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Preliminary measurements of formaldehyde in seawater and edible tissues of farmed gilthead seabream after formalin immersion

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Abstract

This study was performed to measure the possible persistence of formaldehyde, the active substance of formalin solution, in seawater and edible gilthead seabream (*Sparus aurata*) tissues after a formalin bath. The trial was carried out during the summer period as parasitic infestations are of a high frequency. Water samples were taken within or at a short distance from the treatment cages, during and following formalin immersion. Fish fillets were also sampled at the same sampling points. Chromatographic analysis of water and tissue samples was performed with a photodiode array detector (HPLC-PDA). Measured formaldehyde concentrations in gilthead seabream fillets clearly showed no increase due to the immersion or advent of time. Formaldehyde measurements in seawater revealed that after formalin treatments negligible amounts of the substance remain in the aquatic environment and these values seem to be relatively unaffected by depth in the vicinity of the fish cages. Further research is required to investigate the formalin degradation cycle under Mediterranean seawater conditions, including more farm sites and temperature ranges.

Keywords: Formaldehyde residue; formalin bath; gilthead seabream; seawater; edible tissues.

Introduction

The intensive use of sea cages in marine aquaculture has undoubtedly been coupled with the accelerated growth of farmed fish production in the Mediterranean region. The use of fish cages allows larger biomasses of farmed fish, but at the same time, high fish density favours the possible spread of diseases. Poor hygiene combined with adverse environmental conditions triggers the emergence of parasitic diseases that can prove detrimental to farmed fish. Indicatively, the parasitism of gilthead seabream (*Sparus aurata*), one of the most commercialized finfish marine species in the Mediterranean Sea (Stavrakidis-Zachou *et al.,* 2021), by the monogenean parasite *Sparicotyle chrysophrii*, currently represents the most serious and unsolved production concern (Sitjà-Bobadilla *et al.,* 2006; Aslam *et al.,* 2020; Muniesa *et al.,* 2020).

Formalin baths are arguably one of the most common antiparasitic treatments worldwide (Boyd & McNevin, 2015). In several European countries, including Greece, the Aquacen formulation (Cenavisa, Spain) is licensed for use in aquaculture, in concentrations of 100-250 mg L^{-1} and immersion duration of 0.5-1 h. The formalin solution contains mainly 37.5-40% formaldehyde and 12-13.5% methanol, with the first component inducing anti-parasitic properties. Although immersions in licensed formalin solutions have been regularly carried out, for years, consumer concerns have been expressed about the presence of residues in the final products and their possible persistence in the marine environment. Information regarding the environmental toxicity of formaldehyde is relatively limited, although formaldehyde seems to degrade rapidly once it comes into contact with the aquatic environment through various reactions (Guimarães *et al.,* 2012) and no bioaccumulation occurs (bioaccumulation factor, BCF <1) in living organisms (Leal *et al.,* 2018; USA EPA, 2019).

Despite the fact that there is a great number of published studies on the short-term toxicity and other rele-

vant aspects of formaldehyde (reviewed by Leal *et al.,* 2018), measurements of the compound in the vicinity of fish cages and in bathed fish are virtually inexistent or limited in the pertinent literature (Cho &Yang, 1996; Jung *et al.,* 2001; USA EPA, 2017). Thus, the purpose of this study was to evaluate its potential residues in the aquatic environment and fish fillets, resulting from the therapeutic usage of formalin in cultured marine finfish. These objectives were achieved by measuring the concentrations of formaldehyde in seawater samples and edible gilthead seabream tissues, through selected sampling at high temperatures at Greek fish farms.

Materials and Methods

Sampling

Sampling of water and fish tissue during and after formalin immersion, was carried out at a marine fish farm unit in Central Greece during summer (26^{o}C). The choice of the particular fish farm was based on the fact that infestations with *S. chrysophrii* in gilthead seabream are often recorded in the area, especially during periods of warm water temperatures and, consequently, the fish are subjected to repeated formalin immersions. Formalin immersion was carried out during morning hours (10 a.m.), following the usual therapeutic protocol (150 mg L^{-1} for 1 h) (Fig. 1) in a common 80 m diameter cage.

The commercial preparation (Aquacen) was supplied by AQUAVET SA. The water sampling points selected during and after completion of the treatment are described in Table 1. Water samples from 7.5 and 15 m depths were taken by a diver 30 or 50 m away from the treated cage and towards the direction of the dominating sea currents. Also, water samplings were performed 45 days following completion of formalin therapies at three selected sites, namely, inside the treated cage, 30 m, and 50 m away from the cage. The average weight (A.W.) of the gilthead seabreams was 70 g and they were collected from all sampling points for analysis of formaldehyde residues in their edible tissues (fillet) during the trial. Additional water samples (control points) were taken at a distance of 1 km from the fish cages as no formaldehyde residues originating from veterinary purposes or other anthropogenic activities were expected. All the water (50 mL tubes) and fillet samples were stored at 4^{o}C and sent for analysis to the Laboratory of Analytical Chemistry of the National and Kapodistrian University of Athens (NKUA). Sampling and analysis were performed in triplicate.

Reagents

All standards and reagents used were of high purity (> 95%). Acetonitrile (ACN) and methanol were of HPLC-grade (MeOH) and acquired from Fisher Scientific (Geel, Belgium). Phosphoric acid (H_3PO_4) and 2,4-Dinitrophenylhydrazine (2,4-DNPH) were purchased from Sigma Aldrich (Stenheim, Germany), while formaldehyde was provided by lach:ner (Neratovice, Czechia). Ultrapure water was provided by Milli-Q purification apparatus (Millipore Direct-Q UV, Bedford, MA, USA). Regenerated cellulose syringe filters (RC filters, pore size 0.2 μm, diameter 15 mm) and paper filters (No 4) were obtained from Macherey-Nagel (Dóren, Germany). A stock standard solution of formaldehyde was prepared in ultrapure water at a concentration of 1000 mg L^{-1} and stored at -18^{o}C in a 10 mL amber-coloured glass vial. Working solutions of formaldehyde at various concentrations ranging from 0.02 to 10 mg L^{-1} were prepared, following the derivatization process.

Recrystallization of 2,4-DNPH was required prior to each use as follows: 10 g of 2,4-DNPH were dissolved in 100 mL of warm acetonitrile to form a saturated solution. The solution was allowed to cool at room temperature, transferred to dark glass vials and stored at 4^{o}C for 16 h to complete the formation of crystals. The crystals were then collected by vacuum filtration. 2,4- DNPH was used as derivatization solution for both fish and water samples, differing only as regards the dissolving solvent.

To prepare the derivatization solution for the fish samples, 0.150 g of 2,4-DNPH were dissolved with acetonitrile in a 100-mL volumetric flask, 500 μL of concentrated H_3PO_4 were added, and diluted to the mark with acetonitrile (derivatization solution A). To prepare the

Fig. 1: Formalin application in the marine fish farm unit.

Table 1. Description of water sampling points and formaldehyde concentrations in sea bream fillets and water samples after formalin immersion at summer temperatures (n=3).

		SUMMER	
Sampling point	**Site**	**Fillet (mg kg^{-1})**	**Water (mg L^{-1})**
Formalin tank			39.3%±2.3
Prior to bath	Surface	4.2±0.9	<LOD*
	3 m depth in the cage		
30 min after the initiation of treatment	Surface	4.1±0.4	5.9±0.1
	3 m depth in the cage		5.8±0.6
30 min after removal of the tarpaulin bag	surface	4.8±0.5	0.8±0.1
	3 m depth in the cage		0.6±0.1
	8 m depth in the cage		0.7±0.2
4 h after bath	7.5 m depth	5.7±0.5	<LOD
	15 m depth		
	30 m away from the treated cage		
8 h after bath	7.5 m depth	4.8±0.7	<LOD
	15 m depth		
	50 m away from the treated cage		
24 h after bath	7.5 m depth	6.37±2.2	<LOD
	15 m depth		
	50 m away from the treated cage		
Control (1 km)	1 km away from the fish cages		<LOD

*LOD: Limit of detection <0.0066 (mg L^{-1})

derivatization solution for the water samples, 0.150 g of 2,4-DNPH crystals were weighed in a 100-mL volumetric flask, and dissolved with the addition of a mixture of ACN: H_2O in a proportion of (50:50, v/v) (derivatization solution B).

Formaldehyde extraction

Residual formaldehyde in the formalin-treated fish muscle

Sample preparation for fish tissue samples was based on the work of Wahed *et al.* (2016). In a 50-mL centrifuge tube, 5 g of homogenized fish tissue samples was weighed, and 5 mL of acetonitrile was added. Then, the samples were vortexed for 1 min and placed in an ultrasonic bath for 30 min at room temperature. Afterwards, the samples were centrifuged at 4000 rpm for 10 min and the obtained supernatants were filtered through paper filters. A volume of 2.5 mL 2,4-DNPH (derivatization solution A) was added to the filtrates, and vortexed for 1 min. The samples were placed in a 40°C water bath for 60 min. Following that, the organic layer was collected and filtered through RC syringe filters. The extracts were transferred to 2-mL autosampler glass vials and injected to HPLC-PDA. The aforementioned procedure was followed for both spiked samples and the procedural blank. In order to test the derivatization efficiency, a series of working solutions ranging from 0.5 to 20 mg L^{-1} were prepared according to the derivatization process described above.

The quantification of formaldehyde was performed using standard addition calibration curve. Thus, a suitable amount of formaldehyde working solution was spiked to 5 g of fish tissue, and the abovementioned process was applied. Four fortification levels (8 mg kg^{-1}, 15 mg kg^{-1}, 30 mg kg^{-1} and 75 mg kg^{-1}) were prepared and analysed. The equation of standard addition calibration curve was y= (7088 ± 253) x - (20736 ± 6251) and its correlation coefficient was R^2=0.997. The method's detection limit (LOD) was 0.92 mg kg^{-1}, and the method's quantification limit (LOQ) was 2.8 mg kg^{-1}. The obtained recoveries ranged from 88-109%, being fit-for-purpose for this work.

Residues in seawater

The preparation of water samples was based on the work of Abe *et al.* (2021). Thus, a volume of 10 mL of water samples was transferred to 50 mL centrifuge tubes, followed by the addition of 0.5 mL of 2,4-DNPH (derivatization solution B) and 0.2 mL of H_3PO_4 (20%, v/v). The mixture was then vortexed for 1 min and allowed to stand at room temperature for 20 min. The extracts were filtered through RC syringe filters, transferred to 2-mL autosampler glass vials, and injected into the liquid chromatography system.

The derivatization procedure was also carried out for the working solutions, using 0.5 mL of 2,4-DNPH (derivatization solution B), 0.2 mL of H_3PO_4 (20%, v/v), and an appropriate amount of formaldehyde solution. The

concentration of the working solutions was 0.02, 0.1, 0.25, 0.5, 0.75, 1, 2, 5, 10 mg L^{-1}, and their final volume was set at 10 mL. The solutions were stirred in a vortex for 1 min and left for 20 min at room temperature. The derivatized working solutions were injected into the liquid chromatography system and the equation of external standard calibration curve was y= (160344 ± 984) x + (5999 ± 3974). Its linear range was extended from 0.02 to 10 mg L^{-1}, and the correlation coefficient was R^2=0.9999, thus demonstrating satisfactory linearity.

The above-described procedure was also followed for the procedural blank, and the fortified samples. For the procedural blank, an equal volume of ultrapure water (10 mL) was used. Regarding the fortified samples, five fortification levels of the samples were analysed, namely, 0.1, 0.5, 1, 5 and 10 mg L^{-1} and % recoveries ranged from 88-95% at all the examined fortification levels. The obtained % recoveries were considered satisfactory. The method's LOD was calculated from the standard deviation of ten blank samples and found to be 6.60 μg L^{-1}. As a result, the method's LOQ was calculated to be 20.0 μg L^{-1}.

Instrumentation and chromatographic method

The determination of formaldehyde in water and fish samples was performed through a liquid chromatography system equipped with a PDA detector (HPLC Shimadzu LC-2030C 3D Plus). A Zorbax Eclipse XDB-C18 column (250*4.6 mm, 5μm) (Agilent Technologies, Santa Clara, USA) was used for the chromatographic analysis. The column temperature was kept constant throughout the chromatographic analysis at 30°C, and the injection volume was set at 20 μL. The mobile phase consisted of H_2O: MeOH (35:65, v/v) and the elution program was isocratic. The wavelength was set at 355 nm. The total chromatographic run was 15 minutes, while the analyte was eluted at 7.2 min. The representative chromatograms of fish and water samples are depicted in Figure 2.

Results

Formaldehyde concentrations in water samples and edible gilthead seabream tissues after formalin immersion at high temperatures are presented in Table 1. The measured concentrations of formaldehyde in fish fillets clearly show that there is no increase due to immersion and that the residue concentration remains unaffected by time. Regarding seawater measurements, it appears that during formalin immersion, the concentration of formaldehyde is lower than expected. The remaining measurements after the immersion, indicate that undetectable amounts of formaldehyde stand in the aquatic environment. Moreover, formaldehyde was not detected when sampling was carried out >45 days post-formalin treatment in the area.

Discussion

Formaldehyde has wide industrial use due to its special characteristics that mainly include a high degree of reactivity, high purity, and low production cost (Neuss & Speit, 2008). However, according to Regulation (EU) No 528/2012, formaldehyde is a hazardous organic compound that may negatively affect public health and the environment. Apart from the prolonged use of formaldehyde as a disinfectant, its wide application as a component of formalin solution in aquaculture medicine, renders the latter, one of the most widely used fish an-

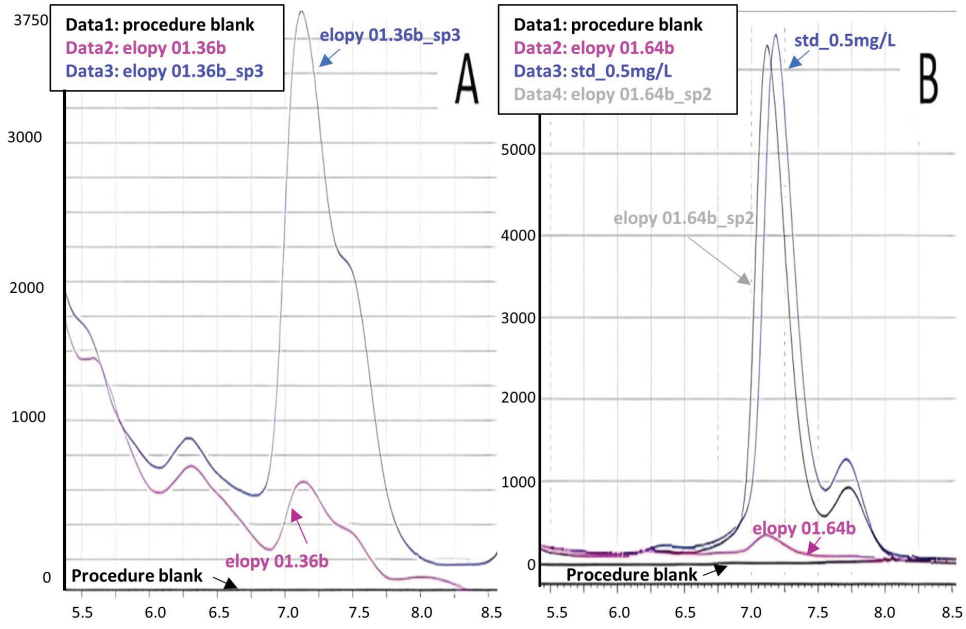

Fig. 2: A. Representative chromatograms of procedural blank (in black), a fish sample (in purple) and a fortified fish sample (30 mg/kg, in blue). B. Representative chromatograms of procedural blank (in black), a standard solution of 0.5 mg L^{-1} (in blue), a water sample (in purple) and a fortified water sample (0.5 mg L^{-1}, in grey).

tiparasitics worldwide (Boyd & McNevin, 2015). Notwithstanding the extensive use of formalin that causes the release of large quantities in the environment, the potential side effects of formaldehyde have not been studied in aquaculture environments. Consequently, formalin baths in aquaculture medicine have raised great concerns and reactions from environmental groups and consumers. The current study is one of the first pilot efforts to evaluate the persistence of formaldehyde in seawater after formalin immersion.

In addition, the use of formalin baths in aquaculture has given rise to strong consumer safety concerns that may be linked to the presence of formaldehyde residues in the final product and its possible carcinogenicity (Liteplo *et al.,* 2002). However, it should be noted that the European Medicines Agency (EMA) has not established a maximum residue limit (MRL) for formaldehyde, perhaps due to the absence of bioaccumulation and rapid degradation (Leal *et al.,* 2018). Therefore, the withdrawal time for the compound in treated products is supposedly zero. Nevertheless, measurement of formaldehyde concentrations in fish fillet was included in our study, although monitoring of formaldehyde residues in the final product of aquaculture is a routine control procedure applied by local fish production companies.

Formaldehyde residues measured in gilthead seabream fillets after completion of immersion were similar to those considered as control values. These results are in agreement with the findings of other studies revealing that the dose and duration of formalin treatment did not affect the accumulation of formaldehyde residues in the fillet of the studied species (Ueno *et al.,* 1984; Xu & Rogers, 1993; Xu & Rogers, 1995). These findings confirm that the formaldehyde levels in fish fillets recorded herein are close to the natural values measured in untreated fish, as the specific substance is a metabolic product of living organisms and is also necessary for the biosynthesis of specific amino acids (Jung *et al.,* 2001).

Similarly, in a published study on formalin-treated halibut (*Paralichthys olivaceus*) (100-300 mg L^{-1} for 1 h), formaldehyde concentrations in the fillets were not greater than those of the control, although residue values were lower (0.8-1.2 mg/kg) (Jung *et al.,* 2001), compared to those measured in this study. The measured formaldehyde values in gilthead seabream fillets are in the range of the concentrations published for food and other productive animals and relatively much lower than those recorded for wild fish species (Table 2). Interestingly, formaldehyde levels have been detected postmortem in the tissues of Pacific cod (*Gadus macrocephalus*), Alaskan cod (*Theragra chalcogramma*), blue shrimp (*Penaeus stylirostris*), and Pacific white shrimp (*Pandalus jordani*) (Amano & Yamada, 1964; Flores & Crawford, 1973; Hose & Lightner, 1980). Moreover, endogenous residues of this substance ranging from 0.1-31.8 mg/kg were measured in various species of teleosts and crustaceans, such as Japanese eel (*Anguilla japonica*) (Ueno *et al.,* 1984), Atlantic seabass (*Morone saxatilis*) (Xu & Rogers, 1993) and banana prawn (*Penaeus merguiensis*) (Yamagata & Low, 1995).

Table 2. Formaldehyde concentrations in various raw food products.

Product	Formaldehyde
	mg kg^{-1}
Meat	5.7-20
Wild fish	6.4-293
Crustacean	1-98
Sugar	0.75
Fruits and vegetables	6-35
	mg L^{-1}
Milk products	0.01-0.80
Coffee	3.4-16
Drinks (alcohol)	0.27-3

(WHO, 1989; EFSA, 2014)

No previous attempts to measure formaldehyde residues in the aquatic environment after formalin bath treatments, have been published in the literature. Notably, preliminary formaldehyde measurements during summer in water samples after treatment (4-24 h) showed small amounts of formaldehyde, which ranged from 0.0164 to 0.0389 mg L^{-1}, at a farming site in a different geographical region (laboratory unpublished data). Nevertheless, formaldehyde concentrations measured after immersion at different distances, depths, and times were not detected herein. This discrepancy indicates that the fate of formaldehyde might depend on the environment subjected to different treatment schedules.

Furthermore, the concentration of formaldehyde was lower than expected during formalin immersion, possibly due to its interaction with natural organic matter (NOM). Meinelt *et al.* (2005) observed a reduction in formalin toxicity using zebrafish (*Danio rerio*) in the presence of NOM, indicating a possible binding of formalin to specific functional groups or structures of NOM. It is well known that NOM binds organic compounds as well as metals to their functional groups and their lipophilic structures (Haitzer *et al.,* 1999; Kopinke *et al.,* 2001).

It is also worth noting that formaldehyde breaks down as soon as it comes into contact with atmospheric oxygen through the process of oxidation to formic acid and with the end products being water and carbon dioxide after the mediation of microorganisms (Kitchens *et al.,* 1976, FDA 1995; Yumura *et al.,* 2002). Formic acid is a natural compound occurring at significant concentrations in aquatic compartments and dissociates into formate anions, which shows a high probability of not being acutely harmful to fish, aquatic algae, and invertebrates (European Chemicals Agency, ECHA, 2022). According to ECHA, formic acid and formate anions have no potential for bioaccumulation in aquatic organisms and not identified as an endocrine disruptor for non-target organisms. A GLP-test (OECD Principles of Good Laboratory Practice) on ma-

rine fish (*Scophthalmus maximus*) showed an extremely high median lethal concentration (LC_{50} =1.700 mg L^{-1}) after 96 hours of formic acid exposure (ECHA, 2022). A high median lethal concentration was also estimated in the brown shrimp *Crangon crangon* (96 h: LC_{50}= 1.308 mg L^{-1}) and in the marine diatom *Skeletonema costatum* (72 h: LC_{50}> 1.000 mg L^{-1}) (ECHA, 2022). Regarding CO_2, one of the end products, its solubility in seawater depends on various physicochemical parameters such as temperature, pressure, salinity, pH, and organic matter concentrations (Teng *et al.,* 1996; Al-Anezi *et al.,* 2008; Dickson, 2011). Hence, no firm conclusion can be drawn about its potential environmental harm and, thus, according to Directive 67/548/EC, it is classified as non-hazardous for the aquatic environment and does not fulfil persistent, bioaccumulative, and toxic (PBT) criteria.

The rate of formaldehyde degradation is mainly influenced by water temperature and the availability of oxygen in the environment (Xu & Rogers, 1995; Jung *et al.,* 2001). In an aquatic environment that contains less oxygen than air, the substance also dissolves rapidly and biodegrades depending on the environmental conditions. For example, for complete degradation of the substance in fresh water under aeration and a temperature of 20°C, 30 h were required, while under anaerobic conditions at 8°C, the necessary degradation period was 3 days (Kamata, 1966). In addition, indirect photodegradation and biodegradation of formaldehyde are important reactions that take place once the substance comes into contact with water (Leal *et al.,* 2018). According to Chinabut *et al.* (1988), formaldehyde concentrations of 25, 50 and 75 mg L^{-1} are completely degraded in 36, 48, and 54 h, respectively, at a temperature of 27-30°C in the presence of oxygen in fresh water. In the same study, in the absence of oxygen, 36, 54, and 60 h were required for complete degradation at the aforementioned concentrations. It should be noted that the majority of formaldehyde studies, either for the evaluation of the degradation time or for toxicity measurement, has been carried out in fresh water environment. An exception is the study by Jung *et al.* (2001), who report that complete degradation of the substance in seawater appears to be slower compared to fresh water. Specifically, 25-200 mg L^{-1} of formaldehyde in seawater tanks in the absence of aeration, took 8-19 days to degrade at 20±1°C, while in the presence of aeration, the degradation was accelerated (6-10 days). Nevertheless, the rate of formaldehyde degradation at temperatures >20°C and specifically at those used for our trials, remains unknown. It would be quite interesting to answer this question, as it has been reported that formaldehyde solutions at high water temperatures degrade at a much faster rate (Xu & Rogers, 1995).

The toxicity of formaldehyde has been evaluated mainly in freshwater organisms and most commonly at short-term levels (reviewed by Leal *et al.,* 2018). The measured concentrations of formaldehyde reported herein appear to be much lower than those published in the literature as toxic (short-term toxicity) to freshwater organisms. Indicatively, the EC_{50} values (half-maximal effective concentration) of formaldehyde were found to be of the order of 19 mg L^{-1}at 3 h for activated sludge (OECD 209), 14.7 mg L^{-1} at 24 h for microalgae (*Scenedesmus quadricauda*) and 5.8 mg L^{-1} at 48 h for crustaceans (*Daphnia pulex*, OECD 202) (Tišler & Zagorc-Končan, 1997). Similar results have been published for marine invertebrates, such as *Pinctada fucata martensii* (one-year-old) in which the 96 h: LC_{50} was found to be 5.3 mg L^{-1}at 25° C (Takayanagi, 2000).

Although formaldehyde after formalin immersions is discharged into the wider marine environment, there is always a risk of partial growth inhibition and/or killing of marine organisms sensitive to this substance, such as phytoplankton organisms, if the exposure exceeds 24 h at concentrations of 100-300 mg L^{-1} (Jung *et al.,* 2001). It should be taken into account that the above values refer to calculations of the short-term toxicity level of formaldehyde, while long-term toxicity should also be taken into account. However, similar studies are absent from the literature. In a single attempt to measure the long-term toxicity of formaldehyde in crustaceans (*D. magna*), the value of the of the no observed effect concentration (NOEC) was determined at 1.04 mg L^{-1} (Assessment, 2017), which is higher than the measured values of this paper (0.71 mg L^{-1}). Based on this parameter, the predicted no-effect concentration ($PNEC_{water}$) calculated for the same exposure with a reduction factor of 100, is 10.4 µg/L for the aquatic environment. While recognizing the significant technical difficulties in performing long-term experiments to ascertain the toxicity of formaldehyde for target organisms, it should be noted that the need for additional relevant knowledge is paramount.

The presence of methanol as a stabilizer in the formalin solution (12-13.5%) and its possible hazardous environmental effects should not be neglected when assessing the overall toxicity of formalin baths, although the LC_{50} of the substance reported in the literature for fish (15.400-29.400 mg L^{-1}; 96 h), is much higher than that measured for formalin (15 µL-225 mL L^{-1}; 96h), indicating a comparatively lower degree of toxicity (Kaviraj *et al.,* 2004; Leal *et al.,* 2018). It should also be mentioned that methanol is systematically used as a solubilizing agent for various substances in laboratory bioassays and cytotoxicity experiments (maximum concentration 5%), which also indicates the low toxicity of this substance. Although methanol degrades rapidly in the environment through photo-oxidation and biodegradation processes, the long-term effects of its use at high concentrations (lethal or sub-lethal) remains unknown (Kaviraj *et al.,* 2004), as toxicity studies have shown a reduction in fish growth and fecundity at a concentration >47.49 mg L^{-1} (Poirier *et al.,* 1986) and relatively high sensitivity to crustaceans of the species *Moina micrura* (96 h: LC_{50}= 4.82 mg L^{-1}) (Kaviraj *et al.,* 2004).

In conclusion, this study showed that the concentrations of formaldehyde in the edible tissues of gilthead seabream remain unaffected by formalin baths and time. Furthermore, the measured concentrations of the substance in the vicinity of the treated cages, during high temperatures appear to be negligible compared to those published in the literature as being toxic, in the short-

term, for marine or freshwater organisms. Furthermore, formaldehyde was unknot detected at the selected sampling points 45 days after completion of the bath treatments. Evaluation of the long-term toxicity of formaldehyde for marine target organisms should be a priority of future research efforts. Consequently, the possible application of a biological, chemical, physical, or mechanical method of formaldehyde deactivation before its environmental removal, deserves further investigation (Hayati *et al.,* 2019). Moreover, the specific duration of complete formaldehyde degradation under Mediterranean seawater summer conditions, where formalin baths are more frequent, should be another subject for future research.

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