RESEARCH ARTICLE

Underwater hyperspectral classification of deep sea corals exposed to 2-methylnaphthalene

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Abstract

Coral reefs around the world are under threat due to anthropogenic impacts on the environment. It is therefore important to develop methods to monitor the status of the reefs and detect changes in the health condition of the corals at an early stage before severe damage occur. In this work, we evaluate underwater hyperspectral imaging as a method to detect changes in health status of both orange and white color morphs of the coral species Lophelia pertusa. Differing health status was achieved by exposing 60 coral samples to the toxic compound 2-methylnaphthalene in concentrations of 0 mg L\(^{-1}\) to 3.5 mg L\(^{-1}\). A machine learning model was utilized to classify corals according to lethal concentration (LC) levels LC5 (5% mortality) and LC25 (25% mortality), solely based on their reflectance spectra. All coral samples were classified to correct concentration group. This is a first step towards developing a remote sensing technique able to assess environmental impact on deep-water coral habitats over larger areas.

Introduction

Coral reefs are the foundation of many marine ecosystems. Corals are found across the world’s ocean, in both shallow tropical and subtropical waters and in deep water. Deep-water corals thrive in cold, dark water at depths of up to 6000 m [1]. They have a cosmopolitan distribution, being particularly abundant in the North East Atlantic, and are for instance found off the coast of Norway and deep underwater in the Mediterranean Sea. In contrast to tropical corals, they are azooxanthellate, meaning that they do not have symbiotic life forms with dinoflagellates and hence do not require direct access to sunlight [2]. Lophelia pertusa (Linnaeus, 1758) is one of the most abundant reef forming scleractinian deep-water corals in cold and temperate regions, with main occurrences at depths ranging from 200 m to 1000 m [3, 4]. The species is found as two color morphs (orange and white) due to different pigment composition causing phenotype specific optical fingerprints [5, 6]. The reef framework offers structural habitat for a
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The exposure experiment received financial support from the project “Species Sensitivity Distribution for Deep Sea Species and Toxicity of continuous and spiked exposures to crude oil at 1 atm for deep sea species”, led by Akvaplan-niva and financed by the American Petroleum Institute (API). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Ecotone AS, Statoil and Akvaplan-niva AS provided support in the form of salaries for authors PAL, IMH, LMSA, IE, RP, LT, LC and JB but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The specific roles of these authors are articulated in the “author contributions” section.

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variety of benthic species [2], including gorgonian corals, sponges, squat lobsters (Munida sarsi) and rosefish (Sebastes viviparous) as well as economically important fish species such as Atlantic cod (Gadus morhua), saithe (Pollachius virens), and cusk (Brosme brosme) [3, 7–9].

The coral reefs are in danger, primarily due to climate change and increased CO₂ levels [10], leading to ocean acidification. The increased CO₂ levels can affect the ability of the coral to form its aragonite skeleton [11, 12]. The combined pressures can result in coral bleaching, slower growth [13] and reproduction rates, and degraded reef structures [14]. Other chemical and physical stressors may also damage corals. The oil industry is moving northwards and many of the drilling areas coincide with habitats listed by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) as being potentially rare or declining, including the cold-water coral reefs and sponge grounds. Corals and sponges are slow growing [15], and their habitats are therefore regarded as vulnerable to the smothering effects from drill cuttings [16]. Coral colonies affected by the Deepwater Horizon oil spill have shown signs of stress and mortality [17, 18]. Mechanical damages from trawling vessels are also considered a threat to the reefs [3, 15].

The Norwegian environmental regulatory authorities have imposed requirements for detailed habitat and organism mapping prior to exploratory drilling, as well as post-drilling surveying to map the distribution of deposited drill cuttings and the extent of possible biological impacts. Further, mapping of sensitive species and habitats to accidental oil pollution is an essential part of contingency plans, and species distribution maps are a crucial tool to assist responders during an incident, especially when the industrial activities are near shore.

Hyperspectral imaging collects and processes incoming light across a range of the optical spectrum. In contrast to ordinary photographic cameras (or similarly, the human eye) which record only three color bands (red, green, and blue), a hyperspectral camera produces a full spectrum of all available wavebands in each pixel in an image. Objects will reflect and absorb light to a varying degree at different wavelengths depending on their color and pigmentation providing a spectral signature of that object. Hyperspectral imaging generates large amounts of data which require sophisticated data analysis and machine learning methods [19]. Multivariate data analysis and machine learning have been used successfully in several marine environmental studies for the interpretation of large data sets, for example in integrated environmental monitoring [20] and for the analyses of photos to assess the potential impact of water-based drill cuttings on deep-water rhodolith-forming calcareous algae [21].

Hyperspectral imaging is widely used as an in-situ and non-invasive sensor for species mapping and detection of ecosystem health status [22]. Hyperspectral imagers are typically operated from satellite or aeroplane [23], for instance, shallow water corals are monitored by remote sensing from satellites [24]. The Underwater Hyperspectral Imager (UHI) used in the present work represents a recent system for identification, mapping and monitoring of objects of interest (OOI) at the seabed [25, 26]. However, underwater spectral measurements have been used to measure changes in coral appearance using point measurements [27], multispectral camera [28] and hyperspectral camera [29], all in the visible part of the electromagnetic spectrum, as both ultra violet and infrared radiation is attenuated in water.

The purpose of the present work was to evaluate the use of UHI and multivariate data analysis to detect changes in health condition of the coral species L. pertusa. Corals were exposed to 2-methylnaphthalene in laboratory experiments in order to provide corals with health condition varying from unaffected to dead. Hyperspectral images of exposed and control corals were then recorded after a recovery period. Finally, classification of these images using machine learning shows in a visual way which spatial areas are affected by exposure to toxic compounds.
Materials and methods

In order to evaluate the use of underwater hyperspectral imaging as a \textit{L. pertusa} health detection tool, corals needed to be sampled and different health conditions established prior to acquisition of hyperspectral images. Hence, the experimental work presented in this study consists of the following activities: collecting and rearing of coral samples, exposure to the toxicant 2-methylnaphthalene, monitoring the corals to determine polyp mortality, and imaging them using UHI. An overview of the timeline is given in Table 1.

Sampling and rearing of corals

Samples of \textit{L. pertusa} were collected at Stokkbergenet in Trondheimsfjorden (Norway, 63.47˚N, 9.91˚E) on September 1st 2015 in collaboration with the Norwegian University of Science and Technology (NTNU) onboard R/V Gunnerus. The site is characterized by a steep rock wall with \textit{L. pertusa} occurring in colonies from 100 m to 500 m depth [30].

The coral samples were collected from four different colonies at depths between 208 m to 235 m. Both white and orange color morphs were sampled. A Sperre Subfighter 7500 remotely operated vehicle (ROV) (“Minerva”) with an attached fish net was used to conduct the sampling. Precaution was taken in order to avoid any damages on other parts of the reefs. This is described in more detail in S4 Fig.

\textit{L. pertusa} has a wide geographical distribution throughout the world, and the density is particularly high along the coast of Norway. The species is not defined as threatened or protected in Norway, but due to the reported decline the last 10 years, they are defined as nearly threatened according to the terms used in the Norwegian Red List for Species. The list is prepared by the Norwegian Biodiversity Information Centre in accordance to the International Union for Conservation of Nature (IUCN). As the sampling was conducted outside of national parks or other type of protected area, no specific permissions were required.

The corals were transported to the Akvaplan-niva Research and Innovation Center Kraknes (RISK) (Tromsø, Norway) by car in a water tank where temperature, oxygen levels and oxygen saturation were frequently monitored. The temperature range and oxygen saturation during transport was 5.1˚C to 9.8˚C and 98% to 113%, respectively. The pieces of \textit{L. pertusa} were divided into smaller samples and placed in silicon tubes attached to steel grids (20 cm × 40 cm) in 500 L tanks with a continuous flow (500 L h\(^{-1}\)) of filtered (60 μm filter) and UV-treated bottom sea water (63 m depth) from the fjord Sandesundet, adjacent to the research facility. One sample refers to a coral branch with 3 to 9 polyps. The temperature, oxygen saturation, and oxygen concentration were monitored on a daily basis during rearing, and the corals were fed three times per week with \textit{Calanus} sp. nauplii from Planktonic AS. The corals were cleaned from sediments once per week.

Table 1. Timeline of experimental activities.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time T (h)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-03-05</td>
<td>-24</td>
<td>Acclimatization start</td>
</tr>
<tr>
<td>2016-03-06</td>
<td>0</td>
<td>Exposure start</td>
</tr>
<tr>
<td>2016-03-10</td>
<td>96</td>
<td>Exposure end</td>
</tr>
<tr>
<td>2016-03-10</td>
<td>96</td>
<td>Recovery period start</td>
</tr>
<tr>
<td>2016-05-03</td>
<td>1392</td>
<td>UHI scanning of all coral samples</td>
</tr>
<tr>
<td>2016-05-03</td>
<td>1440</td>
<td>Classification of polyp mortality</td>
</tr>
</tbody>
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Exposure setup and procedure

2-methylnaphthalene is classified as a low molecular weight poly aromatic hydrocarbon (PAH) with high water solubility. The compound was chosen because it was expected to be sufficiently water soluble and toxic to produce corals with varying health status. In addition, PAHs are abundant constituents in crude oil and are also found in water from oil wells [31].

The set-up comprised five treatment groups: one control group (C0) and four exposed groups (C1, C2, C3 and C4). Each treatment group consisted of four replicates (R1, R2, R3 and R4). Each replicate consisted of one orange and two white pigmented morphs, giving a total of 20 orange and 40 white coral samples. The corals were assessed to be in good condition when sampled, throughout rearing and the 24 h acclimatization before we started the exposure to 2-methylnaphthalene. The samples were randomly distributed in order to represent all four coral colonies at Stokkbergenet.

Each sample comprised a coral branch with 3 to 9 polyps. Briefly, after 24 h acclimatization, L. pertusa were used in a 96 h acute toxicity testing to 2-methylnaphthalene (see Table 1 for a timeline). During acclimatization and exposure, each coral replicate were kept in a square glass aquarium (1.5L). The animals were not fed during the toxicity testing.

The acute toxicity tests to 2-methylnaphthalene involve passive dosing. Based on the principles of passive dosing described by Butler et al. [32], a passive dosage open system with flow-through using silicone O-rings has been developed. Passive dosing was chosen to obtain an as stable concentration of 2-methylnaphthalene as close to nominal concentration as possible. An open system was chosen as the experiment was a dual-project experiment where it was necessary to take into account optimal strategies for other end points than the end point of interest in this particular paper. In detail, the end points we needed to take into consideration were sensitive behavioral end points such as polyp activity. Polyp activity is an endpoint sensitive to sudden changes in water flow and handling, and hence, moving the animals at T0 was not an option, and acclimatization in the exposure beakers was necessary. The O-rings were cleaned before they were loaded with 2-methylnaphthalene with target concentrations in seawater of 0 mg L$^{-1}$, 1.03 mg L$^{-1}$, 2.27 mg L$^{-1}$, 5.00 mg L$^{-1}$, and 8 mg L$^{-1}$ to 10 mg L$^{-1}$ (saturation) for C0 to C4, respectively. After O-rings were loaded, they were set to equilibrate with seawater in 20 L bottles for 24 h, one bottle per treatment group. The final stock solution was pumped into the exposure chambers continuously using a peristaltic pump with the inlet placed in the bottom of the exposure beakers as illustrated in Fig 1. Stock solutions were made in 24 h cycles, and hence, all stock bottles were replaced every 24 h. Approximately 20% (4 L) of the exposure media were left in the bottles at the time the stock bottles were removed and replaced. Overflow ensured that the overhead between water surface and glass lids covering the exposure beakers always was at a minimum. Using O-rings combined with a pump is beneficial as it reduces the interference and stress on the corals during the exposure period. The sensitive behavioral endpoints we needed to take into account also made it necessary to go from exposing the corals to pure sea water during acclimatization until T0, and from there to gradually pump in and replace the sea water with the exposure media with the desired concentrations of 2-methylnaphthalene. Based on a combination of the coral oxygen needs, chemistry and kinetics, tubing capacity, pump capacity and practical reasons, a flow rate of approximately 160 mL h$^{-1}$ was selected. The exchange rate of exposure solvent in the exposure beakers was about 2.5 times per 24 h. In addition to logging of temperature every 30 min throughout the 96 h exposure (Tidbit Temperature Data Logger V2, Onset, Massachusetts), the temperature and oxygen saturation was measured daily throughout the experiment (Oxyguard®). The chambers were covered with a glass lid to reduce evaporation of 2-methylnaphthalene. All stock solution bottles and treatment group replicates were placed on a magnet stirrer throughout the whole
experiment to keep the exposure media homogeneous. The exposure was conducted in a room holding 4 ± 1°C. After the toxicity test was ended, all coral samples were kept for approximately 12 h in individual tanks with pure seawater to provide an initial period of depuration of 2-methylnaphthalene before they were transferred to a single recovery tank of the same type and with similar facilities as they were kept in prior to the toxicity test.

In addition to the 60 samples in the five treatment groups, six coral samples (two orange and four white) referred to as reference alive were also included in the set-up. The reference alive corals originated from the same coral colonies as the samples used in the toxicity test. The reference samples were kept in the rearing tanks with pure seawater while the C0–C4 corals went through the toxicity experiment. Reference alive corals were kept in the same tank as C0–C4 during the recovery period. Hence, reference alive corals were exposed to minimal handling compared to C0–C4 before they were imaged with the UHI. The reference alive group was included as an additional control group in case of accidental contamination of the experimental control group (C0) or in case handling itself affected the corals and their spectral properties.

**Polyp mortality**

Prior to exposure to 2-methylnaphthalene (at time ~24 h, see Table 1 for a timeline), the number of alive polyps on each coral sample was counted; see Fig 2 for an example. Mortality was assessed after recovery and UHI scans (at time 1440 h). This approach was chosen because it

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**Fig 1. Setup for 2-methylnaphthalene exposure.** Based on the principles of passive dosing described by Butler et al. [32], a passive dosage open system with flow-through using silicone O-rings has been developed. The peristaltic pump transfers stock solution from the stock bottle to the exposure chamber containing the test organisms.

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was not possible to determine whether polyps were alive or dead immediately after end of exposure. By keeping the coral samples in a recovery tank over weeks, the samples and individual polyps could be monitored visually for the presence of soft tissue on skeleton and polyps. A polyp was classified as dead when soft tissue was no longer present within the polyp’s calyx. Illustration of live and dead polyps are given in Fig 2. The fraction of dead polyps (i.e., the number of dead polyps divided by the number of alive polyps before toxicant exposure) is presented in results and statistics as polyp mortality.

Chemical analysis of exposure concentration

Preparation of water samples. 10 mL water samples were collected from the exposure beakers and stock bottles in Tromsø, Norway. Water samples were collected from the exposure
beakers at time 0 h, 24 h, 48 h, and 72 h. Water samples were collected also from the stock bottles at time 0 h, 24 h, 48 h, and 72 h, and 96 h. Each stock bottle lasted for 24 h. So in addition to sample all bottles at the beginning of each 24 h cycle when the stock bottles were full (referred to as A for after stock bottle replacement), we also sampled each of the bottles at the end of each 24 h cycle when approximately 20% of the exposure media was left in the bottles (referred to as B for before stock bottle replacement). This was done to monitor the potential impact of decreasing water volumes throughout the 24 h cycle. Samples were acidified to pH 1 using hydrogen chloride (HCl), added methanolic solution (1.8 mL) of perdeuterated naphthalene (Nd8) at the concentration of 100 \( \mu \text{g mL}^{-1} \) and frozen at \(-20^\circ\text{C}\). The final concentration of Nd8 in water samples was 15.3 \( \mu \text{g mL}^{-1} \). The water samples were then sent to Brest, France in a cool box for analysis.

**Extraction of water samples and conditions of analysis.** The water samples were thawed overnight in the fridge, and then extracted with 2 mL of pentane. A calibration curve was established for 2-methylnaphthalene concentrations in the range 0 mg L\(^{-1}\) to 10 mg L\(^{-1}\) with a fixed concentration of Nd8.

Samples were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The GC was an HP 7890 series II (Hewlett-Packard, Palo Alto, CA, USA) equipped with a Multi Mode Injector (MMI) used in the pulsed splitless mode (Pulse Splitless time: 1 min. Pulse Pressure: 103 kPa). The injector temperature was maintained at 300°C and 1 \( \mu \text{L} \) of the extract was injected. The interface temperature was 300°C. The GC temperature gradient was from 70°C (0 min) to 250°C (0 min) at 15°C min\(^{-1}\). The carrier gas was Helium at a constant flow of 1 mL min\(^{-1}\). The capillary column used was a HP 5-ms (Hewlett-Packard, Palo Alto, CA, USA, 30 m in length, 0.25 mm internal diameter, and a film thickness of 0.25 \( \mu \text{m} \)). The GC was coupled to a HP 5975 Mass Selective Detector (MSD) used in the Electronic Impact mode (electronic impact energy 70 eV, system temperatures: 230°C (source) and 150°C (quadrupole)). 2-methylnaphthalene quantifications were done using Single Ion Monitoring mode with respectively the molecular ion of each compound at a minimum rate of 2 s\(^{-1}\) (mass/charge ratio of 142 for 2-methylnaphthalene and 136 for Nd8). 2-methylnaphthalene was quantified relatively to the perdeuterated Nd8 introduced at the beginning of the sample preparation procedure using calibration curves.

**Underwater hyperspectral imaging**

The underwater hyperspectral imager (UHI) is a line camera (often referred to as a “push broom” sensor), consisting of a narrow slit, a spectrograph, and a monochrome 2D camera, mounted in a waterproof housing made of aluminum with a fused silica window. Communication with the camera is achieved through a sub-sea cable.

**Image acquisition.** For capturing images of an area, the UHI is installed on a moving platform, where it captures frames perpendicular to its direction of motion. The UHI was configured to acquire data in the wavelength range 381 nm to 846 nm.

Reflectance is a physical property of an object that can be attributed to material properties. The measured reflectance depends on several parameters such as incident and observation angle, and hence the surface structure of the material in interest. In remote sensing and imaging it is common to assume diffuse reflectance from the targets. This is an assumption about the smoothness of the surface of materials which is surprisingly often valid in nature.

In water, the largest obstacle for achieving accurate reflectance measurements is the optical wavelength dependent attenuation of the water itself. This property alters the apparent color of an object if it is viewed through water at varying depths (optical path length). Water from
different sources in time and space can have non-identical constituents and hence a different attenuation coefficient.

To compensate for the optical attenuation of water, a Spectralon diffuse reflectance standard (Labsphere inc) with dimensions 20 cm × 20 cm × 3 cm was imaged alongside the coral samples. By comparing the measured spectra of the Spectralon, $I_{\text{spec}}(x, \lambda)$, with its calibrated reflectance spectrum, $R_{\text{spec}}(\lambda)$, a conversion factor, $A(x, \lambda)$, was found for every spatial pixel (at position $x$) covered by the Spectralon.

$$A_{\text{spec}}(x, \lambda) = \frac{I_{\text{spec}}(x, \lambda)}{R_{\text{spec}}(\lambda)}$$

(1)

Due to the small size of the reference target a larger PVC plate, roughened with a 500 grit size sandpaper, was used in addition. The reflectance spectrum of the reference target was used to calculate the reflectance spectrum of the PVC plate. The reflectance of the PVC, $R_{\text{PVC}}(\lambda)$, was then calculated by applying the conversion factor, $A_{\text{spec}}(x, \lambda)$, to the spatial pixels in the UHI slit covering the Spectralon, when imaging the PVC. Since Spectralon was 3 cm thick, the reflectance of the PVC plate was calculated for 3 cm as ($N_{\text{spec}}$ is the number of Spectralon pixels):

$$R_{\text{PVC}}(\lambda) = \sum_x \frac{I_{\text{PVC}}(x, z = 3 \text{ cm}, \lambda)}{N_{\text{spec}} A_{\text{spec}}(x, \lambda)},$$

(2)

In order to account for the optical path length of samples of various heights the PVC plate was inclined such that all spatial pixels could be compared to a known reflectance at its own height.

The conversion factors could then be found for all spatial pixels in the slit at any altitude,

$$A(x, z, \lambda) = \frac{I_{\text{PVC}}(x, z, \lambda)}{R_{\text{PVC}}(\lambda)},$$

(3)

The height of the coral samples was measured to be approximately 7 cm using a ruler. The reflectance of the coral samples could thus be obtained using

$$R(x, y, z = 7 \text{ cm}, \lambda) = \frac{I(x, y, \lambda)}{A(x, z = 7 \text{ cm}, \lambda)},$$

(4)

where $I(x, y, \lambda)$ is the recorded intensity, i.e., the hyperspectral image.

The coral samples and reflectance references were placed in a tank of dimensions 2.0 m × 1.0 m × 1.6 m, filled with sea water. The UHI and lighting was mounted on a linear motorized rail; see Fig 3 and S5 Fig. The vertical distance between the spectrograph and coral samples was 0.35 m. Two halogen lamps (Osram Decostar 51 TITAN 50 W 12 V 60˚ GU5.3) with constant power supply were the only light sources during the measurements. Halogen lights were used because of their relative good uniformity across the wavelengths of light for which water is transparent. The whole setup was surrounded by a black tent to avoid light pollution. The UHI was moved along the rail with a constant speed of 0.13 m s$^{-1}$ during imaging. By comparison with focal sheets placed in the bottom of the tank, the spatial resolution of the recorded images was estimated to be approximately 2.5 mm in the center of the slit. The diffraction limited spectral resolution was approximately 5 nm, while the spectra were sampled approximately every 0.5 nm.
Pixel extraction

The reflectance spectra from each coral sample was extracted by manually selecting image pixels; a total of 103 to 718 pixels were selected depending on the size of the coral. Pixels were selected both from the branches and the polyps of the coral. The reflectance spectrum was labelled with the sample ID and stored as an entry in a database. Each entry contained information about the measured reflectance for each pixel at each wavelength from 381 nm to 846 nm, and the pixel position in the original UHI image (corresponding to the position in the tank).

Data used in spectral classification

Spectra were recorded for the 60 coral samples representing the C0 to C4 corals used in the exposure study, as well as for the 6 reference alive corals. Due to poor transmission of ultra violet and near infrared wavelengths even in extremely clean water [33], poor signal quality was experienced in both ends of the spectrum. Therefore, only data from the spectral range 400 nm to 750 nm are included in this study. The spectra recorded are placed in a matrix $X$, where each column corresponds to one wavelength, and each row corresponds to one observation, i.e., a pixel in the hyperspectral image.

The matrix $Y_h$, used for Projection to Latent Structures (PLS) [34] in the preprocessing stage only, consists of the following columns: 2-methylnaphthalene concentration (arithmetic mean of the concentrations measured in the exposure beakers at time 0 h, 24 h, 48 h, and 72 h) and polyp mortality. Each row of the matrix $Y_h$ corresponds to the same row in $X$, i.e., spectra associated with the same coral sample.

The vector $Y_c$, used for classification, consists of one categorical variable, namely, the exposure category of the organism which the spectrum came from. The exposure category is
determined by the LC5 and LC25 concentration (lethal concentration for 5% and 25% of polyps) of 2-methylnaphthalene: the non- or low exposure category was exposed to a concentration \( C \leq \text{LC5} \); the intermediate exposure category was exposed with \( \text{LC5} < C \leq \text{LC25} \); and the high exposure category was exposed with \( C > \text{LC25} \).

**Classification algorithm**

A three-stage machine learning model was applied to classify the exposure level of the coral samples. A flow chart for the data analysis process is given in Fig 4, showing the steps in the data analysis. In all cases, separate models were trained on spectra from white and orange corals, as the pigmentation of the coral can easily be determined using a simple linear classifier.

The stages consist of standardization, PLS and transformation, followed by a \( \nu \)-Support Vector Machine (\( \nu \)SVM) classification algorithm [35–37]. Note that linear classification algorithms were found to be unsuitable to the problem at hand, due to the nonlinear separation of points in the PLS latent variable space. The scikit-learn software package was used for both of the PLS and SVM algorithms [35].

**Standardization of spectra.** Before attempting classification, the model inputs were standardized:

\[
z_i = \frac{x_i - \mu_i}{\sigma_i}
\]

where \( z_i \) is the scaled value, \( x_i \) is the original value, and \( \mu_i \) and \( \sigma_i \) is the mean and standard deviation of feature \( i \) (i.e., the spectrum intensity at wavelength \( i \)), respectively. Standardization implies that all input variables to the model have zero mean and unit variance, thus avoiding influence of, e.g., light intensity variations caused by placement of light sources, unrelated to the reflectance of the imaged object. Each feature corresponds to a wavelength in the spectrum, and a column of the input data matrix \( X \):

\[
X = [x_1 x_2 \ldots x_i \ldots]
\]

where the \( x_i \) are column vectors containing all measurement values. Similarly, standardization was also performed on \( Y_h \) (2-methylnaphthalene concentration and polyp mortality).

**Dimensionality reduction.** Following standardization of inputs, a PLS model relating spectra \( (X) \) to 2-methylnaphthalene concentration and polyp mortality \( (Y_h) \) is constructed:

\[
Y_h = BX + E
\]

where \( E \) is the error term. In the process of constructing the regression coefficient matrix, \( B \), the input matrix \( X \) is transformed to a lower-dimensional (latent) subspace; hence, the name PLS. This is done in a way such that the covariance between the \( X \) and \( Y_h \) matrices is maximized. Furthermore, PLS selects variables that extract the maximum relevant information possible. These latent variables are simply weighted sums of the original variables (e.g., the recorded spectrum intensity at each wavelength). One can think of the colors red, green, and blue as three latent variables used by our eyes to interpret the optical spectrum. The vector of the weights are referred to as loading vectors and reveals which underlying variables (in our case, wavelengths) that have the most explanatory power. Note that the loadings are ordered such that the first PLS component contains the most explanatory power, and subsequent component contain less and less explanatory power between the variables. In our case, a dimensionality reduction from 619 dimensions (wavelengths) to 10 dimensions (i.e., 10 latent variables) was chosen. The number of latent variables was selected using 5-fold stratified cross-validation.
However, we note that the results were not sensitive to the exact number of latent variables, and that classification performed well for 5 to 20 latent variables.

Subsequently, classification is improved both in quality and speed, as noise is removed and the dimensionality of input data is reduced significantly. Also, separation of spectra is improved as the PLS algorithm maximizes covariance between spectra and 2-methylnaphthalene concentration and polyp mortality.

Fig 4. Data analysis flowchart. The flowchart shows the full data analysis pipeline including all steps of the classification model.

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Classification of spectra and samples. The input to the classification stage is, as described above, spectra ($X$) transformed into the 10-dimensional PLS latent variable space, as well as 2-methylnaphthalene concentration and polyp mortality ($Y$). The SVM model constructed uses the parameter $\nu = 0.1$ and a radial basis function kernel [36], as the spectra are not linearly separable in the latent variable space.

After classification of individual spectra, we classify each coral sample to the class with the highest number of single spectra (the majority vote algorithm). This lends significant robustness to incorrect classification, as many individual spectra can be classified incorrectly without affecting overall area coverage.

We note that model training and testing takes on the order of one minute on a desktop computer, and the use of the model to classify new data require only fast and well optimized computational operations, e.g., matrix multiplication. As such, the computational requirements are light, and should not pose any problems for practical use.

Results and discussion
Chemical analysis of 2-methylnaphthalene concentration
Chemical analysis of water samples from the stock bottles confirm that the desired concentrations of 2-methylnaphthalene, by using loaded silicon O-rings, were obtained. The average 2-methylnaphthalene concentration in the water samples from the stock bottles sampled at time 0 h, 24 h, 48 h and 72 h were 123%, 120%, 82% and 102% of nominal concentrations for C1 to C4, respectively (see Table 2 and S2 Table). The 2-methylnaphthalene levels in the C0 stock bottles samples at the same time points were all below 0.033 mg L$^{-1}$ (which was the limit of detection), and hence, regarded as acceptable. In our set-up, the volumes of exposure media in the stock bottles containing the saturated O-rings were reduced by approximately 80% over the 24 h each stock bottle was used. When using passive dosing techniques, a decreasing water volume results in an increased silicon/water ratio, which again and in theory, can increase the water concentration of the chemical absorbed to the silicon. As this was anticipated, a pilot study and calculations were conducted before starting the coral experiment to examine what we could expect in the set-up we used in this particular study. Calculations show that, taking into account the simultaneous decrease of the water:silicone ratio and the amount of 2-methylnaphthalene in silicone, the equilibrium concentrations do not change significantly. These concentrations are in the range 0.17 mg L$^{-1}$ to 0.20 mg L$^{-1}$ for a reduction of water in the stock bottle from 50% to 90%. In addition, these concentrations, slightly lower than the initial one (0.21 mg L$^{-1}$), are liable to be greater due to kinetics effect (the equilibrium is reached only after several hours). Moreover, the pilot study conducted at Cedre enabled the determination of the transfer coefficient for lower temperatures, in agreement with the exposure test, whereas only data at 20˚C is available in the literature. Results of experiments showed that this coefficient determined experimentally was reliable. However, despite the conducted pilot study and calculations, we did collect water samples from the stock bottles both at the start and at the end of each 24 h cycle to confirm that concentration changes due to reduced water volumes in stock bottles were minor (S1 Table). If we exclude the control stock bottle, we have 16 other cases covering the C1 to C4 stock bottles where we could study the changes in concentration over a 24 h cycle (S1 Table). Except from two cases where significant increases were reported, C3 from exposure start to 24 h (70% increase) and C3 from 24 h to 48 h (144% increase), the increase in concentrations were below 23% throughout the 24 h cycle in seven other cases. In four cases the concentrations were unchanged. More unexpected, concentrations were in three cases also reported to drop up to 8%.
Although we cannot explain the variation fully, some of the variation in the 2-methylnaphthalene concentrations are due to other unknown factors, possibly photo degradation of the molecules. We also see variation between stock solutions with identical preparations and with similar nominal concentrations (S1 Table). That we also see variation in the chemical data from parallel samples collected from the same replicate at the same time point, and that we see variation between replicates receiving stock solution from the same stock bottle opens up for multiple but unfortunately unknown explanations to the variation in exposure doses between the replicates in this complex set-up (S2 Table).

The 2-methylnaphthalene concentration in the water samples from the exposure beakers (arithmetic mean of concentrations measured at time 0 h, 24 h, 48 h, and 72 h) were somewhat lower than in the stock bottles and with C1 to C4 calculated to be 44% (replicates ranging from 38% to 54%), 47% (replicates ranging from 40% to 60%), 27% (replicates ranging from 2% to 47%) and 32% (replicates ranging from 26% to 42%) of nominal concentrations, respectively; see Table 2 and S2 Table. However, one of the four C3 replicates (R2) deviated from the others. The replicate C3 R2 contained significantly lower 2-methylnaphthalene concentrations than the other replicates with only 2% of nominal concentration achieved.

Table 2. Concentration of 2-methylnaphthalene (mg L\(^{-1}\)).

<table>
<thead>
<tr>
<th>Exposure group</th>
<th>C0</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal concentration (mg L(^{-1}))</td>
<td>0.0</td>
<td>1.0</td>
<td>2.3</td>
<td>5.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Stock solution</td>
<td>avg</td>
<td>0.0</td>
<td>1.3</td>
<td>2.6</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.2</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>1.0</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.0</td>
<td>1.5</td>
<td>3.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Beakers 1–4 (avg)</td>
<td>avg</td>
<td>0.0</td>
<td>0.4</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.2</td>
<td>0.6</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.1</td>
<td>0.8</td>
<td>2.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Beaker 1</td>
<td>avg</td>
<td>0.0</td>
<td>0.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.3</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>0.0</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.0</td>
<td>0.6</td>
<td>2.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Beaker 2</td>
<td>avg</td>
<td>0.0</td>
<td>0.4</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.0</td>
<td>0.7</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Beaker 3</td>
<td>avg</td>
<td>0.0</td>
<td>0.4</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.2</td>
<td>0.6</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.0</td>
<td>0.7</td>
<td>1.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Beaker 4</td>
<td>avg</td>
<td>0.0</td>
<td>0.5</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>std dev</td>
<td>0.0</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>0.1</td>
<td>0.8</td>
<td>1.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Concentration of 2-methylnaphthalene (mg L\(^{-1}\)) measured in water samples collected from stock solutions and exposure beakers every 24 h in the 96 h exposure period. Concentrations are given as average (avg), standard deviation (std dev), minimum (min), and maximum (max) values based on measured concentration in duplicate water samples from each time points. Nominal concentrations are listed for each exposure group.

https://doi.org/10.1371/journal.pone.0209960.t002
while the remaining three replicates ranged from 39% to 47% of the nominal value (see S2 Table). The deviation of C3 R2 was probably due to a faulty pump channel. The levels of 2-methylnaphthalene in the control (C0) beakers were all below 0.07 mg L\(^{-1}\), and hence, regarded as acceptable. The lower concentration of 2-methylnaphthalene in the exposure beakers compared to the stock bottles is likely explained by evaporation of 2-methylnaphthalene from water masses to air as the systems were not completely closed. PAHs can easily stick to plastic tube walls. To reduce loss of 2-methylnaphthalene when exposure media is transported through the tubes, we used teflon coated tubes and each of the tubes connecting the stock bottles to the exposure beakers were saturated with 2-methylnaphthalene for 24 h prior to exposure start. It is unlikely that bioaccumulation of 2-methylnaphthalene in coral tissue was of significance as the biomass of corals in each replicate beaker was low (ranging from 9 g to 21 g coral, skeleton and soft tissue, in 1.5 L sea water). In all beakers, the concentration of 2-methylnaphthalene was lower at exposure start than measured at later time points (S2 Table). This initial increase is explained by non-contaminated water masses being replaced by contaminated stock solution during the first hours (beaker size of 1.5 L and flow of approximately 160 mL h\(^{-1}\)) of the exposure period. Actually, only 7 of the 16 replicates representing the C1 to C4 beakers show increasing concentrations from the first to the fourth and last water sample (S2 Table). That the concentration fluctuate, indicate that the highest concentration can be any time between a few hours after exposure start and the end of exposure dependent on several factors such as for example stock solution concentration, evaporation rate and flow. Besides what the data in S1 and S2 Tables tells us, it is thereby not possible to conclude when the concentration in the exposure beakers were highest and exactly how it fluctuated in between the measuring points.

**Polyp mortality**

The fraction of dead polyps at the end of the experiment correlated with the 2-methylnaphthalene exposure doses, and based on the sigmoidal dose-response curve the LC5 and LC25 values were determined (Fig 5). Each point in the figure represent one sample (with 3 to 9 polyps), with groups of 3 samples sharing the same 2-methylnaphthalene concentration, as they were placed in the same exposure beaker. Concentrations of 2-methylnaphthalene are based on levels in water samples collected during the exposure period, while the polyp mortality was assessed approximately 8 weeks later. This approach was necessary because it was challenging, if not impossible, to determine whether polyps were alive or dead shortly after exposure was ended. The coral samples could have both live and dead polyps at the time they were assessed. Whether mortality of one single polyp has any impact on neighboring polyps are unknown as there to our knowledge is highly limited knowledge about communication and signaling between polyps of *L. pertusa*. However, it has been shown that there are no nervous communication between polyps in *L. pertusa* colonies [39]. Although no firm conclusion can be given, we believe that there has been no influence of intercommunication between polyps affecting our mortality data.

The variation in polyp mortality was relatively high with a polyp mortality ranging from 0% to 50% for concentrations from 0.9 mg L\(^{-1}\) to 2.3 mg L\(^{-1}\), and a polyp mortality ranging from 0% to 100% for concentrations between 2.3 mg L\(^{-1}\) to 2.7 mg L\(^{-1}\). (Note that the variation in toxin concentration might be a significant cause of variation in observed mortality at a given concentration.) The mortality ranged from 0% to 17% for concentrations of 2-methylnaphthalene ranging from 0.0 mg L\(^{-1}\) to 0.9 mg L\(^{-1}\). The confidence bounds correspond to a 95% confidence interval, computed by bootstrapping (\(N = 10^4\)). The best fit curve is a generalized least squares model with a logit link function, yielding \(R^2 = 0.54\) and was constrained to pass
through the origin. The dependent variable was binarized before fitting the model, as polyp mortality can be seen as an effect on the organism.

Based on the analysis shown in Fig 5, categories of exposure have been chosen with limits corresponding to LC5 and LC25:

- Non-exposed and lowest exposed corals: \( C \leq 1.25 \text{ mg L}^{-1} \)
- Intermediate exposed corals: \( 1.25 \text{ mg L}^{-1} < C \leq 2.30 \text{ mg L}^{-1} \)
- Highest exposed corals: \( C > 2.30 \text{ mg L}^{-1} \)

Note that the inherent uncertainty in the exposure concentration, as well as the lack of a toxicant concentration yielding a consistently high mortality, makes the absolute concentration levels (LC5 and LC25) uncertain. Furthermore, what we here refer to as low, intermediate and high doses, is based on the doses used in the toxicity test to ensure mortality was obtained, and does not refer to what we consider as low, medium, and high concentrations in situ and associated with, e.g., oil spills.

As such, the doses of 2-methylnaphthalene chosen in this study were not primarily chosen to be environmentally relevant. Compared to measured doses of naphthalenes in sea water samples during oil spills, e.g. Deepwater Horizon, the doses used in the present study are considerably higher [40]. Water samples collected and analyzed for oil compounds, including naphthalenes, during or shortly after an oil spill, are generally below the lowest levels of exposure reported in this study. In a study by Guyormach et al. [41], dissolved PAH concentration was monitored during a field trial in the North Sea, with 2 slicks of respectively 1 m\(^3\) and 3 m\(^3\) of Grane crude oil. The maximum concentration, for the sum of 1-methylnaphthalene and 2-methylnaphthalene, was below 2 \(\mu\text{g L}^{-1}\).

![Fig 5. Dose-response curve for 2-methylnaphthalene exposure and polyp mortality (n = 60). The regression line with a 95% confidence interval indicates that there is indeed a positive correlation between polyp mortality and 2-methylnaphthalene exposure. Some data points have similar values, and hence, overlap in the plot. The vertical red dashed lines indicate the 2-methylnaphthalene category thresholds and coincide with the 5% and 25% mortality levels (LC5 and LC25, respectively). The corresponding 2-methylnaphthalene concentrations are 1.25 mg L\(^{-1}\) and 2.3 mg L\(^{-1}\), respectively.](https://doi.org/10.1371/journal.pone.0209960.g005)
For the PLS model, a total of 10 latent variables were retained; cross-validation suggested an essentially flat plateau of model performance for 5 to 20 latent variables. The first six loading vectors are shown in Figs 6 and 7, for white and orange corals, respectively. For reference, some example spectra (i.e., rows in the X matrix) taken from unexposed and high exposure coral samples are shown in S1 Fig. The figures indicate which parts of the spectrum is

**Fig 6. PLS X loadings for white corals.** The PLS X loadings show which parts of the spectrum are significant in explaining covariance between spectra and 2-methylnaphthalene concentration and polyp mortality. Higher order loadings carry less significance in explaining the variation in the corals exposure doses and mortality; this is a property of the PLS algorithm.

https://doi.org/10.1371/journal.pone.0209960.g006

**PLS model**

For the PLS model, a total of 10 latent variables were retained; cross-validation suggested an essentially flat plateau of model performance for 5 to 20 latent variables. The first six loading vectors are shown in Figs 6 and 7, for white and orange corals, respectively. For reference, some example spectra (i.e., rows in the X matrix) taken from unexposed and high exposure coral samples are shown in S1 Fig. The figures indicate which parts of the spectrum is

**Fig 7. PLS X loadings for orange corals.** Same as Fig 6, but created using data from orange corals. A notable difference is the significant noise in loading 6, indicating that explanatory power is decreasing at this point.

https://doi.org/10.1371/journal.pone.0209960.g007
important for explaining covariance between spectra and 2-methylnaphthalene concentration and polyp mortality. Several spectral features, i.e., peaks and dips, can be observed across the spectrum, especially for higher order loadings. Loading 5 and 6 exhibit high frequency noise, indicating that one is approaching an appropriate cutoff in number of latent variables. Some features appear to be present in both color morphs (e.g., the peak/dip at 650 nm for loading 5 in both color morphs), whereas others are observed only in one color morph (e.g., for white corals, a dip is observed in loading 3 at 540 nm, but no such dip is observed for orange corals). Each spectral dip or peak (note that the sign of loading vectors is arbitrary) can potentially be interpreted as absorption by a chemical compound in the coral. It is of interest to examine further the sources of these spectral features, as it would enable the creation of more robust interpretation models for hyperspectral imagery.

After fitting the PLS model to training data, \( X \) scores were extracted. The scores correspond to coordinates in the latent variable space. The first three score components for white coral data can be seen in Fig 8. One dot in the scatter plot corresponds to one coral spectrum, i.e., one hyperspectral image pixel. While the color scale in the figures is continuous, only 20 distinct values appear. This is due to the experimental setups, where each of the five treatment groups consisted of four replicates, giving a total of 20 exposure beakers containing 3 coral samples each. Measurements of 2-methylnaphthalene concentration were done for each replicate.

When examining Fig 8, we observe clustering of low exposure values near the origin, whereas spectra from corals exposed to higher values of 2-methylnaphthalene form scattered clusters outside of the central cluster. We attribute this clustering partly to the 2-methylnaphthalene exposure levels being highly clustered. When rotated in a 3-dimensional view, one can more easily see that the spectra of high exposed corals form a “shell” outside of the central cluster consisting mainly of low exposed corals, and do not take the shape of a simple linear structure. With this in mind, we note that a linear discriminant will not be able to separate the spectra from the lowest and highest concentrations, nor will a linear regression algorithm (including PLS regression) satisfactorily predict 2-methylnaphthalene exposure level. However, as we observe good separation between the non-exposed to lowest exposed corals, the intermediate exposed corals and the highest exposed corals, we have performed spectral classification using the nonlinear classification algorithm SVM.

Score plots analogous to Fig 8, but for orange \( L. \ pertusa \), are given in S2 Fig. Finally, identical score plots but with polyp mortality as the color variable are shown for both color morphs in S3 Fig.

**Classification**

The quality of the classification of exposure category is assessed using the metrics precision, \( P \), and recall, \( R \), which are frequently used in the context of classification. The metrics are defined as

\[
P = \frac{T_p}{T_p + F_p}
\]

and

\[
R = \frac{T_p}{T_p + F_n}.
\]

Here, \( T_p \) is the fraction of true positive classifications, and \( F_p \) and \( F_n \) is the fraction of false positive and false negative classification, respectively. Finally, we give the \( F1 \)-score, which takes
both precision and recall into account as the harmonic average:

\[
F_1 = 2 \frac{PR}{P + R}
\]  

(10)

Note that all quantities $T_p, F_w, F_p, P, R$, and $F_1$ are defined on the interval $[0, 1]$, and that a high score of $P, R$, and $F_1$ equal to 1 is optimal.

**Fig 8. PLS scores for white corals.** (a) shows scores for X latent variable 1 vs latent variable 2, (b) shows scores for X latent variable 1 vs latent variable 3. One dot corresponds to one spectrum (i.e. one hyperspectral image pixel) taken from a coral.

https://doi.org/10.1371/journal.pone.0209960.g008
The input data is split 80–20 into a training data set (80%) and a test data set (20%). The split is performed using random stratified sampling, giving each class (no or low, medium, and high exposure) a number of training data samples proportional to the size of the class. The classification model is fit to the training data set, and all classification results shown below are for the test data set.

Results for the three-class case are given in Table 3. The overall picture is that an accuracy in the range 78% to 97% is achieved using SVM classification. The poorest performance is found for high exposed corals. This is possibly due to the fact that fewer spectra were collected for these classes (see the “pixels” column of Table 3).

Per-organism classification. Obviously, each pixel of each coral cannot have its exposure level be classified separately. Hence, following classification of spectra, i.e., hyperspectral image pixels, we classify the corals at the sample level. This is done by the majority vote algorithm: the coral sample is assigned to the exposure category that most of its pixels are. In this manner, spatial information is used to improve classification, without resorting to advanced image processing algorithms. When this is done, 100% of all samples are classified in the correct exposure category.

An illustration of this final classification step is shown in Fig 9. Here, images of two corals for each of the three exposure categories are shown, with classified pixels shown in the row below. A few scattered pixels are classified incorrectly; e.g., in the center image, a few orange and black pixels are interspersed within the red pixels. By using the majority vote algorithm within each connected region, each Lophelia pertusa sample is classified correctly. This illustrates the power of spatial information when classifying hyperspectral images. Finally, we note that the spatial pattern below is valuable for environmental monitoring, as this technique can be used directly to generate maps of exposed corals.

Reference alive corals

Finally, the 6 reference alive L. pertusa samples (2 orange and 4 white) that were in no way subjected to the handling and experimental conditions in the toxicity test, were scanned with the UHI. Classification on these corals were done with the SVM classifier model trained on data from the main treatment groups C0–C4.

The spectra taken from the orange reference corals classify with a precision of 1.00 and a recall of 0.95, whereas spectra from the white reference corals classify with a precision of 1.00

Table 3. Summary of classification results for single spectra, using the processing pipeline shown in Fig 4. P denotes precision, R recall, and F1 is the harmonic average of P and R.

<table>
<thead>
<tr>
<th>Color morph</th>
<th>Exposure</th>
<th>Pixels</th>
<th>P</th>
<th>R</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>Low</td>
<td>1252</td>
<td>0.87</td>
<td>0.95</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>457</td>
<td>0.87</td>
<td>0.80</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>440</td>
<td>0.92</td>
<td>0.78</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Total/avg</td>
<td>2149</td>
<td>0.88</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>Orange</td>
<td>Low</td>
<td>512</td>
<td>0.85</td>
<td>0.98</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>235</td>
<td>0.94</td>
<td>0.79</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>216</td>
<td>0.97</td>
<td>0.78</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Total/avg</td>
<td>963</td>
<td>0.90</td>
<td>0.89</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Classification results for the three-class case in terms of precision (P), recall (R), and F1-score. An overall F1 score of 0.88 and 0.89 is achieved for white and orange corals, respectively. The “pixels” column shows the number of spectra collected for each class. Note that average values refer to the harmonic average.

https://doi.org/10.1371/journal.pone.0209960.t003
and a recall of 0.88. This is consistent with the classification results of single spectra in the results above, and gives a verification that the experimental conditions have not systematically modified the spectra, at least with respect to classification results.

Finally, at the organism level, 100% of reference coral samples were classified in the category of nonexposed- or lowest exposed corals. This is correct, as the reference corals were kept separately from the coral samples exposed to 2-methylnaphthalene, and should thus be representative of healthy corals in the wild.
Due to the disadvantages of the flow-through system used for the exposure experiment, which was necessary due to the sensitive nature of the species studied, the exposure levels LC5 and LC25 are not as accurately determined as desired. However, the overarching goal of this study is to determine whether underwater hyperspectral imaging has potential as a remote sensing technology to investigate environmental impact over larger areas. The ability of the UHI to classify corals into three exposure categories, where the toxicant exposure concentration are well separated, indicates that this is a promising venue of continued research.

**Conclusion**

Underwater hyperspectral imaging has been shown capable of classifying the cold water coral *L. pertusa* according to their individual exposure to the toxic petroleum compound 2-methylnaphthalene, when categorized according to lethal concentration levels LC5 and LC25 (5% and 25% mortality, respectively). A classification model consisting of projection to latent structures followed by a support vector machine classifier achieves a classification score of 73% to 100% correctness for single spectra. When exploiting the spatial information in the hyperspectral images, a full 100% of *L. pertusa* samples are classified correctly under laboratory conditions. The model has been verified with hyperspectral images of reference coral samples not exposed to any toxic compound.

**Future work**

Scientists have often struggled to provide an integrated and non invasive assessment of coral health status. In that respect, this study represents the first step towards a non-invasive, automated method for in situ mapping of deep-water coral condition. In order to develop exposure mapping using underwater hyperspectral imaging into a field ready method, several challenges remain. Such a tool would be more valuable if several indicator species can be used, not just *L. pertusa*. Further studies should be conducted on environmentally relevant doses of relevant mixtures of toxicants, as these are encountered following oil spills. In a coral physiology perspective, future studies should focus on assessing the underlying biochemical mechanisms for the mortality-linked spectral features, analogous to the widely discussed loss of colors (coral bleaching) caused by the death of symbiont chlorophyll containing algae of tropical corals. Determining whether coral mortality induced by other causes than toxicants, e.g., ocean acidification and smothering by drill cuttings, can be measured using the same hyperspectral technique may lend more generality to the method.

**Supporting information**

*S1 Table. Measured 2-methylnaphthalene concentration in stock bottles.* S1 Table shows the concentration of 2-methylnaphthalene measured in water samples collected from stock solutions every 24 h during the 96 h exposure period. The average (avg) value and corresponding standard deviation (std dev) are also given. Nominal concentrations, $C_{\text{nom}}$, are listed for each treatment group. Each water sample was performed in duplicate, with each duplicate labeled I or II in the table. Regarding the analyses performed on the 2-methylnaphthalene water samples, the limit of quantification is 0.1 mg L$^{-1}$, and the uncertainty is 20% at the limit of quantification. This uncertainty decreases above the limit of quantification.

(PDF)

*S2 Table. Measured 2-methylnaphthalene concentration in exposure beakers.* S2 Table shows the concentration of 2-methylnaphthalene for the exposure beakers. The exposure was
performed with 3 corals in each exposure beaker, across 4 replicates for each of the 5 treatment groups. Regarding the analyses performed on the 2-methylnaphthalene water samples, the limit of quantification is 0.1 mg L\(^{-1}\), and the uncertainty is 20% at the limit of quantification. This uncertainty decreases above the limit of quantification. Additional uncertainty is expected as the magnetic stirring mechanism cannot operate at high frequency, as this would disturb the coral samples.

**S1 Fig.** Example spectra from hyperspectral images of the two color morphs of *Lophelia pertusa*, exposed and unexposed to 2-methylnaphthalene. Reflectance spectra of white unexposed corals (top left), orange unexposed corals (top right), white high exposure corals (bottom left), and orange high exposure corals (bottom right). For each subfigure, all three spectra are taken from the same specimen.

**S2 Fig.** PLS X scores for orange corals. PLS X scores for orange corals are given in S2 Fig. The results correspond exactly to those presented in Fig 8, except that S2 Fig presents results for the orange color morph.

**S3 Fig.** PLS X scores for corals with mortality coloring. PLS X scores colored with the mortality variable are given for orange (A) and white (B) corals are given in S3 Fig. The results correspond exactly to those presented in Fig 8 and S2 Fig, except for the coloring of the dots.

**S4 Fig.** Equipment used for sampling of corals. The ROV was maneuvered towards the rocky wall growing *L. pertusa* colonies, and the fish net was carefully poked towards the corals from below. This resulted in pieces of the coral colony breaking off and landing in the fish net. Precaution was taken to avoid any accidental damage of adjacent colonies.

**S5 Fig.** Experimental setup for UHI image acquisition. S5 Fig shows the experimental set-up for hyperspectral imaging. The photo corresponds to schematic sketch given in Fig 3. Experimental setup for UHI image acquisition. The upper image shows coral samples placed on grids at the bottom of a water filled tank. The UHI with lamps were attached to a scanning mechanism, and was set to image the tank bottom scenery while moving in a “push broom” fashion, keeping constant speed and distance to the tank bottom. A Spectralon reference plate and a PVC plate was used to correct for the inherent optical properties of the water. The lower photo details three coral samples attached to a steel grid.

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