

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☒ ☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☒ ☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

All data-collection was made using custom scripts written in Matlab R2018a (The Mathworks, USA).

Data analysis

Excised larynx and tissue properties data-analysis was made using custom scripts based on previously published methods written in Matlab R2018a (The Mathworks, USA).
The geometry mesh model of the sei whale larynx was reconstructed from the CT scan in Mimics Innovation Suite 24 (Materialise, Leuven, Belgium).
Code is available at Zenodo (DOI:10.5281/zenodo.10390075).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Supplementary Information is available for this paper.

Source data are available in the main text or as supplementary materials.

Raw data is available at Zenodo (DOI:10.5281/zenodo.10390075).

Balaenoptera musculus recording from: Discovery of Sounds in the Sea, <https://dosits.org/galleries/audio-gallery/marine-mammals/baleen-whales/blue-whale/>
 Balaena mysticetus recording from: the Watkins Marine Mammal Sound Database, Woods Hole Oceanographic Institution and the New Bedford Whaling Museum. <https://whoicf2.who.edu/science/B/whalesounds/index.cfm>.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

n.r.

Reporting on race, ethnicity, or other socially relevant groupings

n.r.

Population characteristics

n.r.

Recruitment

n.r.

Ethics oversight

n.r.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences

☐ Behavioural & social sciences

☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No sample size was determined. We gained unique and opportunistic access to fresh larynges of three whale species, including sei (Balaenoptera borealis), common minke (B. acutorostrata) and humpback whales (Megaptera novaeangliae). The difficulty in obtaining very fresh laryngeal tissue from mysticetes is a challenge to the study of voice physiology and biomechanics in these species, therefore the low sample size is sufficient.

Data exclusions

None

Replication

We gained unique access to fresh larynges of deceased sei (Balaenoptera borealis), common minke (B. acutorostrata) and humpback whales (Megaptera novaeangliae). To verify the reproducibility of the experimental findings, we performed successful technical replicated of pressure ramps. After experiments the larynges were stored at -80C and after a day of experiments started to decay and therefore not useful for additional technical replication of the experiments.

Randomization

We randomized frequency settings when possible, including the tissue materials testing (Fig 3e). The excised larynx experiments could not be randomized, as we only had one larynx specimen per species.

Blinding

The excised larynx experiments could not be blinded, as we only had one larynx specimen per species which were very different in appearance.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

No

Wild animals

We gained unique access to fresh larynges of deceased sei (*Balaenoptera borealis*), common minke (*B. acutorostrata*) and humpback whales (*Megaptera novaeangliae*).

Sei whale (*Balaenoptera borealis*), 770 cm male, estimated at 6 months old (Catalog number NHMD-M08-1695, Natural History Museum of Denmark). The individual was first sighted 28 Nov, 2018 in the harbour of Hobro, Denmark, died on December 7, 2018 close to Katbjerg Odde and was brought on land December 8, 2018. Probable cause of death: uncertain, but the animal was emaciated with no major wounds. In this period the average temperature was 5.6 °C and 4.8-5.5 °C (<https://www.dmi.dk/vejrkiv/>) in water and on land respectively, which aided in preserving the tissue. The larynx including hyoid bone was dissected on December 10, 2018 in the harbour of Hobro by CPHE and MW.

Humpback whale (*Megaptera novaeangliae*), 840 cm subadult female, 5480 kg (Catalog number NHMD-M08-1698, Natural History Museum of Denmark). The individual was caught in fishing gear October 7, 2019 near Skagen, Denmark, and in good nutritional state without major wounds. Probable cause of death: drowning. The individual was lifted into a container and covered with ice. In this period the average temperature was 0-8 °C on land. The larynx including hyoid bone was dissected October 9, 2019 at the Nordsøen Oceanarium, Hirtshals by CPHE and MW.

After dissection the sei and humpback whale larynges were immediately placed in ice and transported to the University of Southern Denmark (SDU), where they were flash frozen in liquid nitrogen and stored at -80 °C. The larynges from these two whales were collected under a permit to the Natural History Museum of Denmark, issued by the Danish Environmental Protection Agency as part of the The National Contingency Plan concerning strandings of marine mammals in Denmark.

Minke whale (*Balaenoptera acutorostrata*), 644 cm subadult female (Catalog number M 586/18, Scottish museum). The individual was stranded in Ardesier, Scotland in Sept 2018. Probable cause of death: Meningoencephalitis due to bacterial infection. The larynx and trachea were extracted by Georg Hantke, frozen and transported frozen to Vienna.

Scored on the scale of the U.S. National Marine Fisheries Service, all animals fall well within Code 2: there was no scavenger damage, a fresh smell, no drying and wrinkling of skin, eyes, and mucous membranes, the eyes were clear, the carcass not bloated, the tongue and penis not protruded, the blubber firm and white, the muscles firm, dark red, and well-defined, the viscera intact and well-defined and the gut contained no gas.

Body length was measured as the straight line from the tip of the snout to the notch between the tail flukes.

Reporting on sex

Sex is reported per animal.

Field-collected samples

We worked with fresh tissue of deceased animals.

Ethics oversight

Not relevant.

Note that full information on the approval of the study protocol must also be provided in the manuscript.