**Table S1.** Optimised settings used for the microscopy tissue processor to prepare the samples before resin embedding for transmission electron microscopy (TEM).

|  |  |  |
| --- | --- | --- |
| **Step** | **Type of solution in the station** | **Time (min.)** |
| 1 | Empty | - |
| 2 | 0.13M Sorensen Phosphate buffer | 10 |
| 3 | 0.13M Sorensen Phosphate buffer | 10 |
| 4 | 0.13M Sorensen Phosphate buffer | 10 |
| 5 | 25% EtOH | 30 |
| 6 | 50% EtOH | 30 |
| 7 | 70% EtOH | 40 |
| 8 | 80% EtOH | 40 |
| 9 | 95% EtOH | 40 |
| 10 | Lab grade 100% EtOH | 60 |
| 11 | Lab grade 100% EtOH | 60 |
| 12 | Pure grade 100% EtOH | 60 |
| 13 | Pure grade 100% EtOH | 60 |
| 14 | Propylene oxide | 20 |
| 15 | Propylene oxide | 20 |
| 16 | Propylene oxide 75% / Araldite 25% | 30 |
| 17 | Propylene oxide 50% / Araldite 50% | 60 |
| 18 | Araldite | 150 |
| 19 | Araldite | 150 |
| 20 | Araldite | 150 |

**Table S2.** Model outputs from the linear regression run on axon density. SS; sum of squares, df; degree of freedom, \*; significant variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **SS** | **df** | **F-value** | **p-value** |
| Species | 681.9 | 1 | 909.3 | < 0.001\*\*\* |
| Location | 3729.2 | 1 | 4972.8 | < 0.001\*\*\* |
| Species/Specimen | 196.5 | 4 | 65.5 | < 0.001\*\*\* |
| Location/Type | 4453.5 | 1 | 5938.6 | < 0.001\*\*\* |
| Species:Location | 383.1 | 1 | 510.8 | < 0.001\*\*\* |

**Table S3.** Outputs of *post-hoc* Tukey tests for significant interacting terms tested in the linear model for axon density. CP; *Chiloscyllium punctatum*, CA; *Carassius auratus*, \*; biologically-relevant terms, SE; standard error.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Interaction tested** | **contrast term\*** | **estimate** | **SE** | **p-value (adjusted)** |
| Species\*Specimen | CP4 - CP5 | -0.019 | 0.063 | 0.999 |
|  | CP4 - CP2 | 0.769 | 0.066 | <0.001\*\*\* |
|  | CP5 - CP2 | 0.789 | 0.059 | <0.001\*\*\* |
|  | CA3 - CA2 | -0.184 | 0.058 | 0.018\* |
|  | CA3 - CA4 | 0.205 | 0.057 | 0.005\*\* |
|  | CA4 - CA2 | -0.389 | 0.052 | <0.001\*\*\* |
|  | CA4 - CP4 | 0.208 | 0.063 | 0.013\* |
|  | CA4 - CP5 | 0.188 | 0.057 | 0.012\* |
|  | CA3 - CP4 | 0.413 | 0.069 | <0.001\*\*\* |
|  | CA3 - CP5 | 0.393 | 0.063 | <0.001\*\*\* |
|  | CA3 - CP2 | 1.183 | 0.063 | <0.001\*\*\* |
|  | CA4 - CP2 | 0.978 | 0.057 | <0.001\*\*\* |
|  | CA2 - CP4 | 0.597 | 0.064 | <0.001\*\*\* |
|  | CA2 - CP5 | 0.578 | 0.058 | <0.001\*\*\* |
|  | CA2 - CP2 | 1.367 | 0.059 | <0.001\*\*\* |
| Location\*Type | UM,ON - UM,OP | 1.200 | 0.043 | < 0.001\*\*\* |
|  | M,OP - UM,OP | -3.030 | 0.039 | < 0.001\*\*\* |
| Species\*Location | CA,ON - CP,ON | -0.211 | 0.064 | 0.001\*\* |
|  | CA,OP - CP,OP | 1.524 | 0.040 | < 0.001\*\*\* |

**Table S4.** Model outputs from the linear regression run on axon diameter. SS; sum of squares, df; degree of freedom, \*; significant variables.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | **SS** | **df** | **F-value** | **p-value** |
| Species | 4.121 | 1 | 16.1 | < 0.001\*\*\* |
| Location | 86.435 | 1 | 338.4 | < 0.001\*\*\* |
| Species/Specimen | 0.481 | 4 | 0.4 | 0.757 |
| Location/Type | 167.028 | 1 | 653.9 | < 0.001\*\*\* |
| Species:Location | 6.686 | 1 | 26.1 | < 0.001\*\*\* |

**Table S5.** Outputs of *post-hoc* Tukey tests for significant interacting terms tested in the linear model for axon diameter. CP; *Chiloscyllium punctatum*, CA; *Carassius auratus*, \*; biologically-relevant terms, SE; standard error.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Interaction tested** | **contrast term\*** | **estimate** | **SE** | **p-value (adjusted)** |
| Location\*Type | UM,ON - UM,OP | -0.205 | 0.065 | 0.005\*\* |
|  | M,OP - UM,OP | 1.668 | 0.065 | < 0.001\*\*\* |
| Species\*Location | CA,ON - CP,ON | 0.171 | 0.092 | 0.063 |
|  | CA,ON - CA,OP | -0.750 | 0.079 | < 0.001\*\*\* |
|  | CP,ON - CP,OP | -1.329 | 0.079 | < 0.001\*\*\* |
|  | CA,OP - CP,OP | -0.407 | 0.065 | < 0.001\*\*\* |
|  | CA,ON - CP,OP | -1.157 | 0.079 | < 0.001\*\*\* |
|  | CP,ON - CA,OP | -0.922 | 0.079 | < 0.001\*\*\* |

****

**b.**

**a.**

** **

**Figure S1.** Model fitted mean values for *post-hoc* Tukey tests run on axon density (**a**) and axon diameter (**b**). Error bars indicate standard errors. CP; *Chiloscyllium punctatum* (n=3, CP2, 4, 5), CA; *Carassius auratus* (n=3, CA2-4), ON; olfactory nerve, OP; olfactory peduncle, UM; unmyelinated axon, M; myelinated axons.

**R code**. Custom-written by AR, and used by VCA, to determine the appropriate level of sampling required to detect differences in axon number (UM and M pooled) between the different olfactory tracts of *Carassius auratus*. Tailored power analysis, based on tract size (how many images can fit in the tract zone), descriptive statistics (mean and variance) of preliminary quantitative results (from N=20 images), and number ‘Ni’ of images needed to achieve >99% confidence levels.

##############################################################

### Effect detection simulation

### Author: Alethea Rea

##############################################################

#setwd("~/Documents/Victoria")

rm(list=ls())

## simulation of effect

## compares four populations of photos

## with mean count for each photo (mu1, mu2, mu3, mu4)

## with standard deviations for each pic (sd1, sd2, sd3, sd4)

## with the number of photos that cover each zone (p1, p2, p3, p4)

## with the number of photos that will be sampled (n1, n2, n3, n4)

## simulated s times

ttest\_sim <- function(mu1, mu2, mu3, mu4, sd1, sd2, sd3, sd4,

 p1, p2, p3, p4, n1, n2, n3, n4, s){

 outcome <- mat.or.vec(s,1) ## intialise vector for storage

 for (i in 1:s){ ## for each simulation run

 z1\_simcounts <- rnorm(n = p1, mean = mu1, sd = sd1) ## generate p1 normal vars

 z1\_sampled\_simcounts <- round(sample(z1\_simcounts, n1, replace = F))

 ## sample n of the p vars (i.e. n of the photos)

 ## round to whole numbers

 z2\_simcounts <- rnorm(n = p2, mean = mu2, sd = sd2)

 z2\_sampled\_simcounts <- round(sample(z2\_simcounts, n2, replace = F))

 z3\_simcounts <- rnorm(n = p3, mean = mu3, sd = sd3)

 z3\_sampled\_simcounts <- round(sample(z3\_simcounts, n3, replace = F))

 z4\_simcounts <- rnorm(n = p4, mean = mu4, sd = sd4)

 z4\_sampled\_simcounts <- round(sample(z4\_simcounts, n4, replace = F))

 ##goal: detection of at least one difference between zones 1,2,3 & 4

 outcome[i] <- summary( aov(c(z1\_sampled\_simcounts, z2\_sampled\_simcounts,

 z3\_sampled\_simcounts, z4\_sampled\_simcounts)

 ~ factor(c(rep(1,n1), rep(2,n2), rep(3,n3), rep(4,n4)))))[[1]]$`Pr(>F)`[1]

 }

 return(mean(outcome< 0.05)) ## return the no of sims with a stat sig result

}

**# Example on how to run it in R:**

> mu\_z1m <- … # enter mean axon count values for each zone (tract)

> mu\_z2m <- …

> mu\_z3m <- …

> mu\_z4m <- …

> sd\_z1m <- … # enter standard deviation values for each zone (tract)

> sd\_z2m <- …

> sd\_z3m <- …

> sd\_z4m <- …

> p1 <- … # enter the estimated size of each zone (how many images it fits)

> p2 <- …

> p3 <- …

> p4 <- …

> n1 <- 50 # e.g. arbitrary numbers of images to collect for each zone

> n2 <- 50

> n3 <- 100

> n4 <- 20

>

> s <- 1000 # e.g. number of simulations to run for

>

> ttest\_sim(mu\_z1m, mu\_z2m, mu\_z3m, mu\_z4m, sd\_z1m, sd\_z2m, sd\_z3m, sd\_z4m, p1, p2, p3, p4, n1, n2, n3, n4, s)

[1] 0.991 # result to look for, i.e. >90% confidence level to know how many

 images to collect per zone. Iterate choosing different ni numbers

 until satisfied.