

THE FINAL FRONTIER

WHAT CAN WE LEARN FROM SURVIVING 100 DAYS UNDER THE SEA?

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INTRODUCTION

Since the dawn of human existence, the proximity to and exploration of the oceans have been a wellspring of inspiration for humanity. The vast and mysterious expanse of the ocean, stretching beyond the horizon, has captivated our imaginations, sparking a profound sense of wonder and curiosity. With the prospect of expanding the human race to extraterrestrial locations or even undersea habitats, ongoing research aims to determine the effects of these environments on the human body to assess their feasibility.

NEPTUNE is an acronym which stands for Nautical Experiments in Physiology, Technology and Undersea Exploration and Ecology. The Project NEPTUNE 100 expedition studied the effects of prolonged habitation in an undersea habitat at increased atmospheric pressure. In this groundbreaking endeavor, the project delved into uncharted territories of human physiology, physical adaptation, and psychological well-being by investigating the profound changes experienced by a scientist looking to raise awareness of the potential for undersea habitats, highlight the importance of oceanic health, and examine the effects of extended submersion on a subject.

In the realm of human health, these discoveries may pave the way for novel therapeutic approaches, leveraging hyperbaric oxygen therapy (HBOT) to modulate inflammation, enhance hormonal balance, and promote cellular rejuvenation and healthy aging. As we delve deeper into the synergies between hyperbaric environments and human physiology, the expedition's findings hold promise for advancing our understanding of human adaptation to extreme conditions. Ultimately, this research contributes to the fields of undersea hyperbaric medicine and human health. On a broader scale, these findings are relevant to space exploration, where astronauts face similar challenges related to altered environmental conditions. The expedition sheds light on the adaptability of the human body to extreme environments, offering insights into how individuals may respond to extended space missions.

MISSION OBJECTIVES

Austere environments, such as prolonged underwater submersion, pose unique challenges when it comes to crew health. Measures can be undertaken to prevent physical, physiological, and psychological

pathologies that occur during exposure to extreme environmental conditions. Isolated, confined and extreme environments (ICE) present a unique set of challenges for human physiology and technology assessments. These environments, characterized by their seclusion and demanding conditions, offer an unparalleled opportunity to advance our understanding of how the human body and mind respond to extreme stressors significantly contributing to the field of biomedical engineering.

Current data of physiological changes after multiple days of underwater submersion is drawn from the U.S. Navy and commercial saturation divers. There are studies of 5-30 day submersions at Jules Undersea Habitat, as well as Aquarius Reef Base, and myriad other commercial saturation living facilities. The world record for longest time spent living in an underwater fixed habitat was 73 days set at the same facility prior to this project. During this 73 day stay there was no scientific testing performed or biomedical observations on those individuals. There has never been a study analysing the effects of a human spending 100 days underwater because no one has endeavoured to stay this long before.



Figure 1: Dr. Joseph Dituri in the Jules Undersea Habitat in Key Largo, FL.

The mechanisms of action (MOA) of HBOT on the human body are well-established. This research aims to expand the knowledge for ongoing studies and deeper understanding of these environments. The MOA's that we know include hyperoxygenation, decreased bubble size, reduced lipid peroxidation, vasoconstriction, reduced intravascular leukocyte adherence, toxin inhibition, enhanced leukocyte oxidative killing, antibiotic synergy, angiogenesis, fibroblast proliferation, collagen synthesis, and increased stem cell production. These effects have been demonstrated in numerous studies. For instance, the use of hyperbaric medicine is known to increase CD-34+ progenitor stem cell proliferation.¹¹ Furthermore, various exposures such as ICE, short and long-duration space flights, hyperbaric and hypobaric conditions, and scuba diving have proven to alter immune function^{1, 2, 8}. Other studies found that hyperbaric conditions improved sleep in U.S. service members with post-concussive mild traumatic brain injury, while some discovered improvements in neurocognitive function in post-COVID conditions^{7,12}.

The purpose of this research is to expand the general public's understanding of these mechanisms, offering a novel and effective treatment approach for global health. Additionally, it was designed to emphasize the importance of oceanic health for human survival, demonstrate the effects of prolonged exposure on the human body

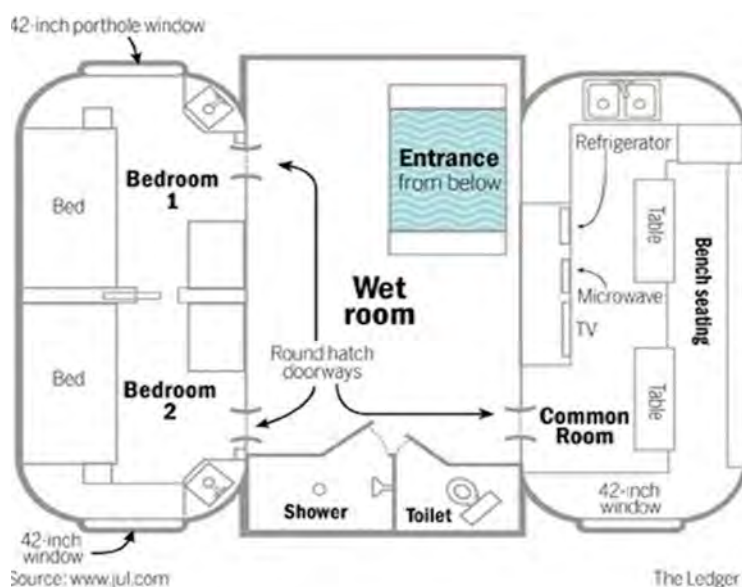


Figure 2: A diagram of the living quarters in Jules' Undersea Lodge located 25 feet under the surface of the sea at Marine Lab in Key Largo.

and mind, and teach the importance of preserving, protecting, and rejuvenating the undersea environment. Furthermore, it aimed to educate children about these critical areas and spark their interest in science, technology, engineering, and mathematics (STEM) fields.

METHODS

This study focuses on a 100-day immersion experience at the Jules Undersea Habitat in Key Largo, Florida at 1.67 ATA (atmospheres absolute) underwater, a setting that mirrors the confinement and isolation challenges that crews in an undersea station or

astronauts may encounter during long-duration missions, particularly those bound for Mars. This included daily SCUBA dives at approximately the same pressure as the habitat making this the endeavor the longest dive experienced by mankind. There was a team of 10 medical doctors, two psychologists, and one psychiatrist that followed closely to monitor and analyze the physiological and psychological changes experienced by a lone researcher (Dr. Joseph Dituri), the subject, during the 100-day underwater immersion.

The subject is a 55-year-old male measuring height 1.85 meters and weight

100 kilograms at the beginning of the research. The study's timeline spanned from several weeks prior to the subject's immersion in water on March 1st to June 9th, totaling 100 days submerged. Tests were conducted before, during, and after the 100-day underwater period using a comprehensive framework, which included blood, urine, and saliva analysis, as well as otological, psychological, emotional, visual, and physiological testing.

This habitat was not only a place of residence but also a dynamic educational and research hub. Over the course of the study, it welcomed a total of 5,579 students and teachers in 125 engaging Zoom calls over the internet, allowing for direct interaction and knowledge sharing. In addition to virtual engagement, the habitat hosted six onsite visits, providing firsthand experiences for those curious about the subject's living conditions. Furthermore, nine overnight Young Explorer Missions were conducted within this space, giving young adventurers a chance to immerse themselves in the STEM experience.

The environment of the habitat and visits had an influence on the subject's physical and mental well-being, as well as provided opportunities for exposure to current surface pathogens.

DISCUSSION

All detailed finding, figures, and graphs can be found on the original article: Dituri, J., Giger, M., Figueredo, J., & Spelsberg, S. (n.d.). Physical, physiological and psychological effects of 100 days in an undersea habitat with a combination of dry and wet diving: Monitoring the NEPTUNE 100 project. Retrieved from <https://underseaoxygenclinic.com/product/neptune-100-research-paper/>.

Biochemical, endocrine and immunological markers were measured, at baseline, during and after the 100 days. These are summarized in Figure 5.

- High-Sensitivity C-Reactive Protein (Hs-CRP)
- Urinary Testosterone
- Cortisol
- Insulin-like Growth Factor (IGF-1)
- Cholesterol
- Dehydroepiandrosterone sulfate (DHEA-S)
- 8-Hydroxy-2'-deoxyguanosine (8-OHdG)
- Creatine Kinase (CK)



Figure 3: Dr. Joseph Dituri receiving a neurological exam after 100-days living underwater.



Figure 4: Dr. Joseph Dituri reviewing blood samples.

- Interleukins (IL)
- Tumour necrosis factor – alpha (TNF-alpha)

Otologic Studies

Any saturation diving environment is a warm, moist, and dark breeding ground for bacteria. The subject's susceptibility to ear infections did not increase. Visualization of the tympanic membrane was repeated consistently and demonstrated no issues with the ear in general. This result occurred without the use of any Domeboro solution

or vinegar and alcohol. The use of Domeboro solution or vinegar and alcohol solutions thins the tympanic membrane, is costly, and is a great time burden for all saturation divers. This information is a critical discovery for saturation diving in the future and hopefully will yield greater understanding of what needs to be done in the future for extended duration diving.

Psychological and Emotional Testing

The results demonstrate a positive trend in psychological well-being during the

BIOCHEMICAL / ENDOCRINE / IMMUNE CHANGES



HIGH SENSITIVITY C-REACTIVE PROTEIN (Hs-CRP)

Baseline measured at 1.6 mg/L, decreasing to 0.6 mg/L then 0.4 mg/L. After resurfacing it increased back to 1 mg/L. Statistically significant decreases observed in hs-CRP suggest potential anti-inflammatory effects of hyperbaric exposure.

URINARY TESTOSTERONE

Baseline measured 3.68 mcg/g, then raised to above 7 mcg/g and stayed increased after resurfacing. The elevation in testosterone suggests a potential adaptation, enhancing human resilience and performance in conditions characterised by altered atmospheric pressure. It may also indicate improvements in his psychological well-being, particularly in terms of sleep.

CORTISOL

Baseline levels measured 5.35 ng/mL, decreased steadily during submersion to 3.53 ng/mL. This increased slightly to 3.95 ng/mL after surfacing but did not return to pre-dive levels. The decrease in cortisol levels, often considered a marker of stress, coupled with increased testosterone production suggests that hyperbaric exposure could have a profound impact on stress management and hormonal balance. This has significant implications for mental health, potentially reducing PTSD, anxiety, TBI, and depression scores.

INSULIN-LIKE GROWTH FACTOR (IGF-1)

Baseline levels measured 149 ng/mL, decreased to 123 ng/mL and then to 121 ng/mL. After resurfacing it stayed low at 118 ng/mL. Statistically significant decreases observed in IGF-1 suggest potential anti-inflammatory effects of hyperbaric exposure.

CREATINE KINASE (CK)

Baseline levels measured 183 U/L decreased to 124 U/L, then increased to 159 U/L after resurfacing. This is significant because CK is elevated in disease processes such as muscular dystrophy, inflammatory myopathies, myocardial infarction, and is implicated in nervous system disorders such as Alzheimer's disease. Further research is needed to determine if CK levels could be reduced long-term, potentially halting the disease process.

CHOLESTEROL

Baseline levels measured 268 ng/dL to 200 ng/dL during submersion. They returned to pre-dive levels several weeks after surfacing (274 ng/dL). The reduction in cholesterol has implications for cardiovascular health, potentially decreasing the risk of heart disease. Hyperbaric conditions has been linked to a decrease in cholesterol levels 6.

INTERLEUKINS

Regrettably, unforeseen circumstances, including shipping and lab errors, resulted in the loss of pre-submersion values for interleukins. Pro-inflammatory interleukins such as IL-alpha, IL-beta, IL-5, IL-6, IL-13 and IL-17 decreased and anti-inflammatory interleukins such as IL-4 and IL10 increased during the study. The observed decrease in pro-inflammatory cells, may be indicative of a mitigated inflammatory response, coupled with a concurrent increase in anti-inflammatory and anti-oxidative stress cells. This included a demonstration of reduced inflammatory markers which has been shown to be reduced up to 50% in other studies. This reduction in inflammation is of paramount importance as chronic inflammation is associated with various chronic diseases, such as cardiovascular disease, diabetes, autoimmunity, and cancer. By elucidating the mechanisms by which hyperbaric environments reduce inflammation, future research will pave the way for potential therapeutic interventions that could have a far-reaching impact on public health.

TUMOUR NECROSIS FACTOR-ALPHA (TNF-alpha)

Baseline was also lost during shipping. The first measurement was 12.5 ng/mL, then increased to 38.1 ng/mL, then decreased after resurfacing to 23.2 ng/mL. The statistically significant decreases observed in TNF-alpha suggest potential anti-inflammatory effects of hyperbaric exposure.

DEHYDROEPIANDROSTERONE SULPHATE (DHEA-S)

Baseline levels measured 123.23 mcg/g, increased to 181.25 mcg/g during submersion and this increase continued to 198.68 mcg/g after resurfacing. This observation suggests a potential influence of sustained elevated atmospheric pressure on the endocrine system, particularly the adrenal glands' production of DHEA. As a steroid hormone is recognized for its diverse physiological roles, including anti-aging effects and overall health promotion. The study's outcome opens avenues for understanding the interplay between environmental factors and hormonal regulation.

8-HYDROXY-2'-DEOXYGUANOSINE (8-OHDG)

Baseline levels measured 5.75 mcg/g, decreased to 0.89 mcg/g then increased to 3.22 mcg/g after surfacing. Although speculative, the observed decline in 8-OHDG may suggest a mitigating effect on oxidative stress. The modulation of reactive oxygen species under increased pressure could be influencing this response. This finding might have significant implications for cellular health and longevity. Hyperbaric conditions has been linked to an increase in telomere length. 4 Longer telomeres are associated with increased cellular longevity and a reduced risk of age-related diseases. In addition, the observed increase in circulating stem cells suggests a potential regenerative effect on tissues and organs, which could have far-reaching implications for various medical treatments, including wound healing and tissue repair 5.

Figure 5: Biochemical / Endocrine / Immune Changes.

immersion period, with improvements in sleepiness, anxiety, depression, and PTSD symptoms. Most measurements returned to pre-immersion levels one month after resurfacing, indicating the adaptability of individuals to the isolated and confined environment. However, the Generalized Anxiety Disorder Assessment (GAD-7) remained elevated at a score of 10, suggesting that some level of anxiety kept low with a slight trend back to normal.

The subjects Epworth Sleepiness Scale and Pittsburgh Sleep Study scores did improve. The data shows a notable improvement in subject's daytime sleepiness as the mission progressed. The authors opine this improvement is attributed to the increase in his sleep patterns as described below. Moreover, the observed doubling of both deep and REM sleep following hyperbaric exposure highlights the potential benefits for individuals suffering from sleep disorders or disturbances.

Electroencephalogram (EEG)

Coherence increased in brainwave activity by 9% and phase lag reduced by 5% with a return to baseline within one month of surfacing. The results were non-statistically significant. The only conclusion to be drawn is that there were no significant changes in brain activity while at this depth and pressure.

Electrocardiograms (EKG)

No statistically significant remarks came from the EKG testing which to the naked eye of several experts appeared largely unchanged day to day. Research using AI analysis to detect subtleties into the EKG and heart rate variability is ongoing. The pressurized environment has little to no impact on EKG tests administered.

Physical monitoring

No statistically significant remarks came from the physical monitoring (blood pressure, heart rate, and O₂ saturation), but the data was correlated with myriad pieces of testing equipment.

Pulmonary Function Tests (PFT)

The FVC and FEV₁ overall, we see that the subject's recorded values were significantly higher than his predicted values. Although both trends are almost identical, it is important to note that the FEV₁ demonstrated a significant drop on



Figure 6: Dr. Joseph Dituri teaching while diver shows marine life to students.



Figure 7: Marine Lab students from Ohio during his daily dives. Picture taken with remotely operated vehicle.



Figure 8: Dr. Joseph Dituri conducting the neurofeedback testing.



Figure 9: Part of the team.

the 25th of April, going from 4.46 on the 16th, 3.62 on the 25th, and back up to 4.69 on the 28th. This extreme spike was seen both in the FEV₁ data and FEV₁/FVC, but there is no spike in the FVC data. This reduction in FVC could be an indicator of pulmonary oxygen toxicity. A reduction in FVC is commonly observed in saturation diving at 50% fraction of inspired oxygen which is why these exposures are commonly limited to approximately 35 days. The fraction of inspired oxygen breathed by the subject was 36%. This decrease in FVC is possibly indicative of a human tolerance limit being reached for human exposure to 36% fraction of inspired oxygen for 100 days.

Muscle size

A 5% weight loss can be seen in the initial 25 days of submersion. The authors attribute this to the increase in metabolism from being in a hyperbaric environment. In order to combat further weight loss, it was necessary to increase from consumption of 1 g protein per kilogram body weight to 1.75 g protein per kilogram body weight. This helped maintain the weight at or around the 5% level without further loss during the study period. The subject effectively preserved muscle mass during the study through the utilization of the KAATSU cuff blood flow restriction device and resistance bands. The KAATSU cuff and resistance bands were efficient and effective for maintaining muscle mass. This approach

demonstrates potential for application in future space missions and ICE.

Visual Acuity Eyesight

The right eye was 20/40 pre-dive and 20/60 post dive. The left eye was 20/35 pre-dive and 20/50 post-dive. Both eyes were 20/30 pre-dive and 20/40 post-dive. All returned to pre-dive metrics six weeks after surfacing. The researchers suggests the progressive myopia was a combination of the exposure to 36% fraction of inspired oxygen combined with myopic focal points. The impact from being in an ICE may have an adverse impact on visual acuity and further mission planning as well as deep space mission should consider carefully this impact. Importantly, there may have been some oxygen-induced myopia coupled with the myopia from short focal point due to the increase in oxygen. There is no way to separate the two. Further investigation is warranted.

MEDICAL CHALLENGES

A multidisciplinary team consisting of 10 physicians, two PhDs in psychology, and one psychiatrist was assembled to identify and mitigate potential risks associated with the mission. The team, led by Dr. Sarah Spelsberg, considered various scenarios which could potentially jeopardize the mission, such as barotrauma, and implemented strategies to avoid them. The subject underwent daily EKGs, PFTs, and other medical assessments

as part of the research and could also detect early signs of any complications. The medical team remained on standby, fully prepared to intervene if necessary.

A comprehensive medical kit was prepared, containing supplies for treating corneal abrasions, ear infections, and fungal conditions—ailments that were anticipated but ultimately did not occur. Despite careful planning for worst-case scenarios and the availability of various medications on-site, none were required during the mission. The most significant health issue encountered was a cracked tooth on day 11, which was not anticipated. The subject endured pain for the remainder of the mission, with temporary relief provided by a numbing mouthwash. An emergency root canal was performed on day 102, two days after the mission's conclusion. The injury was attributed to eating popcorn.

Another critical aspect of the mission was the precise calculation of decompression before resurfacing. This process was calculated by Dr. Dituri at first having completed 16-3rd order partial differential equations and backed up by Dituri's innovative device, which holds a U.S. patent and was tested underwater for the first time during this mission. There were individuals who entered into decompression in the habitat from merely a 5 day stay and also several people staying for a few days in the habitat have been bent prior to the mission. The ultimate decompression strategy involved breathing 100% oxygen for two hours within the habitat, followed by an additional hour at gradually decreasing depths, all while continuing to breathe 100% oxygen. Remarkably, this approach resulted in no detectable bubbles (Doppler score zero) one hour after the dive, despite the 100-day submersion solidifying the adequacy and accuracy of Dituri's new patent/invention. The device, which detects autonomic nervous system stress and predicts oxidative, hypercapnic, and decompressive stress before symptoms appear, proved to be both effective and efficient.

DAILY LIFE

Dr. Joseph Dituri's 100-day underwater journey was a transformative experience that tested his physical and mental limits. The isolation, constant pressure, and lack of sunlight offered a unique perspective on human resilience and adaptation. The following excerpt features Dr. Joseph Dituri

as he gives a brief insight into his 100-day underwater experience:

- As a creature of habit, I started each day at 5 AM with two cups of coffee—after all, science doesn't happen without coffee. Breakfast typically consisted of 3 to 4 eggs with meat and cheese, all prepared in the microwave. Given the constraints of the underwater environment, everything had to be cooked in the microwave or not at all, as open heating elements were too risky in a partial pressure environment. Food was delivered every three days to accommodate these limitations.
- I spent 6 to 8 hours each day on various scientific tasks. Mornings were filled with activities like EEGs, blood and urine analysis, saliva testing, and neurofeedback. Daily vitals were a routine part of my day, while pulmonary function and psychological testing occurred less frequently but were consistently integrated into my 100-day mission. Lunch was often a salad with protein, and dinner typically featured salmon or another protein paired with something green. Again, prepared in a microwave. I can make a mean poached salmon.
- Each day I would spend one hour daily diving at depth within the lagoon, conducting specific activities related to diving, cleaning litter, and admiring the marine life.
- I dedicated about two hours daily to outreach, focusing particularly on STEM-related activities. I had to become a trained Scoutmaster to allow the Scouts to stay overnight in the underwater habitat, an experience they thoroughly enjoyed as we discussed various topics related to underwater exploration and STEM. I also taught a one-hour daily lecture to biomedical engineering students at the University of South Florida, on the topic of bio-transport, which was a rewarding experience. Working with students and outreach programs was the most fulfilling and enjoyable part of my mission.
- Overall, I stayed busy for 12 to 14 hours each day, balancing teaching, media appearances, and scientific research. I initially anticipated challenges with mental health, but I found myself particularly happy while underwater and noticed that I slept better than I

usually did on the surface. I was truly in my element—my 28 years in the Navy had clearly prepared me well.

CONCLUSION

Project NEPTUNE 100 is a modern-day odyssey, with its 100-day immersion into the undersea world. This mission pays homage to our historical relationship with the ocean. It symbolizes the continued human quest for understanding and harnessing the potential of the oceanic environment. In exploring the clinical significance of living in an undersea habitat, we unravel the physiological adaptations of the human body to hyperbaric conditions and ICE. The results of the research show that living in a hyperbaric environment could lead to a decrease in overall cardiovascular and environment-related stress. Project NEPTUNE 100 reaffirms the known clinical insights and supports the known mechanisms of action of HBOT on the human body. Collectively, these findings highlight the multifaceted benefits of hyperbaric exposure on physical, psychological, and physiological well-being, opening doors to innovative therapeutic approaches and lifestyle modifications that can enhance human health and longevity.

The knowledge gained from this undersea study contributes not only to the fields of hyperbaric medicine and human health but also to our future goals of space exploration and living in confined environments. We believe that the undersea environment serves as an effective analogue for the challenges of a mission to Mars. By enhancing our understanding of the physical and mental effects of ICE, we can make significant improvements in future space missions and habitation. Leveraging underwater training, which simulates the unique conditions of space missions, allows astronauts to develop their skills more effectively before venturing into space. Utilizing these environments could also further prepare astronauts, resulting in better physical and mental training and potentially improving their overall health.

This study is a crucial step towards demonstrating the benefits of HBOT and underscores the need for more research in this area.

STUDY LIMITATIONS

The study limitations of the study, most notably sample size, and should be regarded

as an initial step in the scientific process. None of the results presented here should be considered as substantial proof; rather, they are observations of an underwater living experience in preparation for more comprehensive investigations.

For more insights to the study, please watch Dr. Joseph Dituri's Ted Talk: <https://youtu.be/UpnByfqLkmk?si=ZZ5FGBrgu-DH5IFC>



For a tour of the undersea habitat (@drdeepsea): <https://www.youtube.com/watch?v=R59hBduRtNQ>



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