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Article

Article

Microbiological characterization of a rearing system for the common sea urchin *Paracentrotus lividus*: a support to technical production regulations redaction and system monitoring.

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Abstract

Objective: The overexploitation of the sea urchin *Paracentrotus lividus* stock, as a consequence of the growing market demand for roe, has boosted the research for echinoderm rearing. The chemical, physical, and microbiological characteristics of land-based facilities are crucial for sea urchins' health and human consumption of their products. In Italy, health–hygienic regulations for *P. lividus* rearing are still to be perfected by the authorities. In this context, we characterized the microbiological quality of a pilot land-based facility for sea urchin production at the University of Cagliari (Italy) to support the development of technical production regulations.

Materials and Methods: The accredited Hygiene Laboratory of Cagliari University collected and analyzed the samples in June 2023. Mesophilic bacteria, yeasts, and molds were searched for in air and on surfaces. Total coliforms and *Escherichia coli*, Enterococci, Pseudomonadaceae, *Staphylococcus aureus*, sulfite-reducing *Clostridia*, and *Vibrio* spp. were identified in water samples. We searched for *Vibrio* spp. and *Pseudomonas* spp. in the gonads and coelomic fluid of sea urchins.

Results: Although air, surfaces, and water quality were satisfactory overall, some critical points should be monitored more strictly. Enterococci concentration was 250 CFU/100 mL in the water reserve, suggesting animal contamination (other than humans). *Pseudomonas aeruginosa* was the most resistant to filtration processes, with a residual concentration of 6 CFU/250 mL after the second filtration. No colonies of *Vibrio* spp. or *Pseudomonas* spp. were isolated in sea urchins' gonads or coelomic fluid.

Conclusions: Starting from the results, we provided targeted advice for developing technical production regulations, system monitoring, and facility routine maintenance in accordance with the 'best practice' approach. This analysis could be considered a first step toward the elaboration of common regulations about the minimal standards for the breeding environment of *P. lividus* by national and regional authorities.

Keywords: *Paracentrotus lividus*; echinoculture; health hazards; microbial contaminants; production disciplinary; Sardinia.

Introduction

The edible sea urchin *Paracentrotus lividus* (Lamark 1816) is considered overexploited at the local level in Sardinia and in most Atlantic Europe and Mediterranean countries where the 'roe fishery' fuels the demand for its gastronomic use (Boudouresque and Verlaque, 2020; Department of Agriculture and Agropastoral Reform - Sardinia Region, 2024). Its market is mostly traditional in the Mediterranean region, where Italy, France, and Spain are among the top consumers (Stefánsson *et al.*, 2017). The great demand has resulted in high fishing pressure on wild stocks, making harvesting ecologically unsustainable in several countries (Lawrence, 2020). In Italy, this phenomenon is amplified by illegal or unlicensed fisheries, which are thought to represent a relevant part of its current supply (Stefánsson *et al.*, 2017). In Sardinia (southern Italy), sea urchin fishery has a long history in terms of gourmet consumption, resource assessment, and fishery management. According to a recent investigation conducted by the University of Cagliari as part of a project funded by the Fisheries Department of the Autonomous Region of Sardinia, the average production of *P. lividus* (2016–2023) accounts for approximately 28 million urchins/year harvested during the regular fishing season (4 months). This corresponds to 1210 t (±280 t) of wet biomass and 67 t (±16 t) of fresh roe based on an average gonadosomatic index (GSI) of 5% determined during the fishing season (GSI intended as the ratio between the gonad weight and the total weight of the individual). The drastic reduction of the stock and the local extinction of large breeders represent the major ecological issues to the mechanism of self-recruitment for the recovery of the *P. lividus* population (Food and Agriculture Organization of the United Nations, 2021; Giglioli *et al.*, 2021).

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In recent decades, the stock reduction suffered by *P. lividus* has boosted local and international projects dedicated to rearing the species, that is, *echinoculture* (Carboni *et al*., 2012; Fabbrocini *et al*., 2012; Kelly *et al*., 2015; Sartori *et al*., 2015; Williamson, 2015; Carrier *et al*., 2017; Vizzini *et al*., 2018; Castilla-Gavilán *et al*., 2019; Warren-Myers *et al*., 2020). Several aquaculture technologies and techniques for commercial echinoculture are currently under study, representing an alternative to the harvesting of wild specimens and providing biomass to restore the stock with repopulation programs by releasing reared specimens in the environment (Giglioli *et al*., 2021). In this context, the Department of Life and Environmental Sciences of the University of Cagliari (Italy) established an experimental hatchery for echinoderms in collaboration with some local companies belonging to the sea urchins fishery (Prato *et al*., 2018; Giglioli *et al*., 2021; Secci *et al*., 2021; Solari *et al*., 2021). One of the objectives of the rearing projects (EU grant number 606042, FP7-SME-2013; European Maritime and Fisheries Fund, EMFF 2014–2020 Measure: 2.47—Innovation) was the study of land-based breeding technologies and processes, with a view to optimizing the environmental sustainability of sea urchin farming and, in the future, the economic profitability of echinoculture.

When rearing sea urchins is intended for human consumption purposes, like other seafood, compliance with hygienic–sanitary standards is of fundamental importance. Echinoderm production is subject to the provisions contained in Regulation (EC) No. 852/2004 (European Commission, 2004b) on the hygiene of food products. Regulation (EC) No. 853/2004 (European Parliament and Council, 2020) equates echinoderms with live bivalve mollusks for controls carried out along the production chain, except for the provisions relating to purification, as they are grazing herbivores, not filter-feeders. Sea urchins intended for human consumption must meet the following criteria for fecal contamination indicators: *Salmonella* spp. must be undetected in 25 g of product, and *Escherichia coli* must have values not exceeding 230 MPN (most probable number) in 100 g of edible portion (European Commission, 2004a, 2005). Other controls related to microbiological quality, algal biotoxins (e.g. paralytic shellfish poison, diarrhetic shellfish poison, and amnesic shellfish poison), and chemical contaminants (for example, lead, mercury, cadmium) should be regulated by local authorities based on the national guidelines of the Ministry of Health and local health authorities' indications (Permanent Conference for Relations between the State, the Regions and the Autonomous Provinces of Trento and Bolzano, 2010).

In this setting, the characteristics of marine waters of fishing grounds where sea urchins are harvested are one of the requirements of the wild sea urchin supply chain in Sardinia. Here, the coastal waters have been classified by the Autonomous Region of Sardinia as 'A' areas for collecting *P. lividus* (Autonomous Region of Sardinia, 2021), indicating a very low risk of contamination (excluding ports, industrial areas, river mouths, zones of industrial or urban discharges). On the other hand, monitoring the water quality used in land-based facilities has gained a key role both for animal welfare and in the outlook of commercial-scale production. To achieve good production standards, seawater used in land-based facilities should have chemical, physical, and microbiological characteristics that meet precise health and hygiene criteria, following the same criteria for the classification of marine waters.

Starting from these considerations, in the present study, we characterized the microbiological quality of different matrices (water, air, and equipment surfaces) in a land-based facility to produce *P. lividus* biomass for conservative purposes and the exploration of commercial-scale processes. In addition, following the matrices characterization, we analyzed sea urchins' gonads and coelomic fluid for *Vibrio* spp. and *Pseudomonas* spp. This study will support the development of technical production regulations, system monitoring, and routine maintenance of the facility in a view of 'best practice' approach.

Materials and Methods

Description of the facility

The sampling activity was conducted in June 2023 at the 'Living Lab' breeding facility (Figure 1) located in the locality Sa Illetta on the waterfront of the Santa Gilla Lagoon (southern Sardinia, Cagliari, Italy; 39° 13.760′ N; 9° 4.761′ E). The facility consists of three units (Figures 2 and 3). Unit one (1), for phytoplankton production meant to provide food for larval stages of marine invertebrates, such as sea urchins and sea cucumbers, consisted of a system of 12 cylindrical Plexiglas photobioreactors (PBR, 30 L each) for microalgae production in batch (Figure 2). The PBRs were filled with

Figure 1. Panoramic view of the land-based facility for the rearing of echinoderms at the Living Lab of the University of Cagliari.

sanitized culture medium (F/2 medium) and inoculated with the monospecific microalgal culture. When the culture reached the death phase, the PBR was emptied and cleaned. The cleaning consisted of mechanical removal of dirt, rinsing several times, and filling with fresh water containing 0.5 ml/L 5% sodium hypochlorite (bleach) for 24 h before subsequent reutilization.

The second unit (2) is for the storage of the water collected from the lagoon (Figure 3B). Water was stocked in two concrete tanks (7000 L each) and treated with a system of a 60-µm sand filter and a protein skimmer with ozone treatment. The storage unit provided the water for the phytoplankton unit (1) and grow-out unit (3).

The third unit (3) is dedicated to larval settlement and the growth of juvenile sea urchins (Figure 3A). This unit operated with recirculating water consisting of six rearing V-shaped tanks (700 L), each provided with an aeration system with diffusers. The wastewater was collected in the compensation tank and conveyed to the water treatment system provided with a 60-µm sand filter, UV sterilization system, and a chiller to regulate the temperature. At the time of sampling, approximately 500 sea urchins were raised in each tank for a total of 3000 specimens. Sea urchins in the tanks were in good health because they did not show any sign of diseases (for example, 'bald sea urchin', black or green spots on body surfaces, partial spine loss, and discoloration of the peristomium;

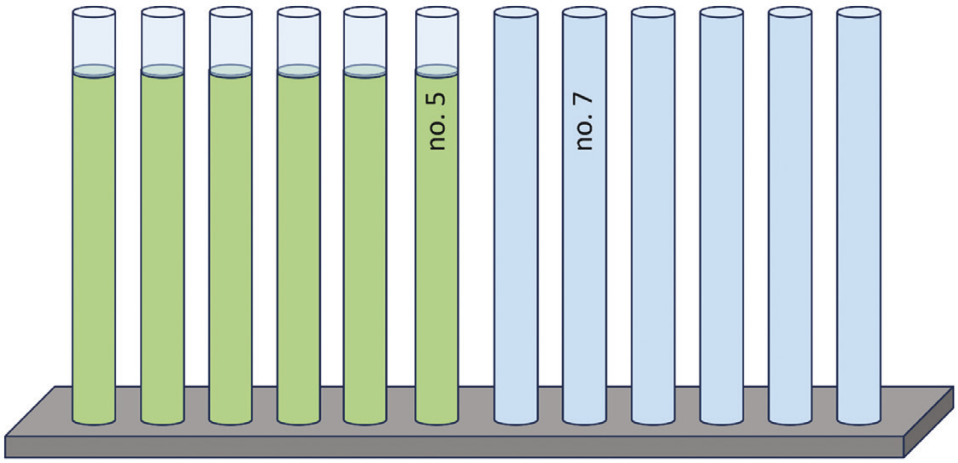

Figure 2. Scheme of the unit (1) for phytoplankton production with sampling points in photobioreactor (PBR) no. 5 and no.7.

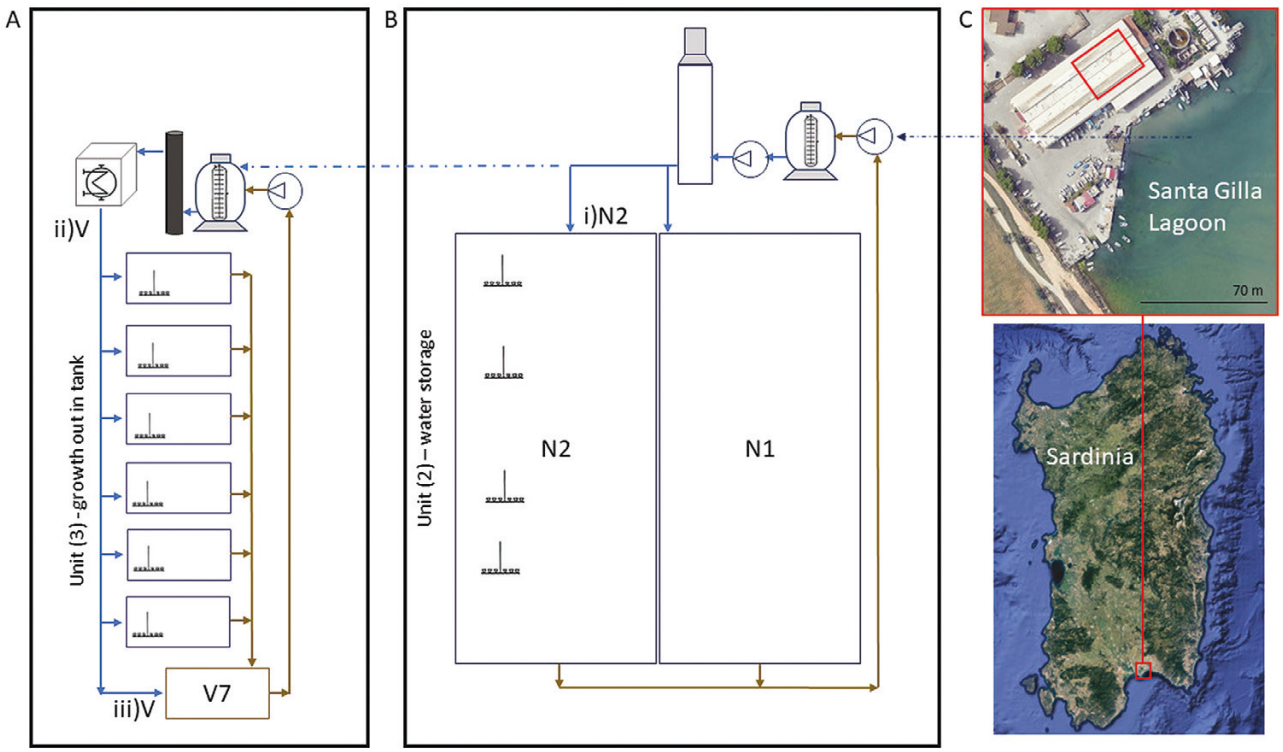

Figure 3. Scheme of units 2 (A) and 3 (B). The seawater is collected from the neighboring lagoon located in the south of Sardinia Island, Mediterranean Sea (C). The different steps for water treatments are reported: 1, water pump; 2, sand filter; 3, protein skimmer; 4, ozone; 5, UV sterilizer; 6, chiller. Sampling points are also indicated: N1, N2, (i)N2, (ii)V, (iii)V, and V7.

Keegan and O'Connor, 1984; McBride, 2005; Becker *et al.*, 2008; Maes and Jangoux, 2020). The whole system was provided by a multiprobe for dissolved oxygen, pH, temperature, and salinity connected to a WiFi for real-time web monitoring (OxyWifi, Tecnos, Chioggia, Italy).

Samplings

The monitoring points involved the sampling of 500 L of air in the center of unit (1), which houses the microalgae cultures (Figure 2) used in feeding the larval stage of the sea urchin and its first stages of development. The ambient temperature at the time of sampling was 22 °C. The active sampling method involved the use of the Air Surface System (ASS) through the conveyance of volumes of air through filters and deposition on specific culture media for researching and enumerating total mesophilic bacteria (UNI EN 13098:2019+UNI EN ISO. 4833-1:2022), yeasts and molds (UNI EN 13098:2019+ISTISAN REP. 160 37/13 13/37). Some surfaces were also sampled for research on mesophilic counts, yeasts, and molds (UNI EN ISO 18593:2018+4833-1:2022 and UNI EN ISO 18593:2018+21527-1:2008): the side wall of the interior of bioreactor no. 7 (after sanitization); the side wall and the interior of photobioreactor no. 5 containing microalgae (Figure 2). The samples were taken to the Hygiene Laboratory of the University of Cagliari (accredited UNI EN UNI IEC 17025:2018) with thermal bags equipped with data loggers.

Water samples before and after purification from units (2) and (3) were taken to evaluate whether the concentration of indicator bacteria (total coliforms and *Escherichia coli*) was effectively reduced. Using sterile bottles with a capacity of 1 L, water was taken from the water reserve in unit (2), identified as N2, contained in a concrete tank (Figure 3B), with air insufflation (salinity 33×10^{-6}). Total coliforms and *E. coli* (UNI EN ISO 9308-1:2017), Enterococci (UNI EN ISO 7899-2:2003), Pseudomonadaceae (UNI EN ISO 16266:2008), *Staphylococcus aureus* (DM 10/02/2015 GU No. 50 02/03/2015 All IV par 2.5), sulfite-reducing *Clostridia* (ISO 15213:2003), and *Vibrio* (ISO/TS 21872-1:2007), were searched for. Furthermore, the mesophilic count was determined at 22 °C (UNI EN ISO 6222:2001). Isolated colonies were identified through the API Biomerieux system (API Staph, API NE, Api 20E). The same procedure was applied to the water reserve tank N1 without insufflation (Figure 3B). The sampling activity continued with the sampling of the delivery water from (i) the water reserve (N2), where the water came out of the tap following an initial purification treatment process with sand filtration, protein skimmer, and ozone procedure (Figure 3B), (ii) the outlet water (V) from the chiller tap of the unit (3) subjected to a second purification process with sand filter and UV sterilization (Figure 3A), and (iii) the tap relating to the second filtration water (V) eliminated due to excess (compensation tank V7; Figure 3A). In these samples, the same microbiological parameters reported for the other waters were searched. The wastewater collected from grow-out tank V7 was also examined via serial dilutions, and the same analytical methods were applied (Figure 3A).

Sea urchins were collected from the 'Living Lab' breeding facility, transported to the laboratory refrigerated (4–8 °C) in seawater, and immediately processed after arrival. Sea urchins were opened with a knife under sterile conditions, and gonads and coelomic fluid were collected. Isolation of *Vibrio* spp. from gonads and coelomic fluid followed the ISO/TS 21872–1:2023 standard. Twenty-five grams of gonads and 25 g of coelomic fluid were enriched in 225 mL of alkaline saline peptone water (ASPW; Microbiol Diagnostici, Uta, Italy). After a first incubation at 37±1 °C/6±1 h, 1 mL of the culture was transferred to 10 mL ASPW and incubated at 41.5±1 °C for 18±1 h. After the secondary enrichment, 10 µL of culture was streaked on TCBS agar and incubated at 37±1 °C for 24±3 h (Microbiol Diagnostici, Uta, Italy). We did not find typical colonies in the samples, and consequently there was no transfer to nutrient saline agar. Enumeration and isolation of *Pseudomonas* spp. followed the ISO 13720:2010 standard. Ten grams of gonads and 10 g of coelomic fluid were homogenized in 90 mL of ASPW. Additionally, a tenfold dilution series was made from the homogenate using a sterile saline solution (Microbiol Diagnostici, Uta, Italy). From the initial suspension and dilutions, 0.1 mL was transferred and spread on CFC agar (Microbiol Diagnostici, Uta, Italy). The isolated colonies were confirmed by oxidase assays and biochemical tests (API NE, bio-Merièux, Marcy l'Étoile, France). To optimize the recovery of *Pseudomonas* spp. and *Vibrio* spp. in the coelomic fluid, we first filtered the remaining homogenate with a 0.22-µm diameter filter (Merck Millipore, Darmstadt, Germany), and the filter was then placed on CFC agar and incubated at 25±1 °C/44±4 h. For *Vibrio* spp., a further 25 mL of coelomic liquid, after 1:10 dilution with ASPW, was filtered through 0.22-µm filters (Merck Millipore, Darmstadt, Germany). Then, the filter was resuspended in 225 mL ASPW, and we proceeded as indicated by the method.

Results

The air in the phytoplankton unit showed a concentration of mesophilic microorganisms and a yeasts and molds count of 120 colony-forming units (CFU)/m^3 and 160 CFU/m^3, respectively. The inside of the post-sanitization photobioreactor showed a total mesophilic count (TMC) <1 CFU/cm^2, whereas the photobioreactor containing the microalgae had a value of 5 CFU/cm^2. From the examination of the presence of *Vibrio* spp. in all waters of the facility, *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damselae*) emerged and was isolated and identified in CN agar culture medium. *Bacillus cereus* and *Clostridium perfringens* were also isolated. The results related to water reserve N2, with insufflation, which showed a TMC concentration of 36 CFU/100 mL at 22 °C, coliforms and *Escherichia coli* equal to 12 CFU/100 mL and 11 CFU/100 mL, respectively, and Enterococci equal to 240 CFU/100 mL and *Pseudomonas aeruginosa* equal to 60 CFU/250 mL. The analysis of water reserve N1, without insufflation and with stasis, showed a TMC concentration of 40 CFU/100 mL at 22 °C, coliforms and *E. coli* equal to 8 CFU/100 mL, Enterococci equal to 250 CFU/100 mL and *P. aeruginosa* equal to 240 CFU/250 mL. Following the first filtration process, the water taken from the tap showed a TMC equal to 32 CFU/mL, and coliforms and *E. coli* equal to 16 CFU/100 mL and 15 CFU/100 mL, respectively. The concentration of Enterococci was 300 CFU/100 mL and that of *P. aeruginosa* was 350 CFU/250 mL. The results of the second treatment, at the outlet of the chiller tap, were TMC at 22 °C <1 CFU/mL; *E. coli*, coliforms, and Enterococci

<1 CFU/100 mL; *P. aeruginosa* 30 CFU/250 mL; and *B. cereus* 6 CFU/100 mL. At the tap outlet, the excess water resulting from the second filtration showed TMC <1 CFU/mL; *E. coli*, coliforms, and Enterococci <1 CFU/100 mL; and *P. aeruginosa* equal to 6 CFU/250 mL. When wastewater left unit (3) and was introduced into the recirculation system, the TMC was 47 CFU/mL; *E. coli*, coliforms, and Enterococci were <1 CFU/100 mL; and *P. aeruginosa* was 64 CFU/250 mL. Table 1 provides a summary of the results.

No colonies corresponding to the targets (*Vibrio* spp., *Pseudomonas* spp.) were isolated in the sea urchins' gonads or coelomic fluid.

Discussion and Conclusions

The supply chain for the consumption of the sea urchin *Paracentrotus lividus*, which is the most economically and commercially important sea urchin species for Mediterranean regions, is assimilated by law for shellfish production. The same rules that govern the production and distribution of live bivalve mollusks intended for human consumption are applied to *P. lividus* sanitation. These requirements are contained in the hygiene package, represented by the following community regulations: (a) No. 852/2004 (European Commission, 2004b) on the hygiene of food products; (b) No. 853/2004 (European Parliament and Council, 2020), which establishes specific hygiene rules for foods of animal origin; and (c) No. 854/2004 (European Commission, 2004c), which establishes specific rules for the organization of officials' controls on products of animal origin intended for human consumption. Although EC Reg. No. 853/2004 refers to echinoderms that live bivalve mollusks, sea urchins are not filtering organisms and cannot be submitted to relaying treatment. Therefore, these data can only be collected in 'A' coastal areas (European Commission, 2005) and are meant for direct human consumption after dispatch center controls, where the minimum sales-size is verified and the health stamp is affixed for marketing.

Because the gonads are usually consumed raw with no intermediate measures applicable to the product, contamination with pathogens represents a significant food safety risk. Current regulations are based on fecal bacteria control in mollusks and do not ensure pathogen-free products. For example, the presence of fecal bacteria is not necessarily related to Vibrionaceae and Pseudomonadaceae, which are usually found in marine environment, or enteric viruses, which are more resistant than bacteria to water treatments, and can be present even in water without fecal bacteria (Croci and Suffredini, 2003; Melis *et al.*, 2014). There are many types of vibrions that are naturally found in aquatic environments, especially in coastal marine waters and estuaries, and approximately half of them have been linked to infections in humans or aquatic animals (Melis *et al.*, 2014). The severity of some Vibrio infections (for example, *V. cholerae* and *V. vulnificus*) and the high number of toxic infections they can cause (for example, *V. parahaemolyticus*) are particularly concerning. The incubation period is between 12 h and 72 h. Watery diarrhea, abdominal pain, vomiting, and fever are the typical symptoms, but in some cases, the infection can manifest as septicemia, cutaneous lesions, and necrosis (Croci and Suffredini, 2003). The infectious dose is approximately 108 CFU/g for immunocompetent subjects, but lower for individuals with hypochlorhydria (Melis *et al.*, 2014). For both bacteria and viruses, the virulence of the etiological agent is not the only factor determining the disease; we should also consider the immunocompetence of the consumer. The cause of *Pseudomonas aeruginosa* infection, for example, is unknown, but it is well known that most infections occur in hospitalized patients, particularly those who are debilitated or immunocompromised (Reynolds and Kollef, 2021; Sathe *et al.*, 2023). Depending on site entry (skin, lungs, and urinary tract), the infection may manifest differently and sometimes cause severe sepsis (Mena and Gerba, 2009). *Pseudomonas aeruginosa* has a high capacity for adaptation, and it is found in all humid environments, surface waters, wastewater, and marine waters, as vibrions and many other pathogens. This aspect should not be overlooked when discussing the safety of sea urchins as food; however, despite the outlined scenario, very few studies have been conducted on the potential role of sea urchin roes as vectors of bacteria and viruses to date (Santos-Ferreira *et al.*, 2020a, 2020b).

Table 1. Microbiological concentrations

Matrices	TMC	Yeasts and molds	*Escherichia coli*	Coliforms	Enterococci	*Pseudomonas*
Air—phytoplankton unit	120 CFU/m^3	160 CFU/m^3				
Photobioreactor (post-sanitization) (no. 7)	<1					
Photobioreactor (microalgae) (no. 5)	5 CFU/cm^2	1 CFU/cm^2	<1	<1	<1	<1
Water reserve N2	36 CFU/100 mL		11 CFU/100 mL	12 CFU/100 mL	240 CFU/100 mL	60 CFU/250 mL
Water reserve N1	40 CFU/100 mL		8 CFU/100 mL	8 CFU/100 mL	250 CFU/100 mL	240 CFU/250 mL
Tap water—post first filtration (i)N2	32 CFU/100 mL		15 CFU/100 mL	16 CFU/100 mL	300 CFU/100 mL	350 CFU 250 mL
Tap water—post second filtration (ii)V	<1		<1	<1	<1	30 CFU/250 mL
Excess water (iii)V	<1		<1	<1	<1	6 CFU/250 mL
Wastewater V7	47 CFU/100 mL		<1	<1	<1	64 CFU/250 mL

Considering *P. lividus*, a brand new chapter is represented by hygienic–sanitary regulations addressed to breeding facilities, which are configured as sites of production of the product 'from scratch'. Currently, there are no *P. lividus* breeding facilities in Sardinia for commercial purposes, and hygienic–sanitary protocols are still needed for land-based facilities both at the national and regional levels. Type and frequency of checks to be carried out during the harvesting of bivalve mollusks and echinoderms are defined by a local regional plan (Regione Autonoma della Sardegna, 2021). However, beyond the monitoring of collection water and final products, *P. lividus* reared in land-based facilities requires hygienic control of the breeding environment due to different contamination risks and the non-filtering nature of the species. Managing health security hazards while also considering animal welfare is essential, particularly for commercial-scale production. Different aspects may impact *P. lividus* production. For example, whether in the wild or closed aquatic systems, sea urchins can be affected by opportunistic bacteria, leading to mass mortality events (Feehan *et al.*, 2013; Wang *et al.*, 2013; Gizzi *et al.*, 2020; Hira and Stensvåg, 2022).

In this study, an evaluation of the health aspects of land-based *P. lividus* production was carried out. We focused on microbiological characterization of the breeding environment (air, water, and surfaces) to provide targeted advice for the development of technical production regulations, system monitoring, and maintenance of on-land implant. The coastal lagoon considered, which provides the water supply for the sea urchin factory, is classified as zone 'B'. Here, live bivalve mollusks are collected and marketed for human consumption only after purification or relaying to satisfy the health standards required for the 'A' class areas (Department of Agriculture and Agropastoral Reform - Sardinia Region, 2019). *P. lividus* cannot undergo relaying procedures and must be collected only in 'A' zones, where live bivalve mollusks and echinoderms can be collected for direct human consumption. Considering this aspect, although the products are not marketed, the sea urchin facility is equipped with a water treatment system to guarantee water quality suitable for breeding *P. lividus*.

The results showed that the air quality of the hatchery is satisfactory and could be further improved by stricter monitoring of air system filters, namely increasing replacement/maintenance frequency. The air introduced through filters is, in fact, the main factor influencing the hygienic quality of bioreactor surfaces, where the simple opening is exposed to the risk of sedimentation at any microbial concentration, mainly represented by molds (*Penicillium* spp., *Cladosporium* spp., *Paecylomices* spp.) and bacteria of the genus *Bacillus*. In the face of TMC of 120 CFU/m^3 and 160 CFU/m^3 of yeasts and molds detected, setting an average concentration of 150 CFU/m^3 of air can be an adequate reference for maintenance standard drafting. The fecal indicator microorganisms in the seawater entering the storage tanks, both with and without insufflation, would need close monitoring to evaluate the effectiveness of the purification system: we suggest a check at least every 6 months. The results show that water purification is obtained as a result of the second filtration process and could be further improved by eliminating a residual concentration of microorganisms represented by colonies of *Bacillus* spp., *Pseudomonas* spp., and *Staphylococcus* spp., isolated in the water from the plant entrance. Concerning this aspect, we suggest an upgrading of the filtration system, which would result in more effective and sustainable purification. This approach would also improve the water recirculation system because the wastewater's initial residual concentration of 6 CFU/250 mL of *P. aeruginosa* increased to 64 CFU/250 mL at the wastewater outlet. In this regard, the water recirculation system should be periodically assessed and monitored, considering the efficiency decrease linked to reuse. As expected, no indicators of fecal contamination were detected in 100 mL of treated seawater. In contrast, in the pretreatment water, high concentrations of Enterococci, indicators of fecal contamination, were measured. Enterococci proportion together with *E. coli* concentrations point towards the origin of the contamination of animals rather than humans. This is an example of contamination classified as an unidentifiable source of pollution (Regione Autonoma della Sardegna, 2021) that derives from the presence of wild animals and birds and represents a lower risk than those arising from human sources. In this regard, physical isolation of the facility would prevent accidental contamination (that is, by birds) of the environment investigated. All access points should also be regularly checked by the staff. Furthermore, the analysis conducted on the gonads and coelomic fluid of sea urchins for *Vibrio* spp. and *Pseudomonas* spp. was negative, confirming that no microbiological hazards were linked to these pathogens and the sea urchins from the 'Living Lab' breeding facility were in good health.

This analysis represents a starting point for redacting best practice guidelines for preventing microbial contamination of land-based facility for the biomass production of the sea urchin *P. lividus*. The rearing of *P. lividus* is a promising reality that could soon establish itself in the Italian aquaculture industry. In view of promoting this new sustainable production and employment opportunity in the sea food market of Sardinia, this study offers support to food operators in characterizing some of the health aspects that guarantee the quality and food safety of the final product. It also represents a first step towards the elaboration of common regulations about the minimal standards of the echinoculture environment of *P. lividus* by national and regional competent authorities. Furthermore, following 'one health' principles (European Commission, 2017; Lindenmayer and Kaufman, 2021), this study contributes to achieving better animal welfare, production, and safety of the final product.

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Author Contributions

All the authors contributed to the design and implementation of the research, the analysis, the evaluation of the results, and the writing and revision of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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