

Autonomic Nervous System characterization in hyperbaric environments considering respiratory component and non-linear analysis of Heart Rate Variability

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Abstract—Objectives: an evaluation of Principal Dynamic Mode (PDM) and Orthogonal Subspace Projection (OSP) methods to characterize the Autonomic Nervous System (ANS) response in three different hyperbaric environments was performed. **Methods:** ECG signals were recorded in two different stages (baseline and immersion) in three different hyperbaric environments: a) inside a hyperbaric chamber, b) in a controlled sea immersion, c) in a real reservoir immersion. Time-domain parameters were extracted from the RR series of the ECG. From the Heart Rate Variability signal (HRV), classic Power Spectral Density (PSD), PDM (a non-linear analysis of HRV which is able to separate sympathetic and parasympathetic activities) and OSP (an analysis of HRV which is able to extract the respiratory component) methods were used to assess the ANS response. **Results:** PDM and OSP parameters follows the same trend when compared to the PSD ones for the hyperbaric chamber dataset. Comparing the three hyperbaric scenarios, significant differences were found: i) heart rate decreased and RMSSD increased in the hyperbaric chamber and the controlled dive, but they had the opposite behavior during the uncontrolled dive; ii) power in the OSP respiratory component was lower than power in the OSP residual component in cases a and c; iii) PDM and OSP methods showed a significant increase in sympathetic activity during both dives, but parasympathetic activity increased only during the uncontrolled dive. **Conclusions:** PDM and OSP methods could be used as an alternative measurement of ANS response instead of the PSD method. OSP results indicate that most of the variation in the heart rate variability cannot be described by changes in the respiration, so changes in ANS response can be assigned to other factors. Time-domain parameters reflect vagal activation in the hyperbaric chamber and in the controlled dive because of the effect of pressure. In the uncontrolled dive, sympathetic activity seems to be dominant, due to the effects of other factors such as physical activity, the challenging environment, and the influence of breathing through the scuba mask during immersion. In sum, a careful description of the changes in all the possible factors that could affect the ANS response between baseline and immersion stages in hyperbaric environments is needed for better interpretation of the results.

Index Terms—electrocardiogram, hyperbaric environment, autonomic nervous system, heart rate variability, principal dynamic mode, orthogonal subspace projection.

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I. INTRODUCTION

Hyperbaric environments are those scenarios in which atmospheric pressure increases, that is, they have pressures greater than 1 atmosphere (atm). The most common exposure to hyperbaric environments is during underwater diving, which became practical with the development of the self-contained underwater breathing apparatus (scuba). During a dive, a descent of 10 meters implies a pressure increase of 1 atm (meaning the ambient pressure is 2 atm), due to water being almost 800 times denser than air. Therefore, the maximum descent for recreational diving is fixed at 40 meters (5 atm). According to some estimates, there were approximately 7 million divers in 2008 and 500,000 new divers taking up the activity every year [1].

However, diving is a challenging activity where some factors have to be monitored closely, for safety. One is the hydrostatic pressure, that increases in proportion to depth. To minimize its effect, a decrease in the heart rate occurs, to maintain an adequate cardiac output [2]. Another is the presence of gases inside the body that can form bubbles during ascent, leading to decompression sickness (DS) [3], [4], whose most common symptom is inner ear disorders [5]. These factors produce a response in the diver's body to maintain homeostasis. This response is reflected in the Autonomic Nervous System (ANS) through the balance between its two branches (sympathetic and parasympathetic or vagal), which reflects the efforts of the body to adapt to new environments. Hence, monitoring ANS response may lead to a better understanding of diving physiology and become a potential diagnostic marker of hazards associated with diving.

The most common non-invasive technique to measure ANS activity is Heart Rate Variability (HRV), extracted from the electrocardiogram (ECG). The power spectral density (PSD) of HRV reveals two main frequency components: low-frequency (LF) components (0.04 - 0.15 Hz), which reflect both sympathetic and parasympathetic activities, and high-frequency (HF) components (0.15 - 0.4 Hz), which reflect parasympathetic activity. Normalized power in the LF band and the ratio between powers in the LF and HF bands have been used as a measure of the sympathovagal balance and power in the HF band has been used as measures of vagal activity [6]. However, while PSD methods are widely used, some limitations have been found: i) PSD is a linear technique, so it fails to account

for non-linear properties of HRV; ii) PSD presents additional limitations when the respiratory rate falls into the LF band, leading to overestimation and underestimation of the power in LF and HF bands, respectively. In the recent years, the PSD's true efficacy as an accurate ANS index has been questioned due to these limitations [7], [8], [9]. To overcome them, two different analyses were performed in this study: i) The Principal Dynamic Mode (PDM) method is able to extract and separate sympathetic and parasympathetic dynamics and handles non-linear relationships [11], as results from human studies have shown [12], [13], [14]; ii) The Orthogonal Subspace Projection (OSP) method is able to separate the linearly related respiratory influences from the heart rate variability, providing information about how much of the respiratory component is reflected in the HRV and therefore leading to a more accurate estimation of the sympathovagal balance [15], [16]. As some parts of the used datasets have a duration of 3 minutes, also the required time duration of the HRV recordings is analyzed in this work, since the Task Force indicates that 2 minutes are needed to address the LF and HF component, while some recent studies suggest that less than 5 minutes recordings may not be sufficient to assess HRV parameters accurately [17], [18].

The ANS response has been analyzed in several works using hyperbaric chamber data. A hyperbaric chamber increases atmospheric pressure without the need for immersion, providing a way to study only the effects of the pressure, without the other external variables involved in a real dive. The results of these studies suggest a reduction in the heart rate and an increase in parasympathetic activity [19], [20], [21], [22], [23], in response to increasing pressure. Studies in hyperbaric chambers only reflect changes due to pressure, but there are many variables that could affect the body's cardiovascular response during a real dive, such as diver body position, diver equipment, visibility, physical activity, water temperature, breathing with a scuba mouthpieces and more [24], [25], [26]. There are only a few studies analyzing ANS response during immersion and they also show dominance of the parasympathetic activity [14], [27], [28], [29]. However, these studies involved lower depths in a pool [27], [28], or only linear relationships were considered in their analysis [27], [28], [29], or they analyzed divers that remained static during the immersion [14].

In light of the above, comparison between PSD, OSP and PDM methods has been performed for data obtained in a hyperbaric chamber, to examine if OSP and PDM can overcome the aforementioned limitations of PSD. Previous PSD results in this hyperbaric database were presented in [23]. Next, comparison between time-domain, frequency-domain, PDM and OSP parameters from 5 minutes and 3 minutes recordings was done in this same dataset to examine the appropriateness of these two time durations. Finally, to complete the analysis of ANS response in hyperbaric environments, time-domain, PDM and OSP parameters have been studied in two additional datasets. The first dataset involved a controlled water immersion where divers remained static in order to minimize the effects of different variables. The second dataset consisted in an uncontrolled water immersion where divers

performed physical activities during the immersion, which is expected to alter ANS dynamics.

II. MATERIALS

Three different datasets were analyzed in this work. The first dataset was comprised of data collected in a hyperbaric chamber. This dataset was used to compare PSD method with both OSP and PDM. In addition, the two different data lengths (5 and 3 minutes) were compared too. For the other two water immersion datasets, the ANS response during real immersions with similar depths (around 20 m or 66 ft) were investigated and compared with the hyperbaric chamber dataset. Subjects in the three datasets were different, but they shared to be military personnel, to be physically active (at least one hour of exercise daily), to be experienced scuba-divers (more than 10 dives in the last year) and to have not taken caffeinated beverages prior to the study.

A. Datasets

- 1) Hyperbaric chamber (HC): 28 subjects (24 males and 4 females) with a mean age of 28.73 ± 6.39 years were recorded inside the hyperbaric chamber of the Hospital General de la Defensa de Zaragoza, with the approval of the ethics committee "Comité de ética de la investigación con medicamentos de la inspección general de sanidad de la Defensa". In this protocol five stages which consisted of 5 minutes stop at 1 atm (which is the pressure at sea level), at 3 atm (simulating 20 meters or 66 feet depth), at 5 atm (simulating 40 meters or 131 feet depth), and then coming back to 3 and 1 atm were studied. These stages were named B1D, B3D, B5, B3A and B1A (B from basal; the number reflects the pressure, n atm; the letter D or A refers to descent or ascent). A schematic representation of the entire protocol is shown in Table I. The test recorded at 1 and 5 atm measured divided attention, and was not analysed because it was not the main focus of the present study. During the basal stages, subjects remained relaxed and sitting comfortably in silence without moving. The hyperbaric chamber was ventilated during the entire test to avoid changes in temperature and humidity. More details of this dataset can be found in [23]. Recordings were performed using a Nautilus device [30], which allows the ECG signal to be recorded with three leads at a sampling frequency (fs) of 2000 Hz, along with the atmospheric pressure (fs=250 Hz) inside the chamber.
- 2) Controlled dive (CD): 11 experienced scuba divers (all men) with a mean age of 41.13 ± 2.03 years were recorded during an immersion in the sea, with the approval of the Worcester Polytechnic Institute's Institutional Review Board. Only two stages have been analyzed in this work, although there are multiple stages in the data (this is a longer database, see details in [14]). In the baseline stage, the divers floated on the surface for 10 minutes with minimal movement, in the supine position, with their faces out of water and breathing through their scuba masks. In the immersion stage,

divers remained at 66 feet (20 m) in a prone body position with minimal movement for 30 minutes, breathing air through their scuba masks. Water temperature at the bottom depth was $12.78 \pm 0.57^\circ\text{C}$ and divers wore a dry suit. With this dataset, the effect of the change of pressure in a semi-controlled real immersion can be analyzed, trying to minimize the influence of the rest of the variables. Recordings were performed using a five-lead digital Holter ECG monitor (RZ153+, Rozinn Electronics, Cleveland, OH), $f_s = 180$ Hz. A diving data logger (GEO, Oceanic, San Leandro, CA) was used to record each diver's dive profile including the dive duration, depth, and water temperature.

- 3) Uncontrolled dive (UD): 15 experienced scuba divers (all men) with a mean age of 28.40 ± 4.95 years were recorded in a real dive in a reservoir, with the approval of the the same ethics committee as the first dataset. Two different stages were analyzed. In the baseline stage, divers remained relaxed and sitting comfortably in silence without moving during 5 minutes outside the water. In the immersion stage, divers immersed for variable durations, but with a 3 minutes stay between 15 and 25 m (49 to 82 ft), breathing air through a scuba mouthpiece, performing physical tasks under low visibility. Water temperature on the surface was 8°C and divers wore dry suits. In this dataset, several factors such as body position, the type of activity, the surroundings during immersion and the way of breathing varied. Therefore, results needed to be carefully analyzed taking into account all these scenarios. Similar to the hyperbaric chamber dataset, recordings were performed using the Nautilus device, so signals from 3-lead ECG and the atmospheric pressure were obtained.

A schematic representation of the three datasets comparison is shown in Table II. From the HC dataset, stages B1D (as baseline) and B3D (as immersion) were selected to study the effect of pressure change, since the rest of variables remained equal.

B. Data extraction

The 5 minutes stops of each stage in the HC dataset were chosen in order to compare PSD vs OPS and PDM methods. Then, the first 3 minutes of each stage were selected to compare the parameters extracted with the 5 minutes recordings, to see if the HRV parameters were reliable and did not change significantly from different signal durations. Finally, the first 3 minutes of each stage in the HC, CD and UD datasets were chosen, in order to be able to compare the ANS response between the three databases. These 3 minutes segments were selected because it was the time that divers remained at the maximum depth during the reservoir immersion.

C. Respiration analysis

In order to better characterize the effect of the way of breathing through a scuba mouthpiece (breath is exaggerated, which can affect the parasympathetic system), an extra dataset was studied. In this fourth dataset, 12 subjects (6 men and 6

women) with a mean age of 27.17 ± 5.22 years were recorded while breathing in a spontaneous way and then while simulating respiration with a scuba mask, breathing deeply and rapidly, with pursed lips. Both stages (breathing styles) had a duration of 3 minutes and subjects remained sitting and relaxed during the entire test. One-lead ECG recordings were taken using an HP 78354A ECG (Hewlett-Packard) and signals were digitized using a PowerLab system at 1000 Hz.

III. METHODS

A. ECG Analysis

For HC and UD datasets, a finite impulse response low-pass filter was applied to the ECG signal to estimate the baseline and then to remove baseline wandering (cut-off frequency of 0.03 Hz) [31]. Heartbeats were detected using a wavelet-based algorithm on the second lead of the recorded ECG signal [32]. In addition, ectopic beats and missed and false detections were identified and corrected [33]. As a result, the QRS complexes could be located in the ECG, and the difference between consecutive R waves was used to generate the RR time series. For the CD dataset, R waves in the ECG recordings were detected using automated software developed for the Rozinn monitor (Holter for Windows+). Any incorrectly recognized R waves were manually corrected. Then, for the three datasets, the time-varying integral pulse frequency modulation model was applied to determine the influence of the ANS on the beat occurrence time series [34]. This model compensates for the influence of the mean heart rate over the modulating signal, thus providing more realistic ANS activity information. Using this model, the instantaneous heart rate signal (HR) was created at a sampling rate of 4 Hz. The mean heart rate (mHR) was obtained by low-pass filtering the HR with a cut-off frequency of 0.03 Hz. Finally, the heart rate variability (HRV) was obtained as the difference between these two terms: $HRV = HR - mHR$.

B. Time-Domain Parameters

Apart from the mHR , measured in beats per minute (bpm), three more time-domain parameters were computed from the beat-to-beat time series:

- $SDNN$ (s): Standard Deviation of the Normal-to-Normal (NN) intervals, as a measure of statistical dispersion. This parameter could be interpreted as an indicator of overall ANS activity [6].
- $RMSSD$ (s): Root Mean Square of the Successive Differences between adjacent NN intervals. This parameter mainly reflects the parasympathetic tone [6].
- pNN 50(%): number of Pairs of successive NN s that differ by more than 50 ms divided by the total number of NN intervals.

C. Frequency-Domain Parameters

As the recordings in the hyperbaric chamber were considered stationary, four frequency domain parameters were calculated based on the PSD analysis of the HRV signal, using Welch's power spectral density estimation, with seven

TABLE I
EXPLANATION OF THE HYPERBARIC CHAMBER PROTOCOL, WITH THE ATMOSPHERIC PRESSURE AND DURATION OF ITS PARTS.

Pressure	1 atm (sea level)			1-3 atm	3 atm	3-5 atm	5 atm		5-3 atm	3 atm	3-1 atm	1 atm (sea level)
Explanation	Audio	Test	Basal	Descending	Basal	Descending	Basal	Test	Ascending	Basal	Ascending	Basal
Duration	5 min	7-8 min	5 min	6-8 min	5 min	6-8 min	5 min	7-8 min	6-8 min	5 min	50-55 min	5 min
Stage	-	-	B1D	-	B3D	-	B5	-	-	B3A	-	B1A

TABLE II
EXPLANATION OF THE COMPARISON OF THE THREE DATASETS, HIGHLIGHTING THE DIFFERENCES BETWEEN THEM.

Immersion factors	Hyperbaric chamber (HC)		Controlled dive (CD) (sea immersion)		Uncontrolled dive (UD) (reservoir immersion)	
Stages	Baseline	Immersion	Baseline	Immersion	Baseline	Immersion
Pressure	1 atm	3 atm	1 atm	3 atm	1 atm	3 atm
Location	Chamber	Chamber	On water surface	Inside the water	Outside the water	Inside the water
Position	Sitting	Sitting	Supine	Prone	Sitting	Activity
Environment	Controlled	Controlled	Controlled	Semi-controlled	Controlled	Challenging
Breathing	Normal	Normal	Diver mask	Diver mask	Normal	Diver mask

1 minute length Hamming windows and an overlap of 50%. The parameters computed were:

- $P_{LF}(ad)$: power inside the LF band (0.04-0.15 Hz). Measurement units: ad , adimensional units.
- $P_{HF}(ad)$: power inside the HF band (0.15-0.4 Hz).
- $P_{LFn}(nu)$: power in LF band normalized with respect to those of the LF and HF bands. Measurement units: nu , normalized units.
- $R_{LF/HF}(nu)$: ratio between LF and HF powers.

About the physiological interpretation of classic frequency components, P_{LF} has been suggested to represent both sympathetic and parasympathetic modulation, whereas P_{HF} has been related only with parasympathetic activity. Then, P_{LFn} and $R_{LF/HF}$ are widely employed as markers of sympathetic activity and sympathovagal balance [35]. Also, other parameter which represents sympathetic activity is the Baevsky's Stress Index (SI) [36], [37], that is going to be used in this work to compare sympathetic activity between the three datasets.

D. Respiratory information extracted from ECG

Respiratory information augments the ANS analysis. The vagal tone reflects respiratory sinus arrhythmia (RSA), which is synchronous with respiration; therefore the relationship between respiratory rate and the parasympathetic system must be taken into consideration during the analysis. The method for estimating respiratory rate from ECG presented in [43] was used. It consists of estimating the respiratory rate from "peaked-conditioned" averaged spectra. This method exploits respiration-induced morphology variations in the ECG signal based on three ECG-derived-respiratory (EDR) signals, namely the R-wave angle, and upwards and downwards of the R wave slope [38]. For each EDR signal, PSD was estimated every 5 seconds and the location of the largest peak closest to the previous respiratory rate estimation inside a reference interval was selected. A measure of peakness was subsequently obtained as the percentage of power around this

peak with respect to the reference interval. Only if this measure was higher than a threshold (if it was peaked enough) the respiratory component was considered clear. Then, a peaked-conditioned average spectra was obtained by averaging those spectra (5 at maximum) which were peaked enough. Finally, the respiratory rate (F_R) was estimated as the maximum of the averaged spectra. Those subjects with F_R lower than 0.15 Hz (upper limit of the LF band) were discarded from the classic PSD analysis of the ANS response to avoid possible misinterpretations [10].

E. Analysis of HRV using Principal Dynamic Modes

The PDM is a method based on extracting only the principal dynamic components of the signal via eigen decomposition. The PDMs were calculated using Volterra-Wiener kernels based on expansion of Laguerre polynomials [39]. A minimum set of basis functions was determined by using principal component analysis, in which the dominant eigenvectors were retained, as they relate more closely to the true characteristics of the signal. In the case of HRV signal, the dominant eigenvectors should reflect the dynamics of the sympathetic and parasympathetic systems. The non-dominant eigenvectors represent noise or non-essential characteristics.

The first step of the PDM method is to obtain a signal with broadband spectral characteristics in order to accurately estimate the Volterra-Wiener kernel. In many instances, significant power in HRV exists in the very low frequency band (VLF, 0-0.04 Hz) compared to the low frequency (LF) and high frequency (HF) bands. Consequently, the method introduced by Tarvainen et al [40] was used with the aim of reducing VLF power to the level of the LF and HF bands so that the overall spectral characteristics are broadband. The result of this process was labeled as HRC [11].

The PDM method requires both the input and output data, but we had only the output signal (HRC), so it was necessary to create an input signal with broadband spectral character-

istics and with a correlation to the dynamics of the heart rate. The TV-OPS algorithm (explained in [41]) was used to create the input signal. This procedure was represented as an autoregressive model where the output signal (HR_c) could be estimated through past samples of this signal (delayed HR_c) expanded onto a set of Legendre polynomials basis functions. Then, a pool of linear independent candidates was selected to estimate the projected coefficients and to reconstruct the output signal (HR_r) minimizing the least-square error. The reconstructed output signal was subtracted from the original output signal to obtain the estimation error signal, labeled HRe : $HRe = HR_c - HR_r$. This error signal, normalized to a unit variance (HR_n), had the broadband characteristics needed for accurate estimation of PDMs. Therefore, HR_n was used as the input signal and HR_c was used as the output signal of PDM model to estimate the Volterra-Wiener kernel, as was used in previous studies [11], [12], [14]. The eigen decomposition of these kernels gave eigenvectors, which were the final values of the PDMs. While the obtained PDMs were in time-domain representation, the FFT transform was used to convert them to the frequency domain. The PDM with the highest power in the HF band was selected to represent the dynamics corresponding to the parasympathetic nervous activity. The power in 0.04 Hz to 0.4 Hz range was computed for this mode (PDM_{para}) since parasympathetic component is reflected in LF and HF classical bands [7], [8], [9]. Then, the PDM with the highest power in the LF band (discarding the one selected as parasympathetic) was chosen as the PDM that represents the sympathetic activity and its power in the LF band was computed (PDM_{symp}). These two components reflect the dynamics of the two ANS branches [14]. This process is illustrated in Figure 1.

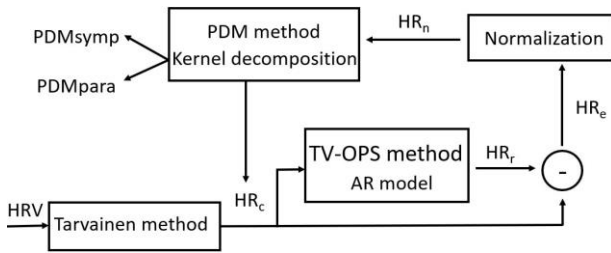


Fig. 1. Diagram of PDM method.

F. Analysis of HRV using Orthogonal Subspace Projections

OSP is a method based on decomposing the HRV signal into two different components: one respiratory component, describing all linearly-related variations associated with respiration, and one residual component, describing all dynamics modulated by other mechanisms different from respiration. In fact, the residual component describes dynamics modulated by the sympathetic nervous system, and other (possible) vagal modulators unrelated to respiration [15], [16].

To apply this method, the respiratory signal and the HRV signal are needed, together with the assumption that the respiratory signal drives changes in the HRV signal [42]. In this

work, respiratory signals were obtained from the ECG using QRS slopes and R-wave angle as described in [43]. However, this method could not be applied in the second dataset (CD), because only RR series were available, not the complete recorded ECG signal, therefore OSP was analyzed only for the HC and UD datasets. In order to extract all dynamics of the heart rate that are linearly related to respiration, a subspace V was constructed using the respiratory signal and its delayed versions. Then, the HRV signal was projected onto the respiratory subspace V . As a result, all dynamics of HRV linearly related to respiration were described in the respiratory component HRV_R . Furthermore, an orthogonal component (residual component), which is related to other heart rate modulators, was computed as $HRV_{\perp} = HRV - HRV_R$. This process is illustrated in Figure 2.

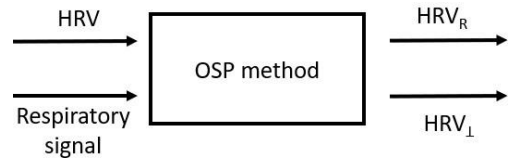


Fig. 2. Diagram of OSP method.

The relative power of each component (P_R for respiratory component and P_{\perp} for the residual component) indicates how much information is shared between respiration and heart rate. For instance, when $P_R \gg P_{\perp}$, most of the variations in the heart rate can be described by changes in the respiration and vice versa. P_R can also be used as an index for respiratory-sinus-arrhythmia assessment, and due to the relationship between RSA and the vagal tone, P_R can be interpreted as a parasympathetic marker. In addition, the power of the residual component in the LF band ($P_{LF \perp}$) could be interpreted as a marker of the sympathetic system, and the power of the residual component in the HF band ($P_{HF \perp}$) as a marker of the parasympathetic system.

G. Statistical analysis

First, a statistical analysis was applied to each time-domain, frequency-domain, OSP and PDM parameter to determine the presence of significant differences between the five stages of the HC dataset. Thus, a Shapiro-Wilk test was applied to check the normal distribution of the parameter, with Student's t-test being applied to every pair of stages if the distribution was normal and the Wilcoxon paired test if not. Then, the correlation of each parameter extracted from 5 and 3 minutes recordings together with a paired sample Student's t-test was performed to study the similarity between these recordings. Finally, statistical analyses were made to compare the baseline versus immersion stages in the three hyperbaric datasets and to compare normal versus simulated scuba mask breathing. A Shapiro-Wilk test was applied to verify the normal distribution of the data, and as these distributions were no-normal, the Wilcoxon paired test was applied. Also, another statistical analysis was made to compare the three baseline stages.

In this case, as they were three independent groups with a non-normal distribution, the Kruskal-Wallis test was applied. Three different p -values, 0.05, 0.01 and 0.001, defined the significance.

IV. RESULTS

ECG recording of one subject stopped in the middle of the HC test, therefore there were 28 subjects for B1D, B3D and B5 stages and only 27 for B3A and B1A stages.

A. HC dataset

Table III shows the respiratory rate in the HC dataset with 5 and 3 minutes recordings. No significant differences were found between stages and between the same stage with different duration. It should be noted that 4 subjects in B1D, 5 in B3D, 4 in B5, 2 in B3A and 3 in B1A had a respiratory rate lower than 0.15 Hz.

TABLE III
MEAN \pm STD OF THE ESTIMATED RESPIRATORY RATE IN THE HC DATASET.

	Duration	B1D	B3D	B5	B3A	B1A
$F_R(\text{Hz})$	5 min	0.22 \pm 0.09	0.22 \pm 0.09	0.22 \pm 0.07	0.24 \pm 0.08	0.23 \pm 0.09
	3 min	0.21 \pm 0.07	0.21 \pm 0.06	0.21 \pm 0.06	0.23 \pm 0.06	0.22 \pm 0.07

Table IV shows the total power of the respiratory and the residual component of the OSP for the HC dataset for 5 and 3 minutes recordings. No significant differences were found between 5 and 3 minutes powers. P_{\perp} was significantly higher than P_R in all stages.

TABLE IV
MEAN \pm STD OF THE TOTAL POWER OF THE RESPIRATORY COMPONENT AND THE RESIDUAL COMPONENT IN THE HC DATASET. SIGNIFICANT DIFFERENCES BETWEEN P_R AND P_{\perp} ARE HIGHLIGHTED WITH A DAGGER (p -VALUE ≤ 0.05).

	5 min		3 min	
	P_R	P_{\perp}	P_R	P_{\perp}
B1D	0.02 \pm 0.02	0.98 \pm 0.02 †	0.02 \pm 0.02	0.98 \pm 0.02 †
B3D	0.02 \pm 0.02	0.98 \pm 0.02 †	0.03 \pm 0.05	0.97 \pm 0.02 †
B5	0.03 \pm 0.03	0.97 \pm 0.03 †	0.05 \pm 0.06	0.95 \pm 0.06 †
B3A	0.02 \pm 0.03	0.98 \pm 0.03 †	0.04 \pm 0.07	0.96 \pm 0.07 †
B1A	0.02 \pm 0.03	0.98 \pm 0.03 †	0.04 \pm 0.04	0.96 \pm 0.04 †

Figure 3 shows the time-domain, the classic frequency-domain, the PDM and the residual OSP parameters for the HC dataset. It should be noted that only for the classic frequency-domain parameters, subjects with a respiratory rate lower than 0.15 Hz were discarded, thus leaving 24 subjects in B1D

and B5, 23 in B3D, 26 in B3A and 25 in B1A, instead of the 28 in B1D, B3D and B5 and 27 subjects in B3A and B1A for the time-domain, the PDM and the residual OSP parameters. Comparison between stages using only the classic frequency-domain parameters were made with 20 subjects in B1D vs. B3D, B1D vs. B1A, B5 vs. B1A; 21 in B1D vs. B3A, B3D vs. B5, B3D vs. B1A, B5 vs. B3A; 22 in B1D vs. B5, B3D vs. B3A; and 23 in B3A vs. B1A. As shown in Figure 3, mHR decreased and $RMSSD$ increased with each stage, whereas $SDNN$ and $pNN 50$ reached a maximum value at stage B5 and B3A respectively. For the frequency-domain results, P_{LF} and P_{HF} increased their value during the descent, reaching a maximum at the deepest stage B5 and then decreasing in value during the ascent. P_{LF} and $R_{LF/HF}$ did not follow a clear trend, but they had their maximum in the B5 stage and their minimum in the B3A stage. Finally, PDM_{symp} , PDM_{para} , $P_{LF\perp}$ and $P_{HF\perp}$ followed the same trend, increasing their value until the B5 stage and then decreasing it until the last stage. The only exception of this path occurred with PDM_{para} , between stages B1D and B3D.

B. 5 minutes vs. 3 minutes recordings

Figure 4 shows the time-domain, the classic frequency-domain, the PDM and the residual OSP parameters extracted from 5 minutes recordings and 3 minutes recordings in the HC dataset. The 3 minutes recordings were the first 3 minutes of the 5 minutes recordings. Correlation between parameters extracted with 5 minutes and with 3 minutes was greater than 90% in all parameters except in two stages of P_{LF} , in one stage of $P_{LF\perp}$ and for PDM_{symp} and PDM_{para} (from 0.55 to 0.89 in these last two parameters). The paired t-test did not show significant differences between the two recordings.

C. Hyperbaric environments comparison

Table V shows the respiratory rate and the percentage of subjects with a respiratory rate lower than 0.15 Hz (that should be discarded for the classic frequency-domain parameters) from baseline and immersion stages in the HC and UD datasets. Note that respiratory rate could not be calculated in CD dataset. Figure 5 shows the time-domain, the PDM, the residual OSP parameters and the Baevsky stress index from baseline and immersion stages in the HC, CD and UD datasets. mHR significantly decreased from baseline to immersion in HC and CD while the rest of the time-domain parameters increased. However, this behavior is the opposite in UD, where mHR significantly increased and $SDNN$, $RMSSD$ and $pNN 50$ decreased. The comparison between the baseline stages of the three datasets found that mHR was significantly higher in CD with respect to the other two baseline stages while the rest of the time parameters were lower in CD. PDM parameters showed that PDM_{symp} was significantly higher during immersion in the CD and UD datasets while PDM_{para} was only significantly higher in UD. Baevsky stress index showed also an increase during immersion in CD and UD datasets, although not significant. Table VI shows the total power of the respiratory and the residual components

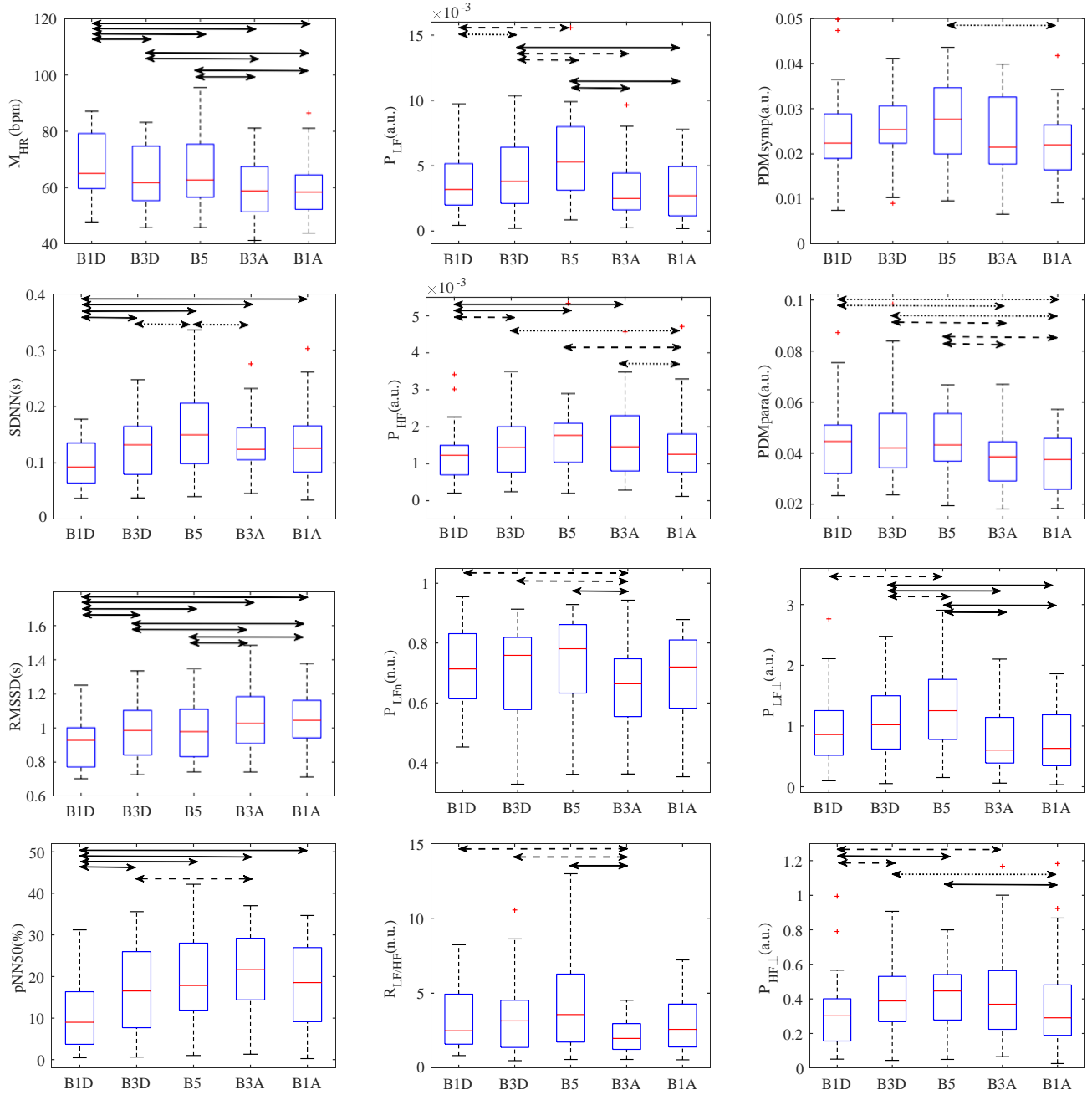


Fig. 3. Boxplots of the time-domain (first column), the classic frequency-domain (second column), the PDM and the residual OSP (third column) parameters in the HC dataset. Significant differences between stages of the same dataset are represented by a double arrow (dotted if p -value ≤ 0.05 , dashed if p -value ≤ 0.01 and solid if p -value ≤ 0.001).

of OSP for the HC and UD datasets. P_{\perp} was significantly higher than P_R in both stages and in both datasets. As shown in Figure 5, power of the residual OSP component (calculated only in HC and UD datasets because of the lack of respiratory signal in CD) showed a significant increase in $P_{LF\perp}$ (only in UD) and in $P_{HF\perp}$ (in both datasets) during immersion.

D. Spontaneous breathing vs. simulated scuba mask breathing

Table VII shows the respiratory rate and the percentage of subjects with a respiratory rate lower than 0.15 Hz (that should be discarded for the classic frequency-domain pa-

TABLE V
MEAN \pm STD OF THE RESPIRATORY RATE AND THE PERCENTAGE OF SUBJECT DISCARDED (WITH A $F_R < 0.15$ Hz) FOR THE HC AND UD DATASETS. SIGNIFICANT DIFFERENCES BETWEEN THE TWO STAGES ARE HIGHLIGHTED WITH A DAGGER (p -value ≤ 0.05).

	Baseline		Immersion	
	F_R	% subjects	F_R	% subjects
HC	0.20 ± 0.09	10.7%	0.21 ± 0.09	14.3%
UD	0.24 ± 0.06	6.6%	0.22 ± 0.08	13.3%

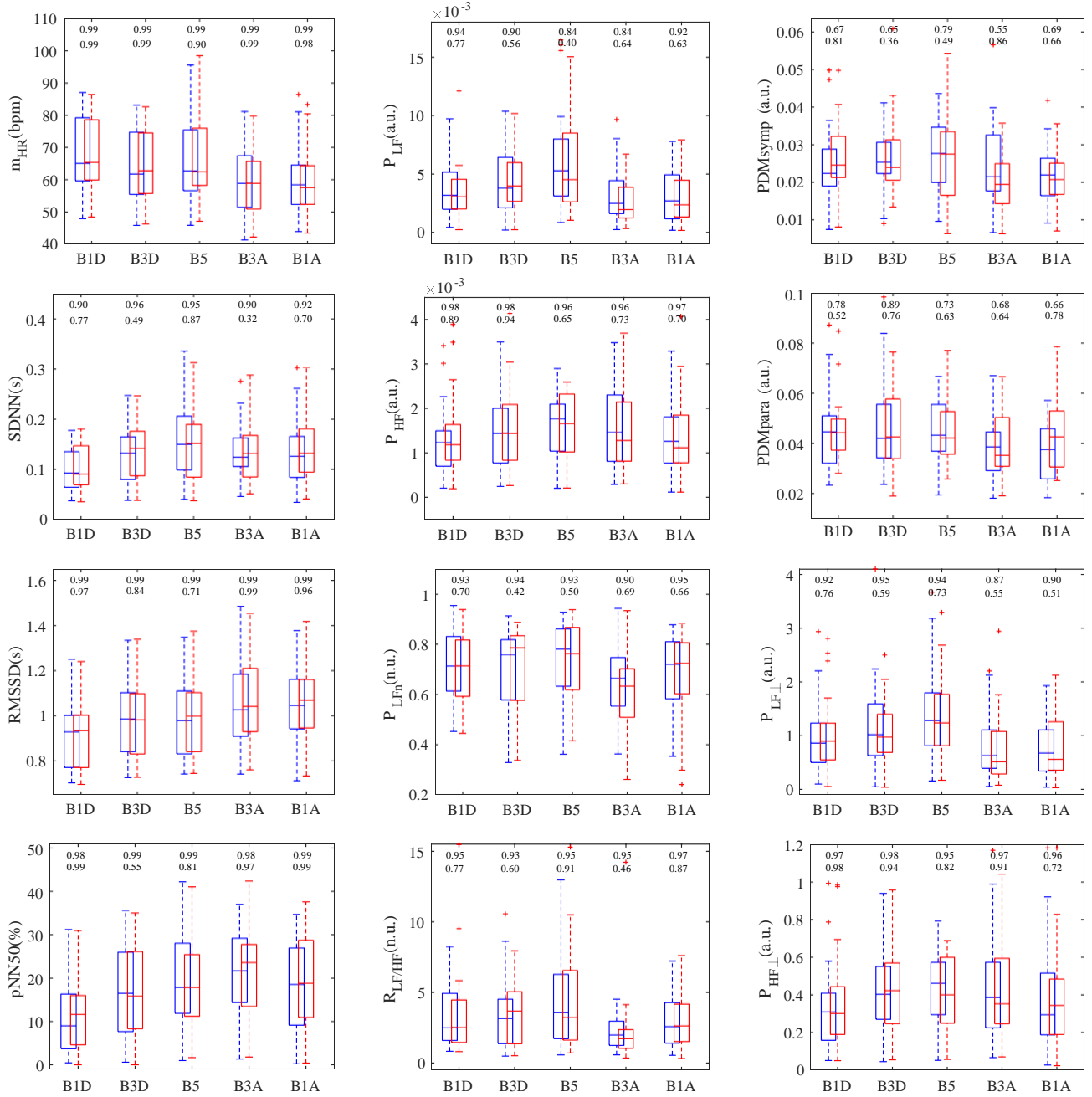


Fig. 4. Boxplots of the time-domain (first column), classic frequency-domain (second column), the PDM and the residual OSP (third column) parameters of 5 minutes recordings (in blue) and 3 minutes recordings (in red) in the HC dataset. The correlation (first row) and the paired t-test (second row) between 5 and 3 minutes measurements are showed on the top.

parameters) during spontaneous breathing and simulated scuba mask breathing. Figure 6 shows the differences between time-domain, the PDM, the residual OSP parameters and Baevsky StresS Index during spontaneous breathing and simulated scuba mask breathing. mHR significantly increased from spontaneous to simulated scuba mask breathing, $SDNN$ also increased but not significantly, $RMSSD$ significantly decreased and $pNN50$ did not change. $PDMsymp$ and SI did not show differences between stages but $PDMpara$ presented a significant increase from spontaneous to simulated scuba mask breathing stage. Regarding OSP parameters, Table VIII shows the total power

of the respiratory and the residual components of OSP for spontaneous vs. simulated scuba mask breathing. P_{\perp} was significantly higher than P_R in both stages. For OSP residual parameters in Figure 6, $P_{HF\perp}$ increased when subjects were breathing through their mouths.

V. DISCUSSION

There were two main goals of this study. The first was to study the ANS response using two different methods, the Principal Dynamic Mode and the Orthogonal Subspace Projection, as they both overcome the limitations of classic

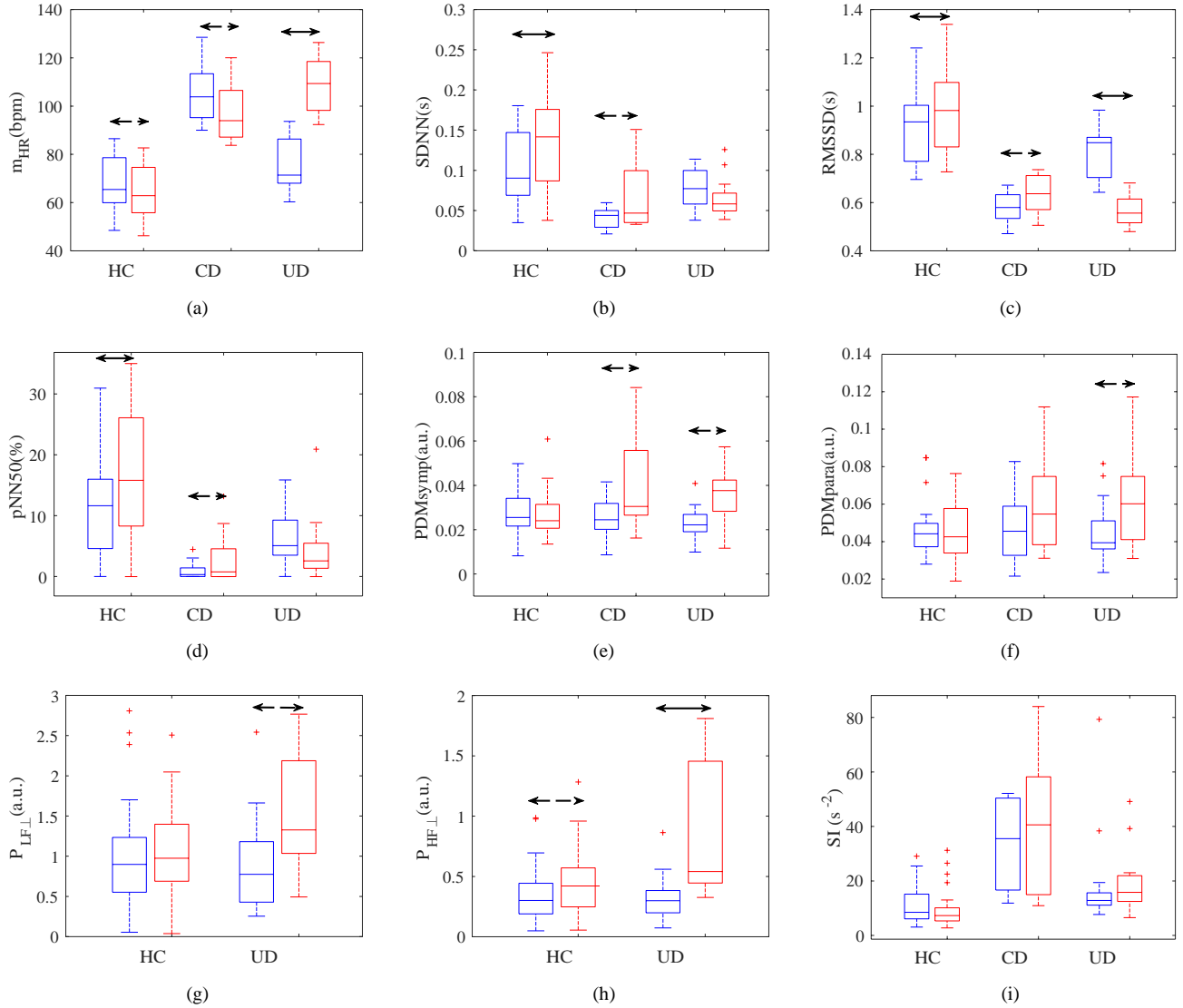


Fig. 5. Boxplots of the time-domain (a-d), the PDM (e,f), the residual OSP (g,h) parameters and the Baevsky stress index (i) of baseline (in blue) and immersion (in red) stages in the HC, CD and UD datasets. Significant differences between stages of the same dataset are represented by a double arrow (dotted if p -value ≤ 0.05 , dashed if p -value ≤ 0.01 and solid if p -value ≤ 0.001).

TABLE VI

MEAN \pm STD OF THE TOTAL POWER OF THE RESPIRATORY AND THE RESIDUAL COMPONENTS OF OSP FOR THE HC AND UD DATASETS. SIGNIFICANT DIFFERENCES BETWEEN P_R AND P_{\perp} ARE HIGHLIGHTED WITH A DAGGER (p -VALUE ≤ 0.05).

	Baseline		Immersion	
	P_R	P_{\perp}	P_R	P_{\perp}
HC	0.02 ± 0.02	0.98 ± 0.02 †	0.03 ± 0.05	0.97 ± 0.05 †
UD	0.05 ± 0.05	0.95 ± 0.05 †	0.02 ± 0.04	0.98 ± 0.04 †

TABLE VII

MEAN \pm STD OF THE RESPIRATORY RATE AND THE PERCENTAGE OF SUBJECT DISCARDED (WITH A $F_R < 0.15$ Hz) FOR SPONTANEOUS VS. SIMULATED SCUBA MASK BREATHING (RESP). SIGNIFICANT DIFFERENCES BETWEEN THE TWO STAGES ARE HIGHLIGHTED WITH A DAGGER (p -VALUE ≤ 0.05).

	Spontaneous		Scuba mask	
	F_R	% subjects	F_R	% subjects
Resp	0.24 ± 0.08	8.3%	0.22 ± 0.11	16.7%

PSD analysis. The second goal was to study the ANS response in three different hyperbaric environments: inside a hyperbaric chamber, where only the pressure varied; during a controlled dive in the sea, where the pressure changed but the effects

of other factors were minimized; and during an uncontrolled dive in a reservoir, where more factors differed from baseline to immersion stage, such as the low visibility environment, the physical activity, the position of the diver and breathing through a scuba mask. A comparison of the HRV features between the two stages (baseline and immersion) in each

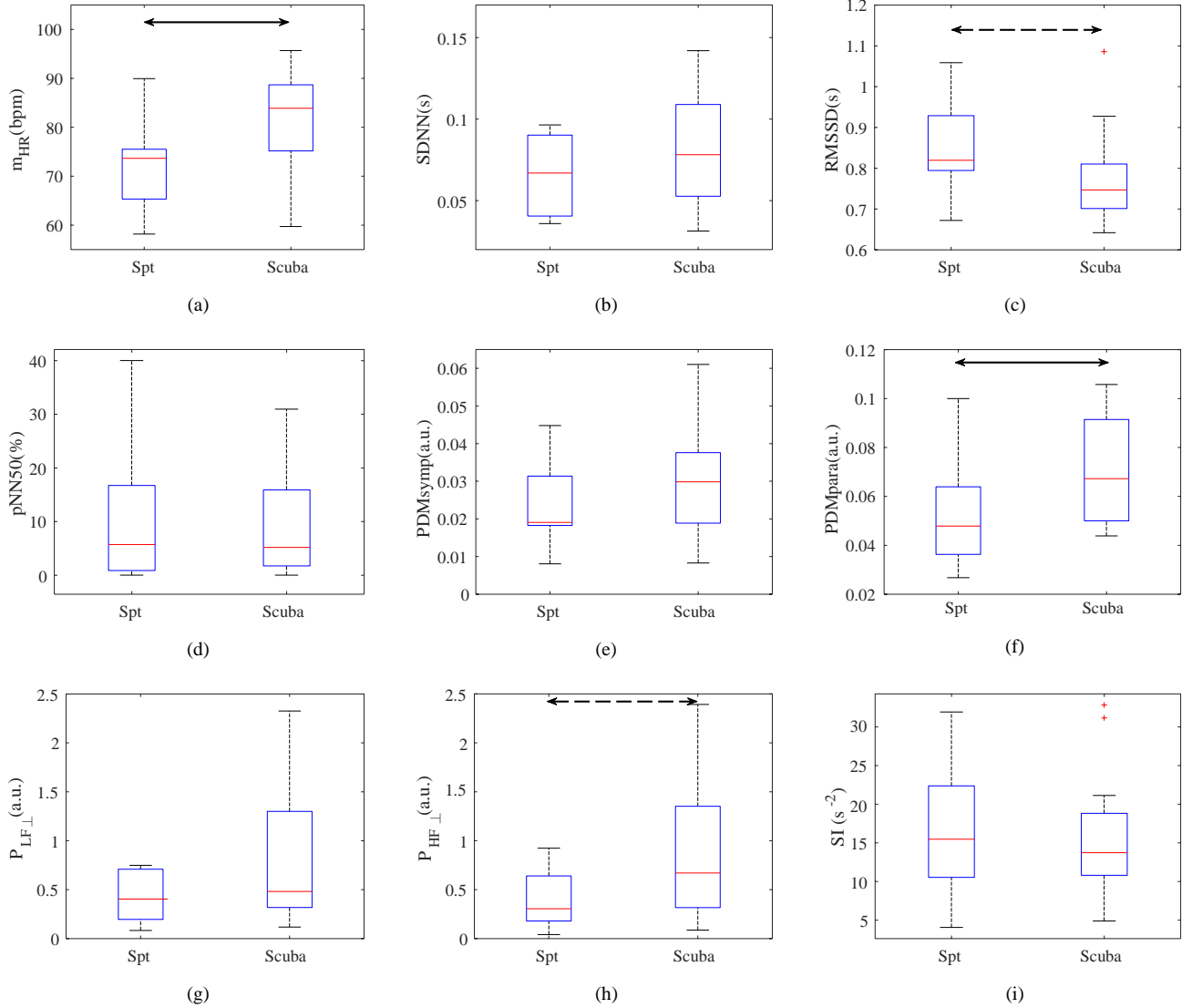


Fig. 6. Boxplots of the time-domain (a-d), the PDM (e,f), the residual OSP (g,h) parameters and Baevsky stress index (i) during spontaneous (Spt) and simulated scuba mask (scuba) breathing. Significant differences between stages are represented by a double arrow (dotted if p -value ≤ 0.05 , dashed if p -value ≤ 0.01 and solid if p -value ≤ 0.001).

TABLE VIII
MEAN \pm STD OF THE TOTAL POWER OF THE RESPIRATORY AND THE RESIDUAL COMPONENTS OF OSP FOR SPONTANEOUS VS. SIMULATED SCUBA MASK BREATHING (RESP). SIGNIFICANT DIFFERENCES BETWEEN P_R AND P_{\perp} ARE HIGHLIGHTED WITH A DAGGER (p -VALUE ≤ 0.05).

	Spontaneous		Scuba mask	
	P_R	P_{\perp}	P_R	P_{\perp}
Resp	0.03 ± 0.03	0.97 ± 0.03 †	0.02 ± 0.02	0.98 ± 0.02 †

dataset was carried out to study how these factors related to scuba diving activity affect the ANS response.

Figure 3 presents the comparison of PSD, PDM and OSP methods for the HC dataset. Although PSD methods are widely spread in the literature, one of their main limitations occurs when the respiratory rate falls into the LF band (a

frequent condition in experiments with scuba divers), since respiration is linked with the parasympathetic branch. It has been shown that changes in respiratory patterns alter the spectral content of HRV [44] and, consequently, the interpretation of sympathetic or vagal activations [45], [46], [47]. In fact, in a previous work with the HC dataset [23], data from subjects with a respiratory rate below 0.15 Hz were discarded to avoid misinterpretations of the ANS response. The same methodology has been used in this study, causing us to discard data from up to 5 subjects in some stages when only the classic frequency-domain parameters were used and leading to comparisons among stages that used 20 subjects instead of 28 (the 71.43% of the total population). To avoid this limitation due to the respiratory rate, the OSP method [15], [16] is used in this work with all the subjects, since it is able to separate respiratory influences from the heart rate. This method was validated for the study of the ANS

response in a previous study in conditions of induced worry and mindfulness [15]] and with pharmacological blockade of the sympathetic and parasympathetic branches [16]. Another limitation of the PSD methods is its incapability to account for the non-linear dynamics of HRV. That is the reason to use the PDM method, described in [14], since it is able to extract and separate the non-linear sympathetic and parasympathetic dynamics of the ANS. This method was validated for the study of the ANS response in a previous study, by the application of the autonomic nervous system blocking drugs atropine and propranolol [11]. Coming back to the results in Figure 3, it can be seen that parasympathetic activity follows the same trend with the three different methods (P_{HF} with PSD, $PDMpara$ with PDM and $P_{HF\perp}$ with OSP), increasing its value until stage B5, and then decreasing until the end of the protocol. Also, sympathetic activity has similar behaviour with the three methods (P_{LF} with PSD, $PDMsymp$ with PDM and $P_{LF\perp}$ with OSP) increasing in value from the beginning to the B5 stage, and then suddenly decreasing their value until its minimum in the two last stages. It should be noted that m_{HR} and $RMSSD$ parameters have a different trend, increasing or decreasing their value during the entire protocol, but this observation is not related with the methodology used. Therefore, HRV analysis with OSP and PDM methods allows us to properly characterize the ANS response inside the hyperbaric chamber, circumventing the respiratory rate limitation that would have forced us to eliminate some data and also allowing us to analyze the non-linear dynamics of the ANS response.

Next, a comparison among parameters extracted from 5 minutes recordings and from 3 minutes recordings was performed. This comparison was done because some recent works suggest that less than 5 minutes recordings may not be sufficient to assess HRV parameters accurately [17], [18], although the Task Force said that 2 minutes would be time enough for frequency-domain parameters [6]. This 3 minutes temporal slot was chosen for comparison among the three datasets, since subjects remained below 15 m for only 3 minutes in the uncontrolled dive in the reservoir. Time-domain parameters did not show differences due to the time slot duration, with a correlation higher than 90% in $SDNN$ and higher than 98% in the other three parameters (see Figure 4). The same trend among 5 and 3 minutes recordings was found in the classic frequency domain parameters extracted from PSD methods, with a correlation higher than 90% in all stages and for the four parameters, except in stages B5 and B3A in P_{LF} , with a correlation of 84%. PDM parameters showed the lowest correlation (from 55% to 89%) between 5 and 3 minutes parameters. This could be due to in 3 minutes there were not enough samples in HC dataset for a reliable measure. Although there is not a standard minimum number of samples for a reliable measure with non-linear parameters [48], in other works that used this PDM technique at least 300 samples were used [11], [14]. This 300 samples were not reached in 3 minutes recordings of HC dataset, but they were reached in 5 minutes recordings of HC and UD datasets. Despite these worse correlation results, the paired t-test did not show significant differences among 5 and 3 minutes recordings in

HC dataset and the trend of $PDMpara$ and $PDMsymp$ among stages was not affected, decreasing or increasing their value in both parameters at the same time. OSP parameters also did not show differences due to the time slot duration, with a correlation higher than 90% in all stages for the two parameters, except in the B3A stage in $P_{LF\perp}$, with a correlation of 87%.

Another difference between this work and other hyperbaric studies was the selection of the temporal window for analyses. While other works has used a middle-to-final time range windows to compare different stages, to account for the adaptation of the diver's body to the hyperbaric conditions [14], [21], [22], [29], in our case we have selected the first 3 minutes. This temporal window allowed us to study how quickly the body adapted to the hyperbaric environment. In [14], a continuous study of a 30 minutes immersion at 66 ft was performed and significant differences with respect to baseline stage were already found in the first 5 minutes, so the selection of the first 3 minutes should not imply a limitation of this study.

One of the main results of this work is the lower power in the OSP respiratory component with respect to the residual OSP component, as Table VI shows. This suggests that cardio-respiratory coupling is reduced in these hyperbaric scenarios, so most of the variations in the heart rate cannot be described by changes in the respiration, but they can be explained by changes in other factors. Therefore, a careful description of each stage in the dataset, with a description of all the changes related to important factors that may affect the ANS response, is needed. The three different analyzed datasets have a similar protocol: one stage of baseline and another stage of immersion, with a pressure around 3 atm. In the two stages of the hyperbaric chamber dataset, subjects remained seated comfortably without talking and the chamber was ventilated to avoid big temperature differences. The only difference between the two stages was the pressure, so its effect on the ANS response can be measured with this dataset. During the controlled dive in the sea, both stages were very similar also: divers remained in horizontal, with all the equipment and breathing through their scuba masks. The only difference was their location: in the baseline stage they were on the water surface, with their heads out of the water, and in the immersion stage they were at a depth of 20 m. It must be highlighted how different this baseline stage was from the hyperbaric chamber baseline stage. Both baseline stages were so different between them because the main aim in these two datasets was to maintain baseline and immersion stages as similar as possible, to study only the pressure differences in the ANS response. However, the possible effects of cold water and currents in the immersion stage in the controlled dive in the sea has to be taken into account. Finally, the uncontrolled dive in the reservoir presents more differences between its two stages. In baseline, subjects remained sitting comfortably, outside the water, without diving equipment and breathing spontaneously, as this stage is similar to the HC baseline but very different to the CD baseline. During reservoir immersion, subjects performed a physical activity in pairs, under the water, with low visibility, with all their equipment, and breathing through scuba masks. Therefore, in our opinion,

in this dataset not only the effect of the pressure, but also the effect of an environment with low visibility, physical activity, body position and scuba mask breathing could be analyzed.

Although pressure changes occurred in all three experiments, it is only in the first two where the effect of pressure can be isolated, to learn how pressure changes affects ANS response. In both datasets, there is a significant decrease in mHR and a significant increase in the rest of time-domain parameters from the baseline to the immersion stage. The heart rate decrease has been reported previously in hyperbaric chamber studies [19], [20], [21], [22] and in immersion data [49]. The possible reason for this bradycardia could be the effect of the diving reflex and the effect of the pressure [24], [50]. The significant increase in the rest of the temporal parameters (especially in $RMSSD$), together with the decrease in the mHR , seems to point out an increase in the parasympathetic activity or a decrease in the sympathetic one. However, PDM results do not show any significant change during the immersion in the parasympathetic activity ($PDMpara$) in HC and CD. The lack of parasympathetic activity due to the pressure in the hyperbaric chamber is in agreement with [23], where no differences were found between the first two stages. One possible factor that may affect this measure is the descent speed. In the HC protocol, it took from 5-7 minutes to transition from 1 atm to 3 atm, although this time was significantly shorter in real immersions (less than 2 minutes for an experimented diver). This slow descent could be less demanding for the subject to adapt to, and this could be a reason why no significant difference in parasympathetic activity was found in the HC data. The descent durations were not reported in the other studies. In CD, there was an increase in $PDMpara$ during immersion, but it was not statistically significant (p -value = 0.1). Concerning the sympathetic activity, a decrease in $PDMsymp$ was found in HC, that could explain the trends in the time-domain parameters. In contrast, an increase in $PDMsymp$ was observed in CD. This increase could be explained by the fact that diving in deeper and colder water with greater current strength will cause substantial stress on even the most experienced divers, so this increase in the sympathetic activity possibly is not related to the pressure changes. Furthermore, the Baevsky's Stress Index, which represents sympathetic activity [36], [37], was applied in both datasets and results showed the same trend, decreasing during the immersion stage in HC and increasing in CD, although not statistically significant. On the other hand, the OSP method shows a lower power in the respiratory component with respect to the residual component for HC data, which indicates that most of the variations in the heart rate are not produced by changes in the respiration. Therefore, the study of the power in the LF and HF bands of the residual component could give some information about the two branches of the ANS. There is an increase in $P_{HF\perp}$ that points to an activation of the parasympathetic activity, and this may be due to the effect of pressure as previous studies suggests [19], [20], [21], [22]. In sum, pressure changes have an impact on time-domain parameters, with a decrease in mHR and an increase in $SDNN$, $RMSSD$ and $pNN 50$, but the effect of pressure changes is not so clear in the frequency-

domain parameters: while $P_{HF\perp}$ from the OSP method could reflect a significant increase in the parasympathetic activity during immersion, $PDMpara$ from the PDM method does not show this change; however, a decrease in $PDMsymp$ is found in HC, and this sympathetic decrease could explain the results of the time-domain parameters.

Finally, results from the uncontrolled reservoir dive show the opposite trend in temporal parameters than those from the hyperbaric chamber and the controlled sea dive. In UD results, a significant increase in mHR and a decrease in the other three time-domain parameters (significant in $RMSSD$) are shown. This suggests an activation of the sympathetic system, as the increases in $PDMsymp$ and $P_{LF\perp}$ confirm (also an increase in the Baevsky's Stress Index was shown). This activation could be explained by different factors: one could be the predominance of the physical activity and the stress of the challenging environment during the immersion. In fact, it has been proved that physical activity during immersion increase the sympathetic tone [51]. Another factor is the stress related to the immersion: some studies have pointed out that an immersion performing a stressful task could increase the heart rate and the sympathetic activity [28]. Therefore, physical activity and stress during the immersion, together with the low visibility environment, that could increase even more the stress of the immersion, and the effect of cold water, that also increases the heart rate [52], [53], contribute to the predominance of the sympathetic activity. There is also an increase in the parasympathetic activity in the UD data. UD is the only dataset with significant differences between stages in $PDMpara$ and in $P_{HF\perp}$ (p value < 10^{-5}). This could imply that other factors, apart from the pressure, have some effect over the vagal tone. In fact, there was a last factor that affects the UD dataset: the difference in the way of breathing, spontaneous in the baseline stage versus through a scuba mask in the immersion stage. It must be highlighted that UD is the only dataset where this difference in the way of breathing between its stages exists, since in the CD dataset subjects were breathing through the scuba mask for both stages. To our understanding, there is no previous works studying the difference between these two types of breathing. This is why we recorded a small dataset of 12 subjects, first breathing spontaneously and then inhaling and exhaling deeply and slightly more rapidly through the mouth, as if they were wearing a scuba mask and breathing through a regulator. As Fig. 6 shows, there was an activation of the parasympathetic activity. $PDMpara$ increases from spontaneous breathing to simulated scuba mask breathing in all the subjects, so this factor could explain, together with the effect of pressure, the increase of the parasympathetic activity in the UD dataset. However, time and frequency domain parameters in the breathing dataset seem to be contradictory. It must be noticed that these results for the way of breathing (increase in mHR and $PDMpara$ and decrease in $RMSSD$) have the same trend as the results of the uncontrolled dive, so may be this factor has more significance in the ANS response than expected. Therefore, the factor of how the divers are breathing during the different stages of a diving protocol should be taken into account for future studies.

In spite of the differences in the datasets, a comparison of

the baseline stages were performed. The baseline stage in the HC data was similar to that in the UD, but both were different with respect to the baseline stage in the CD data. Differences of body position (sitting vs. supine position), environment (on land vs. in cold water) and breathing (spontaneous vs. through a scuba mask) were analyzed in this baseline comparison. An increase in the *mHR* and a decrease in the rest of the time-domain parameters, comparing the first stage of CD with respect to HC and UD, is shown in Fig. 5. According to the literature, there is no significant change in heart rate between sitting and supine position [54] and between being outside the water or immersed with the head out [27]. However, the effect of cold water and the effect of breathing through the scuba mask increases the heart rate, as we have discussed before. Therefore, the effect of breathing through the scuba mask seems to be again an important factor when comparing the baseline stages of the three datasets.

Finally, as a limitation of this study, the number of scuba divers in both immersions (11 and 15) has to be highlighted. Nevertheless, the difficulty of recruiting experienced scuba divers has to be taken into account. In fact, in other hyperbaric studies this problem is recurrent: 10 subjects or less in [19], [20], [21], [29]. Another possible limitation is the fact that divers in the uncontrolled immersion spent only a limited time below 15 m, and because of this, the length of the selected segment for the analysis was restricted. As future studies, the recording of more scuba divers, the recording of a new immersion dataset with more time to be analyzed and the recording of deeper immersions could be done.

VI. CONCLUSION

There were two outcomes of this work. Firstly, the use of OSP and PDM methods was able to overcome the limitations of classic PSD frequency domain parameters when the respiratory rate fell in the LF band and in the account of non-linear properties. Secondly, a comparison of three different hyperbaric environments has been performed, taking into account the effect of pressure, cold water and physical activity during the immersion among other variables. Results show that the respiratory component does not have a great impact on the heart rate variability, so the effect of other factors could explain the differences between the responses of the two branches of the ANS. The effect of pressure can cause an increase in the parasympathetic activity, although this trend is not always found and differences between stages are not always significant. On the other hand, the effect of cold water, together with an environment with low visibility and physical activity during the immersion, may cause an increase in sympathetic activity. Finally, the effect of breathing through a scuba mask may cause an increase in the heart rate, but also an increase in the parasympathetic activity.

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