

## DIAPHONIA

#### Dlagnostic framework to Assess and Predict tHe impact Of underwater Nolse on mArine species



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## **DIAPHONIA PARTNERS**













UNIVERSITÀ DEGLI STUDI di Padova





## **D**NTNU

Norwegian University of Science and Technology





of Ocean Science r Sustainable Development







## The soundscape





#### TODAY'S OCEAN SOUNDSCAPE

#### ANTHROPOGENIC SOURCES

- Acoustic deterrent devices
- Fishing vessels 2
- **Recreational vessels** 3
- Cruise ships
- Commercial shipping 5
- Offshore oil & gas 6
- Seismic airgun surveys 7
- Military & civilian sonar 8
- Offshore renewable energy 9
- Underwater explosions 10
- Construction and pile-driving 11

#### NATURAL SOURCES

- Waves Δ
- Wind R
- Rain С

- Marine mammals D
- Currents
- Underwater landslides, volcanos and earthquakes
- Fishes G
- Invertebrates H













IlFattoQuotidiano.it / Ambiente & Veleni

## Capodogli morti a Vasto, "uccisi dalla ricerca del petrolio in mare"

**AMBIENTE & VELENI** 

Per Legambiente lo spiaggiamento è dovuto alle esplosioni per sondare la presenza di greggio. "Il gas nel sangue avvalora la tesi che i cetacei, disorientati, abbiano fatto una riemersione troppo rapida e questo ha provocato un'embolia"

#### Sound and strandings

4







## **Black Sea Unusual Mortality 2022**



Національний природний парк "Тузлівські лимани" 6 g . 🕤









## What we know and don't know...

Int. J. Environ. Res. Public Health 2015, 12, 12304-12323; doi:10.3390/ijerph121012304

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International Journal of **Environmental Research and Public Health ISSN 1660-4601** www.mdpi.com/journal/ijerph

Review

#### Noise in the Sea and Its Impacts on Marine Organisms

Chao Peng, Xinguo Zhao and Guangxu Liu \*

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### Figure 1. The percentage of studied marine species in different taxa being affected by anthropogenic noise.









# Anatomy and physiology of sound









**Technical Guidance for Assessing the** Effects of Anthropogenic Sound on **Marine Mammal Hearing** 

**Underwater Acoustic Thresholds for Onset of Permanent and Temporary Threshold Shifts** 



U.S. Department of Commerce National Oceanic and Atmospheric Administration National Marine Fisheries Service

NOAA Technical Memorandum NMFS-OPR-55 July 2016



#### Table 3: Summary of weighting and exposure function parameters.

Hearing Group	8	b	f1 (kHz)	<b>fz</b> (kHz)	С (dB)	<b>K</b> (dB)	Weighted TTS onset threshold* (SEL <sub>cum</sub> )
Low-frequency (LF) cetaceans	1.0	2	0.2	19	0.13	179	179 dB
Mid-frequency (MF) cetaceans	1.6	2	8.8	110	1.20	177	178 dB
High-frequency (HF) cetaceans	1.8	2	12	140	1.36	152	153 dB
Phocid pinnipeds (PW) (underwater)	1.0	2	1.9	30	0.75	180	181 dB
Otariid pinnipeds (OW) (underwater)	2.0	2	0.94	25	0.64	198	199 dB
* Determined from minimum value of exposure function and the weighting function at its peak (i.e.,							

mathematically equivalent to K + C.



#### Table ES3: Summary of PTS onset acoustic thresholds.

	PTS Onset Acoustic Thresholds* (Received Level)		
Hearing Group	Impulsive	Non-impulsive	
Low-Frequency (LF) Cetaceans	Cell 1 Lpk,flat: 219 dB LE,LF,24h: 183 dB	<i>Сы! 2</i> <i>L</i> Е,LF,24h: 199 dB	
Mid-Frequency (MF) Cetaceans	Cell 3 Lpk,flat: 230 dB LE,MF,24h: 185 dB	Сай 4 L <sub>E,MF,24h</sub> : 198 dB	
High-Frequency (HF) Cetaceans	Coll 5 Lpk,flat: 202 dB LE,HF,24h: 155 dB	Ся! 6 L <sub>E,HF,24h</sub> : 173 dB	
Phocid Pinnipeds (PW) (Underwater)	Coll 7 Lpk,flat: 218 dB LE,PW,24h: 185 dB	<i>С.# 8</i> <i>L</i> е, рw, 24h: 201 dB	
Otariid Pinnipeds (OW) (Underwater)	Cell 9 Lpk,flat: 232 dB LE,OW,24h: 203 dB	Cell 10 LE,OW,24h: 219 dB	

\* Dual metric acoustic thresholds for impulsive sounds: Use whichever results in the largest isopleth for calculating PTS onset. If a non-impulsive sound has the potential of exceeding the peak sound pressure thresholds associated with impulsive sounds, these thresholds should also be considered.

Note: Peak sound pressure  $(L_{pk})$  has a reference value of 1 µPa, and cumulative sound exposure level ( has a reference value of 1µPa2s. In this Table, thresholds are abbreviated to reflect American National Standards Institute standards (ANSI 2013). However, peak sound pressure is defined by ANSI as incorporating frequency weighting, which is not the intent for this Technical Guidance. Hence, the sub "flat" is being included to indicate peak sound pressure should be flat weighted or unweighted within generalized hearing range. The subscript associated with cumulative sound exposure level thresholds in the designated marine mammal auditory weighting function (LF, MF, and HF cetaceans, and PW and pinnipeds) and that the recommended accumulation period is 24 hours. The cumulative sound exposur thresholds could be exceeded in a multitude of ways (i.e., varying exposure levels and durations, duty c When possible, it is valuable for action proponents to indicate the conditions under which these acoust thresholds will be exceeded.



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## **ANTHROPIC NOISE EFFECTS**



Type of effect



from



## Sounds - effect





ultimate ecological individual effects responses fitness community structure group cohesion distribution habitat avoidance microevolution mortality population dynamics. social bonds predator-prey interactions strandings 8a growth abnormalities community structure immune response population resilience morphology recruitment reproduction  $\approx$ disorientation location in water community structure population dynamics column recruitment malformations mortality strandings









RESEARCH ARTICLE ECOLOGY



KW-mammal

Behavioral responses to predatory sounds predict sensitivity of cetaceans to anthropogenic noise within a soundscape of fear

Patrick J. O. Miller<sup>a,1,2</sup>, Saana Isojunno<sup>a,2</sup>, Eilidh Siegal<sup>a</sup>, Frans-Peter A. Lam<sup>b</sup>, Petter H. Kvadsheim<sup>c</sup>, and Charlotte Curé<sup>d</sup>

Edited by Gerardo Ceballos, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico; received August 12, 2021; accepted January 24, 2022

1-4kHz Sonar

Baseline

Intense foraging Foraging/ exploratory Drift diving KW-mammal Non-foraging active Non-foraging active 2 1-4kHz Sonar Transit diving Near-surface travel Baseline Surface 

## **Behavioral effects**







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Noise in the Sea and Its Impacts on Marine Organisms

#### Chao Peng, Xinguo Zhao and Guangxu Liu \*

Review

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### Impairment of physiological hearing

## and physiological hearing system of marine organisms.

Species	<b>Types of Anthropogenic Noise</b>	Effects	References
M. angustirostris	increased ambient noise	constrains acoustic communication	Southall et al., 2003 [45]
C. chromis S. umbra G. cruentatus	boating and shipping noise	reduces auditory sensitivity and shifts the hearing threshold	Codarin <i>et al</i> ., 2009 [7]
H. didactylus	boating and shipping noise	constrains acoustic communication and shifts the hearing threshold	Vasconcelos <i>et al.</i> , 2007 [46]
P. phocoena	seismic air-gun shooting	shifts the hearing threshold	Lucke et al., 2009 [48]
T. truncatus	experimental noise emanating device	shifts the hearing threshold	Nachtigall et al., 2004 [49]
P. auratus	seismic air-gun shooting	damages the hearing sensory epithelia	McCauley et al., 2003 [37]
L. vulgaris S. officinalis O. vulgaris I. coindetii	experimental noise emanating device	damages the hearing sensory epithelia	André <i>et al</i> ., 2011 [52]
A. dux	seismic air-gun shooting	damage to internal fibers, statocysts,	Guerra et al., 2011 [53]



Table 2. Example studies showing effects of anthropogenic noise on acoustic communication

stomachs, and digestive tracts

### Strandings associated to sounds: military sonars









#### Strandings associated to sounds: military sonars

Pathological changes with gas bubbles consistent with decompression sickness spatially and temporally associated to military exercise

(Jespson et al.,2003; Fernández et al. 2005)









#### Strandings associated to sounds: military sonars





## No mass strandings since sonar ban

Prompt political action may have resulted in a remarkable conservation success for whales and dolphins. The Canary Islands used to be a hotspot for mass strandings, but there have been no mass beachings since the Spanish government imposed a moratorium on naval exercises in these waters in 2004.

Naval sonar operations have long been implicated in mass strandings, particularly of beaked whales (R. Filadelfo *et al. Aquat. Mamm.* **35,** 435–444; 2009). In 2002, the stranding of 14 beaked whales in the Canary Islands was linked to the use of midfrequency naval sonar in the area (P. D. Jepson *et al. Nature* **425,** 575–576; 2003). In 2004, four Cuvier's beaked whales (*Ziphius cavirostris*) were stranded there





#### Strandings associated to sounds: military sonars









## Unkowns...









## **Knowledge gaps & DIAPHONIA**

- 1.Need measurable molecular and structural changes as direct evidence of AOE.
- 2.Need of a fingerprinting counting for several biomarkers in different species.
- 3.Need to differentiate the respective contributions of both sound components in order to model the effects of sound sources in marine organisms.
- 4. Need to determine changes in proteome profiling after sound exposure.



- DIAPHONIA
- will develop a possible diagnostic fingerprinting approach composed of several tissue markers incorporating molecular, metabolomic and microscopic techniques in order to identify functional and morphological changes in the acoustic pathways of invertebrates, fishes and marine mammals.







#### Genomic signaling as early warning signs of anthropogenic stressors

Noise exposure

Endocrine markers

Prestin

ESR1 and TR $\alpha$ 

Involved in hearing loss Reproductive process and fat metabolism



Identifying biomarkers for stress-exposure, mediators of immune system and TTS in marine mammals - WP1





Heat shock protein

Cytokines

HSP70

Stress related protein

Interleukins (IL2 & 10)

Important mediators of the immune system







# Identifying a diagnostic fingerprinting of TTS and effects on CNS functions and morphology (WP1)





Nature Reviews | Neurology





#### **Diagnostic fingerprinting of noise alterations in** the central nervous system





#### **Development of a diagnostic approach to underwater noise impact**



of Ocean Science



## **Knowledge gaps & DIAPHONIA**

5. Data from multiple species regarding anatomy, physiology and behavioural responses to various stimuli are missing. 6.Need to carry out further research on the behavioural responses of a range of fishes to different sound sources, under different conditions. 7.Data are needed on how these responses change over time after the introduction of an anthropogenic source. 8. Links between behavioural and physiological aspects of animals' response to sound are not understood. 9. Long-term exposure to anthropogenic noise on reproduction are not well investigated. 11.Essential to understand which sound types disturb fish most to develop appropriate management and mitigation practices.



- 10. Studies on pile-driving noise during construction are needed that could lead to understanding dose-response relationships of different sound variables.

#### DIAPHONIA

will explore the relationship between behavioural and cellular/molecular/organ effects of both long-term noise exposure of two fish commercial species in different European ocean basins, and short-term noise exposure in invertebrates and fishes, from micro- to macroscale.





People, Buildings & Environments



#### **Impulsive Noise**

The noise from impacts or explosions, e.g., from a pile driver, punch press or gunshot, is called impulsive noise. It is brief and abrupt, and its startling effect causes greater annoyance than would be expected from a simple measurement of sound pressure level.

### Fish & invertebrate in vivo exposure to investigate AOE impact (WP1 & **WP2**)







## Impact assessment of impulsive noise on marine invertebrates

Health status of the exposed individuals (adult, larva and eggs) determined by:

- respiratory rates and metabolisms
- proteomics (MALDI MS)
- electron scan microscopy
- survival rate and hatching success
- behavioral observations











#### Impact assessment of impulsive noise on fishes



Set up the methodology for analysis and selection of a representative number of metabolites



Controlled harmonised exposure experiments to obtain dose-response curves of noise exposure and metabolites concentration in the inner ear of fish













#### - Identify bioindicators for noise exposure in fish - Obtain dose-response curves of noise exposure and metabolites concentration in the inner ear of fish (D1.2)





#### **Metabolomic studies in fish**











#### Linking behavioural and physiological responses









### **Knowledge gaps & DIAPHONIA**

#### 12. The functional anatomy and physiology of the hearing apparatus and the acoustic pathways

DIAPHONIA

will gain insight into the morpho-functionality of the peripheral hearing apparatus in marine man









### Developing new tools and approaches: 3D numerical modelling (WP3)

### **Toothed whale acoustic pathways 3D** reconstruction & FEM wave propagation











### **Knowledge gaps & DIAPHONIA**

### 13. Experimental research using marine animals remains difficult and remains ethically questionab

#### DIAPHONIA

ed organoids from stranded cetaceans and fish fibroblasts to recreate the auditory sensory orgar











# Developing new tools and approaches: 3D cell cultures (WP1)









#### Development of in-vitro model of inner ear of marine mammals and fish













### Integration of results and final outputs (WP4)









B: HTAPF









## **MSFD - 2008/56/EC**

#### Marine Strategy Framework Directive



## DESCRIPTORS

% OF MS THAT WERE ABLE TO CONCLUDE ON CURRENT STATUS





WORKING TO ACHIEVE CLEAN HEALTHY AND PRODUCTIVE EUROPEAN SEAS













#### Anthropogenic Noise

2.16	Assessment and Impact Assessment of Man-Made Noise
3.10	Guidelines to Address the Impact of Anthropogenic Noise
4.17	Guidelines to Address the impact of anthropogenic noise
5.15	Addressing the Impact of Anthropogenic Noise
6.17	Anthropogenic Noise
6.18	Implementation of an ACCOBAMS Certification for High





#### **Methodological Guide:**

#### **Guidance on underwater noise mitigation measures**

V. 3.0.

e on Marine Mammals in the ACCOBAMS Area

on cetaceans in the ACCOBAMS area

ighly Qualified Marine Mammals Observers



of Ocean Science for Sustainable Development





## **Questions?**









## THANK YOU FOR YOUR ATTENTION!

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Cetaceans strandings Emergency Response Team (CERT)

Centro Interuniversitario per la Ricerca sui CEtacei (CIRCE)

International Whaling Commission Strandings Expert Panel Member

IUCN SSC Cetaceans Specialist Group

National Center for Future Biodiversity

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