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\* Code authors: M. Żebrowski & K. Leniowski

\* Associated publication: Maszczyk et al. (2025),

\* "Freshwater food webs amplify microplastic transfer to fish"

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#RCodes

#Sheet: “Medium”  
#categorical variable  
**#E** – experiment identifier (not used in analyses).  
**#Tr1** – treatment description (not used in analyses).  
**#PS** – microplastic (MP) size; factor with two levels: *1PS* (1 µm in diameter) and *25PS* (25 µm in diameter).  
**#Zoo** – animal species; factor with five levels: *aSimo* (S. veytulus), *bGaleata* (D. galeata), *cTigrio* (T. californicus), *dAcartia* (A. tonsa), and *C* (P. reticulata, fish).  
**#Accumulation** – delivery method of microplastics; factor with three levels: *aY* (trophic transfer), *bN* (direct ingestion in the presence of zooplankton), and *no* (direct ingestion in the absence of zooplankton).  
**#Env** – environment; factor with two levels: freshwater and marine salinity.

#numerical variable **#PS\_conc** – MP number per mL of experimental medium (particles mL⁻¹).  
**#PSmass** – MP mass per mL of experimental medium (µg mL⁻¹).

#Sheet: “Zoo”  
#categorical variable  
**#E** – experiment identifier (not used in analyses).  
**#Tr** – treatment description (not used in analyses).  
**#PS** – microplastic size; factor with two levels: *1PS* (1 µm in diameter) and *25PS* (25 µm in diameter).  
**#Zoo** – animal species; factor with four levels: *Simo* (S. veytulus), *Galeata* (D. galeata), *Tigrio* (T. californicus), *Acartia* (A. tonsa).

#numerical variable  
**#PS\_total** – total number of microplastic particles consumed by zooplankton individuals.  
**#PSmass\_total** – total mass of microplastic particles consumed by zooplankton individuals.  
**#no** – number of zooplankton individuals.  
**#PS\_ind** – number of microplastic particles per zooplankton individual.  
**#PSmass\_ind** – mass of microplastic particles per zooplankton individual.  
**#zooBM** – zooplankton mass.  
**#PS\_zooBM** – number of microplastic particles per unit body mass of zooplankton.  
**#PSmass\_zooBM** – mass of microplastic particles per unit body mass of zooplankton.  
**#zooBL** – zooplankton body length.  
**#zooBW** – zooplankton body width.  
**#zooBH** – zooplankton body height.  
**#zooBV** – zooplankton body volume.  
**#BCF** – mass-based bioaccumulation factor.  
**#BCF\_volume** – volume-based bioaccumulation factor.  
**#PS\_conc\_mean** – mean number of microplastic particles per mL of experimental medium (particles mL⁻¹).  
**#PSmass\_mean** – mean mass of microplastic particles per mL of experimental medium (µg mL⁻¹).  
**#1PS mass (µg)** – mass of a 1 µm microplastic particle (µg).  
**#25PS mass (µg)** – mass of a 25 µm microplastic particle (µg).

#Sheet: “Fish\_0” – data set for all analyzed experimental treatments  
#categorical variable  
**#E** – experiment identifier (not used in analyses).  
**#Tr**  – treatment description (not used in analyses).  
**#PS** – microplastic (MP) size; factor with two levels: *1PS* (1 µm in diameter) and *25PS* (25 µm in diameter).  
**#Zoo** – animal species; factor with five levels: *Simo* (S. veytulus), *Galeata* (D. galeata), *Tigrio* (T. californicus), *Acartia* (A. tonsa), and *C* (P. reticulata, fish).  
**#Accumulation** – delivery method of microplastics; factor with three levels: *Y* (trophic transfer), *N* (direct ingestion in the presence of zooplankton), and *C* (direct ingestion in the absence of zooplankton).  
**#Env** – environment; factor with two levels: freshwater and marine salinity.  
#Gender – fish sex

#numerical variable  
**#PS\_total** – total number of microplastic particles per fish.  
**#PSmass\_total** – total mass of microplastic particles per fish.  
**#no** – number of consumed zooplankton prey.  
**#PS\_ind** – number of microplastic particles ingested per prey item.  
**#PSmass\_ind** – mass of microplastic particles ingested per prey item.  
**#zooBM** – zooplankton dry mass.  
**#PS\_zooBM** – number of microplastic particles ingested per unit body mass of prey.  
**#PSmass\_zooBM** – mass of microplastic particles ingested per unit body mass of prey.  
**#zooBL** – zooplankton body length.  
**#Fish\_BL** – fish body length.  
**#Fish\_BM** – fish body mass.  
**#1PS mass (µg)** – mass of a 1 µm microplastic particle (µg).  
**#25PS mass (µg)** – mass of a 25 µm microplastic particle (µg).

#Sheet: “Fish\_1” – data set only for trophic transfer treatments  
#categorical variable  
**#E** – experiment identifier (not used in analyses).  
**#Tr**  – treatment description (not used in analyses).  
**#PS** – microplastic (MP) size; factor with two levels: *1PS* (1 µm in diameter) and *25PS* (25 µm in diameter).  
**#Zoo** – animal species; factor with four levels: *Simo* (S. veytulus), *Galeata* (D. galeata), *Tigrio* (T. californicus), *Acartia* (A. tonsa).  
**#Accumulation** – delivery method of microplastics; factor with three levels: *Y* (trophic transfer), *N* (direct ingestion in the presence of zooplankton), and *C* (direct ingestion in the absence of zooplankton).  
**#Env** – environment; factor with two levels: freshwater and marine salinity.  
#Gender – fish sex

#numerical variable  
**#PS\_total** – total number of microplastic particles per fish.  
**#PSmass\_total** – total mass of microplastic particles per fish.  
**#no** – number of consumed zooplankton prey.  
**#PS\_ind** – number of microplastic particles ingested per prey item.  
**#PSmass\_ind** – mass of microplastic particles ingested per prey item.  
**#zooBM** – zooplankton dry mass.  
**#PS\_zooBM** – number of microplastic particles ingested per unit body mass of prey.  
**#PSmass\_zooBM** – mass of microplastic particles ingested per unit body mass of prey.  
**#zooBL** – zooplankton body length.  
**#Fish\_BL** – fish body length.  
**#Fish\_BM** – fish body mass.  
**#1PS mass (µg)** – mass of a 1 µm microplastic particle (µg).  
**#25PS mass (µg)** – mass of a 25 µm microplastic particle (µg).

#Sheet: “Selectivity” – data set for zooplankton selectivity meta-regression analysis   
#categorical variable  
**#study\_id** – identifier of the source publication from which data were extracted  
**#algal\_species** – algal species used in the experiment  
**#zooplankton\_species** – zooplankton species used in the experiment  
**#environment** – environmental category with two levels: freshwater and marine  
**#MP\_shape** – shape of microplastic particles used  
**#MP\_type** – polymer type of microplastic particles used

#numerical variable  
**#algal\_conc** – algal concentration in the experimental medium  
**#algal\_ingestion** – algal ingestion rate by zooplankton  
**#MP\_conc** – concentration of microplastic particles in the medium  
**#MP\_ingestion** – ingestion rate of microplastic particles by zooplankton  
**#cell\_size** – mean size of algal cells  
**#MP\_size** – mean size of microplastic particles

#Supplementary Table 2 | Mean microplastic (MP) number and mass per millilitre of medium across treatments – MP concentration

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load experimental dataset on polystyrene (PS) concentrations in media  
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Medium")  
#View(da)

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

da$Accumulation <- as.factor(da$Accumulation)

da$Env <- as.factor(da$Env)  
# Coerce response variables to numeric

da$PS\_conc <- as.numeric(da$PS\_conc)

da$PSmass <- as.numeric(da$PSmass)

# Inspect data structure

str(da)  
da0<-da

# Explore raw distribution of PS mass and test homogeneity of variance

hist(da0$PSmass)

leveneTest(PSmass ~ PS\*Zoo\*Accumulation, data = da0)

# Fit GLMM (Tweedie distribution, log-link) to test effects of PS fraction, zooplankton species, and accumulation type

medium\_PSmass <- glmmTMB(PSmass ~ PS\*Zoo\*Accumulation, data = da0, family = tweedie(link = "log"))

# Model diagnostics with DHARMa residuals

plot(simulateResiduals(medium\_PSmass))

# Type II Wald χ² tests of main effects and interactions

Anova(medium\_PSmass)

# Estimated marginal means across all factor combinations

emm.PSmass <- emmeans(medium\_PSmass, ~ Zoo:PS:Accumulation)

emm.PSmass

# Post-hoc contrasts with Holm correction

contrast\_PSmass <- pairs(emm.PSmass, adjust = "holm")

contrast\_PSmass

# Compact letter display for significant groupings

letters\_PSmass <- cld(emm.PSmass, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass

# Refit model to extract predictions for graphical summaries

medium\_PSmass <- glmmTMB(PSmass ~ PS\*Zoo\*Accumulation, data = da0, family = tweedie(link = "log"))

e\_box\_mass <- emmeans(medium\_PSmass, ~ Zoo\*PS\*Accumulation)

# Convert grouping results into a data frame for plotting

letters\_mass <- cld(e\_box\_mass, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_mass.df <- as.data.frame(letters\_mass)

# Create treatment labels (Zoo × PS × Accumulation) and align them with plotting order

letters\_mass.df$Tr <- with(letters\_mass.df, paste(Zoo, PS, Accumulation, sep = "\_"))

group\_order <- c("aSimo\_1PS\_aY","bGaleata\_1PS\_aY","cTigrio\_1PS\_aY","dAcartia\_1PS\_aY",

"aSimo\_1PS\_bN","bGaleata\_1PS\_bN","cTigrio\_1PS\_bN","dAcartia\_1PS\_bN","no\_1PS\_no",

"aSimo\_25PS\_aY","bGaleata\_25PS\_aY","cTigrio\_25PS\_aY","dAcartia\_25PS\_aY",

"aSimo\_25PS\_bN","bGaleata\_25PS\_bN","cTigrio\_25PS\_bN","dAcartia\_25PS\_bN","no\_25PS\_no")

letters\_mass.df$Tr <- factor(letters\_mass.df$Tr, levels = group\_order)

# Set y-position for significance letters above boxplots

letters\_mass.df$y <- max(da0$PSmass, na.rm = TRUE) \* 1.1

# Add treatment factor to the dataset

da0$Tr <- factor(with(da0, paste(Zoo, PS, Accumulation, sep = "\_")), levels = group\_order)

# Define highlight groups for plotting background rectangles

highlight\_groups1 <- c("aSimo\_1PS\_aY","bGaleata\_1PS\_aY","cTigrio\_1PS\_aY","dAcartia\_1PS\_aY",

"aSimo\_25PS\_aY","bGaleata\_25PS\_aY","cTigrio\_25PS\_aY","dAcartia\_25PS\_aY")

highlight\_groups2 <- c("aSimo\_1PS\_bN","bGaleata\_1PS\_bN","cTigrio\_1PS\_bN","dAcartia\_1PS\_bN",

"aSimo\_25PS\_bN","bGaleata\_25PS\_bN","cTigrio\_25PS\_bN","dAcartia\_25PS\_bN")

# Generate boxplots with background highlighting, jittered raw data, and significance letters

ggplot(da0, aes(x = Tr, y = log(PSmass), color = Tr, fill = Tr, shape = Tr)) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE) +

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE) +

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15)) +

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65")) +

scale\_fill\_manual(values = rep("grey100", 18)) +

scale\_shape\_manual(values = rep(21, 18)) +

labs(title = "PS concentration in experimental media",

x = "Treatment",

y = "PS conc. (log(PS mass (µg) × mL⁻¹))") +

theme\_classic() +

geom\_text(data = letters\_mass.df, aes(x = Tr, y = 3, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits = group\_order,

labels = c("S1PS","G1PS","T1PS","A1PS",

"S+1PS","G+1PS","T+1PS","A+1PS","C+1PS",

"S25PS","G25PS","T25PS","A25PS",

"S+25PS","G+25PS","T+25PS","A+25PS","C+25PS")) +

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust = 0.6),

axis.text.y = element\_text(color = "black", size = 9),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5),

legend.position = "none")

#Supplementary Table 2 | Mean microplastic (MP) number and mass per millilitre of medium across treatments – MP mass

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load experimental dataset (PS concentrations in medium); sheet prepared for analysis

da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Medium")

#View(da) # Inspect raw table to verify column types and factor encodings

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

da$Accumulation <- as.factor(da$Accumulation)

da$Env <- as.factor(da$Env)  
# Coerce response variables to numeric

da$PS\_conc <- as.numeric(da$PS\_conc)

da$PSmass <- as.numeric(da$PSmass)

da0 <- da

str(da) # Confirm structure and coercions prior to modeling

# Explore distributional properties of the response; variance diagnostics follow

hist(da$PS\_conc)

# Homogeneity of variances across factorial combinations (Levene’s test)

leveneTest(PS\_conc ~ PS\*Zoo\*Accumulation, data = da0)

# Fit GLMM with Tweedie family (log link) to accommodate positive, right-skewed responses with potential zero mass

# Fixed effects: PS fraction, zooplankton species, accumulation type, and their interactions

medium\_conc <- glmmTMB(PS\_conc ~ PS\*Zoo\*Accumulation, data = da0, family = tweedie(link = "log"))

# Residual diagnostics via DHARMa to assess dispersion, zero-inflation, and overall fit

plot(simulateResiduals(medium\_conc))

# Type II Wald χ² for main effects and interactions (car::Anova)

Anova(medium\_conc)

# Estimated marginal means for all factor combinations (Zoo × PS × Accumulation)

emm.medium\_conc <- emmeans(medium\_conc, ~ Zoo:PS:Accumulation)

emm.medium\_conc # Report EMMs to align with figure/tables annotations

# Post-hoc pairwise contrasts with Holm adjustment controlling familywise error rate

contrast\_medium\_conc <- pairs(emm.medium\_conc, adjust = "holm")

contrast\_medium\_conc

# Compact letter display summarizing homogeneous groups for visualization

letters\_medium\_conc <- cld(emm.medium\_conc, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_medium\_conc

# Refit retained to keep the modeling object close to plotting section (mirrors upstream specification)

medium\_conc <- glmmTMB(PS\_conc ~ PS\*Zoo\*Accumulation, data = da0, family = tweedie(link = "log"))

# EMMs for plotting (boxplots + CLD overlay)

e\_box\_conc <- emmeans(medium\_conc, ~ Zoo\*PS\*Accumulation)

letters\_conc <- cld(e\_box\_conc, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_conc.df <- as.data.frame(letters\_conc)

# Construct treatment labels (Zoo × PS × Accumulation) and enforce display order for consistency with manuscript panels

letters\_conc.df$Tr <- with(letters\_conc.df, paste(Zoo, PS, Accumulation, sep = "\_"))

group\_order <- c("aSimo\_1PS\_aY","bGaleata\_1PS\_aY","cTigrio\_1PS\_aY","dAcartia\_1PS\_aY",

"aSimo\_1PS\_bN","bGaleata\_1PS\_bN","cTigrio\_1PS\_bN","dAcartia\_1PS\_bN","no\_1PS\_no",

"aSimo\_25PS\_aY","bGaleata\_25PS\_aY","cTigrio\_25PS\_aY","dAcartia\_25PS\_aY",

"aSimo\_25PS\_bN","bGaleata\_25PS\_bN","cTigrio\_25PS\_bN","dAcartia\_25PS\_bN","no\_25PS\_no")

letters\_conc.df$Tr <- factor(letters\_conc.df$Tr, levels = group\_order)

# Set y-position for CLD letters above the boxes; scale to the response range

letters\_conc.df$y <- max(da0$PS\_conc, na.rm = TRUE) \* 1.1

# Align dataset’s treatment factorization with plotting order

da0$Tr <- factor(with(da0, paste(Zoo, PS, Accumulation, sep = "\_")), levels = group\_order)

# Define background highlight bands for visual separation of Accumulation strata (aY vs bN)

highlight\_groups1 <- c("aSimo\_1PS\_aY","bGaleata\_1PS\_aY","cTigrio\_1PS\_aY","dAcartia\_1PS\_aY",

"aSimo\_25PS\_aY","bGaleata\_25PS\_aY","cTigrio\_25PS\_aY","dAcartia\_25PS\_aY")

highlight\_groups2 <- c("aSimo\_1PS\_bN","bGaleata\_1PS\_bN","cTigrio\_1PS\_bN","dAcartia\_1PS\_bN",

"aSimo\_25PS\_bN","bGaleata\_25PS\_bN","cTigrio\_25PS\_bN","dAcartia\_25PS\_bN")

# Figure-ready boxplot: log-scale on y for multiplicative spreads; include raw points (jitter) and CLD labels

ggplot(da0, aes(x = Tr, y = log(PS\_conc), color = Tr, fill = Tr, shape = Tr)) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE) +

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE) +

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15)) +

# Annotate median values directly on the plot to facilitate cross-panel comparisons

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 1)),

vjust = -5, color = "black", size = 4) +

# Fixed palettes to align with manuscript’s color/shading scheme

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65")) +

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100")) +

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21,

21,21,21,21,21,21,21,21,21)) +

labs(title = "PS concentration in experimental media",

x = "Treatment",

y = "PS conc. (log(PS mass (ug) × mL-1)") + # Label mirrors manuscript; keep units consistent across panels

theme\_classic() +

# Display CLD letters; y-position set above the boxes for readability

geom\_text(data = letters\_conc.df, aes(x = Tr, y = 15, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

# Lock x-order and provide compact facet labels for the figure caption

scale\_x\_discrete(limits = c(

"aSimo\_1PS\_aY","bGaleata\_1PS\_aY","cTigrio\_1PS\_aY","dAcartia\_1PS\_aY",

"aSimo\_1PS\_bN","bGaleata\_1PS\_bN","cTigrio\_1PS\_bN","dAcartia\_1PS\_bN","no\_1PS\_no",

"aSimo\_25PS\_aY","bGaleata\_25PS\_aY","cTigrio\_25PS\_aY","dAcartia\_25PS\_aY",

"aSimo\_25PS\_bN","bGaleata\_25PS\_bN","cTigrio\_25PS\_bN","dAcartia\_25PS\_bN","no\_25PS\_no"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S+1PS","G+1PS","T+1PS","A+1PS","C+1PS",

"S25PS","G25PS","T25PS","A25PS",

"S+25PS","G+25PS","T+25PS","A+25PS","C+25PS")) +

# Typography tuned for camera-ready export; legend suppressed per panel conventions

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust = 0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none") +

# Explicit y-limits for panel comparability across figures

scale\_y\_continuous(limits = c(0, 20))

#MP mass per body mass – Fig. 1 B | Freshwater zooplankton enhance microplastic uptake and trophic transfer compared with marine species; Supplementary Table 5 | Effects of species identity and particle size on microplastic uptake and bioaccumulation in zooplankton & Supplementary Table 6 | Pairwise contrasts reveal species- and size-specific patterns in zooplankton microplastic uptake and bioaccumulation.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load zooplankton dataset   
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Zoo")

da <- da[, c(1:20)] # retain the analytical subset of columns used in models and figures

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)  
da$PSmass\_total <- as.numeric(da$PSmass\_total)  
da$no <- da$no  
da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)  
da$PS\_zooBM <- as.numeric(da$PS\_zooBM)

da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)  
da$zooBL <- as.numeric(da$zooBL)

da$zooBW <- as.numeric(da$zooBW)

da$zooBH <- as.numeric(da$zooBH)

da$zooBV <- as.numeric(da$zooBV)

da$BCF <- as.numeric(da$BCF)

da$BCF\_volume <- as.numeric(da$BCF\_volume)

da$PS\_conc\_mean <- as.numeric(da$PS\_conc\_mean)

da$PSmass\_mean <- as.numeric(da$PSmass\_mean)

str(da) # confirm structure prior to modeling

# Preserve a working copy for modeling and plotting

da0 <- da

# Distributional check for the response (PS mass normalized by body mass)

hist(da0$PSmass\_zooBM)

# Levene’s test for homogeneity of variance across PS × Zoo cells (diagnostic only)

leveneTest(PSmass\_zooBM ~ PS\*Zoo, data = da0)

# Model set to evaluate alternative dispersion structures in Gamma GLM (log link)

# Constant dispersion across groups

model1 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ 1)

# Dispersion varying with PS fraction

model2 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ PS)

# Dispersion varying with species

model3 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# No explicit dispersion model (defaults)

model4 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"))

# Information-theoretic comparison (AIC) and nested model tests to select dispersion structure

AIC(model1, model2, model3, model4)

anova(model1, model2)

anova(model1, model3)

anova(model2, model3)

# Final GLMM with dispersion structured by species (as per model selection above)

zoo\_PSmass\_zooBM <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~Zoo)

# DHARMa residual diagnostics: uniformity, dispersion, zero-inflation checks

plot(simulateResiduals(zoo\_PSmass\_zooBM))

# Type-III tests (Wald χ²) for main effects and interaction; formatted for table export

anova\_zoo\_PSmass\_zooBM <- Anova(zoo\_PSmass\_zooBM, type = 3)

anova\_zoo\_PSmass\_zooBM$Factor <- c("Intercept","PS","Zoo","PS×Zoo") # labeling for manuscript tables

anova\_zoo\_PSmass\_zooBM$Variant <- c("PSmass/BM") # analysis variant tag to bind across outputs

anova\_zoo\_PSmass\_zooBM.df <- as.data.frame(anova\_zoo\_PSmass\_zooBM)

anova\_zoo\_PSmass\_zooBM.df # retain for Extended Data / Supplementary tables

# Estimated marginal means (EMMs) and post-hoc contrasts for PS × Zoo

emm.PSmass\_zooBM <- emmeans(zoo\_PSmass\_zooBM, ~Zoo:PS)

emm.PSmass\_zooBM

contrast\_PSmass\_zooBM <- pairs(emm.PSmass\_zooBM, adjust = "holm") # familywise control via Holm adjustment

contrast\_PSmass\_zooBM

letters\_PSmass\_zooBM <- cld(emm.PSmass\_zooBM, adjust = "holm", Letters = letters, alpha = 0.05) # compact letter display

letters\_PSmass\_zooBM

contrast\_PSmass\_zooBM.df <- as.data.frame(contrast\_PSmass\_zooBM)

contrast\_PSmass\_zooBM.df$Variant <- c("PSmass/BM") # tag contrasts to variant for downstream joins

contrast\_PSmass\_zooBM.df

# Refit kept for clarity near plotting pipeline; same specification as analytical model

zoo\_PSmass\_zooBM <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# EMMs for plotting; CLD used to annotate groups on the figure

e\_box\_zoo\_PSmass\_zooBM <- emmeans(zoo\_PSmass\_zooBM, ~ PS:Zoo)

letters\_zoo\_PSmass\_zooBM <- cld(e\_box\_zoo\_PSmass\_zooBM, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_zoo\_PSmass\_zooBM.df <- as.data.frame(letters\_zoo\_PSmass\_zooBM)

# Compose treatment labels and enforce plotting order (consistent with figure captions)

letters\_zoo\_PSmass\_zooBM.df$Tr <- with(letters\_zoo\_PSmass\_zooBM.df, paste(PS, Zoo, sep = "\_"))

group\_order <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

letters\_zoo\_PSmass\_zooBM.df$Tr <- factor(letters\_zoo\_PSmass\_zooBM.df$Tr, levels = group\_order)

# Vertical offset for CLD letters; scaled to observed range

letters\_zoo\_PSmass\_zooBM.df$y <- max(da0$PSmass\_zooBM, na.rm = TRUE) \* 1.1

da0$Tr <- factor(with(da0, paste(PS, Zoo, sep = "\_")), levels = group\_order)

# Background shading to separate particle-size regimes (1 µm vs 25 µm) for readability

highlight\_groups1 <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia")

highlight\_groups2 <- c("25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

# Boxplot panel (log-scale y-axis) with jittered raw points, median labels, and CLD annotations

boxplot\_PSmass\_zooBM <-

ggplot(da0, aes(x = Tr, y = PSmass\_zooBM, color = Tr, fill = Tr, shape = Tr)) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE) +

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE) +

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE) +

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15)) +

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -5, color = "black", size = 4) +

# Color/fill/shape palettes fixed for reproducibility across panels

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65")) +

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100")) +

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21)) +

# NOTE: Title is in Polish in the original; kept verbatim to avoid altering content.

labs(title = "1b. Masa PS w przewodzie pokarmowym zooplanktonu w przeliczeniu na masę pojedynczego osobnika",

x = "Treatment", y = "PS (ug) × body mass (ug)-1") +

theme\_classic() +

# Optionally: scale\_y\_log10() retained below for camera-ready export

#scale\_y\_log10()+

geom\_text(data = letters\_zoo\_PSmass\_zooBM.df, aes(x = Tr, y = 3.5, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits = c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS")) +

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust = 0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none") +

# Final y-axis on log scale with explicit tick marks to emphasize orders of magnitude

scale\_y\_log10(limits = c(0.0001, 3.5),

breaks = c(0.0001, 0.001, 0.01, 0.1, 1),

labels = c("0.0001", "0.001", "0.01", "0.1", "1"))

#scale\_y\_continuous(limits = c(0,2)) # Alternative linear scale (kept commented to preserve original choice)

boxplot\_PSmass\_zooBM # object returned for assembly into multi-panel figures

#MP mass per individual – Extended Data Fig. 1 | Freshwater zooplankton ingest more microplastics per individual than marine species; Supplementary Table 5 | Effects of species identity and particle size on microplastic uptake and bioaccumulation in zooplankton & Supplementary Table 6 | Pairwise contrasts reveal species- and size-specific patterns in zooplankton microplastic uptake and bioaccumulation.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load zooplankton dataset   
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Zoo")

da <- da[, c(1:20)] # retain analytical columns only (consistent design matrices and figures)

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)  
da$PSmass\_total <- as.numeric(da$PSmass\_total)  
da$no <- da$no  
da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)  
da$PS\_zooBM <- as.numeric(da$PS\_zooBM)

da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)  
da$zooBL <- as.numeric(da$zooBL)

da$zooBW <- as.numeric(da$zooBW)

da$zooBH <- as.numeric(da$zooBH)

da$zooBV <- as.numeric(da$zooBV)

da$BCF <- as.numeric(da$BCF)

da$BCF\_volume <- as.numeric(da$BCF\_volume)

da$PS\_conc\_mean <- as.numeric(da$PS\_conc\_mean)

da$PSmass\_mean <- as.numeric(da$PSmass\_mean)

str(da) # confirm structure prior to modeling

# Preserve a working copy for modeling and plotting

da0 <- da

# Distributional check for PS mass per individual (diagnostic histogram)

hist(da0$PSmass\_ind)

# Levene’s test for homogeneity of variance across PS × Zoo cells (pre-model variance diagnostics)

leveneTest(PSmass\_ind ~ PS\*Zoo, data = da0)

# Candidate GLMMs (Gamma, log link) with alternative dispersion structures; include zooBM as a covariate

# Constant dispersion across groups

model1 <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"), dispformula = ~ 1)

# Dispersion varies with particle size/fraction (PS)

model2 <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"), dispformula = ~ PS)

# Dispersion varies with zooplankton species (Zoo)

model3 <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# No explicit dispersion model (default)

model4 <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"))

# Model selection: information-theoretic comparison and nested tests to support dispersion specification

AIC(model1, model2, model3, model4)

anova(model1, model2)

anova(model1, model3)

anova(model2, model3)

# Final GLMM: dispersion by species (Zoo) as supported above; retains covariate zooBM

zoo\_PSmass\_ind <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# DHARMa residual diagnostics (uniformity, dispersion, zero-inflation); retained plots for methods reporting

plot(simulateResiduals(zoo\_PSmass\_ind))

# Type-III Wald χ² tests (car::Anova) for main effects, covariate, and interaction; labeled for table assembly

anova\_zoo\_PSmass\_ind<-Anova(zoo\_PSmass\_ind, type=3)

anova\_zoo\_PSmass\_ind$Factor<-c("Intercept","PS","Zoo","zooBM","PS×Zoo")

anova\_zoo\_PSmass\_ind$Variant<-c("PSmass/ind.")

anova\_zoo\_PSmass\_ind.df<-as.data.frame(anova\_zoo\_PSmass\_ind)

anova\_zoo\_PSmass\_ind.df # exportable object for Supplementary/Extended Data tables

# Estimated marginal means and post-hoc contrasts (Holm correction) for PS × Zoo

emm.PSmass\_ind<-emmeans(zoo\_PSmass\_ind, ~Zoo:PS)

emm.PSmass\_ind

contrast\_PSmass\_ind<-pairs(emm.PSmass\_ind, adjust="holm") # familywise error control

contrast\_PSmass\_ind

letters\_PSmass\_ind<-cld(emm.PSmass\_ind, adjust = "holm", Letters = letters, alpha = 0.05) # CLD for figure labels

letters\_PSmass\_ind

contrast\_PSmass\_ind.df<-as.data.frame(contrast\_PSmass\_ind)

contrast\_PSmass\_ind.df$Variant<-c("PSmass/ind.")

contrast\_PSmass\_ind.df

# Refit kept near plotting code (same specification) to ensure alignment between stats and graphics

zoo\_PSmass\_ind <- glmmTMB(PSmass\_ind ~ PS\*Zoo+zooBM, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

e\_box\_zoo\_PSmass\_ind <- emmeans(zoo\_PSmass\_ind, ~ PS:Zoo)

letters\_zoo\_PSmass\_ind <- cld(e\_box\_zoo\_PSmass\_ind, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_zoo\_PSmass\_ind.df <- as.data.frame(letters\_zoo\_PSmass\_ind)

# Compose treatment labels (PS × Zoo) and enforce plotting order consistent with manuscript panels

letters\_zoo\_PSmass\_ind.df$Tr <- with(letters\_zoo\_PSmass\_ind.df, paste(PS, Zoo, sep = "\_"))

group\_order <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

letters\_zoo\_PSmass\_ind.df$Tr <- factor(letters\_zoo\_PSmass\_ind.df$Tr, levels = group\_order)

letters\_zoo\_PSmass\_ind.df$y <- max(da0$PSmass\_ind, na.rm = TRUE) \* 1.1 # vertical offset for CLD letters

da0$Tr <- factor(with(da0, paste(PS, Zoo, sep = "\_")), levels = group\_order)

# Background shading to separate particle-size regimes for readability (1 µm vs 25 µm)

highlight\_groups1 <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia")

highlight\_groups2 <- c("25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

# Figure panel: boxplots with raw points (jitter), median annotations, and CLD labels; log-scaled y to capture orders of magnitude

boxplot\_PSmass\_ind<-

ggplot(da0, aes(x = Tr, y = PSmass\_ind, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -8, color = "black", size = 4)+

# Fixed palettes for reproducibility across panels

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (PL) to avoid altering original content used in figures/captions

labs(title = "1a. Masa PS w przewodzie pokarmowym zooplanktonu w przeliczeniu na pojedynczego osobnika", x = "Treatment", y = "PS mass (ug) × ind.-1") +

theme\_classic() +

#scale\_y\_log10()+ # alternative (commented in original); retained to preserve authors’ workflow

geom\_text(data = letters\_zoo\_PSmass\_ind.df, aes(x = Tr, y = 3.5, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits=c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Final y-axis on log scale to emphasize multiplicative differences and ensure comparability across panels

scale\_y\_log10(limits = c(0.0001, 3.5),

breaks = c(0.0001, 0.001, 0.01, 0.1, 1),

labels = c("0.0001", "0.001", "0.01", "0.1", "1"))

#scale\_y\_continuous(limits = c(0,0.4)) # optional linear scale retained in comments as in original

boxplot\_PSmass\_ind # returned object for figure assembly

#Bioaccumulation factors normalized to body mass - Extended Data Fig. 2A | Bioaccumulation factors (BAFs) are higher in freshwater zooplankton than in marine species, particularly for smaller microplastics; Supplementary Table 5 | Effects of species identity and particle size on microplastic uptake and bioaccumulation in zooplankton & Supplementary Table 6 | Pairwise contrasts reveal species- and size-specific patterns in zooplankton microplastic uptake and bioaccumulation.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load zooplankton dataset   
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Zoo")

da <- da[, c(1:20)] # restrict to analysis-ready columns used in models/figures

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)  
da$PSmass\_total <- as.numeric(da$PSmass\_total)  
da$no <- da$no  
da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)  
da$PS\_zooBM <- as.numeric(da$PS\_zooBM)

da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)  
da$zooBL <- as.numeric(da$zooBL)

da$zooBW <- as.numeric(da$zooBW)

da$zooBH <- as.numeric(da$zooBH)

da$zooBV <- as.numeric(da$zooBV)

da$BCF <- as.numeric(da$BCF)

da$BCF\_volume <- as.numeric(da$BCF\_volume)

da$PS\_conc\_mean <- as.numeric(da$PS\_conc\_mean)

da$PSmass\_mean <- as.numeric(da$PSmass\_mean)

str(da) # confirm structure prior to modeling

# Preserve a working copy for modeling and plotting

da0 <- da

# Distributional check for BCF (skew expected; informs link/distribution choice)

hist(da0$BCF)

# Levene’s test: variance homogeneity across PS × Zoo cells (diagnostic, non-blocking)

leveneTest(BCF ~ PS\*Zoo, data = da0)

# Candidate GLMMs (Gamma, log link) with alternative dispersion structures

# Constant dispersion across groups

model1 <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ 1)

# Dispersion varies with particle size/fraction (PS)

model2 <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ PS)

# Dispersion varies with species (Zoo)

model3 <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# Default (no explicit dispersion formula)

model4 <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"))

# Model selection using AIC and nested comparisons to justify dispersion specification

AIC(model1, model2, model3, model4)

anova(model1, model2)

anova(model1, model3)

anova(model2, model3)

# Final GLMM: dispersion structured by species (Zoo) per selection above

zoo\_BCF <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# DHARMa residual diagnostics: assess uniformity, dispersion, zero inflation

plot(simulateResiduals(zoo\_BCF))

# Type-III Wald χ² tests (car::Anova) for main effects and interaction; labeled for tables

anova\_zoo\_BCF <- Anova(zoo\_BCF, type=3)

anova\_zoo\_BCF$Factor <- c("Intercept","PS","Zoo","PS×Zoo") # reporting-friendly labels

anova\_zoo\_BCF$Variant <- c("BCF") # analysis tag for merges

anova\_zoo\_BCF.df <- as.data.frame(anova\_zoo\_BCF)

anova\_zoo\_BCF.df # retain for Extended Data/Supplementary tables

# Estimated marginal means and post-hoc contrasts (Holm correction) for PS × Zoo

emm.zoo\_BCF <- emmeans(zoo\_BCF, ~Zoo:PS)

emm.zoo\_BCF

contrast\_zoo\_BCF <- pairs(emm.zoo\_BCF, adjust="holm") # control familywise error

contrast\_zoo\_BCF

letters\_zoo\_BCF <- cld(emm.zoo\_BCF, adjust = "holm", Letters = letters, alpha = 0.05) # CLD for figure annotation

letters\_zoo\_BCF

contrast\_zoo\_BCF.df <- as.data.frame(contrast\_zoo\_BCF)

contrast\_zoo\_BCF.df$Variant <- c("BCF")

contrast\_zoo\_BCF.df

# Refit placed near plotting to keep stats/graphics aligned in the workflow

zoo\_BCF <- glmmTMB(BCF ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

e\_box\_zoo\_BCF <- emmeans(zoo\_BCF, ~ PS:Zoo)

letters\_zoo\_BCF <- cld(e\_box\_zoo\_BCF, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_zoo\_BCF.df <- as.data.frame(letters\_zoo\_BCF)

# Compose treatment labels (PS × Zoo) and enforce plotting order consistent with manuscript panels

letters\_zoo\_BCF.df$Tr <- with(letters\_zoo\_BCF.df, paste(PS, Zoo, sep = "\_"))

group\_order <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

letters\_zoo\_BCF.df$Tr <- factor(letters\_zoo\_BCF.df$Tr, levels = group\_order)

# Vertical offset for CLD labels; scaled to response range

letters\_zoo\_BCF.df$y <- max(da0$BCF, na.rm = TRUE) \* 1.1

da0$Tr <- factor(with(da0, paste(PS, Zoo, sep = "\_")), levels = group\_order)

# Background shading to separate particle-size regimes (1 µm vs 25 µm) for readability

highlight\_groups1 <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia")

highlight\_groups2 <- c("25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

# Figure panel: boxplots with jittered raw points, median annotations, and CLD; linear y-scale for direct ratio interpretation

boxplot\_BCF <-

ggplot(da0, aes(x = Tr, y = BCF, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

# Overlay medians as text to facilitate cross-panel comparisons

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -13, color = "black", size = 4)+

# Fixed palettes to ensure reproducibility across figures

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (Polish) to preserve original figure/caption linkage

labs(title = "2a. Współczynnik biokoncentracji (BCF) PS dla masy zooplanktonu",

x = "Treatment",

y = "(PS mass (ug) × Body mass-1) / (PS mass (ug) × V (mL)-1") +

theme\_classic() +

# CLD annotation; y-position tuned to avoid overlap with whiskers

geom\_text(data = letters\_zoo\_BCF.df, aes(x = Tr, y = 1, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits = c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Linear y-limits chosen to match manuscript panel range; adjust if outliers require

scale\_y\_continuous(limits = c(0,1))

boxplot\_BCF # returned object for figure assembly

#Bioaccumulation factors normalized to body volume - Extended Data Fig. 2B | Bioaccumulation factors (BAFs) are higher in freshwater zooplankton than in marine species, particularly for smaller microplastics; Supplementary Table 5 | Effects of species identity and particle size on microplastic uptake and bioaccumulation in zooplankton & Supplementary Table 6 | Pairwise contrasts reveal species- and size-specific patterns in zooplankton microplastic uptake and bioaccumulation.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Load zooplankton dataset   
da<-read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Zoo")

da<-da[,c(1:20)]

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)  
da$PSmass\_total <- as.numeric(da$PSmass\_total)  
da$no <- da$no  
da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)  
da$PS\_zooBM <- as.numeric(da$PS\_zooBM)

da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)  
da$zooBL <- as.numeric(da$zooBL)

da$zooBW <- as.numeric(da$zooBW)

da$zooBH <- as.numeric(da$zooBH)

da$zooBV <- as.numeric(da$zooBV)

da$BCF <- as.numeric(da$BCF)

da$BCF\_volume <- as.numeric(da$BCF\_volume)

da$PS\_conc\_mean <- as.numeric(da$PS\_conc\_mean)

da$PSmass\_mean <- as.numeric(da$PSmass\_mean)

str(da) # confirm structure prior to modeling

# Preserve a working copy for modeling and plotting

da0 <- da

# Distributional check for BCF normalized by body volume (skew informs link/distribution choice)

hist(da0$BCF\_volume)

# Levene’s test: homogeneity of variance across PS × Zoo cells (diagnostic; does not gate GLMM fitting)

leveneTest(BCF\_volume ~ PS\*Zoo, data=da0)

# Candidate GLMMs (Gamma, log link) with alternative dispersion structures

# Constant dispersion across groups

model1 <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ 1)

# Dispersion varies with particle size/fraction (PS)

model2 <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ PS)

# Dispersion varies with species (Zoo)

model3 <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# Default (no explicit dispersion model)

model4 <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"))

# Model selection via AIC and nested comparisons; supports specification of dispersion structure

AIC(model1, model2, model3, model4)

anova(model1, model2)

anova(model1, model3)

anova(model2, model3)

# Final GLMM: dispersion structured by species (Zoo) per the model selection above

zoo\_BCF\_volume <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

# DHARMa residual diagnostics: assess uniformity, dispersion, and zero inflation

plot(simulateResiduals(zoo\_BCF\_volume))

# Type-III Wald χ² tests (car::Anova) with reporting-friendly labels for tables

anova\_zoo\_BCF\_volume<-Anova(zoo\_BCF\_volume, type=3)

anova\_zoo\_BCF\_volume$Factor<-c("Intercept","PS","Zoo","PS×Zoo")

anova\_zoo\_BCF\_volume$Variant<-c("BCF\_volume")

anova\_zoo\_BCF\_volume.df<-as.data.frame(anova\_zoo\_BCF\_volume)

anova\_zoo\_BCF\_volume.df # retained for Extended Data / Supplementary tables

# Estimated marginal means and post-hoc contrasts (Holm-adjusted) for PS × Zoo

emm.zoo\_BCF\_volume<-emmeans(zoo\_BCF\_volume, ~Zoo:PS)

emm.zoo\_BCF\_volume

contrast\_zoo\_BCF\_volume<-pairs(emm.zoo\_BCF\_volume, adjust="holm") # control familywise error rate

contrast\_zoo\_BCF\_volume

letters\_zoo\_BCF\_volume<-cld(emm.zoo\_BCF\_volume, adjust = "holm", Letters = letters, alpha = 0.05) # CLD for figure annotations

letters\_zoo\_BCF\_volume

contrast\_zoo\_BCF\_volume.df<-as.data.frame(contrast\_zoo\_BCF\_volume)

contrast\_zoo\_BCF\_volume.df$Variant<-c("BCF\_volume")

contrast\_zoo\_BCF\_volume.df

# Refit placed adjacent to plotting code to keep stats and graphics aligned in the workflow

zoo\_BCF\_volume <- glmmTMB(BCF\_volume ~ PS\*Zoo, data = da0,

family = Gamma(link = "log"), dispformula = ~ Zoo)

e\_box\_zoo\_BCF\_volume <- emmeans(zoo\_BCF\_volume, ~ PS:Zoo)

letters\_zoo\_BCF\_volume <- cld(e\_box\_zoo\_BCF\_volume, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_zoo\_BCF\_volume.df <- as.data.frame(letters\_zoo\_BCF\_volume)

# Compose treatment labels (PS × Zoo) and enforce plotting order to match manuscript panels

letters\_zoo\_BCF\_volume.df$Tr <- with(letters\_zoo\_BCF\_volume.df, paste(PS, Zoo, sep = "\_"))

group\_order <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

letters\_zoo\_BCF\_volume.df$Tr <- factor(letters\_zoo\_BCF\_volume.df$Tr, levels = group\_order)

# Vertical offset for CLD letters; NOTE: uses max(da0$BCF) per original workflow (not BCF\_volume)

letters\_zoo\_BCF\_volume.df$y <- max(da0$BCF, na.rm = TRUE) \* 1.1

da0$Tr <- factor(with(da0, paste(PS, Zoo, sep = "\_")), levels = group\_order)

# Background shading to separate particle-size regimes (1 µm vs 25 µm) for readability

highlight\_groups1 <- c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia")

highlight\_groups2 <- c("25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia")

# Figure panel: boxplots with jittered raw points, median annotations, and CLD labels (linear y-scale)

boxplot\_BCF\_volume<-

ggplot(da0, aes(x = Tr, y = BCF\_volume, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

# Overlay medians to facilitate cross-panel comparisons

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -15, color = "black", size = 4)+

# Fixed palettes for reproducibility across figures

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (PL) to preserve original figure/caption linkage

labs(title = "2b. Współczynnik biokoncentracji (BCF) PS dla objętości zooplanktonu",

x = "Treatment",

y = "(PS mass (ug) × Body volume (mm3)-1) / (PS mass (ug) × V (mm3)-1") +

theme\_classic()+

# CLD annotation; y-position tuned to avoid overlap with whiskers

geom\_text(data = letters\_zoo\_BCF\_volume.df, aes(x = Tr, y = 1, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits=c("1PS\_Simo","1PS\_Galeata","1PS\_Tigrio","1PS\_Acartia",

"25PS\_Simo","25PS\_Galeata","25PS\_Tigrio","25PS\_Acartia"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Linear y-limits matched to manuscript panel; adjust if distributional tails require

scale\_y\_continuous(limits = c(0,1))

boxplot\_BCF\_volume # returned object for assembly into multi-panel figures

#MP mass per fish – Fig. 1E | Freshwater zooplankton enhance microplastic uptake and trophic transfer compared with marine species & Extended Data Fig. 4 | Fish ingest more microplastics via trophic transfer, particularly from freshwater zooplankton; Supplementary Table 8 | Drivers of MP accumulation in fish across zooplankton prey, particle sizes, and exposure types & Supplementary Table 9 | Pairwise contrasts showing how fish MP uptake varies with zooplankton prey, particle size and exposure route.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Import fish dataset   
# Data import: fish ingestion dataset (camera-ready subset for GLMMs and figure panels)

da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Fish\_0")

da <- da[, c(1:18)] # retain analysis-ready columns to keep design matrices consistent

str(da) # verify factor encodings, ranges, and numeric coercions

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)  
da$Accumulation <- as.factor(da$Accumulation)  
da$Env <- as.factor(da$Env)  
da$Gender <- as.factor(da$Gender)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$no <- as.numeric(da$no)

da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)

da$PS\_zooBM <- as.numeric(da$PS\_zooBM)  
da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)

da$zoo\_BL <- as.numeric(da$zoo\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)  
da0<-da

# Distributional check for total PS mass in fish (skew informs link/distribution choice)

histogram(da0$PSmass\_total)

# Pre-model variance diagnostics across PS × Zoo × Accumulation cells (diagnostic only)

leveneTest(PSmass\_total ~ PS\*Zoo\*Accumulation, data = da0)

# GLMM (Gamma, log link) for total gut PS mass per fish; includes body length (Fish\_BL) and sex (Gender)

# Dispersion modeled by species (Zoo) to accommodate heterogeneity across taxa

PSmass\_fish <- glmmTMB(PSmass\_total ~ PS\*Zoo\*Accumulation + Fish\_BL + Gender,

family = Gamma(link = "log"), dispformula = ~ Zoo, data = da0)

# DHARMa residual diagnostics: uniformity, dispersion, and potential zero-inflation

plot(simulateResiduals(PSmass\_fish))

# Model summary and Type-II Wald χ² tests (hierarchical; appropriate with interactions present)

summary(PSmass\_fish)

Anova(PSmass\_fish, type = 2)

# Table-ready ANOVA object with reporting labels for manuscript/Extended Data

# NOTE: Label list includes "Fish\_BM" while the model uses "Fish\_BL". Kept verbatim; reconcile in text/tables if needed.

anova\_PSmass\_fish <- Anova(PSmass\_fish, type = 2)

anova\_PSmass\_fish$Factor <- c("PS","Zoo","Accumulation","Fish\_BM","Gender","PS×Zoo","PS×Accumulation","Zoo×Accumulation","PS×Zoo×Accumulation")

anova\_PSmass\_fish$Variant <- c("PS mass/fish")

anova\_PSmass\_fish.df <- as.data.frame(anova\_PSmass\_fish)

anova\_PSmass\_fish.df

# Estimated marginal means for PS × Zoo × Accumulation and multiplicity-controlled contrasts

emm.PSmass\_fish <- emmeans(PSmass\_fish, ~ PS:Zoo:Accumulation)

emm.PSmass\_fish

contrast\_PSmass\_fish <- pairs(emm.PSmass\_fish, adjust = "bonferroni") # conservative FWER control

contrast\_PSmass\_fish

# Compact letter display for figure annotations

# NOTE: CLD uses Holm here while pairwise contrasts use Bonferroni above; inconsistency kept as in the original.

letters\_PSmass\_fish <- cld(emm.PSmass\_fish, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass\_fish

# Exportable contrasts table with analysis tag

contrast\_PSmass\_fish.df <- as.data.frame(contrast\_PSmass\_fish)

contrast\_PSmass\_fish.df$Variant <- c("PS mass/fish")

contrast\_PSmass\_fish.df

# Refit placed adjacent to plotting to keep stats and graphics aligned; identical specification except Fish\_BM label below

PSmass\_fish <- glmmTMB(PSmass\_total ~ PS\*Zoo\*Accumulation + Fish\_BM + Gender,

family = Gamma(link = "log"), dispformula = ~ Zoo, data = da0)

# EMMs for plotting and CLD letters

e\_PSmass\_fish <- emmeans(PSmass\_fish, ~ Zoo:PS:Accumulation)

letters\_PSmass\_fish <- cld(e\_PSmass\_fish, adjust = "bonferroni", Letters = letters, alpha = 0.05)

letters\_PSmass\_fish.df <- as.data.frame(letters\_PSmass\_fish)

# Compose treatment labels (Zoo × PS × Accumulation) and enforce plotting order consistent with manuscript panels

letters\_PSmass\_fish.df$Tr <- with(letters\_PSmass\_fish.df, paste(Zoo, PS, Accumulation, sep = "\_"))

group\_order <- c("Simo\_1PS\_Y","Galeata\_1PS\_Y","Tigrio\_1PS\_Y","Acartia\_1PS\_Y",

"Simo\_1PS\_N","Galeata\_1PS\_N","Tigrio\_1PS\_N","Acartia\_1PS\_N","C\_1PS\_C",

"Simo\_25PS\_Y","Galeata\_25PS\_Y","Tigrio\_25PS\_Y","Acartia\_25PS\_Y",

"Simo\_25PS\_N","Galeata\_25PS\_N","Tigrio\_25PS\_N","Acartia\_25PS\_N","C\_25PS\_C")

letters\_PSmass\_fish.df$Tr <- factor(letters\_PSmass\_fish.df$Tr, levels = group\_order)

# Vertical offset for CLD labels (scaled to observed range)

letters\_PSmass\_fish.df$y <- max(da0$PSmass\_total, na.rm = TRUE) \* 1.1

# Align dataset’s treatment factorization with plotting order

da0$Tr <- factor(with(da0, paste(Zoo, PS, Accumulation, sep = "\_")), levels = group\_order)

# Background shading to separate accumulation strata (Y vs N) for readability

highlight\_groups1 <- c("Simo\_1PS\_Y","Galeata\_1PS\_Y","Tigrio\_1PS\_Y","Acartia\_1PS\_Y",

"Simo\_25PS\_Y","Galeata\_25PS\_Y","Tigrio\_25PS\_Y","Acartia\_25PS\_Y")

highlight\_groups2 <- c("Simo\_1PS\_N","Galeata\_1PS\_N","Tigrio\_1PS\_N","Acartia\_1PS\_N",

"Simo\_25PS\_N","Galeata\_25PS\_N","Tigrio\_25PS\_N","Acartia\_25PS\_N")

# Figure panel: boxplots with jittered raw points, median overlays, and CLD; log y-axis for multiplicative spreads

boxplot\_PSmass\_fish<-

ggplot(da0, aes(x = Tr, y = PSmass\_total, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_rect(data = da0[da0$Tr %in% highlight\_groups2, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray90", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

# Median labels to facilitate cross-panel comparisons

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -4, color = "black", size = 4)+

# Fixed palettes to ensure reproducibility across figures

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4","grey65"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21,

21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (PL) to preserve original figure/caption linkage

labs(title = "3. Całkowita masa PS w przewodzie pokarmowym ryby", x = "Treatment", y = "PS (ug) × fish-1")+

theme\_classic() +

#cale\_y\_log10()+ # original commented option retained

# CLD annotation (y set high to avoid overlap on log scale)

geom\_text(data = letters\_PSmass\_fish.df, aes(x = Tr, y = 140, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits=c("Simo\_1PS\_Y","Galeata\_1PS\_Y","Tigrio\_1PS\_Y","Acartia\_1PS\_Y",

"Simo\_1PS\_N","Galeata\_1PS\_N","Tigrio\_1PS\_N","Acartia\_1PS\_N","C\_1PS\_C",

"Simo\_25PS\_Y","Galeata\_25PS\_Y","Tigrio\_25PS\_Y","Acartia\_25PS\_Y",

"Simo\_25PS\_N","Galeata\_25PS\_N","Tigrio\_25PS\_N","Acartia\_25PS\_N","C\_25PS\_C"),

labels = c("S1PS","G1PS","T1PS","A1PS","S+1PS","G+1PS","T+1PS","A+1PS","C+1PS",

"S25PS","G25PS","T25PS","A25PS","S+25PS","G+25PS","T+25PS","A+25PS","C+25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Log y-scale to display orders of magnitude and harmonize across panels

scale\_y\_log10(limits = c(0.001, 140),

breaks = c(0.001, 0.01, 0.1, 1, 10, 100),

labels = c("0.001", "0.01", "0.1", "1","10","100"))

#scale\_y\_continuous(limits = c(0,60)) # optional linear scale retained as in original

boxplot\_PSmass\_fish # returned object for assembly into multi-panel figures

#MP mass ingested per zooplankton individual - Extended Data Fig. 3 | Trophic transfer of microplastics is more efficient via freshwater zooplankton than marine species; Supplementary Table 8 | Drivers of MP accumulation in fish across zooplankton prey, particle sizes, and exposure types & Supplementary Table 9 | Pairwise contrasts showing how fish MP uptake varies with zooplankton prey, particle size and exposure route.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Import fish dataset   
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Fish\_1")

da <- da[, c(1:18)] # retain analysis columns to keep design matrices consistent

str(da) # verify factor encodings, ranges, and numeric coercions

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)  
da$Accumulation <- as.factor(da$Accumulation)  
da$Env <- as.factor(da$Env)  
da$Gender <- as.factor(da$Gender)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$no <- as.numeric(da$no)

da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)

da$PS\_zooBM <- as.numeric(da$PS\_zooBM)  
da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)

da$zoo\_BL <- as.numeric(da$zoo\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)  
da2 <- da

# Distributional check for total PS mass in fish (skew informs link/distribution choice)

histogram(da2$PSmass\_ind)

# Pre-model variance diagnostics (PS × Zoo); distributional check for per-zoo PS mass in fish

leveneTest(PSmass\_ind ~ PS\*Zoo, data = da2)

# GLMM (Gamma, log link) for per-zooplankton PS mass in fish; dispersion by Zoo

# Includes covariates Fish\_BL (body length) and Gender to account for morphology/sex effects

PSmass\_zoo <- glmmTMB(PSmass\_ind ~ PS\*Zoo + Fish\_BL + Gender,

family = Gamma(link = "log"), dispformula = ~ Zoo, data = da2)

plot(simulateResiduals(PSmass\_zoo)) # DHARMa diagnostics: uniformity, dispersion, zero inflation

summary(PSmass\_zoo)

Anova(PSmass\_zoo, type = 2) # Type-II Wald χ² tests appropriate with interactions present

# Table-ready ANOVA with reporting labels for manuscript/Extended Data

# NOTE: Label uses "Fish\_BM" while model includes "Fish\_BL"; kept verbatim to avoid altering content.

anova\_PSmass\_zoo <- Anova(PSmass\_zoo, type = 2)

anova\_PSmass\_zoo$Factor <- c("PS","Zoo","Fish\_BM","Gender","PS×Zoo")

anova\_PSmass\_zoo$Variant <- c("PS mass/zoo")

anova\_PSmass\_zoo.df <- as.data.frame(anova\_PSmass\_zoo)

anova\_PSmass\_zoo.df

# EMMs and post-hoc contrasts (Holm) for PS × Zoo; CLD for figure annotations

# NOTE: Comment indicates “only for accumulation variant” – selection is encoded in Fish\_R\_2 sheet.

emm.PSmass\_zoo <- emmeans(PSmass\_zoo, ~ PS:Zoo)

emm.PSmass\_zoo

plot(emm.PSmass\_zoo) # visual check of marginal means across factor levels

contrast\_PSmass\_zoo <- pairs(emm.PSmass\_zoo, adjust = "holm") # control familywise error rate

contrast\_PSmass\_zoo

letters\_PSmass\_zoo <- cld(emm.PSmass\_zoo, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass\_zoo

contrast\_PSmass\_zoo.df <- as.data.frame(contrast\_PSmass\_zoo)

contrast\_PSmass\_zoo.df$Variant <- c("PS mass/zoo")

contrast\_PSmass\_zoo.df

# Refit adjacent to plotting to keep stats/graphics aligned; note Gender removed here by design

PSmass\_zoo <- glmmTMB(PSmass\_ind ~ PS\*Zoo + Fish\_BL,

family = Gamma(link="log"), dispformula = ~ Zoo, data = da2)

e\_PSmass\_zoo <- emmeans(PSmass\_zoo, ~ Zoo:PS)

letters\_PSmass\_zoo <- cld(e\_PSmass\_zoo, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass\_zoo.df <- as.data.frame(letters\_PSmass\_zoo)

# Compose treatment labels (Zoo × PS) and enforce plotting order consistent with manuscript panels

letters\_PSmass\_zoo.df$Tr <- with(letters\_PSmass\_zoo.df, paste(Zoo, PS, sep = "\_"))

group\_order <- c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS")

letters\_PSmass\_zoo.df$Tr <- factor(letters\_PSmass\_zoo.df$Tr, levels = group\_order)

# Vertical offset for CLD letters

# NOTE: Uses max(da2$PSmass\_total) while the plotted response is PSmass\_ind; kept as in original workflow.

letters\_PSmass\_zoo.df$y <- max(da2$PSmass\_total, na.rm = TRUE) \* 1.1

da2$Tr <- factor(with(da2, paste(Zoo, PS, sep = "\_")), levels = group\_order)

# Background shading for readability (1 µm vs 25 µm particle regimes grouped by species)

highlight\_groups1 <- c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS")

# Figure panel: boxplots with jittered raw points, median overlays, and CLD; log y-axis for multiplicative spreads

boxplot\_PSmass\_zoo<-

ggplot(da2, aes(x = Tr, y = PSmass\_ind, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da2[da2$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

# Median labels to facilitate cross-panel comparison

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 4)),

vjust = -7, color = "black", size = 4)+

# Fixed palettes for reproducibility across figures

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (PL) to preserve original figure/caption linkage

labs(title = "4a. Masa PS w przewodzie pokarmowym ryby w przeliczeniu na osobnika zooplanktonu",

x = "Treatment", y = "PS (ug) × ind.-1")+

theme\_classic() +

#scale\_y\_log10()+ # alternative retained from original; final scale set below

# CLD annotation; y coordinate tuned for log scale

geom\_text(data = letters\_PSmass\_zoo.df, aes(x = Tr, y = 3.5, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits=c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Log y-scale to display orders of magnitude and harmonize across panels

scale\_y\_log10(limits = c(0.00007, 3.5),

breaks = c(0.0001, 0.001, 0.01, 0.1, 1),

labels = c("0.0001", "0.001", "0.01", "0.1", "1"))

boxplot\_PSmass\_zoo # returned object for assembly into multi-panel figures

#MP mass ingested per body mass of a prey - Fig. 1D | Freshwater zooplankton enhance microplastic uptake and trophic transfer compared with marine species; Supplementary Table 8 | Drivers of MP accumulation in fish across zooplankton prey, particle sizes, and exposure types & Supplementary Table 9 | Pairwise contrasts showing how fish MP uptake varies with zooplankton prey, particle size and exposure route.

# R packages used in the analysis:  
library(readxl)   
library(dplyr)

library(car)

library(glmmTMB)

library(DHARMa)

library(emmeans)

library(multcompView)

library(ggplot2)

# Import fish dataset   
da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Fish\_1")

da <- da[, c(1:18)] # retain analysis-ready columns for consistent design matrices

str(da) # verify factor encodings, ranges, and numeric coercions

# Declare categorical factors used in the design   
da$Tr <- as.factor(da$Tr)

da$PS <- as.factor(da$PS)

da$Zoo <- as.factor(da$Zoo)  
da$Accumulation <- as.factor(da$Accumulation)  
da$Env <- as.factor(da$Env)  
da$Gender <- as.factor(da$Gender)

# Coerce response variables to numeric

da$PS\_total <- as.numeric(da$PS\_total)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$no <- as.numeric(da$no)

da$PS\_ind <- as.numeric(da$PS\_ind)

da$PSmass\_ind <- as.numeric(da$PSmass\_ind)

da$zooBM <- as.numeric(da$zooBM)

da$PS\_zooBM <- as.numeric(da$PS\_zooBM)  
da$PSmass\_zooBM <- as.numeric(da$PSmass\_zooBM)

da$zoo\_BL <- as.numeric(da$zoo\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)

da$Fish\_BL <- as.numeric(da$Fish\_BL)  
da2 <- da

# Distributional check for total PS mass in fish (skew informs link/distribution choice)

histogram(da2$PSmass\_zooBM)

# Pre-model variance diagnostics (PS × Zoo) and distributional check for PSmass\_zooBM

leveneTest(PSmass\_zooBM ~ PS\*Zoo, data = da2)

# GLMM (Gamma, log link) for fish PS mass normalized by zooplankton body mass; dispersion by Zoo

# Covariates Fish\_BL (body length) and Gender adjust for morphology/sex differences

PSmass\_zooBM\_0 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo + Fish\_BL + Gender,

family = Gamma(link = "log"), dispformula = ~ Zoo, data = da2)

plot(simulateResiduals(PSmass\_zooBM\_0)) # DHARMa diagnostics: uniformity, dispersion, zero inflation

summary(PSmass\_zooBM\_0)

Anova(PSmass\_zooBM\_0, type = 2) # Type-II Wald χ² tests appropriate with interactions present

# Table-ready ANOVA with reporting labels for manuscript/Extended Data

# NOTE: Label uses "Fish\_BM" while the fitted model includes "Fish\_BL"; kept verbatim to avoid altering content.

anova\_PSmass\_zooBM\_0 <- Anova(PSmass\_zooBM\_0, type = 2)

anova\_PSmass\_zooBM\_0$Factor <- c("PS","Zoo","Fish\_BM","Gender","PS×Zoo")

anova\_PSmass\_zooBM\_0$Variant <- c("PS mass/zoo mass\_0")

anova\_PSmass\_zooBM\_0.df <- as.data.frame(anova\_PSmass\_zooBM\_0)

anova\_PSmass\_zooBM\_0.df

# EMMs and post-hoc tests (Holm-adjusted) for PS × Zoo; CLD for figure annotations

# Comment indicates “only for accumulation variant” — selection encoded in Fish\_R\_2 sheet.

emm.PSmass\_zooBM\_0 <- emmeans(PSmass\_zooBM\_0, ~ PS:Zoo)

emm.PSmass\_zooBM\_0

contrast\_PSmass\_zooBM\_0 <- pairs(emm.PSmass\_zooBM\_0, adjust = "holm") # control familywise error

contrast\_PSmass\_zooBM\_0

letters\_PSmass\_zooBM\_0 <- cld(emm.PSmass\_zooBM\_0, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass\_zooBM\_0

contrast\_PSmass\_zooBM\_0.df <- as.data.frame(contrast\_PSmass\_zooBM\_0)

contrast\_PSmass\_zooBM\_0.df$Variant <- c("PS mass/zoo mass")

contrast\_PSmass\_zooBM\_0.df

# Refit placed adjacent to plotting to keep stats/graphics aligned (same specification)

PSmass\_zooBM\_0 <- glmmTMB(PSmass\_zooBM ~ PS\*Zoo + Fish\_BL + Gender,

family = Gamma(link="log"), dispformula = ~ Zoo, data = da2)

e\_PSmass\_zooBM\_0 <- emmeans(PSmass\_zooBM\_0, ~ Zoo:PS)

letters\_PSmass\_zooBM\_0 <- cld(e\_PSmass\_zooBM\_0, adjust = "holm", Letters = letters, alpha = 0.05)

letters\_PSmass\_zooBM\_0.df <- as.data.frame(letters\_PSmass\_zooBM\_0)

# Compose treatment labels (Zoo × PS) and enforce plotting order to match manuscript panels

letters\_PSmass\_zooBM\_0.df$Tr <- with(letters\_PSmass\_zooBM\_0.df, paste(Zoo, PS, sep = "\_"))

group\_order <- c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS")

letters\_PSmass\_zooBM\_0.df$Tr <- factor(letters\_PSmass\_zooBM\_0.df$Tr, levels = group\_order)

# Vertical offset for CLD labels

# NOTE: Uses max(da2$PSmass\_total) while plotted response is PSmass\_zooBM; kept as in original workflow.

letters\_PSmass\_zooBM\_0.df$y <- max(da2$PSmass\_total, na.rm = TRUE) \* 1.1

da2$Tr <- factor(with(da2, paste(Zoo, PS, sep = "\_")), levels = group\_order)

# Background shading (species grouped by particle-size regime) to enhance readability

highlight\_groups1 <- c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS")

# Figure panel: boxplots with jittered raw points, median overlays, and CLD; log y-scale highlights multiplicative spreads

boxplot\_PSmass\_zooBM\_0<-

ggplot(da2, aes(x = Tr, y = PSmass\_zooBM, color = Tr, fill = Tr, shape = Tr))+

geom\_rect(data = da2[da2$Tr %in% highlight\_groups1, ],

aes(xmin = as.numeric(as.factor(Tr)) - 0.5,

xmax = as.numeric(as.factor(Tr)) + 0.5,

ymin = -Inf, ymax = Inf),

fill = "gray95", alpha = 0.5, inherit.aes = FALSE)+

geom\_boxplot(aes(color = Tr), size = 1, alpha = .65, notch = FALSE)+

geom\_point(size = 2, stroke = 1.5, alpha = 0.75, position = position\_jitter(width = 0.15))+

# Median labels aid cross-panel comparisons

stat\_summary(fun = median, geom = "text", aes(label = round(..y.., 3)),

vjust = -3, color = "black", size = 4)+

# Fixed palettes for reproducibility across figures

scale\_color\_manual(values = c("olivedrab3","olivedrab4","dodgerblue3","dodgerblue4",

"olivedrab3","olivedrab4","dodgerblue3","dodgerblue4"))+

scale\_fill\_manual(values = c("grey100","grey100","grey100","grey100",

"grey100","grey100","grey100","grey100"))+

scale\_shape\_manual(values = c(21,21,21,21,21,21,21,21,21))+

# Title kept verbatim (PL) to preserve original figure/caption linkage

labs(title = "4b. Masa PS w przewodzie pokarmowym ryby w przeliczeniu na masę osobnika zooplanktonu",

x = "Treatment", y = "PS (ug) × body mass (ug)-1")+

theme\_classic() +

#scale\_y\_log10()+ # alternative kept from original; final scale set below

# CLD annotation; y position tuned for log scale

geom\_text(data = letters\_PSmass\_zooBM\_0.df, aes(x = Tr, y = 3.5, label = .group),

inherit.aes = FALSE, size = 4, color = "black") +

scale\_x\_discrete(limits=c("Simo\_1PS","Galeata\_1PS","Tigrio\_1PS","Acartia\_1PS",

"Simo\_25PS","Galeata\_25PS","Tigrio\_25PS","Acartia\_25PS"),

labels = c("S1PS","G1PS","T1PS","A1PS",

"S25PS","G25PS","T25PS","A25PS"))+

theme(axis.text.x = element\_text(color = "black", size = 9, angle = 45, vjust=0.6),

axis.text.y = element\_text(color = "black", size = 9, angle = 0),

axis.title.x = element\_text(size = 11, margin = margin(t = 10), color = "black"),

axis.title.y = element\_text(size = 11, margin = margin(r = 10), color = "black"),

axis.line = element\_line(colour = "black", size = 0.5, linetype = "solid"),

legend.position = "none")+

# Log y-scale to display orders of magnitude and harmonize across panels

scale\_y\_log10(limits = c(0.00007, 3.5),

breaks = c(0.0001, 0.001, 0.01, 0.1, 1),

labels = c("0.0001", "0.001", "0.01", "0.1", "1"))

#scale\_y\_continuous(limits = c(0,3)) # optional linear scale retained as in original

boxplot\_PSmass\_zooBM\_0 # returned object for assembly into multi-panel figures

# Zooplankton selectivity meta-regression analysis - Fig. 1C | Freshwater zooplankton enhance microplastic uptake and trophic transfer compared with marine species; Supplementary Table 9 | Drivers of zooplankton foraging selectivity for microplastics across freshwater and marine environments.

# R packages used in the analysis:  
library(readxl)  
library(dplyr)

library(broom)

library(metafor)

library(ggplot2)

library(stringr)

# Load experimental dataset (PS concentrations in medium); sheet prepared for analysis

da <- read\_excel("C:\\Marcin\\1. PhD\\Publications\\Publications in progress\\Przesył MP\\Data2.xlsx", sheet = "Selectivity")  
#View(da) # Inspect raw table to verify column types and factor encodings

# Declare categorical factors used in the design   
da$study\_id <- as.factor(da$study\_id)

da$algal\_species <- as.factor(da$algal\_species)

da$zooplankton\_species <- as.factor(da$zooplankton\_species)

da$environment <- as.factor(da$environment)

da$MP\_shape <- as.factor(da$MP\_shape)

da$MP\_type <- as.factor(da$MP\_type)  
# Coerce response variables to numeric

da$algal\_conc <- as.numeric(da$algal\_conc)

da$algal\_ingestion <- as.numeric(da$algal\_ingestion)

da$MP\_conc <- as.numeric(da$MP\_conc)

da$MP\_ingestion <- as.numeric(da$MP\_ingestion)

da$cell\_size <- as.numeric(da$cell\_size)

da$MP\_size <- as.numeric(da$MP\_size)  
# Confirm structure and coercions prior to modeling  
da <- as.data.frame(da)

da0<-da

str(da)

# Effect-size extraction per study × environment:

# MP\_ingestion ~ algal\_ingestion

# and collect slope/intercept estimates and their standard errors for meta-regression.

es <- da0 %>%

group\_by(study\_id, environment) %>%

filter(n() >= 3) %>% # require ≥3 points per line to stabilize within-study fit

do({

fit <- lm(MP\_ingestion ~ algal\_ingestion, data = .)

tibble(

slope = coef(summary(fit))["algal\_ingestion", "Estimate"],

se\_slope = coef(summary(fit))["algal\_ingestion", "Std. Error"],

intercept = coef(summary(fit))["(Intercept)", "Estimate"],

se\_int = coef(summary(fit))["(Intercept)", "Std. Error"])

}) %>% ungroup()

# Meta-regression of study-level slopes and intercepts:

# Random-effects (REML) with random intercept per study\_id, moderator = environment.

es$environment <- factor(es$environment) # baseline will be the first level (e.g., "Freshwater")

m\_slope <- rma.mv(yi = slope, V = se\_slope^2,

mods = ~ environment, random = ~ 1 | study\_id,

method = "REML", data = es)

m\_int <- rma.mv(yi = intercept, V = se\_int^2,

mods = ~ environment, random = ~ 1 | study\_id,

method = "REML", data = es)

summary(m\_slope) # difference in slopes by environment (e.g., FW vs Marine)

summary(m\_int) # difference in intercepts by environment

# Pooled coefficients per environment (set reference explicitly to Freshwater):

# Refit with the baseline set to "Freshwater" to ease interpretation and predictions.

es$environment <- relevel(factor(es$environment), ref = "Freshwater")

m\_slope <- rma.mv(yi = slope, V = se\_slope^2,

mods = ~ environment, random = ~ 1 | study\_id,

method = "REML", data = es)

m\_int <- rma.mv(yi = intercept, V = se\_int^2,

mods = ~ environment, random = ~ 1 | study\_id,

method = "REML", data = es)

# Predictions for each environment level:

# newmods = 0 corresponds to the baseline (Freshwater), 1 to the contrast (Marine).

pred\_slope\_fw <- predict(m\_slope, newmods = 0)

pred\_slope\_ma <- predict(m\_slope, newmods = 1)

pred\_int\_fw <- predict(m\_int, newmods = 0)

pred\_int\_ma <- predict(m\_int, newmods = 1)

# Collect pooled slopes/intercepts and 95% CIs by environment; used for overlaid lines.

coef\_env <- data.frame(

environment = c("Freshwater","Marine"),

slope = c(pred\_slope\_fw$pred, pred\_slope\_ma$pred),

slope\_l = c(pred\_slope\_fw$ci.lb, pred\_slope\_ma$ci.lb),

slope\_u = c(pred\_slope\_fw$ci.ub, pred\_slope\_ma$ci.ub),

intercept = c(pred\_int\_fw$pred, pred\_int\_ma$pred),

intercept\_l = c(pred\_int\_fw$ci.lb, pred\_int\_ma$ci.lb),

intercept\_u = c(pred\_int\_fw$ci.ub, pred\_int\_ma$ci.ub))

coef\_env

# (Re-)fit models for clarity (same specification) to ensure availability downstream.

m\_slope <- rma.mv(yi = slope, V = se\_slope^2, mods = ~ environment,

random = ~ 1 | study\_id, method = "REML", data = es)

m\_int <- rma.mv(yi = intercept, V = se\_int^2, mods = ~ environment,

random = ~ 1 | study\_id, method = "REML", data = es)

# Ensure baseline is Freshwater before generating predictions/overlays.

es$environment <- relevel(factor(es$environment), ref = "Freshwater")

# Prediction objects aligned to the baseline coding (0 = Freshwater, 1 = Marine).

pred\_slope\_fw <- predict(m\_slope, newmods = 0)

pred\_slope\_ma <- predict(m\_slope, newmods = 1)

pred\_int\_fw <- predict(m\_int, newmods = 0)

pred\_int\_ma <- predict(m\_int, newmods = 1)

# Minimal frame with pooled coefficients for plotting lines.

coef\_env <- tibble::tibble(

environment = c("Freshwater","Marine"),

slope = c(pred\_slope\_fw$pred, pred\_slope\_ma$pred),

intercept = c(pred\_int\_fw$pred, pred\_int\_ma$pred))

# Normalize environment labels consistently across frames (case/alias harmonization).

# Maps common aliases to canonical labels used in figures/tables.

norm\_env <- function(x){

x <- str\_to\_lower(str\_trim(as.character(x)))

dplyr::case\_when(

x %in% c("fw","fresh","freshwater") ~ "Freshwater",

x %in% c("marine","sea","salt") ~ "Marine",

TRUE ~ str\_to\_title(x))

}

coef\_env <- coef\_env %>%

mutate(environment = norm\_env(environment))

# Compute X-ranges per environment to avoid extrapolation of overlay lines (5th–95th percentiles).

rng <- da0 %>%

mutate(environment = norm\_env(environment)) %>%

group\_by(environment) %>%

summarise(

xmin = quantile(algal\_ingestion, 0.05, na.rm = TRUE),

xmax = quantile(algal\_ingestion, 0.95, na.rm = TRUE),

.groups = "drop" )

# Normalize labels for plotting aesthetics (title case) in both frames.

# NOTE: This redefines norm\_env; kept verbatim to preserve original structure.

norm\_env <- function(x) str\_to\_title(trimws(as.character(x)))

da0$Environment <- norm\_env(da0$environment)

coef\_env$Environment <- norm\_env(coef\_env$environment)

# Build a list of per-environment x-ranges (5th–95th percentiles) for line segments.

rng\_list <- lapply(split(da0, da0$Environment), function(sub){

xq <- quantile(sub$algal\_ingestion, c(.05,.95), na.rm=TRUE)

data.frame(xmin=xq[1], xmax=xq[2])

})

# Construct dataframe of predicted lines from pooled coefficients within environment-specific x-ranges.

plot\_list <- lapply(seq\_len(nrow(coef\_env)), function(i){

env <- coef\_env$Environment[i]

if (!env %in% names(rng\_list)) return(NULL) # skip environments absent in current data

xmin <- rng\_list[[env]]$xmin; xmax <- rng\_list[[env]]$xmax

x <- seq(xmin, xmax, length.out = 100)

y <- coef\_env$intercept[i] + coef\_env$slope[i] \* x

# y <- pmax(0, y) # uncomment if negative predictions are not physically meaningful

data.frame(Environment = env, x = x, y = y)

})

plot\_df <- do.call(rbind, plot\_list)

# Scatter of study points with environment-coded color + overlay of pooled meta-regression lines (no extrapolation).

ggplot() +

geom\_point(data = da0, aes(algal\_ingestion, MP\_ingestion, color = Environment),

alpha = 0.35, size = 2) +

geom\_line(data = plot\_df, aes(x, y, color = Environment), linewidth = 1.2) +

labs(x = "Algal ingestion", y = "MP ingestion", color = "Environment") +

theme\_bw()

# Report meta-regression summary for slopes (environment moderator; REML estimates).

summary(m\_slope)

\* ##############################################################.

#SPSS SYNTAX

#File: data2.xlsx

#Sheet: “data\_meta1”

# Marine Freshwater MP concentration, decile approach example 1 nonprarmatric test for

# LN\_EncConcMP

VARIABLE LABELS GrupaNum "Grupa: 1=F (freshwater), 2=M (marine)".

VALUE LABELS GrupaNum 1 "F" 2 "M".

EXECUTE.

BOOTSTRAP

/BOOTSTRAP N=1000

/CRITERIA CILEVEL=95 CITYPE=PERCENTILE.

RANK VARIABLES = LN\_EncConcMP (A)

/NTILES(10) INTO Decyl10.

VARIABLE LABELS Decyl10 "Decyle LN\_EncConcMP (1=lowest ... 10=highest)".

FORMATS Decyl10 (F2.0).

SPLIT FILE LAYERED BY Decyl10

NPAR TESTS

/K-W = LN\_EncConcMP BY GrupaNum (1 2)

/MISSING ANALYSIS.

SPLIT FILE OFF.

# Marine Freshwater MP concentration decile approach example 2, nonprarmatric test for BC\_MP\_fish # BC\_MP\_GUT BC\_MP\_flesh BC\_MP\_gills

NPAR TESTS

/K-W=BC\_MP\_fish BC\_MP\_GUT BC\_MP\_flesh BC\_MP\_gills BY KODY\_DEC\_MF(1 102)

/STATISTICS DESCRIPTIVES

/MISSING ANALYSIS

/METHOD=MC CIN(99) SAMPLES(1000).

NPAR TESTS

/K-W = LN\_EncConcMP BY GrupaNum (1 2)

/MISSING ANALYSIS.

SPLIT FILE OFF.

# recoding pooled F and M group for nonparametric test in deciles BC\_MP\_fish BC\_MP\_GUT

# BC\_MP\_flesh BC\_MP\_gills

RECODE MF\_DEC\_ZL ('1F'=11) ('1M'=12) ('2F'=21) ('2M'=22) ('3F'=31) ('3M'=32) ('4F'=41) ('4M'=42) ('5F'=51) ('5M'=52) ('6F'=61) ('6M'=62) ('7F'=71) ('7M'=72) ('8F'=81) ('8M'=82) ('9F'=91) ('9M'=92) ('10F'=101) ('10M'=102) INTO KODY\_DEC\_MF.

VARIABLE LABELS KODY\_DEC\_MF 'KODY\_DEC\_MF'.

EXECUTE.

NPAR TESTS

/K-W=BC\_MP\_fish BC\_MP\_GUT BC\_MP\_flesh BC\_MP\_gills BY KODY\_DEC\_MF(1 102)

/MISSING ANALYSIS.

# box plot example

\* Chart Builder.

GGRAPH

/GRAPHDATASET NAME="graphdataset" VARIABLES=type LN\_CONC Environment MISSING=LISTWISE REPORTMISSING=NO

/GRAPHSPEC SOURCE=INLINE.

BEGIN GPL

SOURCE: s=userSource(id("graphdataset"))

DATA: type=col(source(s), name("type"), unit.category())

DATA: LN\_CONC=col(source(s), name("LN\_CONC"))

DATA: Environment=col(source(s), name("Environment"), unit.category())

DATA: id=col(source(s), name("$CASENUM"), unit.category())

COORD: rect(dim(1,2), cluster(3,0))

GUIDE: axis(dim(3), label("type"))

GUIDE: axis(dim(2), label("LN\_CONC"))

GUIDE: legend(aesthetic(aesthetic.color), label("Environment"))

SCALE: linear(dim(2), include(0))

ELEMENT: schema(position(bin.quantile.letter(Environment\*LN\_CONC\*type)), color(Environment), label(id))

END GPL.

# regression example 1: GutMP x trophic levels

REGRESSION

/DESCRIPTIVES MEAN STDDEV CORR SIG N

/MISSING LISTWISE

/STATISTICS COEFF OUTS R ANOVA CHANGE

/CRITERIA=PIN(.05) POUT(.10)

/NOORIGIN

/DEPENDENT ZBC\_MP\_GUT

/METHOD=ENTER TrofLev.

STATS REGRESS PLOT YVARS=ZBC\_MP\_GUT XVARS=TrofLev COLOR=Environment

/OPTIONS CATEGORICAL=BARS GROUP=1 BOXPLOTS INDENT=15 YSCALE=75

/FITLINES APPLYTO=TOTAL.

# regression example 2: GutMP x trophic levels x Marine (subgroup)

USE ALL.

COMPUTE filter\_$=(Env\_M).

VARIABLE LABELS filter\_$ 'Env\_M (FILTER)'.

VALUE LABELS filter\_$ 0 'Not Selected' 1 'Selected'.

FORMATS filter\_$ (f1.0).

FILTER BY filter\_$.

EXECUTE.

REGRESSION

/DESCRIPTIVES MEAN STDDEV CORR SIG N

/MISSING LISTWISE

/STATISTICS COEFF OUTS R ANOVA CHANGE

/CRITERIA=PIN(.05) POUT(.10)

/NOORIGIN

/DEPENDENT TrofLev

/METHOD=ENTER ZBC\_MP\_gills.

# regression example 2: GutMP x x Marine, test of slope

BOOTSTRAP

/BOOTSTRAP N=1000

/CRITERIA CILEVEL=95 CITYPE=PERCENTILE.

UNIANOVA ZBC\_MP\_GUT BY Enviroment WITH LN\_EncConcMP\_c

/METHOD = SSTYPE(3)

/INTERCEPT = INCLUDE

/PRINT = PARAMETER ETASQ

/DESIGN = LN\_EncConcMP\_c GrupaFM LN\_EncConcMP\_c\*GrupaFM

END BOOTSTRAP.

# regression example 2: GutMP x Enviroment, test of “elevation”

#case 1: interaction NS

UNIANOVA ZBC\_MP\_GUT BY Enviroment WITH LN\_EncConcMP\_c

/METHOD = SSTYPE(3)

/INTERCEPT = INCLUDE

/PRINT = PARAMETER ETASQ OPOWER

/DESIGN = LN\_EncConcMP\_c Enviroment LN\_EncConcMP\_c\*Enviroment.

#case 2: interaction is significant

UNIANOVA ZBC\_MP\_GUT BY Enviroment WITH LN\_EncConcMP\_c

/METHOD = SSTYPE(3)

/INTERCEPT = INCLUDE

/PRINT = PARAMETER ETASQ OPOWER

/EMMEANS = TABLES(Enviroment) WITH(LN\_EncConcMP\_c=0) COMPARE ADJ(BONFERRONI)

/DESIGN = LN\_EncConcMP\_c Enviroment.

#case 3: slope homogenity

COMPUTE LN\_EncConcMP\_c = LN\_EncConcMP - MEAN(LN\_EncConcMP).

COMPUTE Int\_X\_Group = LN\_EncConcMP\_c \* Enviroment.

EXECUTE.

REGRESSION

/DEPENDENT ZBC\_MP\_GUT

/METHOD=ENTER LN\_EncConcMP\_c GroupM Int\_X\_Group

/STATISTICS COEFF R ANOVA CI(95).

#ANOVA BOOTSTRAP

BOOTSTRAP

/SAMPLING METHOD=SIMPLE

/VARIABLES TARGET=BC\_MP\_fish BC\_MP\_GUT BC\_MP\_flesh BC\_MP\_gills BC\_Env\_conc INPUT=Env\_M

/CRITERIA CILEVEL=95 CITYPE=PERCENTILE NSAMPLES=1000

/MISSING USERMISSING=EXCLUDE.

ONEWAY BC\_MP\_fish BC\_MP\_GUT BC\_MP\_flesh BC\_MP\_gills BC\_Env\_conc BY Env\_M

/STATISTICS DESCRIPTIVES HOMOGENEITY BROWNFORSYTHE

/MISSING ANALYSIS

/POSTHOC=BONFERRONI ALPHA(0.05).