± 24.7	0.99 ± 0.2	3.384 ± 0.8
		Winter	Dec	<1.4	27.86 ± 32.9	7.1 ± 10.8	0.16 ± 0.16	181.8 ± 84.92	2.119 ± 3.7	235.2 ± 218.4	1.476 ± 0.8	<0.5
	2017	Spring	May	6.5 ± 12.9	<8.0	<2.00	0.04 ± 0.04	216.8 ± 78.88	6.248 ± 4.0	2.159 ± 4.3	0.941 ± 0.3	<0.5
		Summer	Jul	84.3 ± 82.1	<8.0	<2.00	0.29 ± 0.26	52.27 ± 31.37	1.049 ± 1.3	<0.40	0.225 ± 0.1	0.364 ± 0.3
S3	2014	Summer	Jul	<1.4	<8.0	6.7 ± 9.9	0.04 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.
			Aug	16.1 ± 14.7	9.398 ± 0.59	4.2 ± 2.5	0.11 ± 0.06	n.a.	n.a.	n.a.	n.a.	n.a.
		Autumn	Sep	<1.4	<8.0	6.1 ± 4.4	0.04 ± 0.01	n.a.	n.a.	n.a.	n.a.	n.a.
	2015	Spring	Apr	5.4 ± 8.1	<8.0	3.8 ± 2.6	0.05 ± 0.03	n.a.	n.a.	n.a.	n.a.	n.a.
		Summer	Jul	171.1 ± 233.6	<8.0	<2.00	0.56 ± 0.73	76.09 ± 30.15	11.16 ± 7.8	2.181 ± 4.4	0.358 ± 0.1	1.771 ± 1.6
			Aug	52.1 ± 37.5	<8.0	<2.00	0.19 ± 0.12	74.45 ± 24.02	2.26 ± 2.5	<0.40	0.322 ± 0.1	<0.5
		Autumn	Nov	<1.4	<8.0	13.6 ± 28.1	0.07 ± 0.09	144.7 ± 30.55	4.959 ± 1.7	<0.40	0.626 ± 0.1	<0.5
	2016	Winter	Feb	<1.4	<8.0	<2.00	<0.004	240.2 ± 39.07	4.681 ± 4.2	<0.40	1.03 ± 0.2	3.966 ± 2.2
		Spring	May	53.6 ± 31.4	<8.0	4.6 ± 7.9	0.20 ± 0.11	<1.0	6.739 ± 14.0	288 ± 130.7	0.879 ± 0.4	1.774 ± 0.7
		Summer	Jul	31.9 ± 32.9	<8.0	3.4 ± 3.8	0.13 ± 0.10	51.21 ± 46.36	<1.00	62.1 ± 138.4	0.403 ± 0.5	3.572 ± 0.6
		Autumn	Oct	34.8 ± 48.2	<8.0	<2.00	0.13 ± 0.15	181.6 ± 108.5	<1.00	207.6 ± 140.4	1.39 ± 0.6	4.588 ± 1.4
		Winter	Dec	10.5 ± 22.0	40.92 ± 50.95	<2.00	0.23 ± 0.23	147.8 ± 35.35	18.44 ± 21.2	137.5 ± 157.3	1.085 ± 0.4	0.506 ± 0.2
	2017	Spring	May	16.8 ± 36.1	<8.0	<2.00	0.08 ± 0.11	128.7 ± 54.98	1.581 ± 1.6	<0.40	0.55 ± 0.2	<0.5
		Summer	Jul	82.0 ± 27.2	<8.0	<2.00	0.28 ± 0.09	35.18 ± 14.23	49.94 ± 22.2	<0.40	0.283 ± 0.1	<0.5
S4	2017	Spring	May	162.6 ± 115.3	<8.0	<2.00	0.53 ± 0.36	199.5 ± 117.1	<1.00	7.134 ± 10.1	0.866 ± 0.5	0.563 ± 0.2
		Summer	Jul	<1.4	<8.0	2.2 ± 2.5	0.03 ± 0.01	212.1 ± 130.4	1.566 ± 2.4	104.3 ± 27.5	1.213 ± 0.5	0.505 ± 0.6
		Autumn	Sep	<1.4	<8.0	<2.00	<0.004	15.33 ± 6.353	<1.00	98.95 ± 68.1	0.361 ± 0.2	0.392 ± 0.2
S5	2017	Spring	May	231.1 ± 67.2	19.51 ± 34.67	3.0 ± 4.3	0.83 ± 0.22	283.2 ± 70.14	<1.00	50.97 ± 16.5	1.352 ± 0.3	0.519 ± 0.4
		Summer	Jul	<1.4	<8.0	<2.00	<0.004	190.2 ± 79.1	2.027 ± 2.5	<0.40	0.811 ± 0.3	0.725 ± 0.7
		Autumn	Sep	<1.4	<8.0	2.3 ± 2.7	0.01 ± 0.02	30.18 ± 18.35	1.626 ± 2.6	89.28 ± 40.0	0.398 ± 0.1	0.573 ± 0.6
S6	2017	Spring	May	<1.4	<8.0	<2.00	<0.004	197.8 ± 35.82	1.041 ± 1.1	60.19 ± 43.3	1.019 ± 0.2	2.113 ± 0.9
		Summer	Jul	<1.4	<8.0	<2.00	<0.004	128.8 ± 55.88	4.691 ± 5.8	96.12 ± 34.4	0.845 ± 0.2	<0.5
		Autumn	Sep	<1.4	<8.0	2.0 ± 1.7	0.01 ± 0.01	34.7 ± 30.31	<1.00	20.52 ± 9.2	0.209 ± 0.2	0.395 ± 0.3
S7	2016	Summer	Jul	52.9 ± 71.5	<8.0	2.1 ± 2.1	0.18 ± 0.24	73.58 ± 76.26	4.516 ± 9.0	165.5 ± 70.6	0.817 ± 0.5	5.619 ± 3.7
S8	2016	Summer	Jul	75.4 ± 57.9	<8.0	<2.00	0.26 ± 0.18	157 ± 105.8	4.97 ± 8.3	39.9 ± 36.6	0.797 ± 0.4	2.83 ± 0.9
S9	2016	Winter	Dec	<1.4	22.99 ± 26.01	5.5 ± 2.9	0.13 ± 0.13	76.61 ± 59.85	<1.00	<0.40	0.326 ± 0.3	1.967 ± 3.8
S10	2016	Winter	Dec	6.9 ± 13.8	12.28 ± 18.52	4.0 ± 6.6	0.09 ± 0.09	47.43 ± 17	<1.00	<0.40	0.203 ± 0.1	1.029 ± 0.5
S11	2016	Winter	Dec	<1.4	14 ± 22.37	9.4 ± 8.1	0.09 ± 0.13	46.37 ± 21.82	2.784 ± 5.2	<0.40	0.204 ± 0.1	<0.5
S12	2016	Winter	Dec	<1.4	17.45 ± 12.52	<2.00	0.08 ± 0.07	123.7 ± 26.1	<1.00	<0.40	0.526 ± 0.1	<0.5
	2017	Autumn	Sep	<1.4	<8.0	<2.00	<0.004	147.2 ± 37.74	5.275 ± 1.9	12.6 ± 9.3	0.674 ± 0.2	3.496 ± 0.7
S13	2017	Spring	Mar	280.1 ± 161.8	<8.0	<2.00	0.90 ± 0.51	563.3 ± 196.2	11.79 ± 8.7	<0.40	2.416 ± 0.8	1.067 ± 0.6
		Summer	Aug	<1.4	<8.0	<2.00	<0.004	53.37 ± 40.03	4.82 ± 2.7	24.83 ± 24.2	0.313 ± 0.2	0.557 ± 0.2
S14	2017	Spring	Mar	<1.4	<8.0	<2.00	<0.004	309.2 ± 208.1	1.413 ± 2.1	205 ± 48.5	1.922 ± 1.0	<0.5
		Summer	Aug	<1.4	<8.0	<2.00	<0.004	39.84 ± 18.22	6.719 ± 4.8	8.622 ± 6.3	0.212 ± 0.1	0.438 ± 0.3

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