# Modifiable clinical dental impression methods to obtain whole-mouth and detailed dental traits from non-livestock vertebrates

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# Abstract

Dental impressions, important for capturing oral characteristics in clinical settings, are seldom used in research on non-livestock and non-mammalian vertebrates. Live animal dentition studies usually require euthanasia and specimen dissection, microCT and other scans with size and resolution tradeoffs, and/or ad-hoc individual impressions or removal of single teeth, preventing in-vivo studies that factor in growth and other chronological changes, and separating teeth from the context of the whole mouth. Here, we describe a non-destructive method for obtaining high resolution dentition traits that can be used on both living animals and museum specimens, involving a customizable and printable dental impression tray. This method can repeatedly and accurately capture whole-mouth morphology and detailed features at high resolution using living *Polypterus senegalus* in a laboratory setting. It can be used for comparative morphology and to observe temporal changes such as microwear, tooth replacement rates, and bite and morphological changes through ontogeny.

# Summary statement

This study presents a new tool and method to measure dental traits in living animals accurately.

# Introduction

Vertebrate dentition provides essential information on the ecology and evolution of species. In fishes alone, dental traits have been used to investigate wide-ranging topics such as development (Hung et al., 2015), replacement (Carr et al., 2021), dental microwear analysis (Purnell and Darras, 2015), jaw evolution (Ronco et al., 2021), feeding characteristics (Streit et al., 2015), biomechanics and bite force (Deng et al., 2022) and diversification of the oral apparatus (Burress, 2016). However, all these studies used either dead specimen or isolated teeth (Burress, 2016; Streit et al., 2015), and euthanasia was often necessary to obtain samples (Hung et al., 2015; Ronco et al., 2021). Furthermore, dissection of the jaw and/or removal of teeth is typically performed, damaging the original sample. The same constraints limit *in vivo* studies of other vertebrate dentitions and oral traits, apart from those on livestock

and a handful of primates for whom clinical dental practices exist (Hoffman et al.,
2015; Teaford and Oyen, 1989).

In order to avoid destructive practices in obtaining morphological data, scanning
methods (e.g. optical, microCT, laser, and Synchrotron) have been increasingly used
on full specimens (Akhter and Recker, 2021). However, scanning approaches also
have constraints which limit their utility in many vertebrates. Optical scanning, as of
2024, lacks the resolution necessary to see fine details such as wear (L.S. and T. K.
personal observation). MicroCT and Synchrotron X-ray scanning imposes size
limitations on specimens and have trade-offs between field of view, processing time,
and image resolution, and of course take place under high radiation (Sun et al.,
2022). Usage of X-ray scanning therefore requires dead and or dissected samples in
most cases and/or considerable preparations and planning at limited facilities for
limited *in vivo* work to date (Gradl et al., 2018).

Here, we propose and demonstrate a noninvasive alternative methodology to obtain
high resolution dental and oral trait information. Based on human clinical practice, we
designed 3D printed, adjustable dental impression trays which can be used with
readily available, inexpensive non-toxic impression materials to take multiple whole
intraoral records from living and dead vertebrates. Direct application of dental
impression material to non-human teeth has been shown to accurately capture fine
surface details (Purnell and Darras, 2015; Sawaura et al., 2022). However, this ad-
hoc application was limited to fixed dead and skeletal animal specimens or fallen
teeth. The use of a species-customized tray allows for repeated use with live
animals, for tracking of chronological changes, and exact capture of whole mouth
morphology.

Here, we demonstrate the utility of our modifiable, freely printable tray in taking
detailed oral impressions from non-human vertebrates (see below and
supplementary figure 1 for download and adjustment details). We developed and
tested this methodology on live and preserved specimens of a non-model but
increasingly used laboratory organism, the non-teleost fish *Polypterus senegalus*
Cuvier. Below, we describe the details of our tray, its proper usage, and the results
obtained by this method.

# Materials and methods

## Impression tray design

A dual arch impression tray system (Figure 1A) with a handle was chosen as the basis for our design, eliminating the need for each animal to undergo multiple sessions to take impressions for both maxillary/upper and mandibular/lower arches. The tray design was built to include intraoral and extraoral components based on trials with live fishes and human dental tray designs. We placed a crescent-shaped trench of 0.5-1 mm in depth and of uniform on both maxillary and mandibular aspects of the tray to allow extra occlusal clearance for dentition to impress the material (Figure 1A). The tray was parameterized to enable customization of size and shape (Figure 1B).

An extraoral perforated retaining wall in front of the trench allows for firm stabilization during impression sequences (Figure 1C-F), as movement by the animal or the operator would otherwise negatively affect accuracy and material distortion. Our wall element extends posteriorly and past to the angle of the mouth in order to affix and stabilize the head. Perforations were included in the design of the retaining wall to aid in attaching the impression material to the tray without the use of adhesives. The tray wall can be customized to match the external border and size of the jaw (Figure 1A, right). The intraoral portion of the tray includes occlusal stops to avoid tooth contact with tray during impression and prevent posterior sliding (Figure 1A, middle). Incorporating stops based on oral morphology ensures a uniform thickness (Terry et al., 2010) and minimizes distortion (Ortensi and Strocchi, 2000). In our *Polypterus* test animals, the centrally located parasphenoid bone on the oral roof and the "tongue" (basihyal) in the mandibular floor were designated as hard/soft tissue stops. We inserted a centralized raised stop behind the trench on maxillary section of the tray and a smaller raised central lingual floor on the mandibular side in our test trays, both of which can be removed before printing (Supplementary figure 1).

The final version of our adjustable, 3D-printable impression trays was designed using Autodesk Fusion360 and parameterized using Blender 4.0. Inclusion of sliders and checkboxes in Blender to adjust the size, relative dimensions, and intraoral and extraoral features (stops, holders, etc.) enables application to a wide range of mouth shapes and allows the tray to be modified without technical skills in 3D modeling.

120  The model is freely available and archived on Zenodo (doi:
121  10.5281/zenodo.12524788). The steps to modify the tray are outlined in
122  supplementary figure 1.

## Fish husbandry

*Polypterus senegalus* Cuvier specimens of 20-25cm size of random sex were
acquired from local pet shops. 100 L water tubs with Eheim Classic 2215 external
filters (Eheim, Germany) are used to house up to 2 *Polypterus senegalus* Cuvier
specimens of 20-25cm size. The water temperature is controlled to be 25°C. Feeding
is performed 3 times a week using 2 pellets per fish of Hikari Crest Freak Bottoms
(Kyorin, Japan), however any other food for bottom dwelling carnivorous fish should
be admissible. 10-20% water change is performed every week, removing detritus
and uneaten food. Water quality, including pH, nitrate and nitrite contents are
monitored after every feeding, changing 10-20% of water when nitrate or nitrite
levels show higher than around 25ppm and 1ppm respectively.

## Fish sedation

Tricaine mesylate (MS-222) (Sigma-Aldrich, USA) was used for fish sedation with
twice in mass of $NaHCO_3$ (Nacalai Tesque, Japan) as buffer, following previous
reported sedation of *Polypterus* (Whitlow et al., 2022). Fish was transferred to a
smaller tank of 2 L of water with dissolved buffered MS-222 (Figure 2B).
Optimization of fish sedation was performed by gradually changing the MS-222
concentration in increments of 50 mg $L^{-1}$, starting from 100 mg $L^{-1}$ with 5 minutes of
exposure, and measuring the time before the fish starts moving outside the water.
For approximately 25 cm *Polypterus senegalus* Cuvier, 5 minutes of exposure to 300
mg L-1 of MS-222 was enough to keep the fish sedated for at least 5 minutes. We
used 1.5 mg L-1 of MS-222 to keep the fish sedated for 30 minutes, enough to
obtain 5 impressions.

## Intraoral impression process

147  The following protocol was developed through testing with live and preserved
148  *Polypterus sp.* This protocol can be modified for other species and to use equivalent
149  impression materials. We recommend consultation with a veterinarian, Animal Care

150   and Use Committee, and/or published, established protocols to determine

151   appropriate husbandry and sedation of other species.

**Scanning of dental impressions**

Obtained dental impressions were scanned using a confocal laser microscope (VK-

X210, Keyence, Japan) with a violet laser (408nm) and an ultra-long range 100x lens.

Molds were cut along the line connecting mesio-distal section of each tooth using a

scalpel and dental loupes, so that both buccal/labial and lingual/palatal surfaces of

teeth can be observed in the impression. The tips of teeth were scanned as this area

contact most intensively with food and likely have more dental microwear on the basis

of work with other non-mammalian tetrapods (Winkler et al., 2019). The three-

dimensional surface models and two-dimensional images were generated using the

photo-simulation function of the laser microscope.

**Adjusting and printing impression trays**

We first adjust our tray models using Blender to the specifications for species and

individuals. We then 3D print trays using an Ultimaker S5 (Ultimaker, Netherlands)

with Ultimaker PLA material (Ultimaker, Netherlands), but any printer can be used

with PLA material from any source. Ultimaker Cura is used to slice the STL files

generated from Blender, using the default preset "extra fine" with 0.06 mm layer

height. We find that the tray prints best with the support overhang angle at 80° and

brim build plate adhesion type, printing the structure upright with the handle on top

and the mouthpiece at the bottom. Less Z axis resolution can be used with larger

impression trays.

**First impression to remove debris**

After preparation (Figure 2A) and confirming the animal has been completely

sedated if alive and not domesticated (Figure 2B), tweezers are used to gently pry

the mouth open to expose the teeth. Compressed air is used to dry the dentition to

remove residual moisture (Figure 2C). First impression is taken with Vinyl

Polysiloxane Putty Type (we used ExaFine, GC, Japan), which is mixed according to

manufacturer directions and then directly applied to dentition to remove and reduce

enamel pellicle (Figure 2D-E). Gentle pressure is used to close the mouth on the

putty material 2-3 times ensuring that the teeth are fully submerged while the putty is

relatively soft. Putty is removed in about 1 minute prior to complete hardening to
reduce the risk of enamel damage.

**Final impression sequence**

To prepare for the final impression using a more accurate impression material, an
Automix tip is affixed to a Polyvinyl siloxane (PVS) cartridge (we used Dr. Silicon
regular, BSA Sakurai, Japan) to ensure proper mixing of impression components.
This material has been shown to cure quickly and has high accuracy (Sawaura et al.,
2022), suitable for a wide range of applications including dental microwear study,
and is routinely available from dental suppliers (Supplementary table 1). A portion of
the impression material is exuded from the tip prior to filling the impression tray to
ensure that only properly mixed material is injected. To avoid air pockets, the
impression dispenser Automix tip remains submerged inside the impression material
as it is ejected into the tray (Figure 2F).

The loaded impression tray is placed into the mouth of the animal, where both jaws
are pressed together to simulate a bite (Figure 2G). Animal and impression tray are
held together securely yet gently to avoid harm until the impression material is
completely set (Figure 2H). Curing time was set to 5 minutes or more to let the
material harden (Sawaura et al., 2022), but this may vary with PVS from other
manufacturers. The live animal is then returned to its original confines and monitored
until it regains motor functions. This two-impression process takes approximately 10
minutes.

# Geometric Morphometric Analysis

We imaged the dental impressions using Hirox digital microscope HRX-01 (Hirox,
Japan) with a high-range motorized zoom lens 20x-2500x. As there are no
standardized oral soft or hard tissue landmarks for *Polypterus*, fourteen set landmarks
and ten semi-landmarked curves were chosen around the parasphenoid and
ectopterygoid bones, representing common and reproducible points on different
impressions. Two-dimensional morphometric data was collected from the digitized
dental impressions in R version 4.3.1 using the StereoMorph package v1.6.7 (Olsen
and Westneat, 2015). Procrustes distances and PCA analyses were subsequently
performed using the package borealis v2022.10.27

(https://github.com/aphanotus/borealis). Disparity is calculated as the Procrustes
variance using the diagonal elements from the group covariance matrix divided by the
number of observations and was derived using the function "morphol.disparity()" from
the package geomorph v4.0.7 (Baken et al., 2021). The codes and raw data used for
this analysis are available and archived on Zenodo (doi: 10.5281/zenodo.12524788).

## Ethics statement

218 This research was approved by the ethics committee of the Okinawa Institute of
219 Science and Technology under the protocol number ACUP-2023-006-2.

# Results

## Trait resolution (Based on *Polypterus)*

Using this method, we were able to produce impressions that accurately captured
dentition and intraoral morphology of very small live specimens of *Polypterus* (Figure
3A-B). We scanned the impressions taken from different individuals (see Materials
and Methods for details of scanning and image production) and effectively captured
dental structures less than 1mm across the entire jaw (Figure 3C-F), a higher
resolution than a previous study using direct manual measurements (Mihalitsis and
Bellwood, 2019). Further, we identified enamel damage on some teeth by quantifying
the depths of the ridges on the surface of the teeth (Figure 3E-F). From 38
impression attempts on 12 individuals, there was no incident of specimen mortality or
injury due to the impression process.

## Reproducibility

As this is a qualitative method, we tried to assess the reproducibility and accuracy of
obtaining shape information through geometric morphometric analysis of
maxillary/upper impressions (Figure 4, Supplementary figure 2-3). This allowed
quantification of impression method error, landmark assignment operator error, and
individual variation, based on generating 5 sets of landmarks for the parasphenoid
and associated soft tissues in each of 4 maxillary impressions on each of 2 different,
very small (approximately 20cm) individuals in one sedation session (40 total
landmark sets) (Supplementary figure 2, Figure 4A). We performed principal
component analysis (PCA) (Figure 4B-C, Supplementary figure 3), and found that
PC1 was associated with differences between individuals, while PC2 was associated

with some impression method error (the difference between a tight clustering of most
impressions and an outlier). Outliers were not related to order of impression and did
not include any initial impressions. Using the same parasphenoid landmarks, we also
conducted a geometric morphometrics analysis of all available whole maxillary
impressions from 7 individuals taken over multiple sessions, spread over the multi-
month course of method development and data collection for another project (Figure
4C, Supplementary figure 3C-D). Our PCA results for all individuals further
demonstrated that variation between different individuals or the same individual's
growth over time was much greater than any method error or operator error
generated in a single session. This suggests that this method can quantify the gross
oral morphology of individual specimens reproducibly, and capture variation over
time within individuals and across populations.

We calculated disparity values for landmark sets from duplicate images of single
impressions, multiple sequential impressions of one individual, and from all 7 study
individuals (Figure 4D). Levels of variance between duplicate images of one
impression were comparable, representing similar levels of operator error between
observations. Disparity was highest for all impressions from individuals (reflective of
individual variation) and lower for multiple impressions from one individual even
including outliers on PC2 (Figure 4B), reflective of method error and not the
sequence in which impressions were taken. This indicates the method can typically
accurately detect and represent distinctions between individual variations with high
fidelity and a low rate of error in shape.

# Discussion

As above, this method can be used to non-destructively and cheaply obtain whole
mouth oral traits at high resolution from both live and dead specimens. Therefore, it
can be used for any morphological studies involving oral traits, occlusion, and
dentition in place of existing methods that are destructive (e.g. dissection), expensive
(e.g. microCT), and/or provide high resolution for a much smaller area (e.g.
Synchrotron, SEM), as well as methods with less control (e.g. direct application of
putty), or reduced resolution (e.g, optical scanning). One of the most important
benefits of the new method is that it allows production of time series for
morphological changes across the entire dentition and oral-pharyngeal cavity of

living animals. This is useful for studies of dental microwear analysis (Hoffman et al., 2015) as well as growth, replacement, and development (Carr et al., 2021; Streelman et al., 2003; Trapani et al., 2005). Dental impressions can also record detailed intraoral soft tissue anatomy of living specimens, including features previously could only be evaluated on dead specimens (Luczkovich et al., 1995).

## Potential applications

Some possible applications of this tray-mediated impression method include the production of physical and 3D digital models from live or rare specimens which cannot be prepared for high resolution scans by microCT or other means. Impressions can be used for geometric morphometric analyses of dentitions in a range of species. Occlusion patterns preserved in impressions can provide an alternative approach to study bite articulation and jaw biomechanics (Wilga and Ferry, 2015). Soft tissue and high resolution dental models created from scans of impressions can also be of use with the physical replication of skeletal movements and feeding mechanics for (XROMM) studies of individuals, which typically depend on whole body CT scans at low resolution (Camp and Brainerd, 2015). Although tooth replacement has been extensively studied from a histological and developmental perspective there is very little data on tooth growth and replacement on living non-mammal individuals (Carr et al., 2021), especially with time series from a single individual. The recurring creation of whole-dentition tooth records via dental impression provides an opportunity to not only study replacement patterns but also examine external factors that can affect it. We are positive that researchers focused on the ecology and evolution of vertebrates will find many additional uses for the data captured by our method.

## Material sourcing and cost considerations

Costs are reduced by using existing, consumer-grade, and readily available 3D printers and material. In principle, most rigid 3D printing materials can be used, here we opted to use poly-lactic acid as it is one of the most available and cheap material. Dental impression compounds can also be selected based on the purpose. For fish, the use of MS-222 anesthetic can be costly, thus clove oil can be used as a substitute due to its potency and cost (Javahery et al., 2012). The same anesthesia mix can also be reused for multiple aquatic individuals, as it is mediated by water.

307 The breakdown of the materials and costs for our tests are available in
308 supplementary table 1.

## Method limitations and considerations

**Sedation of live animals**

Changes to size or species might require modification of the sedation method to
accommodate time required for material polymerization. Air breathing *Polypterus*
have an advantage in surviving outside of water. When applying this method to other
aquatic animals, physiology is crucial in determining survivability and thus should be
thoroughly investigated. Due to the hydrophilic nature of PVS, the body can be
submersed under water and only the jaw exposed to air during the impression
process.

Maintenance of sedation might be required when more time is needed to take
impressions, while optimization of anesthesia concentration should be performed
depending on species and size. If longer time is required, maintenance of sedation
by running oxygenated water with anesthetics through the gills might be required.

**Mitigating the enamel pellicle and biofilm**

In-vivo dental microwear analysis is complicated by pellicle (protein film) formation
on enamel, which can affect impression accuracy and obscure microwear details
(Hoffman et al., 2015). Operational time constraints necessitated that we have a
quick and safe process to remove biofilm prior to impression. Using diluted bleach
can have negative effects on PVS material (Hamalian et al., 2011) and teeth (Sim et
al., 2001), while brushing can deform enamel surface (Wiegand et al., 2007).
Therefore, we opted for a dual impression technique to remove biofilm.(Hamalian et
al., 2011; Sim et al., 2001; Wiegand et al., 2007)

**Tooth and animal size**

PVS dental impression materials are mainly used to precisely replicate human teeth
with high reproducibility. In the case of smaller oral morphology, molding success
becomes technique-dependent, making reproducibility challenging. Therefore, it is
recommended to obtain impressions from multiple specimens, and repeatedly on a
single specimen to determine the best material and confirm accuracy in different
species. Further, morphometric data or external photos or optical scans of

specimens can be used to help modify the custom trays for other species and individual animals, reducing the need for iterative printing and testing.

**Pharyngeal and extraoral teeth**

It is possible to produce pharyngeal teeth impressions with customization of the tray to extend intraorally, and we have included options for this within our model. However, precautions must be taken to avoid injury or ingestion of the impression material. Due to the lack of deep pharyngeal teeth, this could not be tested with *Polypterus.* However, internal gill morphology deep within the pharynx was captured alongside the marginal dentition (Figure 3A-B)

**Interpretation of soft tissue results in preserved specimens**

While this method can be easily applied to dead and preserved specimens, users are advised to use caution in the interpretation of soft-tissue dependent whole mouth traits such as occlusion patterns. It is likely that post-mortem and preservation changes can affect the movement of muscles, allowing the jaws to move into positions not possible in life.

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# Competing interests

The authors declare no competing interests.

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# Data availability

Parameterized blender file and STL files for the impression trays used in this project are available on https://anonymous.4open.science/r/impressiontray-24FE/README.md and is archived on Zenodo (doi:10.5281/zenodo.12524788).

# Author contributions

J.N.W., R.S., T.K., and L.S. conceived the project. J.N.W. and R.S. conceived the method and performed overall direction and planning. J.N.W., T.K., and R.S., performed molding trials. J.N.W. designed and P.P. parameterized and visualized the impression tray. T.K. and R.S. performed microscope scanning of obtained molds. T.O. performed morphometric analyses. J.N.W., T.K., R.S. and L.S. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our research integrates authors from different countries, including the country where the study was conducted.

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475

# Figure legends

## Figure 1. Schematic Representation of the Impression Tray and Usage

Schematic representation of the dental impression tray and impression process, using *Polypterus* as an example. (A) 3D rendered image of the impression tray showing mandibular/lower side, maxillary/upper side, and lateral view. (B) Impression trays in multiple different sizes. (C-D) Impression material is applied to both sides of the impression tray. (E-F) *Polypterus* biting on the impression material.

## Figure 2. Example Impression Sequence Using *Polypterus*

Molding sequence of *Polypterus*. (A) Preparation of materials before starting the procedure (B) Individual in buffered MS-222 bath. (C) Removal of residual moisture using compressed air duster. (D-E) Removal of impurities from teeth surface using putty. (F) Application of PVS material on the surface of the impression tray. (G-H) Molding of the teeth by firmly pushing the jaw onto the impression tray.

## Figure 3. Example impressions from *Polypterus*

PVS mold obtained upon the completion of impressions. (A-B) Undetached impression from (A) maxillary side and (B) mandibular side. (C-D) Lingual scan of impression obtained from the maxillary side for (C) posterior tooth and (D) anterior tooth and (E-F) of the mandibular side for a middle tooth showing detailed enamel damage, (E) photo-simulation and (F) topology showing the elevation at the area where enamel was flaked off. The size of photo-simulations (C-E) is 100 micrometers (Y axis) times 140.5 micrometers (X axis).

## Figure 4. Geometric morphometrics analysis

(A) Landmarks used to perform morphometrics analysis. 42 landmarks were placed in total; white numbers are fixed landmarks, and red numbers are semi-landmarked curves. (B) PCA plot of geometrics morphometrics analysis using landmarks from 4 maxillary/upper impressions of each of 2 individuals. 5 replicate landmark sets were digitized from each impression (represented by red convex hulls). Trial numbers are indicated over each hull group. (C) PCA plot of geometrics morphometrics analysis of all available whole mouth impressions (N=13) from 7 individuals used in method

development. Some impressions were omitted when they were deemed to be

deformed due to contact between animal and tray (eliminating putty) and could not

be landmarked, or when they were cut to obtain details on tooth impressions

featured in Fig. 3A. (D) Disparity calculations for image duplicates and impression

duplicates. "Overall" disparity shows procrustes variance across impressions taken

from individuals 1-7. "Method" disparity shows procrustes variance between all

impressions taken from specimen 1 and specimen 2 respectively. "Operator"

disparity shows the difference between all duplicated images from each impression

used in the principal components and represents operator error.

## Supplementary Figure 1. Tray customization

Customization of the impression tray using Blender. (A) The supplementary (or

downloadable) file parametric_tray.blend can be loaded into Blender. (B) Relative

parameters can be adjusted depending on the target species by adjusting the values

or the slider on the pane on the right side of the window. (C) Perforations need to be

cut after adjustments and before exporting to STL as it is a computationally intensive

process. (D) STL can be exported by selecting export > Stl (.stl).

## Supplementary Figure 2. Impression taken from two individuals for morphometrics analysis

525    Impressions obtained from 2 different individuals in single sedation sessions (~20

526    minutes total). (A-D) Impressions taken from individual 1 representing trials 1, 3, 4, 5

527    respectively. (E-H) Impressions taken from individual 2 representing trials 2, 3, 4, 6

528    respectively.

## Supplementary Figure 3. Geometric morphometrics analysis

531    Geometric morphometrics analyses. (A-B) PCA plot (N=40) showing (A) PC1 and

532    PC2 and (B) PC3 and PC4 from the morphometrics analysis of 2 individuals. Each

533    impression is represented by the red convex hull. Trial numbers are indicated over

534    each hull group. (C-D) PCA plot (N=65) showing (D) PC1 and PC2 and (E) PC3 and

535    PC4 from the morphometrics analysis of 7 different individuals.

## Supplementary Table 1. Impression material cost

The approximate cost of impression material for the experiments performed in (A) this study and (B) alternative cost for impression materials with a more cost-effective option.

# Figure 1

# Figure 2

# Figure 3

# Figure 4

# Supplementary figure 1

A

B

C

D

# Supplementary figure 2

# Supplementary figure 3

# Supplementary table 1

## A

| Type | Item | Manufacturer | Approximate cost in USD | Use per experiment | Approximate cost per experiment in USD | Note |
|------|------|--------------|--------------------------|---------------------|-----------------------------------------|------|
| Impression material | Dr. Silicone regular type (medium body) 50ml cartridges | BSA sakurai | 10 per cartridge | ~5ml per animal | 1 | Tray size 2 cm by 2 cm deep and included filling palatal and mandibular sides. |
| Impression material | Exifone putty 556 ml (combined base and catalyst) | GC Japan | 60 | 2.8 ml per animal | 0.3 | A quarter of a mix of 1 scoop of base and 1 scoop of |
| Anesthesia | Ethyl m-Aminobenzoate Methanesulfonate (MS-222) 25g | Nacalai Tesque | 108 | 0.6g in 2L of water | 2.6 | |
| 3D printing | | Ulti Maker | 50 | 1g | 0.1 | |

## B

| Type | Item | Manufacturer | Approximate cost in USD | Use per experiment | Approximate cost per experiment in USD | Note |
|------|------|--------------|--------------------------|---------------------|-----------------------------------------|------|
| Anesthesia | Clove oil C8392-100ML | Sigma Aldrich | 55 | Approximately less than 0.2ml | 0.1 | We assume that ethanol that is used to dissolve the clove oil is already present in most labs |
| 3D printing | Generic PLA 1kg | various | 20-30 | 1g | less than 0.1 | |
| 3D printing | Generic ABS 1kg | various | 20-30 | 1g | less than 0.1 | |
| 3D printing | Generic PETG 1kg | various | 20-30 | 1g | less than 0.1 | |
| 3D printing service | 3D printing service | various | 1-2 | - | 1-2 | |