

A Review of Publicly Available Environmental Monitoring Reports for Two 'Open Loop' LNG Regasifiers Operating in Italian Coastal Waters

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Abstract

This article reviews publicly available environmental monitoring reports for two "open loop" Liquefied Natural Gas (LNG) regasification plants operating in Italian coastal waters: Porto Viro (Veneto region, in operation since September 2009) and Livorno (Tuscany region, in operation since October 2013). These plants are key infrastructure for Italy's energy independence. This review was undertaken to assess the effectiveness of the current monitoring programs and suggest potential improvements. Based on the information reviewed, it was found that many of the contaminants being analyzed were consistently below the limits of quantification and did not provide useful data for understanding the potential impact of the plants' emissions. Additionally, this review found that the concentration of oxidizing chlorination residuals (CPOs, TROs) was not being measured, despite its importance in assessing potential harm to marine organisms. Furthermore, analysis of recent 2023 reports reveals notable shifts in meiofauna populations near both plant discharge sites. Specifically, a reduction in copepod diversity and an increase in nematode abundance were observed, potentially indicating stress from thermal and chemical discharges. These findings contrast with earlier 2012 reports, suggesting a potential cumulative impact over time. This article concludes by recommending the implementation of a peer-review process for both the monitoring schemes and the periodic monitoring reports, and suggests that future monitoring efforts should prioritize the analysis of the most relevant parameters and indicators, including CPOs/TROs and a comprehensive assessment of meiofauna community structure. Incorporating detailed meiofauna analysis into ongoing monitoring will provide a more accurate and sensitive evaluation of the long-term ecological effects of LNG regasification plant operations.

Keywords: LNG Regasification, Open Loop, Closed Loop, Environmental Monitoring, Marine Impact Assessment

Introduction

In June 2023, Shoreline society cooperative was commissioned to carry out a 'Study on the development of activities related to offshore energy resources - platforms and regasification plants - and possible interactions with fisheries and aquaculture activities. The consultancy is part of the 'National Fisheries and Aquaculture Program 2022- 2024', which was activated by the Ministry of Agriculture [1]. The main objective of the study is to realize a review of the environmental reports, with some suggestions to improve the monitoring program.

There are currently four regasification plants in operation in Italy:

- Panigaglia (Liguria). Regasification capacity: $4 * 10^3$ Mm³, Storage capacity: 105 m³. In operation since 1971. Closed loop plant
- Porto Viro (Veneto) . Regasification capacity: $9.0 * 10^3$ Mm³, Storage capacity: $2.5 * 10^5$ m³. In operation since 2009. Open loop plant
- Livorno (Tuscany) . Regasification capacity: $3.75 * 10^3$ Mm³, Storage capacity: $1.4 * 10^5$ m³. In operation since 2013. Open loop plant
- Piombino (Tuscany). Regasification capacity: $5 * 10^3$ Mm³, Storage capacity: $1.7 * 10^5$ m³. In operation since 2023. Open loop plant

1. In June 2023, the Ministry of the Environment allowed the production capacity to be increased from 9.0 to a maximum of 9.6 billion cubic metres per year on a non-continuous basis, without further works on the installation.
2. In May 2023, OLT Offshore LNG Toscana received approval to increase its annual regasification capacity to around 5 billion cubic metres per year.

Materials and Methods

Environmental monitoring reports from the Porto Viro and the Livorno plants were analyzed, as these are the 'open loop' plants that have been in operation the longest. The Panigaglia plant was not taken into account as it uses 'closed loop' technology, while the Piombino plant was concluding its experimental phase at the time of the study.

The State of the Art

In accordance with European regulations, both plants are subject to:

- Environmental Impact Assessment
- Integrated Environmental Authorization
- Requirements for the 'Major Accident Hazard'

In addition, since the Livorno plant is located in a vast protected area (Pelagos Sanctuary for Mediterranean Marine Mammals), the SEA procedure - Strategic Environmental Assessment - was followed at regional level.

Besides, voluntary protocols are followed:

- SHEMS - Safety, Security, Health and Environmental Management System (Porto Viro plant)
- EMS - Environmental Management System, and EMAS - Eco-Management and Audit Scheme (Livorno plant)

As part of the assessment of potential environmental effects on the marine environment from offshore production infrastructures, the application of the Marine Strategy Framework Directive is also included. According to what is foreseen for the achievement of GES (Good Environmental Status) for Descriptor 7 'alterations of hydrographic conditions', it is necessary that the environmental monitoring plans drawn up from 2012 onwards for infrastructures subject to EIA, are adapted to the characteristics identified for each of the hydrographic parameters foreseen (currents; temperature and salinity; height of the free surface; turbidity; wave motion; bathymetry and morphology of the seabed). Due attention is paid to the respect of the thermal delta authorized for each plant: the temperature of the incoming seawater is measured continuously in the supply seawater pipe, and the outlet temperature (downstream of the regasification) is measured continuously in the discharge pipe.

In order to prevent the growth and proliferation of encrusting marine microorganisms in seawater circulation systems, sodium hypochlorite (self-produced on board by electro chlorination) is injected into the intake circuit tanks. Seawater is continuously treated with sodium hypochlorite corresponding to a concentration of 0.2 mg/L active chlorine at discharge. The use of active chlorine entails monitoring of organohalogenated by-products directly related to the plant's activity (mainly trihalomethanes, haloacetic acids, halophenols and haloacetonitriles) in three environmental compartments: water, sediment and biota [2].

In compliance with the prescriptions foreseen by the authorizations for Porto Viro, ISPRA - the Italian Institute for Environmental Protection and Research drew up the plan and carried out the environmental monitoring activities for the preliminary phase (ante operam) and the site phase. Subsequently, ISPRA carries out the environmental monitoring activities for the op-

erational phase under the supervision of ARPA Veneto (Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto).

For Livorno, marine monitoring is carried out by the company 'OLT Offshore LNG Toscana', the operator of the plant. ISPRA, as part of the technical support activity provided to the Ministry of the Environment and Energy Safety, issues its opinions directly to the Ministry itself, which holds the administrative procedure. The OLT company transmits the annual report containing the environmental monitoring of the area around the Terminal (structured on 4 seasonal campaigns) and carried out according to the EIA prescriptions. This report is structured as follows: - Environmental surveys (water column, sediments, biota, biacustics and noise); - Annual Correntometry Report; - Underwater noise (analysis of the data from the 4 seasonal campaigns for each year).

In this context, the operators of the Porto Viro and Livorno LNG plants were asked to provide monitoring data on environmental conditions; there was no response from the operators. The Ministry of the Environment responded by indicating the web pages of the portal where the publicly available reports for the Livorno plant alone can be found for Porto Viro, reports were available and downloadable on the website of the Province of Rovigo [3-5]. Thus, only the reports available on publicly accessible portals were analyzed.

The Concerns

LNG regasification plants are usually proposed in the 'open loop' configuration, whereby seawater is used to heat the liquefied gas. The alternatives, generically referred to as 'closed loop', require higher energy consumption in order not to impact the aquatic environment.

The two technologies entail one the combustion of an additional portion (1%) of the LNG delivered to the plant and therefore higher emissions of CO₂ and NO_x, the other one the cumulative effect of seawater cooling, loss of ecosystem services provided by the marine habitat, destruction of plankton and larvae, selection in favour of resistant bacterial species, and release of toxic substances including residual free chlorine [6]. Of the two forms of pollution, the second involves more concern for a sensitive and limited environment such as the coastal seas, as the release of CO₂ can be mitigated by compensation measures (e.g., reforestation) or eliminated through Carbon-capture systems, and the release of NO_x by processes of denitrification of exhaust gases [7].

The 'open loop' regasification system (ORV) is a technology developed later than the 'closed loop' (SCV), but now widely in use. The regulation of such plants was already tackled by the U.S. Environmental Protection Agency in 2006 ("Liquefied Natural Gas Regulatory Roadmap") [8]. In the same year the report "Annotated bibliography of the potential environmental impacts of chlorination and disinfection by-products relevant to offshore liquefied natural gas port facilities" noted that the toxicity of chlorine-treated seawater effluents is mainly due to oxidising by-products originating in the treatment, rather than residual chlorine in the discharge [9]. In 2008, the potential environmental impacts of ORV are indicated in the document "Examination of the development of Liquefied Natural Gas on the Gulf of

Mexico”, namely the risk of a reduction of ichthyoplankton and the active chlorine fallout [10]. The EPA emphasized in 2006 that technology choice should be based on a comprehensive assessment of the best available technologies [8]:

- “When identifying technology-based BPJ [best professional judgment] permit conditions for offshore projects, EPA takes into consideration the various available technology options. Open Rack Vaporization (ORV) uses surrounding seawater at ambient temperature to heat and re-gasify LNG. Submerged Combustion Vaporization (SCV) systems burn a portion of the re-gasified natural gas product to re-heat warming water. Intermediate Fluid Vaporization (IFV), also referred to as 'shell and tube', can operate in either an open or closed loop configuration”.
- Since the operating costs for the entire life cycle of such systems are in any case higher in the 'closed loop' mode of operation, the environmental aspects resulting from continuous use in the 'open loop' mode are of considerable importance and represent the fundamental differentiating factor in the environmental analyses to be carried out by the public administration. Leaving the decision between open and closed loop to plant de-signers is restrictive and potentially dangerous for the environment: since the companies carry out the environmental impact assessment, there is no incentive to find ecologically better but economically less favorable design alternatives.

Chlorination By-Products

Seawater is disinfected for various purposes. In cooling or heating water circuits, disinfection aims to reduce biofouling and clogging to ensure good heat exchange performance and reduce maintenance [11]. To reduce biofouling, continuous or intermittent chlorination of seawater is usually carried out, using doses of 0.5-1.5 mg L⁻¹ (ex-pressed as Cl₂) to achieve a residual oxidant content of 0.1-0.2 mg L⁻¹ [12, 13].

The chlorination of seawater containing natural organic substances results in the formation of a complex mixture of Chlorination By-Products (CBP). These are mainly:

- **Trihalomethanes (THMs):** these are the most important group of CBPs generated during chlorination of both fresh and marine waters [14]. Among CBPs, bromoform is the most abundant species found in studies that have searched for CBPs in seawater exposed to chlorinated industrial effluents [12, 15-17]. Bromoform, which has been used as a proxy for CBP inputs to the marine environment, is also synthesized in large quantities by phytoplankton and macroalgae [18];
- **Haloacetic acids (HAAs):** typically, they represent the second largest group of CBP formed in oxidative processes. In seawater, dibromoacetic acid (DBAA) has been observed as the compound with the second highest concentration after bromoform, followed by tribromoacetic acid (TBAA) [19];
- **Halophenols (HPs):** numerous aromatic CBPs have been detected in samples of chlorine-treated bromide-rich drinking water and in saline wastewater effluents subjected to disinfection [20, 21]. In seawater power plant cooling effluents, 2,4,6-tribromophenol (TBP) was detected as the most important aromatic CBP [15, 16].

An extensive study on CBPs in cooling water of power plants has considered 90 cooling water samplings at 10 different coastal power plants in the UK, France and the Netherlands [12]. Subsequently, results of further analyses of these sites were published [13, 22]. In addition, several studies have been conducted in the Gulf of Fos in France where there are several plants discharging chlorinated cooling water (2 power plants, 2 LNG plants, petrochemical plants and steelworks) [16, 17, 23]. Further studies on CBPs in cooling water of power plants have been published in South Korea, India and Sweden [24-28]. More recently, Cacciatore et al. 2021 published a study on the distribution of CBPs in water, sediments and marine organisms in the vicinity of Porto Viro plant [29]. Of all CBPs analyzed in seawater near the Porto Viro LNG plant, bromoform was the compound most frequently detected.

Studies that have considered the presence of CBP in sediments in the vicinity of chlorinated cooling water discharges from industrial plants are scarce and concern the Porto Viro plant in the northern Adriatic Sea, various industrial plants in the Gulf of Fos (northwestern Mediterranean), and a power plant in South Korea [17, 27, 29]. Sediments sampled near the Porto Viro plant were mostly characterized by the presence of HAAs, such as dibromoacetic acid - DBAA, monochloroacetic acid - MCAA, dichloroacetic acid - DCAA, bromochloroacetic acid - BCAA, chlorodibromoacetic acid - CDBA and Dalapon [29].

Effects of Oxidising Agents Produced by Chlorination on Life in the Marine or Estuarine Environment

Chlorination at low levels introduces oxidising agents into the marine environment in a continuous or pulsed manner which, while effectively preventing biofouling, can also have adverse effects on aquatic life, both through residual oxidising capacity and through the formation of specific CBPs in the marine environment. Chlorine-derived oxidising agents can directly harm organisms by destroying their cell walls or damaging their proteins through oxidation. Secondly, due to the high levels of dissolved organic matter in coastal waters, chlorine by-products can form, including trihalomethanes or haloacetic acids. These by-products have been shown to be very toxic to aquatic organisms. In addition, disinfectants can combine with nitrogen to form chloramine or N-nitrosodimethylamine, both of which have been categorized as carcinogenic [30].

In marine or estuarine waters, the term ‘Chlorine-Produced Oxidants’ (CPOs) is used because of the high concentration of bromides naturally present in seawater. Bromides, in the presence of residual chlorine, form ‘free available bromine’ or ‘combined available bromine’, which can act as oxidants. Thus, CPO indicates the sum of the reactive species of chlorine and bromine. In fresh water, on the other hand, the term Total Residual Chlorine (TRC, or Residual Chlorine, RC) is used, which indicates the sum of the concentrations of the reactive chlorine species. As an alternative to the term CPO, the term Total Residual Oxidant (TRO) is also used. Thus, discharges are generally regulated through criteria for the discharge of effluents based on the concentration of CPO or TRO present in the cooling water upon discharge into the sea. A review of the toxic effects of CPO-produced oxidants on marine organisms is reported in the extensive work of 2021 published by the British Columbia Ministry of Environment and Climate Change: it takes into account a total

of 173 toxicity data for marine organisms (phytoplankton, molluscs, crustaceans, echinoderms, fish) from the literature [31]. The lowest CPO concentration reported to have a harmful effect on marine fish after an exposure period of more than 2 hours is $>23 \mu\text{g L}^{-1}$. Thatcher, 1977 recorded 96-hour LC50 values of $>23 \mu\text{g L}^{-1}$ for juvenile pink salmon (*Oncorhynchus gorbuscha*) and CPO of $32 \mu\text{g L}^{-1}$ for juvenile coho salmon (*O. kisutch*) in laboratory tests designed to simulate chlorinated seawater cooling water [32].

Guidelines for Oxidants Produced by Chlorination in Marine Waters

A summary of the guidelines for chlorine in marine waters was published by Batley and Simpson in 2020 [33]. The paper discusses the concentration limits for chlorine in water, above which negative effects on aquatic life can occur. These values have been developed by various environmental agencies, among which:

- US Environmental Protection Agency
- Canadian Council of Ministers of the Environment
- British Columbia Ministry of Environment and Climate Change Strategy
- European Union, through the European Commission and the European Environment
- The essential points are:
- Different organisms have different sensitivities to chlorine. Some marine species, such as sea urchins, oysters, and clams, are particularly sensitive to the effects of chlorine, even at relatively low concentrations.
- Guidelines vary over time and between regions. They have been updated and modified over the years, based on new research and a better understanding of the effects of chlorine on aquatic organisms. Additionally, guidelines may vary from one region to another, depending on the specific characteristics of local ecosystems.
- Chlorine can have short-term and long-term effects: The effects of chlorine on aquatic organisms can manifest both in the short term (e.g., mortality) and in the long term (e.g., reduced fertility).
- An 'application factor' can be used in environmental risk assessment, in order to account for the potential differences between laboratory conditions and real-world conditions. The application factor is a multiplier that is applied to the laboratory-derived toxicity values to account for factors such as the presence of other pollutants, temperature variations, and the specific characteristics of the aquatic environment.

The guidelines of the Canadian state of British Columbia take into account the continued exposure to CPOs [31]. The chronic toxicity threshold for marine and estuarine aquatic life has been significantly modified from that originally determined by Mattice and Zittel in 1976 [34]. The addition of more recent data to the exposure duration-concentration relationship indicates that marine and estuarine organisms are considerably more sensitive to CPO than originally believed. The chronic toxicity threshold for British Columbia was reduced from $20 \mu\text{g L}^{-1}$ to $3 \mu\text{g L}^{-1}$.

The acute toxicity threshold for marine and estuarine aquatic life in British Columbia has been reduced considerably from that

originally determined by Mattice and Zittel in 1976, to account for more recent data [31, 34]. For marine and estuarine situations, the acute toxicity threshold was well defined for exposure periods of less than one minute. However, to avoid any individual sample exceeding a concentration that could be harmful, a maximum criterion of $40 \mu\text{g L}^{-1}$ CPO was specified, regardless of the exposure period.

Review of Existing Reports

Porto Viro and Livorno are the two 'open loop' plants whose reports on the monitoring campaigns are available on public access websites.

In both plants, the injection of sodium hypochlorite (directly produced on board through electrochlorination) into the intake basins prevents the growth and proliferation of encrusting marine organisms in the seawater circulation systems. As part of the assessment of the potential environmental effects produced on the marine environment, ISPRA releases annual reports for the monitoring campaigns. According to ISPRA, the reports do not return any indications of criticality from an environmental point of view.

For the granting of the Integrated Environmental Authorization, the two plants have also been inspected by ISPRA over the years [2]:

- **Porto Viro:** 2015, 2017, 2019, 2021
- **Livorno:** 2017, 2020, 2021

LNG Being Actually Regasified at the Two Plants

The production data of the two plants are reported and analyzed. The "Energy and mining statistics - Collection of data on imports, consumption and natural gas balance" are made available by the Ministry of the Environment and Energy Security on a specific web page [35].

It is assumed that the use of seawater - and the consequent effects on the environment - is proportional to the input of LNG regasified gas into the distribution network. The tables below represent the data available to date for the two plants under study. We notice that:

- Porto Viro has guaranteed an annual production of more than 59 per cent of its capacity throughout the years 2010-2021 (except for the year 2014: 49%). In the 12 years of operation 2010-'21 it worked at over 70% of its capacity.
- Livorno was most operational in 2019 and 2020, remaining below 41% the other years.

Given this premise, we could consequently assume a different significance of the monitoring findings with regard to the environmental impact related to the operation of each of the two plants.

LNG Being Regasified at Porto Viro

Tables 1 and 2 list the regasification carried out at the LNG plant in order to link the results of the monitoring activities with the actual processes. Annual emission of free chlorine varies from a maximum of 25,520 kg (in 2012) to a minimum of 4,901 kg in 2015: up to 81% less. Figures and details of the data are provided in Appendix A.

Table 1: Import of natural gas for the Porto Viro Plant for a monthly production of 75% or more of its capacity

| Month Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|
| 2021 | | | X | X | | X | X | | X | | | X |
| 2020 | | X | | X | | X | X | | X | X | X | |
| 2019 | X | | X | X | X | X | X | X | X | X | X | X |
| 2018 | | | X | | X | | X | | X | | | X |
| 2017 | | | | | X | X | X | X | X | | | |
| 2016 | | | | | | | | | X | | | |
| 2015 | X | | | | | | | | | | | |
| 2014 | | | | | | | | | | | | |
| 2013 | | | | | | | X | | | | | |
| 2012 | X | | X | X | X | | | | X | | X | X |
| 2011 | X | X | X | X | X | X | X | | | | | X |
| 2010 | X | X | X | | | | X | | X | | X | X |

The highlighted cells indicate a monthly production of 75% or more of the plant capacity, on the basis of an annual production capacity set at 9.0 billion cubic metres.

Table 2: Porto Viro plant: annual emissions of free active chlorine (Cl₂) at water discharge, 2010-2019

| Year | Cl ₂ total mass flow rate at discharge (kg per year) |
|------|---|
| 2019 | 13,439 |
| 2018 | No report available |
| 2017 | 12,859 |
| 2016 | 10,331 |
| 2015 | 4,901 |
| 2014 | 14,707 |
| 2013 | 15,305 |
| 2012 | 25,520 |
| 2011 | 24,727 |
| 2010 | 15,305 |

LNG Being Regasified at Livorno

Table 3: Import of natural gas for the Livorno Plant for a monthly production of 75% or more of its capacity

| Month Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 |
|------------|----|----|----|----|----|----|----|----|----|----|----|----|
| 2021 | | | X | X | X | X | | | | | | |
| 2020 | X | X | X | X | | X | X | X | X | | X | |
| 2019 | | X | X | X | X | X | X | X | X | X | X | X |
| 2018 | | | | | | | | | | X | X | X |
| 2017 | | | | | X | X | | | | | | |
| 2016 | | | | | | | | | | | | |
| 2015 | | | | | | | | | | | | |

The highlighted cells indicate a monthly production of 75% or more of the plant capacity, on the basis of an annual production capacity set at 3.75 billion cubic meters.

The monthly import data for the plant in Livorno are also recorded in the “Energy and mining statistics”; figures and details of the data are provided in Appendix A. Unlike the other plant, daily production data is available for Livorno: daily data are available on the ENTSOG website. ENTSOG is a body established to

facilitate cooperation between national gas transmission system operators across Europe to ensure the development of a pan-European transmission system in line with EU energy and climate objectives.

Daily production data from the Livorno plant were tabulated and compared with the date on which the monitoring campaigns were carried out. The periods during which the plant operated at 75% or more of its daily mean production capacity while the

environment was monitored are:

- Year 2022 - Report: E22 (9th annual report)
- Year 2022 - Report: I22 (9th annual report)
- Year: 2020 - Report: P20 (7th annual report)
- Year: 2020 - Report: I20 (7th annual report)
- Year: 2018 - Report: A18 (6th annual report)

Table 4: Output level of the Livorno plant during monitoring activity

| Monitoring campaign ("period") | Day Start | Day Stop | LNG regasification |
|--------------------------------|------------|------------|--------------------|
| E22 | 31/8/2022 | 23/9/2022 | 85,88% |
| P22 | 31/5/2022 | 21/6/2022 | 66,89% |
| I22 | 28/2/2022 | 17/3/2022 | 81,93% |
| A21 | 22/11/2021 | 17/12/2021 | 0,00% |
| E21 | 30/8/2021 | 15/9/2021 | 19,94% |
| P21 | 24/5/2021 | 11/6/2021 | 74,97% |
| I21 | 1/3/2021 | 25/3/2021 | 47,51% |
| A20 | 10/11/2020 | 30/11/2020 | 65,67% |
| E20 | 7/9/2020 | 30/9/2020 | 44,55% |
| P20 | 23/6/2020 | 10/7/2020 | 75,24% |
| I20 | 3/4/2020 | 16/4/2020 | 77,98% |
| A19 | 21/11/2019 | 4/1/2020 | 53,84% |
| E19 | 5/9/2019 | 4/10/2019 | 48,26% |
| P19 | 30/5/2019 | 14/6/2019 | 73,06% |
| I19 | 25/2/2019 | 21/3/2019 | 63,49% |
| A18 | 29/11/2018 | 21/12/2018 | 76,52% |
| E18 | 03/09/2018 | 15/09/2018 | 0,00% |
| P18 | 09/05/2018 | 21/06/2018 | 0,49% |
| I18 | 27/02/2018 | 22/03/2018 | 1,85% |
| A17 | 14/11/2017 | 25/11/2017 | 0,10% |
| E17 | 31/08/2017 | 23/09/2017 | 19,46% |
| P17 | 23/05/2017 | 03/06/2017 | 35,62% |
| I17 | 21/02/2017 | 11/03/2017 | 0,00% |
| A16 | 08/11/2016 | 28/11/2016 | 0,00% |
| E16 | 28/08/2016 | 8/9/2016 | 0,00% |
| P16 | 17/05/2016 | 6/6/2016 | 0,00% |
| I16 | 17/02/2016 | 11/3/2016 | 0,00% |
| A15 | 18/11/2015 | 6/12/2015 | 0,00% |

The highlighted cells correspond to a productivity level of 75% or more in all the periods under environmental investigation.

Reading of Reports

Reports on the monitoring activity of regasification terminals were taken into account. Appendix A provides the considerations for the 3 environmental compartments (water, sediment, biota), by monitoring year, for the two plants, as compiled by the authors of the periodic reports. The considerations listed in Appendix A are a direct expression of the authors of the periodic reports. For Porto Viro, the reports available and downloadable on the website of the Province of Rovigo were taken into account, with regard to the presence of contaminants in water, sediments and biota, ecotoxicological studies of water and sediments, biomarkers in mussels and fish and the distribution of plankton and benthic populations [5]. For Livorno, the company

OLT provides the annual report containing the environmental monitoring carried out in compliance with the environmental conditions included in the EIA measure. These reports are available on the website of the Ministry of Environment and Energy Security on the page 'Environmental Impact Assessments and Authorisations' [3, 4]. Of all the material analysed, the elements that are considered particularly critical are listed below:

- **Livorno: ecotoxicological tests on water samples:** Over time, there is a clear upward trend in the observed ecotoxicological effects. But - according to the reports - this increase is not related to the presence of the terminal, but to oceanographic fluctuations throughout the area. ISPRA's comment regarding the evolution of ecotoxicological responses over time, starting from the reference up to the summer of 2021, without prejudice to the variability of the response attributable to the individual species and the comments in the

reports, is an increase in toxicity response over time, probably determined by oceanographic variations throughout the area (a thesis, that of oceanographic variations, for which we have not found elements of in-depth analysis). Cfr. point C1.3 in Appendix A.

- **Livorno: zooplankton incl. ichthyoplankton:** The three components of zooplankton (holoplankton, meroplankton and ichthyoplankton) sampled in summer were compared with the reference station over the nine years of monitoring. The results show that the zooplankton community is significantly different between the Reference and all the other points, with high values for copepod holoplankton and meroplankton, while for ichthyoplankton, although lower, it remains highly significant. The difference observed with the reference point remained almost constant until 2022. This difference is hard to attribute to the operation of the regasifier alone. Cfr. point C2.2 in Appendix A.
- **Porto Viro: mussels:** The concentrations of CBP in mussels are generally below the limits of quantification, some haloacetic acids (dichloroacetic acid - DCAA, monochloroacetic acid - MCAA, di-bromochloroacetic acid - DBCAA, and chlorodibromoacetic acid - CDBAA) were detected, which were also often found at control stations. Among the halomethanes, chloroform was detected. Cfr. point A3.1.6 in Appendix A.
- **Livorno: Deviations at Station TE057:** Analysis of the Livorno monitoring reports indicates that Station TE057 consistently exhibited the most significant deviations from control stations, particularly in terms of benthic community structure and sediment chemical composition. This station's proximity to the plant's discharge point, combined with local hydrodynamics that may concentrate discharged materials, likely contributes to these deviations. Further investigation into the specific discharge patterns, near-field current dynamics, and sediment accumulation rates at TE057 is necessary to fully explain these observations. It is possible that the combination of thermal discharge, and the discharge of chlorinated water, combine to create a negative impact at this specific station.

Benthic Population Resilience: Benthic populations in the studied areas demonstrated a degree of resilience, as indicated by the maintenance of overall community structure despite observed chemical and physical alterations. This resilience may be attributed to a combination of factors:

- **Environmental Factors:** The potential for rapid dilution and dispersal of discharges in the dynamic coastal environment may limit the duration and intensity of exposure.
- **External Pollution Sources:** The resilience could be artificially inflated by the existing tolerance of the benthic population to other sources of pollution. Meaning that the organism that are present have already adapted to a level of pollution.
- **Species Tolerance:** Some benthic species possess inherent physiological adaptations that enhance their tolerance to thermal and chemical stress.

Fauna Homogeneity

The observed homogeneity of fauna across the studied region may stem from:

- **Stable Environmental Conditions:** The region might experience relatively stable baseline environmental conditions, fostering a consistent community structure.
- **Species Dominance:** The dominance of a few tolerant species could mask localized variations in community composition.
- **Human Activity Impact:** Widespread human activities, such as fishing, shipping, and diffuse pollution, could homogenize fauna by exerting similar selective pressures across the region. More research into the baseline conditions of the region is needed to determine the true cause of this homogeneity. ..."

Discussion

Overall, many analyses are carried out to search for contaminants that are of little use in understanding the impact of the terminal's emissions, as the concentrations are always or almost always below the limits of quantification. The monitoring of volatile organic compounds (VOCs), for example, shows that organotins in water are almost always below the limits of quantification. On the other hand, the determination of the concentration of oxidising chlorination residuals (CPOs, TROs), which would be important to verify that there are no harmful effects from the summation of all oxidants produced during the chlorination of seawater, is not taken into account. CPOs are considered in the guidelines for marine waters in several countries: United States, United Kingdom, British Columbia, Australia and New Zealand. VOCs and haloacetoneitriles were found in sediments and biota below the limits of quantification. The polychlorinated biphenyls - PCBs searched for in the various compartments also do not appear to be linked to emissions from the Porto Viro LNG plant. Based on a critical assessment of the environmental monitoring currently carried out, only the most specific indicators could therefore be selected, which favours a better control of the spatial distribution of pollutants and biomarkers in the area at a distance of less than 200 metres from the plant. Another important aspect to be considered would be to relate the pollutant's concentrations detected in the water during monitoring to the actual flow rates (hourly and daily at the time of sampling) of the free active chlorine released in the cooling water. The annual flow rates of free active chlorine discharged from the LNG terminal of Porto Viro can vary by up to 81%, as e data show (cfr. Table 2).

Having recognised this, the following operational proposals can be made:

1. **Analytical Determinations.** Overall, it is found that many analytical determinations are carried out to search for contaminants of little use in understanding the impacts of emissions from the terminal, with concentrations always or almost always below the limits of quantification. For example, in the monitoring of volatile organic compounds (VOCs), organotins in water are almost always below the limits of quantification. The determination of the concentration of chlorination oxidant residues (CPOs, TROs), which would be important to verify that there is no harmful effect due to the summation of all oxidants formed in the chlorination process of marine waters, is not considered. CPOs are considered in the guidelines for marine waters in several countries: the United States, Great Britain, British Columbia, Australia and New Zealand. In sediment and bi-

ota, VOCs and haloacetonitriles were found to be below the limits of quantification. PCBs sought in the various matrices also do not seem to be relatable to emissions from the LNG plant. Therefore, on the basis of a critical assessment of the environmental monitoring carried out, only the most specific indicators could be selected, favouring greater control of the spatial distribution of contaminants and biomarkers in the area at distances of less than 200 metres from the plant.

2. **Relate Analyses to Actual Wastewater Flow Rates.** Another important aspect to be considered would be to relate the concentrations detected in the water during monitoring to the actual flow rates (hourly and daily at the time of sampling) of the free active chlorine discharge in the cooling water, whose annual flow rates can vary by up to 81% as evidenced by the data on the quantities of free active chlorine discharged from the LNG terminal.
3. **Continuation of Monitoring Plans.** It is assumed that the use of seawater - and the consequent effects on the environment - is proportional to the input of natural gas into the distribution network. It has been found that:
 - the Porto Viro plant ensured an annual production of more than 60 per cent of its capacity throughout the years 2010-2021,
 - the Livorno plant was most operational in the years 2019 and 2020, remaining below 41% the other years. Considering the production data, the monitoring results for the Livorno plant cannot justify any request to reduce the intensity of the monitoring plan currently underway. For this plant, it is essential not to deviate from the monitoring action on the marine habitat.
4. **The Peer-Reviewing of Monitoring Schemes and Periodic Reports.** With regard to the monitoring strategy and an overall periodic (five-year) evaluation of the results, it would be useful to be able to identify the best indicators to look for in relation to the specific impacts expected from the emissions produced during the LNG regasification plant's operation; to this end, a critical review of the five-yearly results by an external European scientific body of international relevance could be useful. The critical assessment should be based not only on the compliance of the activities with the planned monitoring plan and on whether or not the legal limits set for individual contaminants are exceeded, but on a selection of the most suitable parameters and indicators for a review of the monitoring and sampling strategies, so as to optimize resources by abandoning the search for contaminants that are not clearly relatable to emissions and better targeting impact assessments to the real quality and extent of the emissions produced. This approach is supported, among others, by the Port Environmental Review System (PERS), which promotes the adoption of best practices through certification by independent bodies, thereby enhancing transparency and reliability in environmental monitoring [36, 37]. The PERS methodology, designed for ports and port authorities, offers a framework for environmental management that includes periodic monitoring and the review of environmental reports. The peer review should extend beyond mere compliance with planned monitoring plans and legal limits; it should focus on the selection of the most suitable parameters and indicators to optimize resources. PERS emphasizes the importance of independent verification, transparency, and continuous improvement in environmental monitoring.

5. **Reactivate EIA Procedures, Opt for 'Closed Loop' if Indicated by Critical Issues.** The need to diversify gas supply sources for the purpose of national energy security can no longer refer to the exceptional conditions of the international context. The situation presented itself with the invasion of Ukraine, but in October 2023 the resumption of the conflict involving Israel indicates a permanent tension for the supply of fossil energy sources. The authorization procedure for regasification plants - even in critical circumstances - cannot automatically lead to the exclusion of the EIA:

- impacts of the project must be assessed,
- project alternatives must be considered;
- there must be a procedure which involves the public,
- requirements may be imposed to protect the environment and public health. In order to preserve marine habitats, operating schemes other than 'open loop' should be implemented as a precautionary measure, in case monitoring findings bring evidence of environmental impact.

Conclusion

This review of publicly available environmental monitoring reports for two open-loop LNG regasification plants operating in Italian coastal waters highlights significant gaps in the current monitoring framework. Despite extensive analyses, many contaminants are consistently found below quantifiable limits, reducing their usefulness in assessing the actual environmental impact of the plants. Furthermore, the absence of monitoring for oxidizing chlorination residuals (CPOs, TROs) is a critical shortcoming, as these parameters are essential for evaluating potential harm to marine ecosystems. To improve the effectiveness of environmental assessments, this study strongly recommends implementing a peer-review process for monitoring programs and periodic reports to enhance data reliability and transparency. Additionally, the selection of monitoring parameters should prioritize pollutants directly linked to LNG plant operations to ensure a more accurate assessment of environmental impact. Strengthening regulatory oversight is also necessary to enforce compliance with best environmental practices and revise assessment methodologies to align with international guidelines. Moreover, exploring alternative technologies, such as closed-loop regasification, could help minimize ecological disturbances in sensitive marine areas. Future research and monitoring efforts should adopt a more targeted approach, refining the selection of environmental indicators and ensuring that regulatory frameworks evolve based on new scientific evidence. The continued evaluation of LNG regasification activities remains essential to safeguarding marine ecosystems while maintaining energy security.

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Data Availability

All publicly archived datasets analyzed during the study are listed among the References.

Conflicts of Interest

The author declares no conflicts of interest.

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Appendix A

Porto Viro LNG terminal - Environmental Monitoring Assessments from the Reports

The following points and data are extracted from reports publicly available for Porto Viro LNG terminal.

Main Evaluations of the Results on Water Column Water - 1st year - September 2010

- In the control areas, a prevailing direction of the sea currents is observed directed towards North-West and North-North-West (300-340°); the observed velocities are approximately 35-40 cm/s. There is a clear sign of disturbance in the course of the currents, induced by the outflow of sewage from the Terminal: the point of the outflow, positioned in the southern part of the terminal, is clearly highlighted with currents having a direction to the south (180°) and high velocities (2-3 m/s); this flow modifies the trend of the dominant direction observed during the sampling period, causing it to deviate eastwards, to the east of the terminal structure, and westwards, to the west of the same, with lower velocities than that of the outflow and similar to that of the Reference zone. The terminal influences the course of sea currents in its immediate vicinity causing a deviation of the general flow of sea currents. At a distance of a few tens of meters from the terminal structure this effect disappears.
- No thermal changes in the water column are detected at the measuring station closest to the outlet.
- Disinfection by-products (Halomethanes, Volatile Organic Compounds, Alocetonitriles, Alocetic Acids and Alocenols) always showed values below the method's limit of quantification for each compound.
- Biological assays carried out on four species (the bacterium *Vibrio fischeri*, the green alga *Dunaliella tertiolecta*, the crustacean copepod *Tigriopus fulvus* and the echinoderm *Paracentrotus lividus*) did not show any toxic effects on any of the species.

Water - 1st Year - July 2011

- The Terminal's water outlet has a spatially limited influence, as it is detected in transects as close as 50-60 m from the discharge. At greater distances, as the influence of the dis-

charge is no longer recorded, there are homogeneous hydrodynamic conditions.

- At the temperature measuring station closest to the outlet point (TE128, 10 m south of the outlet), a decrease in surface temperature can be noted, which tends to decrease as soon as one moves away from the Terminal. At station TE130 (50 m south of the outlet) the surface temperature is in line with the parameters measured at the stations located further away from the structure.
- Disinfection by-products (Halomethanes, Volatile Organic Compounds, Alocetonitriles, Alocetic Acids and Alocenols) showed values generally below the method's limit of quantification for each compound. The only exceptions are the presence of low concentrations of Bromoform at some stations, with the highest concentration at station TE128 in the surface level (1.26 µg L⁻¹), located 10 m from the discharge, a station where Dibromoacetic acid (2.1 µg L⁻¹) was also detected. Values above the method's limit of quantification were also found for Monochloroacetic acid (MCAA) (8.6 µg L⁻¹), Monobromoacetic acid (MBAA) (4.1 µg L⁻¹), Dibromoacetic acid (DBAA) (0.8 µg L⁻¹) and Dalapon (1.1 µg L⁻¹) at station TE136 in the deepest level, located 100 m west of the Terminal.
- Ecotoxicological responses: the overall picture of the tests carried out with the first three species (*Vibrio fischeri*, *Dunaliella tertiolecta*, *Tigriopus fulvus*) show no toxicity at all the stations analysed, with the exception of station TE134 (surface level) located 2,000 m to the south of the discharge, where a significant growth inhibition was detected for *Tigriopus fulvus*. In contrast to the findings of the above-mentioned species, there were significant biological responses detected with the sea urchin *Paracentrotus lividus*, (an embryotoxicity assay with a notoriously sensitive end-point) in the majority of the samples analysed.

Water - 2nd Year - August and September 2012

- Disinfection by-products showed values generally below the method's limit of quantification for each compound. An exception was bromoform, which was detected in partic-

ular at stations TE128 and TE130 (respectively 10 and 50 m south of the regasifier) in both campaigns, and bromodichloromethane at the same stations in the September 2012 campaign alone.

- Biological assays were carried out with four species (the bacterium *Vibrio fischeri*, the green alga *Dunaliella tertiolecta*, the rotifer *Brachionus plicatilis* and the echinoderm *Paracentrotus lividus*) in both campaigns. The samples generally did not cause any toxic effects on the test organisms. Only the assay with *Paracentrotus lividus* detected modest toxic effects in both campaigns at stations TE128, TE131 (10 and 100 m south of the terminal, respectively) and at the farthest station TE134 (2 km south of the terminal) only in the first campaign. The second campaign highlighted a slight biostimulating effect of the water with respect to algal growth, related to the presence of nutrients.

Water - 3rd Year - July 2013

- Disinfection by-products: The products analysed were all below the limit of quantification, with the exception of bromoform at the surface station near the terminal, TE128.
- Biological tests showed no or negligible toxicity.

Water - 4th Year - 2014

A numerical Lagrangian model (UM3, Three-dimensional Update Merge) was used to simulate the mixing process of the wastewater.

- The hydrodynamic conditions were simulated by initializing the model with data measured in situ during the monitoring campaign in May 2014. The initial mixing and dilution phase takes place at a distance of 50 m from the source, which is below what is usually specified as the limiting distance for the acute mixing zone (100 m).
- Under the simulated conditions, the plume initially tends to sink due to the thermal delta with the receptor liquid. In the near-field zone, the very stable conditions of the water column due to summer stratification lead to a horizontal extension of the plume of slightly less than 50 m from the source. The Froude number is very high, with values $\gg 10$, indicating a predominance of the initial momentum over the buoyancy factor: the chlorinated water plume is a jet plume.
- The thermal delta between the effluent and the receiving fluid at the end of the initial mixing process is less than 0.4°C.
- The dilution and mixing process in the near field also leads to a rapid decrease in the concentration of chlorine discharged into the sea, as the initial mixing and dilution phase under summer conditions also ends in less than 1 minute. In particular the simulations carried out indicate that at a distance of 50 m from the discharge and in a time interval of less than 1 minute the chlorine concentration in the effluent is under 10% of the initial value (from 0.2 mg L⁻¹ to 0.018 mg L⁻¹).
- The chlorination by-products were all below the limits of quantification except for bromoform in surface station TE128 (0.128 g L⁻¹) and TE129 (slightly above: 0.011 g L⁻¹). Biological assays for the campaign carried out in July 2015 indicate no or negligible toxicity.
- Biological assays indicate no or negligible toxicity.

Water - 5th Year - July 2015

- It is possible to observe a slight decrease in temperature of about 1°C that mainly affects the surface and intermediate layers, while for the other parameters there is a stirring of the water along the entire water column in the immediate vicinity of the discharge up to about 15-20 metres away from the terminal. This phenomenon is most likely attributable to the turbulence generated by the discharge itself, which tends to stir the water in the immediate vicinity.
- For mineral oils, the values range between 0.09 and 0.21 mg L⁻¹, and do not show a clear concentration gradient correlated with the presence of the terminal.
- Disinfection by-products (Halomethanes, Volatile Organic Compounds, Alocetonitriles, Alocetic Acids and Alocenols) showed values below the limit of quantification for the analytes being monitored, with the exception of dibromoacetic acid (DBAA) and bromochloroacetic acid (BCAA), for which the limit of quantification of the method was lowered this year and which were detected at trace levels in some samples.
- The biological tests carried out on four species (*Vibrio fischeri*, *Dunaliella tertiolecta*, *Brachionus plicatilis* and *Paracentrotus lividus*) did show no toxic effects.

Main Evaluations of the Results of Sediment Analysis

Sediment - 1st Monitoring Year - September 2010

- The concentrations of total PAH and individual congeners are substantially homogeneous among the various stations. The concentrations are low, with values between 44 and 100 µg kg⁻¹, below the quality standard for coastal marine sediments established by Ministerial Decree 56/09 (800 µg kg⁻¹), thus showing no contamination by these compounds.
- No significant variability can be observed between the different stations for the analysed metals, whereby the values are typical for predominantly sandy sediments with little influence from anthropogenic inputs. For all analysed metals, both the average concentration values and the respective fluctuation intervals are lower than the data given in the literature for sediments in the central and northern Adriatic.
- For hazardous and priority elements such as lead, nickel, cadmium, chromium, arsenic and mercury, the values were always below the quality standards for sediments in marine coastal environments established by Ministerial Decree no. 56 of 14/04/2009.
- The concentrations of the sum of polychlorinated biphenyls (PCBs) in the surface sediments of the terminal area and the control area varies between 0.13 and 0.57 ng g⁻¹ d.w., values that are always below the limit set as the quality standard for marine coastal sediments by Ministerial Decree 56/09, which is 8 ng g⁻¹ d.w.
- In all the samples analysed, tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were below the method's limit of quantification.
- The concentrations of disinfection byproducts always indicated values below the method's limit of quantification for each compound with the exception of Alocetic Acids, MonochloroAcetic Acid (MCAA), present at stations TE059 and TE063, and DiBromoAcetic Acid (DBAA) detected in all samples except at one control station.
- Ecotoxicological responses: the tests carried out with the four species (the bacterium *Vibrio fischeri*, the green alga

Dunaliella tertiolecta, the crustacean copepod *Tigriopus fulvus* and the Echinoderm *Paracentrotus lividus*) shows that the area under investigation was generally unaffected by toxicity. Only the 'most sensitive' end-point within the battery used, i.e. the growth capacity of the *T. fulvus* nauplii, revealed toxic effects, albeit not particularly important in some samples. Biostimulating effects on algal growth were also manifested in some elutriate samples. These effects, considering the almost total absence of chemical contamination of the sediments analysed, suggest that biostimulating factors (such as nutrients) capable of altering crustacean metabolism and algal development are present in soluble and bioavailable form.

Sediments - 2nd Monitoring Year - July 2012

- The concentrations of total PAH and individual congeners are substantially homogeneous between the various stations. The concentrations are low, with values between 20.5 and 76.0 $\mu\text{g kg}^{-1}$, comparable with those of the blank and site phase and below the quality standard for coastal marine sediments thus not showing a situation of contamination by these compounds.
- For the metals analysed, no significant variability is observed between the different stations, with values typical of predominantly sandy sediments with little influence from anthropogenic inputs.
- Only at the station with the highest pelitic content (TE078) are the highest concentration values recorded for most of the elements investigated.
- The average concentration values of all metals and their respective fluctuation ranges are lower than the data reported in the literature for sediments in the Central-Northern Adriatic and comparable with the data from the blank phase.
- The concentrations of the sum of polychlorinated biphenyls (PCBs) in the surface sediments of the Terminal area and the control area, expressed as the sum of the congeners analysed, varies between 0.05 and 0.53 $\mu\text{g kg}^{-1}$ d.w., lower than the values found in the blank phase.
- In all the samples analysed, tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were below the method's limit of quantification.
- The concentrations of the disinfection by-products always showed values below the method's limit of quantification for each compound with the exception of a few stations where dibromoacetic acid (DBAA), bromochloroacetic acid (BCAA) and dalapon were detected with values just above the limits of quantification.
- Total hydrocarbons always present concentrations below the method's limit of quantification of 20 mg kg^{-1} d.w.
- Ecotoxicological responses: the area investigated was generally not affected by biologically relevant and widespread toxic effects. Only the assay with *Paracentrotus lividus* revealed toxic effects, albeit of a relatively modest extent in two samples (TE077 and TE078).

Sediments - 5th Monitoring Year - July 2015

- The concentrations of total PAH and individual congeners are essentially homogeneous between the various stations. The concentrations are low, with values between 20.7 and 53.1 $\mu\text{g kg}^{-1}$ around the terminal and between 21.2 and 86.4 $\mu\text{g kg}^{-1}$ in the control area, comparable with those found in

the previous blank, site and operational campaigns.

- The values determined for the metals are typical for predominantly sandy sediments and are little influenced by anthropogenic inputs; no significant variability can be observed between the different stations. The average concentration values of all metals and their respective fluctuation ranges are below the data reported in the literature for sediments in the central and northern Adriatic. For hazardous elements such as Pb, Cd, Cr, As and Hg, values are below the environmental quality standards.
- The concentrations of the sum of polychlorinated biphenyls (PCBs) in the surface sediments varied between 1.47 (TE080) and 3.42 ng g^{-1} d.w. (TE071) in the samples from the area affected by the structure and between 1.43 and 1.92 ng g^{-1} d.w. in the samples from the control area. This time, the concentrations are on average slightly higher than those found in the blank phase.
- The concentrations of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were below the quantification limits of the method at all stations.
- The concentrations of disinfection by-products showed values below the method's limit of quantification for the analytes monitored, with the exception of carbon tetrachloride, quantified in traces, at station TE064 only, and some haloacetic acids for which the method's limit of quantification was lowered this year. In particular, Monochloroacetic acid (MCAA), Dichloroacetic acid (DCAA) and Dibromoacetic acid (DBAA) were detected in some samples.
- Total hydrocarbons showed concentrations below the method's limit of quantification (5 mg kg^{-1} d.w.) at all stations, which was lowered in this campaign, with the exception of the values found at three stations, with values not exceeding 10.2 mg kg^{-1} d.w.
- Ecotoxicological responses: the area investigated was generally not affected by toxic effects. Only two stations (TE057 and TE079) showed moderate toxicity in the fertilisation test with the sea urchin *P. lividus*, which is known to be more sensitive than the other species.

Main Evaluations of the Results of Bioaccumulation and Biomarker Surveys in *Mytilus Galloprovincialis*

Bioaccumulation and Biomarkers in *Mytilus Galloprovincialis* - 1st Year of Monitoring

Bioaccumulation and biomarker investigations were carried out on individuals of *Mytilus galloprovincialis* reared in a control area and relocated to biomonitoring facilities in the vicinity of the Terminal's area, during the four campaigns of the first year of provisional operation.

- Total PAH are generally low, showing no contamination situations at the various stations; in particular, fluctuations in PAH levels in mussels are evident in relation to the organisms' seasonal reproductive cycle. The lowest average concentrations are found in October (range 22-33 ng g^{-1}) while the highest are found in December (range 88-92 ng g^{-1} d.w.). The values are lower than the values found in the blank and site phase and comparable with the values at the control station.
- The metal concentrations show fluctuations, which are mainly related to the reproductive cycle of the organisms. In general, higher average values are recorded in the fourth campaign (December 2011), in particular for manganese,

mercury, cadmium and copper, while iron shows higher values in the first two campaigns (September and October 2011). Nickel and chromium always present values below the method's detection limit in all four survey campaigns. For cadmium, values are higher in the fourth campaign at all stations than in any other campaigns, although the comparison with the control is not significant (the average value at the control station is even higher); arsenic and lead, on the other hand, show much lower average values in the fourth campaign than in the previous campaigns. The concentrations of all metals are in any case lower than or comparable with the values recorded in the blank phase, with the sole exception of lead, which is also higher in the control station in the interim phase. The fluctuations recorded over the four campaigns are, however, part of the natural variability linked to the physiology of the organism. The differences recorded compared to the control do not reveal any criticalities linked to the Terminal's activity.

- The concentration values of polychlorinated biphenyls (PCBs) showed rather homogenous values between the different stations and in the different campaigns. The average concentrations found were in the range of 15-26 ng/g, values that are generally comparable with the data acquired during the blank campaigns.
- The organostannic compounds in *Mytilus galloprovincialis* show the predominance of TBT (tributyltin), compared to DBT (dibutyltin) and MBT (monobutyltin), which were generally below the method's detection limits. TBT concentrations were low at all the stations and in the different campaigns, with average values between 5 and 8 ng Sn g⁻¹ d.w., lower than the values found in the blank phase campaigns.
- Disinfection by-products (Halomethanes, Alocetonitriles, Volatile Organic Compounds and Alocetic Acids) provided values generally below the quantification limits. However, some Alocetic Acids were also detected in the organisms of the control station. This phenomenon requires further analytical investigation in the next monitoring campaigns.
- The overall results of the biomarker analyses showed some slight oxidative effects in the organisms translocated to the Upper Adriatic sites during the third and fourth campaigns. The main changes involved an increase in metallothioneins, acetyl CoA oxidase and acetylcholinesterase activity, as well as a change in some antioxidant defenses and a decrease in lysosomal membrane stability. However, this slight oxidative disturbance is not believed to be correlated with the terminal's operating activities, but rather with environmental characteristics of the Upper Adriatic. The variations observed did not lead to the alteration of biologically relevant parameters such as the decrease in total antioxidant capacity, the appearance of genotoxicity phenomena or lipid peroxidation, thus not compromising a good state of health of the transplanted organisms. The overall processing of the results by means of a risk analysis model essentially excluded biologically relevant effects in the biomarkers of the transplanted mussels in the vicinity of the Terminal and attributable to the operating activities.

Bioaccumulation and Biomarkers in *Mytilus Galloprovincialis* - 2nd Year of Monitoring

- Total PAH concentrations in mussels show some fluctuations in relation to the organisms' seasonal reproductive

cycle. The lowest average concentrations were found in December 2012 (range 115-124 ng g⁻¹ d.w.) and the highest in February 2013 (range 143-186 ng g⁻¹ d.w.). The values were on average higher than the values found in the monitoring of the first year of operation, but comparable to the values found in the blank phase of the winter period and in general with those of the control station.

- Homogeneous average concentrations are observed for almost all metals. The fluctuations in the values recorded during the two campaigns are part of the natural variability linked to the physiology of the organism. The differences recorded with respect to the control are not significant and comparable concentrations are generally observed between the blank phase and the operating phase, showing no criticalities linked to the operation of the Terminal.
- The concentration values of polychlorinated biphenyls (PCBs) showed rather homogenous values between the different stations and in the different campaigns (concentration range: 19-29 ng g⁻¹). These concentrations were comparable with the values recorded in the control organisms in both campaigns (concentration range: 23-25 ng g⁻¹).
- The concentrations of TBT (tributyltin) alone were low in all the stations considered and in the different campaigns, with average values between 3 and 8 ng Sn g⁻¹ d.w., values comparable to or lower than those found in the winter campaigns of the blank phase. Both DBT (dibutyltin) and MBT (monobutyltin) were below the limit of quantification of the analytical method at all stations in both campaigns.
- The analytical determinations of disinfection by-products provided concentration values generally below the limits of quantification of the applied methods. Only chloroform and dichloroacetic acid were detected in some samples from both the control station and the Terminal area in quantities at or above the limit of quantification.
- The analyses of biomarkers carried out in the organisms translocated to the Upper Adriatic sites during the second year of provisional operation showed (mainly in the first campaign) a slight oxidative disturbance (GST, TOSCA ROO, GSH) associated with significant variations in the stability of lysosomal membranes and the degree of DNA fragmentation. However, this oxidative-type disturbance does not appear to be linked to the Terminal's operating activities, but rather to environmental characteristics of the Upper Adriatic, as highlighted by the risk analysis for this area.

Bioaccumulation and Biomarkers in *Mytilus Galloprovincialis* - 5th Year of Monitoring

- The concentrations of individual PAHs were homogeneous between the pools at each station in both the first and second campaigns, showing some fluctuations in relation to the organisms' seasonal reproductive cycle. In both campaigns, concentrations are particularly low both in the stations close to the Terminal and in the control station, with concentrations in the first campaign (May 2015) higher (30.2 - 44.4 ng g⁻¹ d.w.) than those found in the second campaign in July 2015 (15.9 - 22.1 ng g⁻¹ d.w.), which can be related with the mussels' seasonal biological cycle. The values were on average comparable or lower, in both sampling periods, to those determined in previous interim years as well as to those of the blank and site campaigns.

- The concentrations of the metals were on average higher in the first campaign in May 2015 than in the following one in July 2015, highlighting a natural variability due to the physiological characteristics of the organism and seasonality. For almost all metals, concentrations were generally comparable to those found in the blank phase and in the previous years of provisional operation, showing no particular criticalities linked to the Terminal's activity.
- The concentration values of polychlorinated biphenyls (PCBs) show average concentration ranges of 7.5 - 12.7 ng g⁻¹ d.w. in the first campaign (May 2015) and 3.5 - 14.5 ng g⁻¹ d.w. in the second campaign (July 2015). These concentrations are comparable with each other and with the values measured in the previous monitoring campaigns and in the reference campaign.
- TBT (tributyltin) concentrations were low at all stations, with average values between 3 and 5 ng Sn g⁻¹ d.w. in the first campaign in May 2015 and 2 and 5 ng Sn g⁻¹ d.w. in the second campaign in July 2015. The values are in line with the trend observed during all previous campaigns of the interim phase and comparable or lower than those of the blank phase. Both DBT (dibutyltin) and MBT (monobutyltin) were always below the limit of quantification of the analytical method.
- The analytical determinations of all disinfection by-products always provided concentration values below the limits of quantification of the applied methods.
- **Biomarkers:** the mollusc *Mytilus galloprovincialis*, transplanted at two different times of the year (May and July 2015) in the LNG Terminal area, showed a 'good' physiological state in general. Only the mussels used in the first campaign in May 2015 showed a 'slightly more stressed' physiological state, attributable to the spawning condition. Although effects at the level of acetylcholinesterase, peroxisomal proliferation and metallothioneins were detected in the May campaign, these were not such as to induce effects at the level of the antioxidant system nor genotoxicity effects. These results, similar to the results of the monitoring carried out in previous years during the provisional operating phase, make it possible to exclude, also for the 5th year of monitoring, the presence of relevant biological effects in mussels transplanted in the vicinity of the facility attributable to the terminal's operating activities.

Bioaccumulation and Analysis of Biomarkers in *Mytilus Galloprovincialis* - 1st Year, 2nd Quinquennium

For the bioaccumulation investigations and the analysis of biomarkers in *Mytilus galloprovincialis*, native organisms were sampled from two stations in the LNG Terminal area, one located on the N-NW side of the terminal and the other at the Meda, approximately 590 m south-east of the regasifier; a site located approximately 10 km north of the terminal, at the disused Campo Ada structure, was chosen as a 'control'. Sampling was carried out seasonally, in June and September 2018 and in March 2019.

- With regard to bioaccumulation assessments in *Mytilus galloprovincialis*, the seasonal mean concentrations of total PAH were low and homogenous among the organisms sampled at the different sites, varying between spring minima (13.3±0.9 ng g⁻¹) and winter maxima (17.7±1.4 ng g⁻¹). Slightly higher levels of PAH always characterised the control sites (16.8±2.6 ng g⁻¹). In all samples, the highest

contributions to total PAH were due to phenanthrene (35 ± 4 %), naphthalene (29 ± 3 %), fluoranthene (8 ± 2 %) and pyrene (8 ± 2 %). The concentrations of fluoranthene, referred to wet weight, were always below the environmental quality standard (30 µg kg⁻¹ w.w.), varying between a minimum of 0.14 ± 0.01 µg kg⁻¹ w.w., and a maximum of 0.38 ± 0.03 µg kg⁻¹ w.w., detected in June 2018 and March in control mussels, respectively. Among the PAH compounds, Benzo(a)pyrene is the only one on the lists of EC Regulation 1881/2006, which defines maximum levels of certain contaminants in food; the concentrations of this analyte (Middlebound: 0.25 ng g⁻¹ d.w.), in relation to the wet weight of the organisms, were markedly lower (≈0.05 µg kg⁻¹ w.w.) than the limit defined for bivalve molluscs (10 µg kg⁻¹ w.w.); the environmental quality standard set by DLGS 172/2015 for benzo(a)pyrene (EQS: 5 µg kg⁻¹ w.w.) was also never exceeded.

- For most of the metals sought, the accumulation levels in mussels followed a seasonal trend, with lower late spring (June 2018) values and increases between late summer (September 2018) and winter (March 2019). In particular, the highest mean levels of zinc (79.6 ± 1.8 µg g⁻¹), manganese (12.96 ± 0.58 µg g⁻¹), cadmium (0.76 ± 0.04 µg g⁻¹), and mercury (0.087 ± 0.002 µg g⁻¹) were detected in March 2019 in the control organisms sampled 10 km north of the terminal (Campo Ada). At the same time, the lowest values of mercury (0.057 ± 0.004 µg g⁻¹), cadmium (0.38 ± 0.03 µg g⁻¹), and zinc (58.5 ± 4.7 µg g⁻¹) were observed in the native mussels of Meda. Only vanadium reached the highest values during late spring (June 2018), in particular in samples taken in the terminal area (≈1.6 µg g⁻¹). For all metals, the bioaccumulation levels detected are comparable to those found between spring and summer 2015 (5th year of monitoring, 1st five-year period of operation) and in line with, or lower than, the values in the blank phase and in the previous years of provisional operation, thus not highlighting any particular criticality related to the Terminal's activity. The concentrations of cadmium, mercury and lead, identified as contaminants of foodstuffs by current European legislation (EC No. 1881/2006), referred to wet weight, were always below the limits established by the regulation (Cd: 1 mg kg⁻¹ w.w., Hg: 0.5 mg kg⁻¹ w.w., Pb: 1.5 mg kg⁻¹ w.w.).
- Samples of *Mytilus galloprovincialis* taken from natural populations in the terminal area and in Campo Ada showed no contamination by chlorination by-products (halophenols, haloacetic acids, halomethanes and haloacetonitriles) and organostannic compounds (TBT, DBT and MBT). The values obtained for these compounds were always below the relative quantification limits of the method, except for tributyltin (TBT) which showed values (5 ng g⁻¹) slightly above the LOQ in mussels taken from the terminal, on the North Northwest side in September 2018.
- In general, the majority of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), including the most toxic congeners (2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD and 2,3,4,7,8-PentaCDF) were below the respective method quantification limit; 1,2,3,4,7,8-HexaCDD was sporadically detected in mussels taken from the Meda in June 2018 (0.55 pg g⁻¹ d.w.), 2,3,7,8-TCDF, in all samples in March 2019, with values ranging from 0.80 pg g⁻¹ d.w. to 0.92 pg g⁻¹ d.w. and the 1,2,3,7,8,9-HexaCDF, in

control mussels in June 2018 (0.45 pg g⁻¹ d.w.). In contrast, with regard to dioxin-like polychlorinated biphenyls (PCB DLs), only PCB 81 and PCB 169 were below the method's detection limit in all samples analysed. The congeners that reached the highest concentrations, i.e. PCB 118, PCB 105, PCB 77, PCB 167 and PCB 156, which are considered to be of low toxicity, showed the generally highest values in the organisms taken in March 2019. In all samples, the summation of PCDDs, PCDFs and dioxin-like PCBs, in terms of toxicity equivalent, was markedly below the limit set (EQS: 6.5 pg TE g⁻¹ p.u.) by DLGS 172/2015, varying between a minimum of 0.36 pg (TE) g⁻¹ w.w. in control mussels in June 2018, and a maximum of 0.67 pg (TE) g⁻¹ w.w. in organisms sampled from the terminal (TE045T) in September 2018.

- Evaluations of biological stress indices (biomarkers) conducted on the *Mytilus galloprovincialis* species indicated an overall good physiological state for native organisms sampled in the terminal area and comparable to that of the control mussels, sampled from the abandoned structures of the Ada Field, located 10 km north of the terminal. Compared to the reference mussels, significant but sporadic alterations were mainly observed in the organisms sampled on the North side of the terminal, on generic stress indicators (i.e. catalase and malondialdehyde in September 2018) and specific indicators (i.e. acetylcholinesterase, in June 2018 and March 2019). This variability may also be induced by factors not correlated to the presence of contamination but rather to variations in environmental parameters, such as temperature and salinity, which influence, both directly and indirectly, the biological cycle of the organisms. At the same time, no major alterations to the antioxidant system or genotoxicity effects were observed.

Main Evaluations of the Results of Bioaccumulation and Biomarker Surveys Carried out on Species of Interest for Fisheries

Bioaccumulation and Biomarker in Species of Interest in Fisheries - 1st Year of Monitoring

With regard to the bioaccumulation and biomarker surveys carried out on the species of fishing interest caught in both the March and December 2011 campaigns, the following was reported with regard to the species *Squalus acanthias*, the only species caught in both monitored stations:

- The average concentrations of total PAHs found in the muscles and livers of *Squalus acanthias* in the sampling campaign carried out in March 2011 were relatively low, ranging between 40 and 63 ng g⁻¹ d.w. for muscle and between 122 and 134 ng g⁻¹ d.w. for liver. In the December 2011 sampling campaign, the average concentrations of total PAH in muscle tissue for both stations were around 40 ng g⁻¹ d.w., while those in livers were higher at around 210 ng g⁻¹. Overall, the mean concentrations of total PAH are higher in livers than in muscle. Comparison of the concentrations detected in the Terminal area and in the control, area indicate no significant differences in bioaccumulation between the two stations for either muscle or liver tissue. With regard to chemical risk, the Benzo(a)pyrene value reported in EC Regulation 1881/2006 in fish muscle of 2 ng g⁻¹ w.w. is never exceeded.

- With regard to metals in the tissues of *Squalus acanthias*, barium, chromium, manganese and nickel, concentrations are always below the limit of quantification. In general, it is observed that cadmium, copper and iron tend to accumulate mainly in liver tissue, while arsenic and mercury, in the muscle tissue of fish. For the first campaign, mercury and arsenic show rather high concentrations in the muscle at both stations, already reported in the blank and site phase for cartilage species: mercury at the station close to the Terminal (TE042) reaches a maximum concentration of 5.631 mg kg⁻¹ d.w. and in the control 2.863 mg kg⁻¹ d.w., while arsenic shows a maximum concentration in the control station (TE043) of 303.240 mg kg⁻¹ d.w. and 130.089 mg kg⁻¹ d.w. in the station close to the Terminal. In the second campaign, mercury reaches an average concentration of 4.057 mg kg⁻¹ d.w. in the station close to the Terminal, while in the control station it had an average concentration of 2.218 mg kg⁻¹ d.w. The arsenic values found in both stations of the second campaign were considerably lower than in the first, being between 20-50 mg kg⁻¹ d.w. With regard to chemical risk, Regulation (EC) No. 1881/2006 indicates a threshold value of 1 mg kg⁻¹ w.w. for mercury in shark muscle. The concentrations detected, making the due dry weight/wet weight conversion equal to 0.2, reveal a slight exceeding of the limit (average value of 1.12 mg kg⁻¹ w.w.) only in the first campaign at the station near the Terminal (TE042). For arsenic, the data available in the literature show great variability in fish products in the Adriatic, depending on different dietary habits (Istisan Reports 04/4), recording values in the Adriatic of arsenic as high as 52 mg kg⁻¹ w.w., equal to 208 mg kg⁻¹ d.w. (Storelli et al., 2004). The differences found in the two campaigns, particularly for As, and to a lesser extent for Cu, Fe and Zn, can probably be attributed to seasonality rather than to impacts linked to the LNG Terminal's activity.
- The concentration range of total PCBs detected in the muscle tissue in the first campaign ranges from 32.10 to 78.58 ng g⁻¹ in the station closest to the Terminal and the control station respectively, while the amount determined in the liver is much higher and ranges from 475.94 to 933.85 ng g⁻¹ in the control and the station closest to the Terminal respectively. In the second campaign, concentrations in muscle tissue ranged from 68.37 ng g⁻¹ at the station closest to the Terminal to 207.24 ng g⁻¹ at TE043, whereas the amount determined in the liver ranged from 932.01 to 1341.07 ng g⁻¹ at the control and the station closest to the Terminal, respectively. The high concentrations found in the livers of the species analysed, is a characteristic that has already been observed, albeit to a lesser extent, in the previous blank and site campaigns; subsequent monitoring will allow further analytical verifications to be carried out on this parameter.
- In the muscle and liver samples analysed, only TBT was detected among the organostannic compounds, while DBT and MBT concentrations were generally below the limit of determination, with the exception of the muscle sample from the control station of the first campaign. The tendency for preferential accumulation of TBT in muscle over liver for shark species, already highlighted in the blank and site campaigns, emerged. The levels of TBT contamination found in the two matrices analysed (muscle and liver) were low and comparable to the data reported in the scientific

literature on the accumulation of organostannic compounds in related fish species. There were also no significant differences in concentrations of *Squalus acanthias* between the different stations in both tissues analysed.

- The analytical determinations of disinfection by-products (Halomethanes, Haloacetonitriles, Volatile Organic Compounds and Haloacetic Acids) performed on the muscle, yielded concentration values generally below the quantification limits of the applied methods. However, some Haloacetic Acids were also detected in the control station. This phenomenon requires further analytical investigation in the next monitoring campaigns.
- As far as biomarkers are concerned, the results obtained showed certain oxidative effects in the elasmobranchs sampled (*Squalus acanthias*), which led to a decrease in total antioxidant capacity, peroxisomal activity and an increase in lipid peroxidation. The magnitude of these changes is not particularly high, making it possible to exclude biologically relevant effects on the physiology, ecology or health of these organisms. However, the overall picture of all biological responses processed by means of a risk analysis model provided a summary index of biomarker alterations of 'moderate' in the transition from the reference to the operating phase, thus suggesting the need to continue monitoring activities on these elasmobranch species.

Bioaccumulation and Biomarkers in Species of Interest in Fisheries - 2nd Year of Monitoring

The following was reported with regard to the species *Squalus acanthias*, the only one caught in both November and December 2012 campaigns:

- The average concentration of total PAHs found in the muscle (23 ng g⁻¹ d.w.) and liver (161 ng g⁻¹ d.w.) of *Squalus acanthias* in the sampling campaign carried out in November 2012 was higher than in the December 2012 campaign, in which an average value of 13 ng g⁻¹ d.w. was found in the muscle and 91 ng g⁻¹ d.w. in the liver. The average concentrations of total PAH were, as already shown in previous monitoring, higher in the liver than in the muscle. As far as chemical risk is concerned, the value of Benzo(a)pyrene listed in EC Regulation 1881/2006 for fish muscle (i.e. 2 ng g⁻¹ d.w.) is never exceeded.
- With regard to metals in the tissues of *Squalus acanthias*, barium, chromium, iron, manganese and nickel concentrations are below the limit of quantification. Cadmium and copper accumulate mainly in liver tissue, while arsenic and mercury in fish muscle tissue. For the first campaign, mercury and arsenic show rather high concentrations in the muscle of cartilaginous species, reported already in the reference station and first year of provisional operation: mercury at control station TE043 reaches the highest average concentration of 3.1 mg kg⁻¹ d.w., while arsenic shows an average concentration at the same station of 176 mg kg⁻¹ d.w. In the second campaign, mercury reaches a maximum average concentration of 3.5 mg kg⁻¹ d.w., and arsenic 234 mg kg⁻¹ d.w. at station TE042. As far as chemical risk is concerned, Regulation (EC) No. 1881/2006 reports a threshold value of 1 mg kg⁻¹ w.w. for mercury in shark muscle: the concentrations detected, making the due dry weight/wet weight conversion, do not exceed the value reported in the Regulation. For arsenic, the data in literature show great variability

in fish products in the Adriatic Sea, depending on different dietary habits (Istisan Reports 04/4), indicating values as high as 52 mg As kg⁻¹ w.w. in the Adriatic, equal to 208 mg kg⁻¹ d.w. (Storelli et al., 2004).

- The average concentration of total PCBs measured in the muscle of *Squalus acanthias* in the first campaign is 64 ng g⁻¹ d.w. while the amount determined in the liver is considerably higher at 629 ng g⁻¹ d.w. In the second campaign, an average concentration of 81 ng g⁻¹ d.w. was found in muscle, while in the liver was 649 ng g⁻¹ d.w. The high concentrations found in the liver is a characteristic already found in the previous reference campaigns, at the construction site and in the first year of temporary operation.
- In the muscle and liver samples of *Squalus acanthias*, only tributyltin (TBT) was detected among the organostannic compounds, while the concentrations of DBT and MBT were always below the method's limit of determination. The tendency for preferential accumulation of TBT in muscle compared to the liver, which had already been highlighted in the blank, site and exercise campaigns, emerged. The levels of TBT contamination found in the two matrices muscle and liver (values in the two campaigns in the range of 6-7 ng Sn g⁻¹ d.w. and 3-5 ng Sn g⁻¹ d.w. respectively) are low when compared to the data reported in the literature on the accumulation of organostannic compounds in related fish species.
- The analysis of disinfection by-products (halomethanes, haloacetonitriles, volatile organic compounds and haloacetic acids) carried out on the muscle of *Squalus acanthias* provided values generally below the limits of quantification. However, chloroform and dichloroacetic acid were detected in both campaigns.

Bioaccumulation and Biomarkers in Species of Interest for Fisheries - 5th Year of Monitoring

With regard to the bioaccumulation and biomarker studies carried out on the species of interest to fisheries (two campaigns in December 2015), the following information has been provided only for *Squalus acanthias*, as it was the only species sampled in both campaigns and stations (near the terminal and in the control) and the only one for which a comparison with previous monitoring campaigns could also be made.

- The average concentration of total PAHs found in the muscle of *Squalus acanthias* was always low, below 20 ng g⁻¹ d.w., and with considerably higher values in the live liver (range 74.5-142.7 ng g⁻¹ d.w.), as already highlighted in the previous monitoring campaigns. The values found in both tissues at the station in the vicinity of the Terminal and at the control station were comparable and fell within the concentration range found in the previous reference, site and interim campaigns. The Benzo(a)pyrene value, set by EC Regulation 1881/2006 in fish muscle at 2 ng g⁻¹ w.w. is never exceeded.
- With regard to metals, as in the previous campaigns it is confirmed that cadmium, copper, iron, manganese and zinc accumulate mainly in liver tissue. Conversely, mercury and arsenic accumulate preferentially in muscle tissue. In the muscle of *S. acanthias*, mercury and arsenic reach rather high concentrations. With regard to mercury, in the first campaign a concentration of more than 3 mg kg⁻¹ d.w. was recorded at station TE042, and in the second campaign

high values were recorded with maximum concentrations at control station TE043 (6.7 mg kg⁻¹ d.w.). The Directive 2013/39/EU, and its Italian transposition (D. lgs 172/2015), set the Environmental Quality Standard for mercury in biota at 20 µg kg⁻¹ w.w. Regulation (EC) No. 1881/2006 establish the limit for human consumption of Hg, Cd and Pb in mussels and fish. These values are expressed in wet weight, while the campaign's values are expressed in dry weight (the dry weight/wet weight conversion factor is approximately 0.2). The conversion of the data indicates that, for Hg in both campaigns, concentrations always exceed the reference EQS and sometimes even exceed the health limit (0.50 mg kg⁻¹ w.w. for fish muscle and 1 mg kg⁻¹ w.w. for shark muscle) at both stations TE042 and TE043.

For Cadmium and Lead, values were always below the threshold value of Reg. 1881/2006. For arsenic in *Squalus acanthias*, the highest value of 264 mg kg⁻¹ d.w. was found at point TE042 in the first campaign. In the literature, arsenic levels show great variability in fish products from the Adriatic Sea: Storelli et al. (2004) record arsenic concentrations of up to 52 mg kg⁻¹ w.w., equal to 208 mg Kg⁻¹ d.w. For most of the analytes, no significant variations are found between the average values of the concentrations determined in the reference phase compared to the provisional operating phase. However, some fluctuations found in the bioaccumulation values in the muscle and liver tissue of this species are presumably linked to the specific natural variability of the individuals analysed during the various campaigns and the seasonality of the sampling.

- The average concentration of total PCBs detected in the two tissues of *Squalus acanthias* was, in both campaigns, higher in the control station than in the one near the Terminal, with average values higher in the latter than in the former campaign. In particular, in the first campaign at the control station TE043, an average concentration of 106 ng g⁻¹ d.w. was found in muscle, which was higher than at the station near the Terminal TE042 (56 ng g⁻¹ d.w.), as well as in livers (control station TE043: 1901 ng g⁻¹ d.w., TE042: 740 ng g⁻¹ d.w.). In the second campaign, an average concentration of 165 ng g⁻¹ d.w. was found in muscle at control station TE043 compared to the station near Terminal TE042 (108 ng g⁻¹ d.w.) as well as in livers (control station TE043: 2582 ng g⁻¹ d.w., TE042: 1297 ng g⁻¹ d.w.). The high concentrations found in the livers is a characteristic already observed in the previous blank, site and first four years of temporary operation. The values found were on average higher than the values found in the reference phase in muscle and liver at both stations.
- Tributyltin (TBT) was detected in all samples, both muscle and liver, with rather low and comparable values between the station close to the terminal and the control station, while the concentrations of DBT and MBT were always below the method's limit of quantification. The levels of TBT contamination found in the two tissues muscle and liver (values for both in the range of 4-5 ng Sn g⁻¹ d.w.) are low compared to the data reported in the scientific literature on the accumulation of organostannic compounds in related fish species. The levels of TBT are comparable to those found during previous campaigns and lower than those determined in some reference campaigns.

- The levels of disinfection by-products (halomethanes, haloacetonitriles, volatile organic compounds and haloacetic acids) carried out on the muscle of *Squalus acanthias* in the first and second fishing season yielded concentration values always below the method's limit of quantification for each compound analysed.
- Analyses of biomarkers carried out on *Squalus acanthias* have confirmed, also with data from the latest campaigns in 2015, that some of the observed oxidative effects are mild and transient. However, the magnitude of these changes is such that biologically relevant effects on the physiology, ecology and health of these organisms are excluded.

Main Evaluations of the Results of Planktonic Population Surveys

Phytoplankton and Zooplankton - 1st Year of Monitoring - September 2010

The phytoplanktonic community presents both in terms of abundance and biomass (µg C l⁻¹) at all stations a prevalence of flagellates followed by diatoms, dinophyceae and coccolithophorids. The species observed are typical of late summer, when the water column presents stratified conditions. Rather unusual are the higher concentrations at 12m than at the surface, found in TE135 and TE136 on the west side with a preponderant contribution of phytoflagellates (around 90%). On the south side of the regasifier, station TE131 located at 100m and TE133 located at 1000m present, on the contrary, the highest concentrations at the surface and slightly lower at 12m mainly due, also in this case, to phytoflagellates. The specific composition includes heterotrophic species and also toxic species in particular (Diarrhetic Shellfish Poisoning).

The zooplanktonic population appears unevenly distributed among the various stations, and in particular is strongly more abundant in the stations located to the south and east of the system, with a substantial reduction in the stations located to the north. Over 60% of the zooplanktonic population consists of copepods. Species of cladocerae and larval forms are also present. The stations furthest from the structure on the north (TE137) and west (TE135) show the least diversity. There is a different specific composition of the zooplanktonic population at the different stations. A possible concentration effect of zooplankton in the vicinity of the structure will have to be confirmed in future samplings.

Ichthyoplankton - 1st Year of Monitoring - September 2010

With regard to ichthyoplankton (eggs and larvae of teleosts), a preponderant presence of anchovy (*Engraulis encrasicolus*) eggs was detected. However, the number of anchovy larvae was always modest. For the eggs of other teleosts, it was only possible to identify a few morphological types without arriving at a systematic recognition. It should be noted that some eggs sampled at stations TE131 (100 m south of the terminal), TE137 and TE138 (1000 m and 100 m north of the terminal respectively) were in an obvious state of degeneration (possibly because they were not fertilized).

When comparing the egg concentrations, the samples from stations TE138 and TE140 (100 m north and east of the terminal, respectively) showed higher values than those from stations TE131 and TE136, which are located 100 m south -under the

outlet flow- and west -on the side of the water inlet-, respectively. In general, a diverse occurrence of ichthyoplankton was observed around the terminal, which needs to be confirmed during the next monitoring activities.

Phytoplankton and Zooplankton - 1st Year of Monitoring - July 2011

Phytoplankton shows a predominance of phytoflagellates in the stations south of the regasifier, while diatoms predominate in the stations west of it; dinoficeae and coccolithophorids are hardly present in quantitative terms. The largest quantities of phytoflagellates and diatoms of the genus *Pseudo-nitzschia* are found in the stations west of the plant (TE135 and TE136 at 1000 m and 100 m from the terminal respectively, both at the surface and at 12m depth); at the surface, the stations south of the plant (TE131 and TE132 at 100m and 500m from the plant respectively) have the lowest abundances. At 12m depth, there are no major differences between the four stations, with the exception of a slight diatom dominance at station TE136, which is located west of the terminal. It is worth noting that in the northern Adriatic, in terms of annual phytoplanktonic succession, the genus *Pseudo-nitzschia* usually occurs with bloom phenomena, but usually in late winter-spring and after nutrient inputs due to precipitation typical of this period. In contrast, the sampling carried out in September 2010 showed a community completely dominated by phytoflagellates, with a clearer distinction in terms of abundance between the stations in the south of the site, which were the most abundant, and the stations in the west, where abundance was low.

The zooplankton is characterized by a significant presence of cladocerans, which are typical of summer and make up on average 47% of the total population, and copepods, which make up on average 37% of the population. Zooplankton biomass is generally lower than that measured in September 2010, with a minimum at station TE131 100 m south of the estuary and a maximum at station TE140 100 m east of the structure. The high biomass observed at station TE140 appears to confirm the concentration of zooplankton near the eastern side of the structure, which was already observed during the September 2010 sampling, although this is not confirmed by the abundance data, which are higher at the station 1000 m north of the structure.

Ichthyoplankton - 1st Year of Monitoring - July 2011

As for the larvae, abundances in the range of 11.34-330.42/100 m³ were found, mainly belonging to the anchovy (*Engraulis encrasicolus*). A few larvae of horse mackerel (*Trachurus* sp.) were found in the samples, but none of *Sardinella*. However, the clear predominance of anchovy larvae over eggs (medians of 15.47 eggs/100 m³ and 54.36 larvae/100 m³) shows that the sampling took place during a period of low spawning. In general, the morphological appearance of the larvae and eggs was 'normal' (i.e. comparable to what is normally found in similarly sampled ichthyoplankton specimens), with the exception of the anchovy. Even the proportion of 'dead' or 'damaged' anchovy eggs was not high, considering that many eggs of this species were at an early stage of development and therefore characterized by higher mortality. The ichthyoplankton samples taken therefore do not appear to be characterized by particularly high morphological or embryonic development abnormalities. The abundance levels calculated at each station show that teleost eggs and larvae are

significantly more abundant near the LNG terminal, presumably due to the attraction that submerged structures can exert on many fish. The continuation of monitoring activities will allow further assessment elements to be recorded over the years to verify the possible influence of the structure on the planktonic populations.

Phytoplankton and Zooplankton - 2nd Year of Monitoring - July 2012

The phytoplankton community consisted mainly of diatoms and flagellates, which predominated over dinoflagellates and coccolithophores in terms of both abundance and biomass. Station TE145, located 20 m southwest of the terminal, was the one with the highest abundance, while the stations south of the terminal (TE134 -12 m at 2 km and TE131 at 100 m) had the lowest abundance. In terms of species diversity, diatoms and dinoflagellates had the greatest number of taxa, with no differences between stations; station TE145 again proved to be the 'richest'. The overall abundance values were comparable to those of the previous sampling carried out 12 months earlier. The diatom community was characterised by different species of the genus *Chaetoceros*, while the genus *Pseudo-nitzschia* dominated during the sampling in July 2011. In general, a specific variability within the analysed phytoplankton community is confirmed.

The zooplankton population sampled in July 2012 showed the typical composition of the summer season, characterised by the strong presence of cladocerans, followed by copepods. At all stations, cladocerus *Penilia avirostris* was the most abundant species, almost always followed by the other cladocerus species and juvenile forms of copepods of the genus *Paracalanus* and *Paracalanus parvus*. As with the last monitoring in July 2011, the values measured for specific diversity are within the range measured for this index in the upper Adriatic. The analysis of the specific composition of the mesozooplanktonic population did not reveal any particular differences between the stations, while confirming that the stations east/southeast and southwest of the site seem to differ quantitatively from the others by having the highest abundances and biomasses, suggesting an effect of zooplankton concentration in the vicinity of the facility.

Ichthyoplankton - 2nd Year of Monitoring - July 2012

With regard to ichthyoplankton (eggs and larvae of teleosts), numerous eggs were found with abundances in the range 399.37-965.88 eggs per 100 cubic metres of filtered water, significantly higher than those found in the 2011 monitoring (17.06-276.60/100 m³). Among the eggs definitely identified there were small batches of those of anchovies (*Engraulis encrasicolus*), with small abundances of 4.30-18.57 eggs/100 m³ (median: 8.98 eggs/100 m³) and somewhat larger batches of those of sardinella (*Sardinella aurita*), at levels ranging between 4.74 and 41.62 eggs/100 m³ (median: 27.35 eggs/100 m³). The presence of eggs and the simultaneous absence of sardinella larvae is interesting in that it is presumably linked to high water temperatures, since the females of this species mainly spawn at over 23-24 °C (temperatures measured during monitoring with the multi-parameter probe), i.e. at much higher temperature ranges than those of anchovies.

If the comparison is limited to the presence of *E. encrasicolus* alone, the situation is reversed, as anchovy eggs and larvae were hardly present in the samples from July 2012, whereas abun-

dances of 0.00-31 eggs/100 m³ and 8.67-313.55 ind./100 m³ were calculated for the larvae of the eight analysed stations during the previous monitoring twelve months earlier. However, the presence of sardine eggs in the samples, a phenomenon that indicates high temperatures in the upper part of the water column, suggests that the anchovy spawners had stopped spawning due to the unfavourable temperatures for this species. However, it cannot be ruled out that the anomalous atmospheric temperature trend in 2012, with high values in late spring and summer, led to a 'slippage' in the anchovy spawning season compared to the period that has prevailed in recent years.

Phytoplankton and Zooplankton - 5th Year of Monitoring - July 2015

The phytoplankton community shows typical characteristics of the phytoplankton population of the northern Adriatic Sea in summer, both in terms of abundance and specific composition. It consisted mainly of diatoms and phytoflagellates, which were dominant in terms of abundance. Regarding the contribution of each group to the biomass, diatoms are the dominant group, followed by dinoflagellates, which are present with relatively low abundance values but contribute significantly to the biomass. Phytoflagellates, although present in high densities, contribute to the biomass to a much lesser extent. There is no clear trend in the phytoplankton population in terms of the number of taxa, abundance and biomass in relation to the presence of the regasifier.

The zooplankton population shows a slight deviation in composition from the summer populations typically described for the northern Adriatic. The summer mesozooplankton is normally dominated by cladocerans, especially the species *Penilia avirostris*. The samples of the present study, collected in July 2015, are mainly composed of copepods (67-87%) and larvae (7-21%), while Cladocerae show a relative abundance between 4-13% (with *P. avirostris* dominating). However, the planktonic communities generally show strong interannual variations in abundance and specific composition. In this context, it is important to note that 2015 was a year of thermal anomalies in the Adriatic Sea, with lower rainfall and freshwater inputs from the Po River, which may have affected the composition of the mesozooplankton and delayed the transition from copepods to cladocerans. There is no clear trend in the zooplankton population in terms of the number of taxa, the number of individuals and the biomass in relation to the presence of the regasifier.

Ichthyoplankton - 5th Year of Monitoring - July 2015

In the ichthyoplankton (eggs and larvae of Teleostei), both eggs and larvae of Teleostei were found in all samples in July 2015; in the species *Engraulis encrasicolus* (anchovy) in particular, the eggs were almost completely absent and the larvae were rare or not very common everywhere. The abundance of anchovy eggs and larvae appears to be similar to the ichthyoplankton samples collected in July 2012 and 2014 (but not in July 2013, when many eggs but no larvae were found). The presence of some eggs (but not larvae) of *Sardinella aurita* had also been detected in some of the sampling from previous years. As for the heterogeneous group 'other Teleostei' (i.e. all Teleostei species except the Mediterranean anchovy and the Clupeidae), most of the eggs of this large assemblage of bony fishes were assigned to the genus *Arnoglossus* (which includes species of little or no commercial value in this sea).

In contrast to the eggs, the larvae of the 'other Teleostei' group were scarce, but similar situations were also found in the ichthyoplanktonic samples from July 2012 and September 2011. The presence of larvae of certain species (e.g. *Buglossidium luteum*) also corresponded to what was observed in the samples from previous years' summers. As with phytoplankton and zooplankton, there is no clear trend in ichthyoplankton in terms of the abundance of eggs and larvae of bony fish (especially anchovies) in relation to the presence of the regasifier.

Main Evaluations of Survey on Macrozoobenthic and Meio-benthic Populations

Macrozoobenthic and Meiobenthic Populations - 1st Year of Monitoring - September 2010

When analyzing the composition of the population in the three campaigns (the Reference campaign (November 2006), the Site-construction campaign (October 2008) and the Operational campaign (September 2010), the following was established:

- in the Operational phase, the numerical abundance of each of the studied tax an increase compared to the previous two phases;
- the abundance of polychaetes increases substantially in the Operational phase;
- molluscs reach almost three times their original abundance values in the Operational phase;
- crustaceans and echinoderms, which had declined sharply during the Site-construction phase, return to abundance values comparable to those of the Reference phase during the Operational phase.

The comparison of the species richness of the taxa and the analysis of the differences between the Reference, the Site-construction phase and the operational phase have shown that:

- the species richness of polychaetes increases during the Operational phase compared to the previous two phases;
- molluscs and crustaceans, which were represented by fewer species during the Site-construction phase, again reach values comparable to those of the Reference phase;
- echinoderms, which were represented by a small number of species in the Reference phase, show a slight increase in values in the Site-construction phase and Operational phases.

As far as the stations in the Control area are concerned, they are never connected to the stations located in the Terminal area of the corresponding campaign, but are always slightly detached from them. This can be interpreted as evidence that a change has also taken place at these stations, suggesting changes related to natural causes rather than the direct influence of the Terminal structure.

A comparison of the structural parameters in the three different campaigns (see above) also shows that the Control stations are affected by fluctuations in the main ecological indices analyzed, further evidence of changes in the community structure. It is interesting to note that in the case of the Shannon-Wiener specific diversity index, the value of the index returns to almost identical values in the Operational phase as in the Reference phase.

As for the meiobenthic population, the average density values are within the range reported for other sites in the Mediterranean.

nean, with a high homogeneity of the fauna. The meiobenthic biocoenosis consists of organisms belonging to eighteen main groups. Nematodes are the dominant group and generally account for more than 90% of the total population, followed by harpacticoid copepods, polychaetes and nauplii. Higher densities of harpacticoid copepods and nauplii were found at stations TE038 (control), TE078 and TE079, which may be related to differences in the presence of nutrients specific to this taxon or other factors selectively affecting these three sites. The ecological indices and the Nematode/Copepod ratio showed that there were changes in terms of fauna at site TE075, where the population was almost exclusively characterized by nematodes. It is possible that the sediment was in a highly hypoxic state; however, based on current knowledge, it is not possible to hypothesize that this possible change in the seabed is related to the operation of the LNG terminal.

Macrozoobenthic and Meiobenthic Populations - 1st Year of Monitoring - July 2011

The macrozoobenthic population of July 2011 campaign is characterized by a few species associated with the biocenosis of well-calibrated fine sands (such as the polychaete *Owenia fusiformis*), with a prevalence of mysticolous species (such as the mollusc *Phaxas adriaticus*), of species with a greater affinity for muddy bottoms (the holothuroid *Labidoplax digitata*, the polychaetes *Goniada maculata* and *Ampharete acutifrons*, and the amphipod *Ampelisca typica*), and species related to the coastal-detritic biocenosis (the polychaetes *Galathowenia oculata* and the amphipod *Ampelisca tenuicornis*). In spatial terms, this campaign revealed differences in both univariate and multivariate indices between the community of the stations in the Terminal area and that of the control stations, a fact that was already highlighted in previous monitoring campaigns. In particular, in terms of abundance and number of species, the control stations appear to be characterized by lower values than those found in the Terminal area, while for the Simpson evenness and diversity indices, higher values were observed in the control stations than in the Terminal area.

From a temporal point of view, a comparison of the structural parameters of the previous Reference, Site-construction and Exercise campaigns with the campaign in July 2011 shows that both the control stations and the stations in the Terminal area show changes in the most important ecological indices, which indicate changes in the structure of the community compared to the previous campaigns. The changes in the community also observed at the control stations could be mainly due to seasonal fluctuations, which are probably due to environmental stress of a transient nature and not directly related to the offshore structure. In general, coastal marine benthic populations in large parts of the Upper Adriatic are naturally exposed to environmental stresses, both natural and anthropogenic. The macrobenthic community is able to respond to such disturbances with a high resilience, i.e. to survive the changes even under unfavourable conditions, and resilience, i.e. recovering almost completely the original ecological balance in a relatively short time.

The meiofauna of the seabed near the LNG terminal during the Reference phase, analysed in July 2011, shows average densities in the range reported for other sites in the Mediterranean. The meiobenthic biocoenosis consists of organisms belonging

to twenty main groups. Nematodes are the predominant group and generally account for about 90% of the total population, followed by harpacticoid copepods, nauplii and polychaetes. The area under investigation shows a high homogeneity of the fauna, so that it is not possible to detect significant differences between the sites selected as controls and the stations close to the Terminal. However, the density of nematodes in the control stations is significantly lower than in the Terminal area, a discrepancy that currently seems to be related to edaphic factors or trophic aspects of the seabed. Subsequent monitoring activities will lead to a better understanding of trends in the meiobenthic community.

Macrozoobenthic and Meiobenthic Populations - 2nd Year of Monitoring - 2012

The following remarks should be made on the surveys of benthic populations (macrozoobenthos and meiobenthos) carried out in July and September 2012 for the second year of monitoring of the Operational phase.

During the July 2012 campaign, some homogeneity was observed between the macrozoobenthic community in the area near the Terminal and the control station, while during the September 2012 sampling, some differences appeared in relation to two of the control stations (TE037 and TE038), probably related to seasonal variations in the macrozoobenthic population structure.

As for the temporal comparison between the campaigns of the Reference phase (November 2006) and those of the second year of the Operational phase (July/September 2012), a certain similarity can be observed in terms of community structure indices (abundance of individuals A, specific richness S, Shannon-Wiener specific diversity H', Pielou's equitability J'). In fact, the values in this monitoring campaign are comparable to those of the Reference phase. The statistical analysis also showed no significant changes in the structure of the soft-bottom macrozoobenthic community between the two periods.

The benthic populations of coastal marine areas in large parts of the upper Adriatic are naturally exposed to environmental pressures, both of natural origin and due to human activities. The macrobenthic community is able to respond to such disturbances with high resilience, i.e. to survive the changes even under unfavourable conditions, and with high resilience, i.e. to almost completely restore the original ecological balance in a relatively short time.

The meiofauna of the seabed near the LNG terminal in the second year of monitoring during the Operational phase in July and September 2012 shows average densities that are within the range reported for other sites in the Mediterranean. The meiobenthic biocoenosis consists of organisms belonging to a total of twenty main groups. Nematodes represent the dominant taxon with more than 80% of the total population, followed by harpacticoid copepods, their juvenile stages (nauplii) and polychaetes. The area under investigation shows a good faunistic similarity (similarity of about 86%), although univariate analyses revealed some differences in the values of the structural indices. In particular, the control sites (TE037 and TE038) showed higher values of the diversity and evenness indices, lower values of the dominance index and the nematode/copepod ratio compared to some stations in the terminal area. The largest differences observed

in both campaigns compared to the control stations were at station TE057, located 100 m south-east of the plant. Despite these results, the multivariate analyses (cluster, MDS, ANOSIM) showed a high similarity of fauna between the samples analyzed.

Macrozoobenthic and Meiobenthic Populations - 5th Year of Monitoring

As far as the macrozoobenthos is concerned, the ecological and structural indices showed no significant differences between the terminal and control communities during the campaign in July 2015. In the second campaign (October 2015), however, the values of the ecological and structural indices, J' , $H'(\log_e)$ and $1-\lambda$, were on average higher at the control stations than in the Terminal area. In contrast, the nMDS analyses of the abundance matrices and the Cluster analyses for both campaigns (July and October 2015) showed a difference in community structure between the control stations and those in the Terminal area. However, the data available so far show that these differences are largely natural and not due to the presence of the terminal. In general, it can be stated that after a period of disturbance due to the activities of the construction phase, the benthic populations recovered in a relatively short time to conditions of abundance and species richness comparable to those of the blank phase.

This type of process is typical of marine coastal areas in large parts of the upper Adriatic, which are naturally exposed to environmental pressures that are both of natural origin (Steckbauer et al., 2011) and caused by anthropogenic activities (Simonini et al., 2005; Morello et al., 2005). Macrobenthic communities are indeed able to respond to such disturbances with high resilience (i.e. they survive unfavourable conditions) and resistance (i.e. they almost completely restore the original ecological balance in a relatively short time). The subsequent temporal evolution of the population composition during the Operation phase in the study area therefore seems to depend essentially on environmental conditions at the macro-scale in the North Adriatic coastal area and not on the presence or activities of the Terminal.

The meiofauna of the seabed near the LNG terminal showed average densities during the campaign in July 2015 that were in the range of values reported for other sites in the Mediterranean. The meiobenthic biocoenosis consists of organisms belonging to 19 main groups. Nematodes are the dominant group, often accounting for almost 90% of the total population, followed by copepods, harpacticoids, polychaetes and nauplii. The area under investigation shows a good faunistic homogeneity. Nevertheless, at some stations near the terminal and at a control site, a certain variability of the population was observed (this has already been noticed during previous monitoring) and which does not appear to be directly related to the operation of the Terminal.

Concluding Remarks

The simulations carried out with numerical Lagrangian models indicate that the initial mixing and dilution phase of the chlorinated water with the surrounding liquid takes place within a horizontal distance of 50 meters from the source: The measurements carried out with multiparametric probes have confirmed that the surface temperature already at the station 50 m south of the discharge matches the parameters measured at the stations further away from the structure.

The modelling simulations carried out show that the chlorine concentration in the wastewater at a distance of 50 m from the discharge and in a time interval of less than 1 minute is under 10% of the initial value (0.2 mg L⁻¹ to 0.018 mg L⁻¹). The concentrations of chlorination by-products support this prediction, as the concentrations of the most important by-products are generally below or slightly above the limit of quantification.

Chlorination by-products are the only contaminants directly associated with the cooling water discharged from the LNG plant into the sea after the chlorination process. The compounds found in the water at concentrations above the LOQ are generally halomethanes, mainly bromoform, and haloacetic acids, mainly DBAA. Contamination by haloacetonitriles and volatile organic compounds (VOCs, of which halomethanes are excluded) in water is not reported.

Other pollutants detected in seawater such as metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organostannic compounds and volatile organic compounds (VOCs) do not appear to be linked to emissions from the LNG plant. The volatile organic compounds (VOCs) were always below the limits of quantification for each analyte, as were the organostannic compounds in almost all cases.

Biological assays on water, carried out with four species (*Vibrio fischeri*, *Dunaliella tertiolecta*, *Brachionus plicatilis* and *Paracentrotus lividus*), generally showed no or only negligible toxicity. Only the test with *Paracentrotus lividus* showed moderately toxic effects in some cases, and the test with *Dunaliella tertiolecta* showed biostimulatory effects in a few cases.

The concentrations of PAHs and PCBs in the sediments, expressed as the sum of the analysed compounds, were below the EQS. PCBs showed quite homogeneous values and were generally comparable to the data collected during the Reference campaigns. Organostannic compounds in the sediments showed concentrations below the limits of quantification. If TBT was present, it was generally below the concentrations measured during the Reference tests.

The concentrations of CBP in the sediments were generally below the limits of quantification, some haloacetic acids (dichloroacetic acid - DCAA, monochloroacetic acid - MCAA, bromochloroacetic acid - BCAA, dibromochloroacetic acid - DBCAA, chlorodibromoacetic acid - CDBAA, and Dalapon) were detected, which were generally also found in the control stations. Contamination by haloacetonitriles and volatile organic compounds (VOCs, of which halomethanes are excluded) was not detected in the sediments.

Biological assays out on the sediments with four species (the bacterium *Vibrio fischeri*, the green alga *Dunaliella tertiolecta*, the rotifer *Brachionus plicatilis* and the echinoderm *Paracentrotus lividus*) showed that the area investigated was generally not affected by biologically relevant and widespread toxic effects. Only the test with *P. lividus* showed toxic effects in some cases, albeit to a relatively small extent.

With regard to the bioaccumulation of PAHs, PCBs, metals and organostannic compounds in mussels, no critical problems were identified in connection with the operation of the LNG terminal.

CBP concentrations in mussels are generally below the limit of quantification, some haloacetic acids (dichloroacetic acid - DCAA, monochloroacetic acid - MCAA, dibromochloroacetic acid - DBCAA, and chlorodibromoacetic acid - CDBAA) were detected, which were also frequently found in the control stations. Chloroform was also detected among the halomethanes.

Slight oxidative effects were observed in some campaigns; biologically relevant effects are excluded in the biomarkers of mussels transplanted near the terminal and attributable to operational activities. Overall, no relevant biological effects were observed in mussels transplanted in the vicinity of the facility and attributable to the operational activities of the terminal.

Regarding bioaccumulation in fish, mercury and arsenic in the muscle of *Squalus acanthias* show relatively high concentrations both near the terminal and in the control area in the Operational phase, which were already previously detected in the Reference and Site-construction phases.

For the chemical risk, Regulation (EC) No 1881/2006 specifies a threshold value of 1 mg kg⁻¹ wet weight for Hg in shark muscle. The measured concentrations indicate a slight exceedance of the threshold value (average value of 1.12 mg kg⁻¹ wet weight) only in the first campaign at the station near the terminal. High levels were also found in the liver of *Squalus acanthias*, which were higher near the terminal than at the control station. The high concentrations found in the liver of *S. acanthias* are a feature already observed, albeit to a lesser extent, in the Reference and Site-construction campaigns. There does not appear to be any evidence that these results are related to the discharges from the LNG terminal.

Dibutyltin and monobutyltin were generally below the limits of quantification in the analysed shark tissues, with tributyltin (TBT) preferentially accumulating in the muscle of *Squalus acanthias* compared to liver, which was also detected in Reference and Site-construction campaigns.

The CBPs analysed were generally below the limits of quantification of the individual compounds in the muscle and liver tissue

of *S. acanthias*; however, some haloacetic acids such as MCAA, DCAA, BCAA, CDBAA were detected.

Occasionally, some oxidative effects were detected in *Squalus acanthias*, leading to a decrease in total antioxidant capacity, peroxisomal activity and an increase in lipid peroxidation.

If we look at the plankton population from the available reports, there are no fluctuations in the phytoplankton population, both in terms of the number of taxa, abundance and biomass, that can be linked to the presence of the regasifier. There are also no changes in the zooplankton population in terms of the number of taxa, the number of individuals and the biomass that can be linked to the presence of the regasifier.

No negative effects on the ichthyoplankton were observed. The abundance values show that eggs and larvae of teleosts are significantly more abundant in several campaigns near the LNG Terminal, which is probably due to the attraction that submerged structures exert on many fish. There is no clear trend in ichthyoplankton abundances of eggs and larvae of bony fish (especially anchovies) in relation to the presence of the LNG facility.

In general, it can be stated that after a period of disturbance caused by the activities of the construction phase, the benthic populations recovered in a relatively short time to a state of abundance and species richness comparable to that of the Reference phase. The subsequent temporal development of the composition of the populations during the Operational phase thus appears to depend essentially on the macro-scale environmental conditions of the northern Adriatic and not on the presence or activities of the Terminal.

The area investigated around the terminal shows a good homogeneity of the meiofauna. However, at some stations close to the terminal and at a control site, a certain variability of the population - already found in previous monitoring campaigns - was highlighted, which would appear not to be directly related to the operation of the terminal.

Porto Viro LNG Terminal - Monthly Import of Natural Gas from the LNG Facility

The monthly import of natural gas is recorded into the “Energy and mining statistics - Collection of data on imports, consumption and natural gas balance”.

Data are indicated as millions of cubic metres (Mm3).

Table 1: Porto Viro LNG Terminal - Monthly import of natural gas in the years 2010-2021

| Month/ Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | Total per year (Mm3) |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------------|
| 2021 | 527 | 539 | 627 | 825 | 455 | 805 | 627 | 536 | 628 | 543 | 541 | 716 | 7,368 |
| 2020 | 529 | 630 | 448 | 630 | 517 | 657 | 757 | 217 | 622 | 714 | 616 | 446 | 6,783 |
| 2019 | 629 | 512 | 713 | 623 | 616 | 587 | 742 | 703 | 612 | 624 | 662 | 608 | 7,629 |
| 2018 | 544 | 355 | 703 | 509 | 573 | 474 | 616 | 448 | 621 | 533 | 672 | 620 | 6,671 |
| 2017 | 493 | 406 | 536 | 541 | 618 | 577 | 651 | 621 | 583 | 559 | 535 | 528 | 6,648 |
| 2016 | 535 | 449 | 451 | 531 | 445 | 535 | 444 | 268 | 628 | 446 | 345 | 363 | 5,439 |
| 2015 | 623 | 531 | 419 | 531 | 447 | 507 | 261 | 534 | 356 | 532 | 452 | 531 | 5,724 |

| | | | | | | | | | | | | | |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| 2014 | 352 | 363 | 265 | 360 | 536 | 442 | 536 | 179 | 115 | 510 | 353 | 437 | 4,448 |
| 2013 | 544 | 539 | 448 | 448 | 444 | 448 | 623 | 361 | 273 | 486 | 321 | 442 | 5,377 |
| 2012 | 598 | 493 | 657 | 601 | 581 | 497 | 396 | 269 | 594 | 401 | 556 | 563 | 6,206 |
| 2011 | 630 | 565 | 620 | 710 | 711 | 617 | 613 | 439 | 439 | 534 | 540 | 619 | 7,035 |
| 2010 | 603 | 614 | 619 | 556 | 550 | 522 | 613 | 523 | 604 | 534 | 613 | 721 | 7,072 |

Source: Italian Ministry of Economic Development - DGSAIE - <https://sisen.mase.gov.it/dgsaie/bilancio-gas-naturale>

In June 2023, the Ministry of the Environment approved the update of the integrated environmental authorization for Porto Viro plant. This allows production capacity to be increased from 9.0 to a maximum of 9.6 billion cubic meters per year on a non-continuous basis without further works on the installation.

The following tables indicate the level of production capacity achieved each month. They are set either for a monthly capacity of 750 million cubic meters (1/12 of 9.0 billion) or 800 million cubic meters (1/12 of 9.6 billion). The percentage values indicated correspond to the monthly production figure divided by one-twelfth of the nominal annual capacity.

Table 2: Porto Viro LNG Terminal - Level of production in the years 2010-2021 for a capacity of $9.0 \cdot 10^3$ Mm3

| | Annual production capacity: $9.0 \cdot 10^3$ Mm3 | | | | | | | | | | | | |
|----------------|--|--------|--------|---------|--------|---------|---------|--------|--------|--------|--------|--------|-------------------------------------|
| Month/ Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 1/12 of annual capacity (Mm3) |
| 2021 | 70.27% | 71.87% | 83.60% | 110.00% | 60.67% | 107.33% | 83.60% | 71.47% | 83.73% | 72.40% | 72.13% | 95.47% | 750 |
| 2020 | 70.53% | 84.00% | 59.73% | 84.00% | 68.93% | 87.60% | 100.93% | 28.93% | 82.93% | 95.20% | 82.13% | 59.47% | 750 |
| 2019 | 83.87% | 68.27% | 95.07% | 83.07% | 82.13% | 78.27% | 98.93% | 93.73% | 81.60% | 83.20% | 88.27% | 81.07% | 750 |
| 2018 | 72.53% | 47.33% | 93.73% | 67.87% | 76.40% | 63.20% | 82.13% | 59.73% | 82.80% | 71.07% | 89.60% | 82.67% | 750 |
| 2017 | 65.73% | 54.13% | 71.47% | 72.13% | 82.40% | 76.93% | 86.80% | 82.80% | 77.73% | 74.53% | 71.33% | 70.40% | 750 |
| 2016 | 71.33% | 59.87% | 60.13% | 70.80% | 59.33% | 71.33% | 59.20% | 35.73% | 83.73% | 59.47% | 46.00% | 48.40% | 750 |
| 2015 | 83.07% | 70.80% | 55.87% | 70.80% | 59.60% | 67.60% | 34.80% | 71.20% | 47.47% | 70.93% | 60.27% | 70.80% | 750 |
| 2014 | 46.93% | 48.40% | 35.33% | 48.00% | 71.47% | 58.93% | 71.47% | 23.87% | 15.33% | 68.00% | 47.07% | 58.27% | 750 |
| 2013 | 72.53% | 71.87% | 59.73% | 59.73% | 59.20% | 59.73% | 83.07% | 48.13% | 36.40% | 64.80% | 42.80% | 58.93% | 750 |
| 2012 | 79.73% | 65.73% | 87.60% | 80.13% | 77.47% | 66.27% | 52.80% | 35.87% | 79.20% | 53.47% | 74.13% | 75.07% | 750 |
| 2011 | 84.00% | 75.30% | 82.70% | 94.70% | 94.80% | 82.30% | 81.70% | 58.50% | 58.50% | 71.20% | 72.00% | 82.50% | 750 |
| 2010 | 80.40% | 81.90% | 82.50% | 74.10% | 73.30% | 69.6% | 81.70% | 69.70% | 80.50% | 71.20% | 81.70% | 96.10% | 750 |

Table 3: Porto Viro LNG Terminal - Level of production in the years 2010-2021 for a capacity of $9.6 \cdot 10^3$ Mm3

| | Annual production capacity: $9.6 \cdot 10^3$ Mm3 | | | | | | | | | | | | |
|----------------|--|--------|--------|---------|--------|---------|--------|--------|--------|--------|--------|--------|-------------------------------------|
| Month/ Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 1/12 of annual capacity (Mm3) |
| Month/ Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 1/12 of annual capacity (Mm3) |
| 2021 | 65.88% | 67.38% | 78.38% | 103.13% | 56.88% | 100.63% | 78.38% | 67.00% | 78.50% | 67.88% | 67.63% | 89.50% | 800 |
| 2020 | 66.13% | 78.75% | 56.00% | 78.75% | 64.63% | 82.13% | 94.63% | 27.13% | 77.75% | 89.25% | 77.00% | 55.75% | 800 |
| 2019 | 78.63% | 64.00% | 89.13% | 77.88% | 77.00% | 73.38% | 92.75% | 87.88% | 76.50% | 78.00% | 82.75% | 76.00% | 800 |
| 2018 | 68.00% | 44.38% | 87.88% | 63.63% | 71.63% | 59.25% | 77.00% | 56.00% | 77.63% | 66.63% | 84.00% | 77.50% | 800 |
| 2017 | 61.63% | 50.75% | 67.00% | 67.63% | 77.25% | 72.13% | 81.38% | 77.63% | 72.88% | 69.88% | 66.88% | 66.00% | 800 |
| 2016 | 66.88% | 56.13% | 56.38% | 66.38% | 55.63% | 66.88% | 55.50% | 33.50% | 78.50% | 55.75% | 43.13% | 45.38% | 800 |
| 2015 | 77.88% | 66.38% | 52.38% | 66.38% | 55.88% | 63.38% | 32.63% | 66.75% | 44.50% | 66.50% | 56.50% | 66.38% | 800 |
| 2014 | 44.00% | 45.38% | 33.13% | 45.00% | 67.00% | 55.25% | 67.00% | 22.38% | 14.38% | 63.75% | 44.13% | 54.63% | 800 |
| 2013 | 68.00% | 67.38% | 56.00% | 56.00% | 55.50% | 56.00% | 77.88% | 45.13% | 34.13% | 60.75% | 40.13% | 55.25% | 800 |
| 2012 | 74.75% | 61.63% | 82.13% | 75.13% | 72.63% | 62.13% | 49.50% | 33.63% | 74.25% | 50.13% | 69.50% | 70.38% | 800 |
| 2011 | 78.75% | 70.63% | 77.50% | 88.75% | 88.88% | 77.13% | 76.63% | 54.88% | 54.88% | 66.75% | 67.50% | 77.38% | 800 |
| 2010 | 75.38% | 76.75% | 77.38% | 69.50% | 68.75% | 65.25% | 76.63% | 65.38% | 75.50% | 66.75% | 76.63% | 90.13% | 800 |

Livorno LNG Terminal - Environmental Monitoring Assessments from the Reports

The following points and data are extracted from reports publicly available for Livorno LNG Terminal.

Water Column

Hydrological Profiles

Oceanographic parameters (temperature, salinity, pH, turbidity, oxygen saturation, chlorophyll a, oxidation-reduction potential) were measured at 14 monitoring stations from autumn 2013 to summer 2022. These data were compared to pre-regasifier measurements (August 2012) to assess any terminal-related effects. The analysis focused on depth-specific data (12-13 meters) where the greatest impact was anticipated. A non-parametric ANOVA test (Scheirer-Ray-Hare) was used to determine if time (year) or distance from the discharge point significantly influenced the measured parameters. The essential points are:

- **Temperature:** A strong seasonal pattern was observed with winter lows around 14-15°C and summer highs between 22.5 and 25.5°C.
- **Salinity:** Values remained relatively stable around 38.1%, except for slightly lower readings in 2014 and 2015 when the plant was not at full capacity.
- **pH:** Consistent values were recorded between 7.6 and 8.3, with minor seasonal variations.
- **Turbidity:** Low and constant levels (<1 NTU) were observed in most seasons, except for winter when values ranged from 1 to 4 NTU.
- **Oxygen Saturation:** No significant inter-annual or seasonal variations were noted, with average values consistently above 80%.
- **Chlorophyll:** Similar to oxygen saturation, chlorophyll levels showed no significant trends.
- **ORP:** A more pronounced inter-annual variability was observed in ORP values, ranging from 100 to 240 mV.
- **Statistical Analysis:** the ANOVA analysis revealed a strong effect of time (year) on the measured variables but no specific trend. While minimal effects were observed for distance from the terminal and the interaction between distance and year, these findings suggest that the terminal's presence had a negligible impact on the physical properties of the water column at the depth studied.

Physical, Chemical and Microbiological Characteristics

To assess the Terminal's impact on various parameters, including irradiance, nutrients, suspended solids, particulate organic matter, dissolved chromophoric organic matter, chlorophyll a, and pigment diversity, data were analyzed from the "Reference" campaigns (E12, E14-E22). One-factor ANOVA tests were conducted on log-transformed data to evaluate the effects of sampling year, distance from the Terminal, and sampling depth. The salient points are:

- **Irradiance and Spectral Irradiance:** No significant alterations were observed due to the Terminal's presence.
- **Inorganic Nutrients, TSM, POM, and CDOM:** While the bathymetric profiles of TSM indicated low concentrations with minimal variations throughout the water column, the organic fraction (POM) accounted for approximately 40% of TSM. CDOM concentrations were within a narrow range, suggesting contributions from seasonal phytoplankton blooms or resuspended detrital material.

- **Chlorophyll and Pigment Diversity:** Chlorophyll a concentration showed similar profiles at all stations, with some diatom blooms. The Terminal's presence had no significant impact on these parameters.
- **Surfactants, Chloroderivatives, Total Hydrocarbons, and Microbiological Analyses:** For non-ionic surfactants and chloroderivative compounds, no anomalies were reported. Anionic surfactants were below the limit of quantification in all monitoring campaigns. Halomethanes and VOCs were generally below the limit of quantification, except for some exceedances in the E21 campaign. Bromoform was detected in all campaigns. Total hydrocarbons varied between stations and campaigns, with a general increase in concentrations observed in E21 compared to the "Reference" phase. C>12 hydrocarbons showed a notable increase in the 2018 spring campaign but subsequently declined.

Ecotoxicological Tests on Water Samples

The results of three ecotoxicological tests (Absent, Low, Average) were categorized as Presence-Absence data (0-1). A generalized linear model (GLM) with binomial distribution was used to analyze the test response (dependent variable) in relation to year, distance to the Terminal, and season.

- **Statistical Analysis:** The analysis revealed that only the factors "year" and "season" significantly influenced the test response. Depth, distance, and the interaction between distance and year had no significant effects.
- **Coefficient Estimation:** The coefficient estimates indicate a significant negative relationship between season and test response, suggesting lower winter toxicity. Additionally, a positive trend in test response over time was observed, unrelated to the Terminal's presence.

ISPRA's 2021 comment highlighted a general increase in toxicity response over time, likely driven by broader oceanographic variations in the area. This trend is evident despite individual species variability and comments made in the reports.

Plankton

Phytoplankton

Seasonal trends in phytoplankton were analyzed based on total phytoplankton abundance and four taxonomic groups: diatoms, dinoflagellates, coccolithophores, and "other plankton." ANOVA tests were conducted to assess the effects of sampling year, distance from the Terminal, and sampling depth. The key findings are:

- **Seasonal Cycle:** Phytoplankton abundance and taxonomic groupings exhibited a consistent seasonal pattern, aligning with typical variations in Ligurian-Tyrrhenian waters. Winter maxima and summer minima were observed, with a noticeable increase in "Other plankton" from E20 to E22.
- **ANOVA Analysis:** Inter-annual variability was significant for total phytoplankton abundance and all taxonomic groups, indicating fluctuations from year to year. Distance from the Terminal had no significant effect on any parameter. Depth, however, was significant for all groups except "other plankton."
- **ANOSIM and SIMPER:** ANOSIM analysis revealed significant differences in taxonomic composition between the Reference campaign and the nine summer campaigns. The

greatest dissimilarity occurred between Reference and the last two campaigns (E20 and E21). SIMPER analysis further confirmed this dissimilarity, with a higher than 43% difference attributed to quantitative contributions of taxa rather than substitution.

The distance from the FSRU Terminal did not significantly affect phytoplankton population composition or any of the analyzed environmental and biological parameters. In contrast, the time factor (year) was a significant determinant of changes in environmental parameters, phytoplankton biomass, and community composition.

Zooplankton

The analysis of three zooplankton fractions (copepod holoplankton, meroplankton, and ichthyoplankton) aimed to assess the FSRU Toscana's potential effects on zooplankton composition and structure. Bray-Curtis similarity/dissimilarity measures were used to compare samples across different temporal (seasons) and spatial (depth) factors. ANOSIM and pairwise tests were employed to evaluate significant differences between the "Reference" phase and subsequent summer campaigns, as well as among summer seasons. The salient features are:

- **Seasonal Influence:** Seasonality is a crucial factor influencing zooplankton distribution, driven by physical and ecological factors.
- **Long-Term Monitoring:** The study area's mesozooplankton population has been extensively monitored, providing valuable insights into population dynamics, biomass, rare species presence, and potential disturbances.

- **Spatial Factors:** Depth is another factor influencing zooplankton distribution. Some species prefer deeper water layers, while others thrive near the water-air interface.
- **Bray-Curtis Analysis:** Significant differences were observed between the "Reference" campaign and all subsequent summer campaigns, particularly for copepod holoplankton and meroplankton. Ichthyoplankton also showed significant differences, although to a lesser extent.
- **R Trend Comparison:** The R trend comparison between the "Reference" phase and summer campaigns revealed consistent differences, suggesting that the observed differences between the "Reference" phase and E14 persisted throughout the monitoring period. This indicates that these differences are not likely attributable to the regasifier's operation.

Figure 1 shows the R-trends of the three zooplankton components when comparing the "Reference" and summer seasons over the nine years of monitoring. The results show that the zooplankton community is significantly different between the reference campaign and all others, with high values for copepod holoplankton and meroplankton, while the value for ichthyoplankton is lower but remains highly significant. When comparing the trend of R resulting from the comparison of B vs Ex, the values were found to vary little, indicating that the difference already observed between "Reference" and E14 has remained more or less constant until 2022. This shows that this difference is difficult to attribute to the operation of the LNG plant.

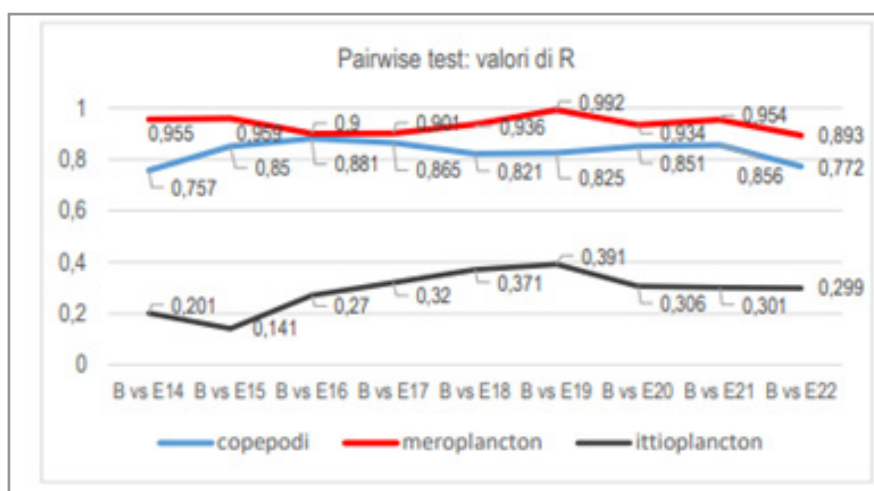


Figure 1: R-values of the pairwise tests comparing the "Reference" phase (B) and summer operation phase (E) (from Operation Phase IX Annual Report - Volume 2)

Sediments

The reports highlight that the area where the Terminal is installed was previously used for port dredging mud spills. This historical contamination can significantly influence sediment particle size and contaminant concentrations, often leading to high spatial variability and exceeding national reference chemical levels (L1 or L2 DM 173/16) or DM 56/2009 Environmental Quality Standards.

Physical, Chemical and Microbiological Characteristics

The analysis of sediment data collected from "Reference" (B) to Summer 2022 (E22) revealed a complex pattern of spatial variability in grain size and contaminant concentrations, but no clear temporal trends or patterns that could be definitively attributed to the presence of the Terminal. While individual stations showed notable variations in the relative proportions of different sediment fractions, the overall average remained consistent with the reference data. Additionally, statistical analysis comparing

standard deviations between stations near and far from the Terminal revealed no significant differences, suggesting that the fluctuations in grain size fractions are comparable throughout the study area, regardless of their proximity to the Terminal.

The elements monitored by the control bodies are TBTs, some metals (As, Cr, Ni, Pb) and PAHs, which had higher concentrations than the corresponding EQS specified in Ministerial Decree 260/2010 and Legislative Decree 172/2015. In addition, polycyclic aromatic hydrocarbons are widespread and have the highest values at the Terminal. Chlorinated compounds (Haloacetic acids, Alocetonitriles, Halomethanes and VOCs, Halophenols), on the other hand, were always below the limit of quantification, with the exception of compounds such as 1,1,2 Trichloroethane, 1,2 Dibromo-3 Chloro propane and Bromoform at some stations.

Ecotoxicological Tests on Sediment Samples

The bioassay with *Corophium orientale* revealed a fluctuating trend in the first 5 years of operation (until Summer 2018) fol-

lowed by a clear deterioration from E19 onwards (for 3 out of 6 stations, 2 of which are close to the terminal). In the subsequent 2 summers, the toxicity level increased significantly for all stations, regardless of their distance from the terminal. This worsening is attributed to an increased bioavailability of contaminants in the area, which was already heavily contaminated due to past port sludge spills. However, in the last published summer campaign, toxicity values dropped to low or even zero.

The test with *Vibrio fischeri* consistently showed no variation over time or with respect to terminal distance, with toxicity always absent except at station MG13 in E22.

The test with *Paracentrotus lividus* generally showed no to low toxicity. However, in E22, there was a slight improvement at two stations near the terminal and at the control station (MG10).

Table 4: Results of ecotoxicological tests conducted from the "Reference" campaign to Summer 2022. Absent toxicity=A; Low toxicity=B; Medium toxicity=M; High toxicity=High). MG6, MG7, MG12, MG13 are the stations in the vicinity of the Terminal (from Operation Phase IX Annual Report - Volume 2).

| | <i>P. lividus</i> | | | | | | | | | | | <i>C. orientale</i> (cronico) | | | | | | | | | | | <i>V. fischeri</i> (fase solida) | | | | | | | | | | |
|------|-------------------|-----|-----|-----|-----|------|-----|-----|-----|-----|---|-------------------------------|------|-----|-----|-----|------|------|------|-----|---|-----|----------------------------------|-----|-----|-----|-----|-----|-----|-----|---|--|--|
| | B | E14 | E15 | E16 | E17 | E18 | E19 | E20 | E21 | E22 | B | E14 | E15 | E16 | E17 | E18 | E19 | E20 | E21 | E22 | B | E14 | E15 | E16 | E17 | E18 | E19 | E20 | E21 | E22 | | | |
| MG6 | B | A | A | B | A | A | B | B | B | B | M | M | A | M | B | B | M | Alta | Alta | A | A | A | A | A | A | A | A | A | A | A | A | | |
| MG7 | B | B | B | B | B | B | B | B | B | B | M | M | B | M | A | A | A | Alta | Alta | A | A | A | A | A | A | A | A | A | A | A | A | | |
| MG9 | B | A | B | M | A | B | B | B | B | B | B | B | B | M | A | B | M | Alta | Alta | A | A | A | A | A | A | A | A | A | A | A | A | | |
| MG10 | B | B | B | M | B | M | B | M | B | A | M | B | B | M | A | B | Alta | Alta | Alta | A | A | A | A | A | A | A | A | A | A | A | A | | |
| MG12 | B | M | B | B | B | Alta | B | B | M | A | M | B | Alta | M | A | B | Alta | Alta | Alta | B | A | A | A | A | A | A | A | A | A | A | A | | |
| MG13 | B | A | B | B | A | B | B | B | M | A | B | B | M | M | A | B | Alta | Alta | Alta | A | A | A | A | A | A | A | A | A | A | A | B | | |

The Monitoring Plan for the Marine Environment Year 9 - Volume 2 emphasizes the importance of including the acute test, along with other tests, to create a comprehensive battery of assays. Starting from E22, the acute test was added to the chronic test to enhance the overall interpretation of the test battery. This battery requires at least 3 organisms from distinct taxonomic groups, combined with at least one solid-phase assay, one liquid-phase assay, and one chronic effects assay. The test with *V. fischeri* on liquid phase was introduced to complement the existing sediment-only test.

The ecotoxicological hazard classification using SediQualSoft 109.00 software, based on 2 acute tests (*V. fischeri* and *C. orientale*) and 1 chronic test (*P. lividus* and *V. fischeri*), indicates an absent hazard (less than 1) for most stations. However, station MG13 shows a low hazard (21-1.5) with an HQRamry of 1.01, according to DM 173/16.

Following the opinion expressed by ISPRA, the monitoring did not reveal clear temporal patterns of ecotoxicological hazards, neither near nor far from the FSRU. This suggests that the observed variations are not directly attributable to the Terminal.

Biota

Macrozoobenthos

The analysis of macrozoobenthos populations revealed significant changes over time, as indicated by PERMANOVA analysis. While distance from the Terminal had a significant effect on the overall distribution of organisms, there was no clear interaction between time and distance, suggesting that the initial differences observed in the "Reference" phase persisted throughout the monitoring period. This indicates that the Terminal's influence is not directly causing these changes.

Additionally, the Shannon-Wiener biodiversity index and specific richness index showed a general increasing trend, suggesting a positive impact on biodiversity. While distance from the Terminal had significant effects on biodiversity and specific richness, the overall trend was for higher values near the Terminal and in the intermediate area compared to the control area.

Seasonal effects were also observed, with significant differences in biodiversity and specific richness during winter and spring compared to autumn. These findings suggest that the presence of the Terminal does not appear to have a direct impact on macrozoobenthos populations, and the observed changes are likely

attributable to natural variability, including seasonal fluctuations and long-term ecological trends. ISPRA has corroborated in its latest technical opinion that the populations near the Terminal have evolved over time (from 2012 to 2021) in a similar way to those present in the Control areas, which are very distant from the source of disturbance (gas tanker), thus showing patterns attributable to normal natural variability.

Meiobenthos

The fauna found during the 19 sampling campaigns (Reference, I14, E14, I15, E15, I16, I17, E17, I18, E19, I20, E20, I21, E21, I22, E22) consisted of organisms belonging to a total of 29 taxonomic groups, of which 7 were present in all the monitoring campaigns. The Gastrotrichi were only present in the Reference campaign, while Hydrozoa, Rotifers, Oligochaetes, Sipunculidae, Bryozoa, Solenogastri, Scaphopods, Acari, Ophiuroids and Tunicates were found in one (Scaphopods and Tunicates) or more of the campaigns of the Exercise phases. The mean total meiofauna density of the investigated area was 358.8 ± 168.3 ind./10cm in the "Reference" samples, and varied during the Exercise phases from a minimum of 45.4 ± 74.8 ind./10cm in E21 to a maximum of 360.7 ± 271.4 ind./10cm in E17. Nematodes were always the dominant taxon, with percentages varying between 71.8% in I21 and 85.8% in E19, followed in order by Copepods (range: 3.7-10.1%), Polychaetes (range: 3.9-9.1%) and, finally, Nauplii (range: 1.8-5.3%). The first four groups make up a total of 95-98% of the meiobenthic biocoenosis, depending on the campaigns. The other taxa are present in very low densities and percentages, with only Ostracods, Scaphopods, Turbellarians, Oligochaetes, Bryozoans and Ciliates reaching 1.0% of the total on some occasions.

The PERMANOVA analysis of the summer campaign data shows that the factor 'Time' is significant, indicating that the natural stands, as expected, evolve over time. In contrast, the factor 'Distance' is non-significant, indicating that the stands at different distances from the Terminal do not differ. This condition does not change over time; in fact, the 'Time x Distance' interaction is not significant.

However, a significant difference emerged between sites resulting mainly from reduced densities of Copepods, Nauplii, Ciliates and, to a lesser extent, Polychaetes. This revealed some significant variations in the meiobenthic community in the area investigated. The explanation given is a general reduction in abundance that occurred over time, not clearly attributable to the presence and operation of the FSRU Terminal but due to other, unspecified causes.

Bioaccumulation

Bioaccumulation analyses on transplanted mussels shows no particular contamination issues, despite some signs of an increase in contaminants compared to "time zero".

- C<10 hydrocarbons were all below or close to the method's limit of quantification (0.5 mg/kg), with a few exceptions in P20 and P21.
- Hydrocarbons C10-C40 generally present values below the limit of quantification of the analytical method used. In the I20 campaigns (except at station B) P20, P21 and I22 all stations present values above the limit of quantification (5 mg/kg) with values generally comparable between all stations.

In E22, the values are still above the limit but markedly lower than in the other campaigns.

- For metals in the 5 seasons in which the terminal was at full capacity out of the 12 metals analysed in I20 and P20, there was an increase in Arsenic and Zinc: in I20 at one station, a higher value was also found for Barium, and in P20 for Cadmium. These four elements, although not at all stations, were also slightly above the threshold in P21 and I22. In E22, Arsenic was higher at only one station, Barium was within the threshold values, while Lead showed higher values. This non-constant trend between seasons, also corroborated by the comparison of the concentrations of mussels placed along the Terminal with respect to T0 and those between the mussels placed in Gorgona ("Reference") and T0, is insufficient to prove the existence of a causal relationship with the activities of the Terminal. Moreover, the time trend of the concentrations of zinc found in the mussels does not demonstrate the existence of effects attributable to the presence of the sacrificial anodes located along the hull of the Terminal.
- The concentrations of PAHs in all campaigns were generally close to or below the quantification limits of the analytical method. However, in the campaigns that coincided with at least 75% functionality of the Terminal, these alterations were found:
 - in the I20 campaign, higher values are found for the congeners Phenanthrene and Naphthalene;
 - In the P20 campaign, values above the respective limits were found for Acenaphthene, Acenaphthylene, Phenanthrene, Floranthene, Fluorene and Naphthalene.
 - In the P21 campaign, values slightly above the respective limits of quantification were found for Acenaphthylene and Naphthalene.
 - In the I22 campaign, values slightly above the respective limits of quantification were found for Acenaphthylene, Phenanthrene and Naphthalene.
 - In the E22 campaign, higher values of Acenaphthylene and Naphthalene are found at station B, which were declared not to be attributable to the FSRU since they also appear in the control in Gorgona ("Reference").
- The values of the chlorine compounds were generally below or close to their respective limits of quantification. The only exceptions are 2,4,6-Trichlorophenol and 2,4-Dichlorophenol in the I20 and P20 campaigns at only a few stations where Pentachlorophenol was also found at Time 0 and Station A.
- Organotin compounds (TBT, DBT and MBT) were always below the method's limit of quantification in all campaigns. Finally, no signs of microbiological contamination by total and faecal coliforms and faecal streptococci were detected.

Biomarkers

The linear regression analysis updated to E22 showed no significant trend for any of the three biomarkers analysed (Neutral Red Retention, Comet assay and Histological analysis of gill tissue). The genotoxicity assessment in terms of the degree of DNA fragmentation, which had been reported to increase in the years 2016 and 2017, was not confirmed in the last two years of activity. The results of the Sediquelsoft processing, which integrates data for all biomarkers investigated, for the 33 monitoring campaigns carried out since the start showed that only in one

campaign (I15) was there a moderate level of environmental risk, while in all other cases the risk was slight or absent. The overall assessment of the data for the three biomarkers therefore shows that the FSRU Terminal had a negligible effect on the health status of the mussels analysed.

In the ISPRA opinions, although it is reiterated that there are no particular observations or indications to be reported, it is suggested that further investigations into DNA damage be provided, such as an analysis of the frequency of micronuclei.

Fish Fauna

Benthonectonic Fish Fauna

Surveys conducted on the fishery (carried out with both bottom trawls and bottom-set gillnets) indicate no particular criticalities or effects due to the presence of the Terminal. Statistical analyses on some species and main groupings (Bony Fish, Cartilaginous Fish, Crustaceans- Decapod, Molluscs- Cephalopod) showed either no effect from the Terminal or for some species caught by trawling such as hake, *Merluccius merluccius*, mud mullet, *Mullus barbatus*, and dogfish, *Scyliorhinus canicula*, or a positive effect (increase in biomass expressed as kg/km). In general, it is reported that there is no effect of the presence of the Terminal on the indices of specific diversity and species richness analysed over the years.

ISPRA observed potential improvements in the sampling methodology, particularly in relation to haul allocation and distance from the terminal. The monitoring body (CIBM) highlighted that all sampling stations, including controls, were situated within the no-fishing zone (4-mile radius) to prevent a "reserve effect." Placing controls outside the terminal could introduce further disturbances (fishing activity), making it challenging to attribute any observed differences solely to the terminal's presence.

Pelagic Fish Fauna

The low catches with this gear (drift net) in all the monitoring years show that the concentration of pelagic species is generally

very low and, in any case, organized in shoals that do not have a continuous distribution.

Cetaceans and Sea Turtles

Livorno terminal is located within the Pelagos Sanctuary, an area known for its high concentration of marine mammals and reptiles. Despite this, sightings of bottlenose dolphins were infrequent during the nine years of sampling. Only three out of nine campaigns (30%) recorded sightings within a 1-mile radius of the terminal, with two out of five of these occurring within 100 meters. Most sightings were reported within a 6-mile radius, particularly in the north and east, which is also a popular trawling area. This suggests that fishing activity may influence dolphin behavior and habitat choice.

Regarding sea turtles, only three sightings were recorded in the first two campaigns. This lack of sightings is likely due to the turtles' behavior, including long apneas, brief surface times, and rapid diving to avoid disturbances. Their preference for neritic waters (near the coast) may also explain the limited sightings. However, the presence of *Caretta caretta* has increased in Tuscan waters over the past decade, with nesting observed in the northern area (Cinquale and Marina di Pietrasanta).

ISPRA's opinion emphasises what has already been expressed in the previous reports regarding the methodology, the implementation of monitoring and the production of data, which cannot currently provide strong results indicating the presence of cetaceans in the area. It has been suggested to:

- distribute the monitoring within the seasons, since the survey conducted on a single day provides only a punctual indication,
- rethink the survey strategy and its frequency, in order to make it better adapted to the specific case, is therefore reiterated.

Livorno LNG Terminal - Monthly Import of Natural Gas from the LNG Facility

The monthly import of natural gas is recorded into the "Energy and mining statistics - Collection of data on imports, consumption and natural gas balance".

Data are indicated as millions of cubic meters (Mm3).

Table 5: Livorno LNG Terminal - Monthly import of natural gas in the years 2015-2021

| Month/ Year | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | Total per year (Mm3) |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------------|
| 2021 | 0 | 32 | 268 | 302 | 238 | 270 | 99 | 86 | 96 | 0 | 0 | 46 | 1,437 |
| 2020 | 271 | 272 | 347 | 287 | 173 | 429 | 380 | 266 | 261 | 159 | 252 | 79 | 3,176 |
| 2019 | 186 | 268 | 386 | 285 | 270 | 356 | 353 | 277 | 248 | 346 | 287 | 329 | 3,593 |
| 2018 | 0 | 0 | 58 | 0 | 88 | 0 | 0 | 0 | 0 | 239 | 431 | 371 | 1,186 |
| 2017 | 0 | 0 | 0 | 179 | 236 | 236 | 90 | 0 | 85 | 0 | 0 | 163 | 989 |
| 2016 | 0 | 0 | 0 | 28 | 0 | 82 | 170 | 141 | 20 | 0 | 0 | 67 | 509 |
| 2015 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 61 | 61 |

Source: Italian Ministry of Economic Development - DGSAIE - <https://sisen.mase.gov.it/dgsaie/bilancio-gas-naturale>

Table 6: Livorno LNG Terminal - Level of production in the years 2015-2021 for a capacity of $3.75 \cdot 10^3$ Mm3

| Month/ Year | Annual production capacity: $3.75 \cdot 10^3$ Mm3 | | | | | | | | | | | | |
|----------------|---|-------|--------|-------|-------|--------|--------|-------|-------|--------|--------|--------|-------------------------------------|
| | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 1/12 of annual capacity (Mm3) |
| 2021 | 0.0% | 10.2% | 85.8% | 96.6% | 76.2% | 86.4% | 31.7% | 27.5% | 30.7% | 0.0% | 0.0% | 14.7% | 312.5 |
| 2020 | 86.7% | 87.0% | 111.0% | 91.8% | 55.4% | 137.3% | 121.6% | 85.1% | 83.5% | 50.9% | 80.6% | 25.3% | 312.5 |
| 2019 | 59.5% | 85.8% | 123.5% | 91.2% | 86.4% | 113.9% | 113.0% | 88.6% | 79.4% | 110.7% | 91.8% | 105.3% | 312.5 |
| 2018 | 0.0% | 0.0% | 18.6% | 0.0% | 28.2% | 0.0% | 0.0% | 0.0% | 0.0% | 76.5% | 137.9% | 118.7% | 312.5 |
| 2017 | 0.0% | 0.0% | 0.0% | 57.3% | 75.5% | 75.5% | 28.8% | 0.0% | 27.2% | 0.0% | 0.0% | 52.2% | 312.5 |
| 2016 | 0.0% | 0.0% | 0.0% | 9.0% | 0.0% | 26.2% | 54.4% | 45.1% | 6.4% | 0.0% | 0.0% | 21.4% | 312.5 |
| 2015 | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 19.5% | 312.5 |

Table 7: Livorno LNG Terminal: average production level during the monitoring campaigns A15 to E22.

| Monitoring Campaign (Period) | Day start | Day stop | Duration (number of days) | Mean daily output in the period (kWh/d) | Maximum daily output in the period (kWh/d) | Peak daily output recorded in the periods (kWh/d) | Productivity in the period (%) Mean daily output in the period / Peak daily output recorded in the periods |
|------------------------------------|--------------|------------|---------------------------------|--|---|--|--|
| E22 | 31/8/2022 | 23/9/2022 | 23 | 139711737 | 155017762 | | 85.88% |
| P22 | 31/5/2022 | 21/6/2022 | 21 | 108829005 | 155005552 | | 66.89% |
| I22 | 28/2/2022 | 17/3/2022 | 17 | 133281426 | 155021043 | | 81.93% |
| A21 | 22/11/2021 | 17/12/2021 | 25 | 0 | 0 | | 0.00% |
| E21 | 30/8/2021 | 15/9/2021 | 16 | 32440081 | 144944173 | | 19.94% |
| P21 | 24/5/2021 | 11/6/2021 | 18 | 117247626 | 150022913 | | 74.97% |
| I21 | 1/3/2021 | 25/3/2021 | 24 | 77303937 | 155037000 | | 47.51% |
| A20 | 10/11/2020 | 30/11/2020 | 20 | 106843138 | 155040704 | | 65.67% |
| E20 | 7/9/2020 | 30/9/2020 | 23 | 82577482 | 155808230 | | 44.55% |
| P20 | 23/6/2020 | 10/7/2020 | 17 | 122418406 | 155017603 | | 75.24% |
| I20 | 3/4/2020 | 16/4/2020 | 13 | 126864186 | 155011244 | | 77.98% |
| A19 | 21/11/2019 | 4/1/2020 | 44 | 100524621 | 155053277 | | 53.84% |
| E19 | 5/9/2019 | 4/10/2019 | 29 | 78518326 | 155019152 | | 48.26% |
| P19 | 30/5/2019 | 14/6/2019 | 15 | 120099750 | 155023050 | | 73.06% |
| I19 | 25/2/2019 | 21/3/2019 | 24 | 103292797 | 155014634 | | 63.49% |
| A18 | 29/11/2018 | 21/12/2018 | 22 | 124499827 | 160065167 | | 76.52% |
| E18 | 03/09/2018 | 15/09/2018 | 12 | 0 | 0 | | 0.00% |
| P18 | 09/05/2018 | 21/06/2018 | 43 | 19625146 | 102004057 | | 0.49% |
| I18 | 27/02/2018 | 22/03/2018 | 23 | 24680084 | 162691890 | 162691890 | 1.85% |
| A17 | 14/11/2017 | 25/11/2017 | 11 | 156623 | 1879471 | | 0.10% |
| E17 | 31/08/2017 | 23/09/2017 | 23 | 58586881 | 110797002 | | 19.46% |
| P17 | 23/05/2017 | 03/06/2017 | 11 | 57963173 | 110797002 | | 35.62% |
| I17 | 21/02/2017 | 11/03/2017 | 18 | 0 | 0 | | 0.00% |
| A16 | 08/11/2016 | 28/11/2016 | 20 | 0 | 0 | | 0.00% |
| E16 | 28/08/2016 | 8/9/2016 | 11 | 0 | 0 | | 0.00% |
| P16 | 17/05/2016 | 6/6/2016 | 20 | 0 | 0 | | 0.00% |
| I16 | 17/02/2016 | 11/3/2016 | 23 | 0 | 0 | | 0.00% |
| A15 | 18/11/2015 | 6/12/2015 | 18 | 0 | 0 | | 0.00% |

Source: ENTSG (https://transparency.entsg.eu/)

The highlighted cells correspond to a productivity level of 75% or more