Soil redox potential as a predictor of endobenthos species composition in an intertidal zone

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Abstract

Soil redox potential measurements can be done without much physical effort in a short amount of time, whereas measuring endobenthos species diversity involves more effort and time. This study explored if soil redox potential can be used as a sole predictor of the abundance of endobenthos. Both variables were measured at an intertidal zone at Schiermonnikoog at a transect from salt marsh to mudflat. Inundation was expected to be influential in species composition, and to correct for this, species abundances and redox values were obtained from two depths at each site. It was found that redox potential alone cannot be used as predictor of endobenthos composition.

Introduction

Species composition is one of the first and most vital pieces of data a field-based ecological research needs to gather. Any way to reliably conclude the same information with less work will save researchers both time and resources. One piece of information that is easy to obtain is a soil its redox potential. Redox potential is not just a passive abiotic factor; it is a value of biological importance. Among others, redox potential can be a measure of oxygen in the soil, and it is known that plant roots can raise the oxygen level in the soil (and thus its redox potential) [4]. This positive correlation, however, is not always correct, because at least one other study finds a negative correlation [5]. As far as I know, using redox potential as a predictor of bethos species being present or not, has not yet been investigated.

As redox potential is positively correlated with soil oxygen concentration, and all animals need oxygen for respiration, I expect some benthos animals to respond to soil redox potential. The most likely candidates are animals that live in the soil and breathe through their skin, as these animals might choke from a too low oxygen concentration and will probably move to a habitat with a higher oxygen concentration instead. Additionally, it might be that microbenthos serving as prey aggregrates at a certain oxygen concentration, which might cause its predator to follow this distribution.

This study investigates if soil redox potential can be used as a sole predictor of benthos species abundance. Or: would it be that some species abundances are distributed normally around a certain redox potential?

Materials and method

This study was carried out at the intertidal zone of Schiermonnikoog. In the Southwest of this island, a 2400 meter long transect was set up, from salt marsh to mudflat. The elevations of the transect range from 270 cm to -80 cm NAP. All measurements were done at September 9th and 10th. At those days, inundation times of the sample sites ranged from from 1-80% of a tidal period.

Soil samples of 20 cm deep were taken at different distances. The top 5 cm was separated. Both parts of the soil sample were scored for species.

Redox values were measured by a potentiometer using 4 platinum-tip electrodes and a solution of KCl as a reference. The electrodes were put in at two depths: 2 cm and 10 cm, in this sequence. The potential read is the value that remained constant, when placing or changing the electrodes. The values read were transformed to use earth as a reference point, using the formula $V = 1.8847 \cdot V_{measured} - 53.201$.

In this study, the redox potential at 2 cm depth is coupled to the benthos species diversity at depth 0-5 cm, where the potential at 10 cm depth is coupled to the diversity of depth 5-20 cm.

Of all collected species, only species with at least 3 individuals at both depths were taken into account. The minimum value of 3 was chosen, because it is the minimum number to test for normality. The requirement for a species to be at two depths is to disrupt the effect of inundation, as there can be similar redox potentials for different inundation times.

For each species, a Shapiro-Wilk normality test was used to determine if abundance is distributed normally around a certain redox potential. This test is chosen, as it has the best power for a given significance [1].

The script to analyze the data is written in R and can be downloaded at `https://github.com/richelbilderbeek/EvoEcoResearchCourse2014`.

Results

The redox potentials measured can be seen in figure 1.

863 individuals of 18 different species were collected at the site (see figure 1). Of these species, 8 species had at least 3 individuals at both depths. Out of these 8 species, only 4 could be used, as not all sites had their redox potential measured. From the 4 species left, only the 2 species occurring at multiple redox potentials were analyzed. These two species were *Hydrobia ulvae* and *Nereis diversicolor*. Figure 2 shows the abundance of both species at different

Species name	Depth: 2 cm	Depth: 10 cm
Arenicola marina	12	1
Bathyporeia	2	0
Carcinus maenas	3	14
Cerastoderma edule	5	22
Crassostrea gigas	4	6
Eteone longa	0	5
Gammarus locusta	1	3
Hemigropsus takanoi	4	11
Heteromastus filliformis	1	13
Hydrobia ulvae	131	369
Lanice conchilega	2	31
Littorina littorea	11	51
Macoma balthica	0	31
Mytilus edulis	7	78
Nereis diversicolor	14	10
Nereis virens	3	0
Scoloplas armiger	1	16
Scrobicularia plana	1	0

Table 1: All 18 species and the number of individuals found per species per depth.R

Name	n	p	significance
Hydrobia ulvae	294	$<$ 2.2e-16	***
Nereis diversicolor	9	0.04965	*

Table 2: Shapiro-Wilk normality test of the species abundances on redox potential. n: number of individuals. p: chance the species abundances do not follow a normal distribution for a redox potential.

redox potentials. A Shapiro-Wilk normality test shows that both species have a significant probability of not following a normal distribution ($p_{Hydrobia} < 0.001$, $p_{Nereis} < 0.05$, see table 2 for exact values).

Discussion

This study makes a strong case that soil redox potential cannot be used to predict species abundances, for both *Hydrobia ulvae* and *Nereis diversicolor*.

As *Hydrobia ulvae* is an epibenthic grazer [2], it seems rather obvious that is not influenced by the oxygen level of the soil underneath it. Less obvious is that individuals were found in benthos 5 cm below the surface. This finding appears not to be an experimental error, as *Hydrobia ulvae* is found in deeper soil samples at multiple distances. It might be that the *Hydrobia ulvae* found

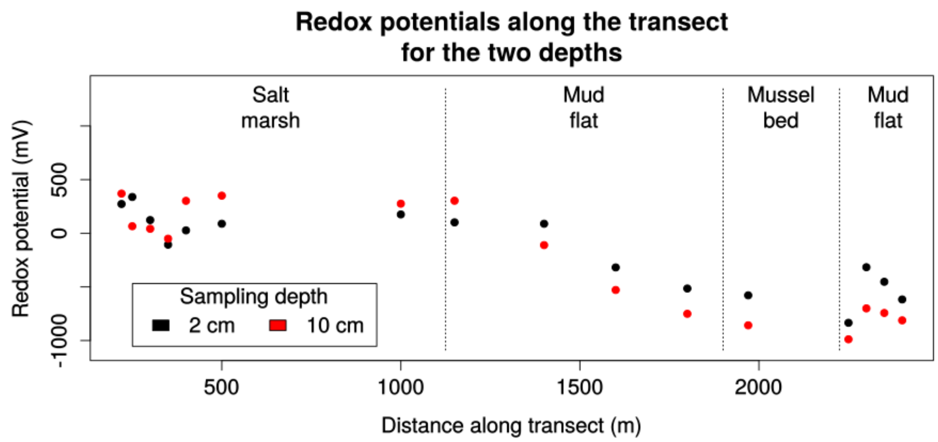

Figure 1: Redox potentials along the transect.

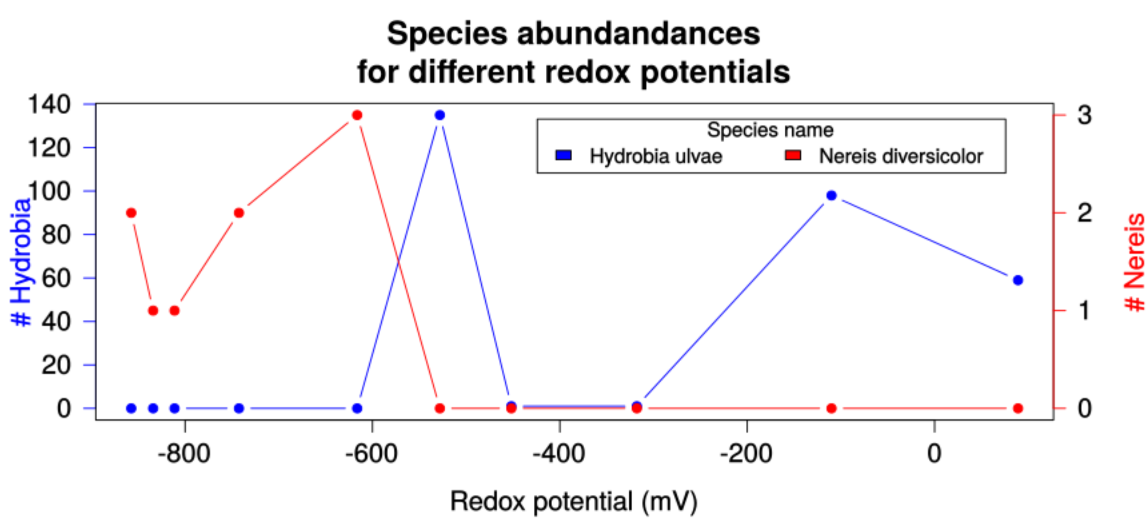

Figure 2: Number of individuals at the different redox potentials.

Distance	Orderedness
220	LTH
250	UNO
300	UNO
350	LTH
400	LTH
500	LTH
1000	UNO
1150	LTH
1400	HTL
1600	HTL
1800	HTL
1970	HTL
2250	HTL
2300	HTL
2350	HTL
2400	HTL

Table 3: The way the redox potentials are ordered when measuring a redox potential at 2, 5 and 10 cm in this order. LTH: low-to-high (the lowest redox potential was measured at 2cm deep, the heighest at 10 cm), HTL: high-to-low, UNO: unordered.

in the deeper soil were not individuals, but only the shells left.

Nereis diversicolor creates burrows in the mud and is a predator and scavanger [6]. As it does not live in the soil itself, nor does it feed on something in the soil itself, it is not surprising that also this species is unaffected by soil redox potential.

Coupling a redox potential at a single depth to species abundances at a range of depths may have been too much of a simplification. Redox potential changes when probing at different depths in the soil, but whether this change is monotonous was unknown. After drawing the conclusions, the change of redox potential was analyzed for its orderedness. Because in the experiments also the redox potential at 5 cm deep was measured, it could be tested if redox potential changes monotonously for depths 2, 5 and 10 cm. It was found that this was the case in 10 out of 13 locations (see table 3). Thus, in 3 out of 13 cases, the redox potential at intermediate depth was the heighest or lowest value measured at that location. There can be two explanations for this unexpected pattern: (1) the soil redox potential is a complex abiotic variable that does not follow a monotonic change, or (2) the noise in the redox measurement is higher than the change in 'true' redox potential between depths.

References

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[3] Rauschenplat, E (1901). Ueber die Nahrung von Thierer aus der Kielerbucht. Wiss. Meeresunters. 5(2):85-151.

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[5] Dong, B; Han, R; Wang, G; Cao, X (2014). O2, pH, and Redox Potential Microprofiles around *Potamogeton malaianus* Measured Using Microsensors. PLoS ONE 9(7): e101825. doi:10.1371/journal.pone.0101825

[6] Witte, F; Wilde, de, PAWJ (1979). On the ecological relation between *Nereis diversicolor* and juvenile *Arenicola marina*. Netherlands Journal of Sea Research 13(3/4): 394-405

Appendix

```
# Manuscript, script to generate figures and tables for the Evolution & Ecology
    Research course 2014
# Copyright (C) 2014 Richel Bilderbeek
#
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# along with this program. If not, see <http://www.gnu.org/licenses/>.
#
# File can be downloaded from https://github.com/richelbilderbeek/
    EvoEcoResearchCourse2014
#
# Research question: Can I predict where species are from a redox potential?
# Experiment:
# * Couple shallow (0-5 cm) benthos abundances with redox measurement of 2 cm
# * Couple deep (5-20 cm) benthos abundances with redox measurement of 10 cm
# Answer:
# * No

rm(list = ls())
setwd("~/GitHubs/EvoEcoResearchCourse2014")
library(reshape2)
library(testit)

# Create benthos data as such:
#  dist_m depth_cm species_name
#  2350   2        Bathyporeia_spec.
#  2400   10       Nereis_diversicolor
# Removes species at unknown depths
CreateDataBenthos <- function()
{
  data_benthos <- read.table("benthos_species_diversity.csv",header=TRUE,sep=",")
  #Remove useless columns
  data_benthos <-data_benthos[ c("dist_m","soil_layer","species_name") ]
```

```
  # Only keep rows with 'D' or 'S' (for deep and shallow/top part of the core)
  data_benthos<-data_benthos[data_benthos$soil_layer != "U" &
       data_benthos$soil_layer != "?", ]
  # Drop the unneeded levels
  data_benthos$soil_layer <- droplevels(data_benthos$soil_layer)
  #Of data_benthod, change the column 'soil_layer' to 'depth_cm':
  # if soil_layer == "S" -> depth_cm = 2
  # if soil_layer == "D" -> depth_cm = 10
  names(data_benthos) <- c("dist_m","depth_cm","species_name")
  data_benthos$depth_cm <- as.factor(data_benthos$depth_cm)
  # Rename the levels
  levels(data_benthos$depth_cm) <- c(2,10)
  data_benthos
}

# Count the species occurring at two depths
# species_name       2  10
# Arenicola_marina  12  1
# Bathyporeia_spec. 2   0
GetSpeciesCountAtDepths <- function()
{
  species_to_depth <- dcast(
    CreateDataBenthos(),
    species_name ~ depth_cm,
    value.var = "species_name",
    fill = 0,
    fun.aggregate=length # fun.aggregate may also be mean
  )
  species_to_depth
}

# Obtain the species names occuring at least thrice in both of the two depths in the
     soil
# Will be this:
# [1] Carcinus_maenas      Cerastoderma_edule   Crassostrea_gigas
    Hemigropsus_takanoi Hydrobia_ulvae
# [6] Littorina_littorea   Mytilus_edulis       Nereis_diversicolor
# 8 Levels: Carcinus_maenas Cerastoderma_edule Crassostrea_gigas Hemigropsus_takanoi
     ... Nereis_diversicolor
GetSelectedSpecies <- function()
{
  species_to_depth <- GetSpeciesCountAtDepths()
  selected_species_to_depth <- subset(species_to_depth,
      species_to_depth$"2" > 2 & species_to_depth$"10" > 2)
  selected_species_to_depth <- droplevels(selected_species_to_depth)
  # Collect all species' names
  species_list <- levels(selected_species_to_depth$species_name)
  selected_species <- selected_species_to_depth$species_name
  selected_species <- droplevels(selected_species)
  selected_species
}

GetDataBenthosSelected <- function()
{
  data_benthos <- CreateDataBenthos()
  selected_species <- GetSelectedSpecies()
  #data_benthos_selected <- data_benthos[ data_benthos$species_name %in%
       selected_species ,]
  data_benthos_selected <- data_benthos[ data_benthos$species_name %in%
       GetSelectedSpecies() ,]
  data_benthos_selected
}

# Prepare the redox data
# dist_m depth_cm replicate redox_calib
# 220    2        1         304.89200
# 220    2        2         302.63036
CreateDataRedox <- function()
{
  data_redox <- read.table("Redox.csv",header=TRUE,sep="\t")
  data_redox <- subset(data_redox,depth_cm != 5)
  data_redox
  #Remove the replicate column
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  # Take the average redox values, so that every distance and depth has a single
       redox value
  data_redox <- aggregate(data_redox,list(data_redox$depth_cm,data_redox$dist_m),
       mean)
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  data_redox
}

CreateDataRedoxAll <- function()
{
  data_redox <- read.table("Redox.csv",header=TRUE,sep="\t")
  #Remove the replicate column
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  # Take the average redox values, so that every distance and depth has a single
       redox value
  data_redox <- aggregate(data_redox,list(data_redox$depth_cm,data_redox$dist_m),
       mean)
```

```
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  data_redox
}

GetRedoxPerDistance <- function()
{
  data_redox <- read.table("Redox.csv",header=TRUE,sep="\t")
  #Remove the replicate column
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  # Take the average redox values, so that every distance and depth has a single
      redox value
  data_redox <- aggregate(data_redox,list(data_redox$depth_cm,data_redox$dist_m),
      mean)
  data_redox <- data_redox[c("dist_m","depth_cm","redox_calib") ]
  data_redox
}

# Get the 32 redox values used in this study
# [1] -987.68238 -857.44961 -834.07933 -811.22734 -750.16306 -742.48291 -699.70022
    -616.30224 -577.85436 -528.05116
# ...
# [31]  350.83156  370.14974
GetRedoxValues <- function()
{
  redox_values <- subset(CreateDataRedox(),select="redox_calib")
  redox_values <- unique(redox_values)
  redox_values <- sort(redox_values$redox_calib)
  redox_values
}

# Obtain the distances of all redox sites
#  [1]  220  250  300  350  400  500 1000 1150 1400 1600 1800 1970 2250 2300 2350
    2400
GetDistances <- function()
{
  distances <- subset(CreateDataRedox(),select="dist_m")
  distances <- unique(distances)
  distances <- sort(distances$dist_m)
  distances
}

# Get all species per redox potential
# redox_calib   species_name
# -78.26751 Hydrobia_ulvae
# -78.26751 Hydrobia_ulvae
GetDataCombined <- function()
{
  data_combined <- merge(CreateDataRedox(),GetDataBenthosSelected(),by=c("dist_m","
      depth_cm"),all=FALSE)
  data_combined$species_name <- droplevels(data_combined$species_name)
  data_combined <- data_combined[ c("redox_calib","species_name") ]
  data_combined
}

# Get the redox potentials of Hydrobia
# [1] -78.26751 -78.26751 -78.26751 -78.26751 -78.26751 -78.26751 -78.26751
    -78.26751 -78.26751 -78.26751
GetRedoxesHydrobia <- function()
{
  redoxes_hydrobia <- subset(GetDataCombined(),species_name == "Hydrobia_ulvae")
  redoxes_hydrobia <- subset(redoxes_hydrobia,select=c("redox_calib"))
  redoxes_hydrobia
}

GetRedoxesNereis <- function()
{
  redoxes_nereis <- subset(GetDataCombined(),species_name == "Nereis_diversicolor")
  redoxes_nereis <- subset(redoxes_nereis,select=c("redox_calib"))
  redoxes_nereis
}

TallySpeciesPerRedox <- function()
{
  redox_to_species <- dcast(
    GetDataCombined(),
    redox_calib ~ species_name,
    value.var = "species_name",
    fill = 0,
    fun.aggregate=length # fun.aggregate may also be mean
  )
  redox_calib <- redox_to_species$redox_calib
  # Note that not all species are present anymore that often. This is due to that
      not all distances are redoxed
  redox_to_species
}

TallySelectedSpeciesPerRedox <- function()
{
  redox_to_species <- TallySpeciesPerRedox()
  redox_to_species <- redox_to_species[, colSums(redox_to_species) > 6]
  redox_to_species <- cbind(TallySpeciesPerRedox(),redox_to_species)
```

```
  redox_to_species <- redox_to_species[, c("redox_calib","Hydrobia_ulvae","
       Nereis_diversicolor")]
  redox_to_species
}

# Can the redox potentials at a certain distane be assumed to be linear at a certain
     depth?
CalcOrderednessPerDistance <- function()
{
  orderedness_per_distance <- data.frame(dist_m = numeric(),order = factor())
  for (i in GetDistances())
  {
    data <- subset(CreateDataRedoxAll(),dist_m == i)
    low <- subset(data,depth_cm == 2)$redox_calib
    mid <- subset(data,depth_cm == 5)$redox_calib
    high <- subset(data,depth_cm == 10)$redox_calib
    if (low < mid && mid < high)
    {
      orderedness_per_distance <- rbind(orderedness_per_distance,data.frame(dist_m =
           i,order = "LTH"))
    } else if(low > mid && mid > high)
    {
      orderedness_per_distance <- rbind(orderedness_per_distance,data.frame(dist_m =
           i, order = "HTL"))
    } else  {
      orderedness_per_distance <- rbind(orderedness_per_distance,data.frame(dist_m =
           i, order = "UNO"))
    }
  }
  orderedness_per_distance
}

assert("CreateDataBenthos: 863 individuals were scored at known depths",length(
     CreateDataBenthos()$dist_m) == 863)
assert("GetSpeciesCountAtDepths: All 863 individuals must be seperated correctly at
     their depths",length(CreateDataBenthos()$dist_m) == sum(GetSpeciesCountAtDepths
     ()$"2") + sum(GetSpeciesCountAtDepths()$"10"))
assert("GetSpeciesCountAtDepths: 20 species were scored at all depths",length(
     GetSpeciesCountAtDepths()$species_name) == 20)
assert("GetSelectedSpecies: 8 species were found at both depths, at each depth
     occurring at least thrice",length(GetSelectedSpecies()) == 8)
assert("GetDataBenthosSelected: 740 individuals of the 8 selected species were
     scored at known depths",length(GetDataBenthosSelected()$species_name) == 740)
assert("CreateDataRedox must only consider depths of 2 and 10 cm",length(subset(
     CreateDataRedox(),depth_cm != 2 & depth_cm != 10)$depth_cm) == 0)
assert("GetRedoxValues: 32 redox potentials are investigated",length(GetRedoxValues
     ()) == 32)
assert("GetDistances: 16 distances are investigated",length(GetDistances()) == 16)
assert("GetDataCombined: needs to be 1176 Hydrobia",length(subset(GetDataCombined(),
     species_name == "Hydrobia_ulvae")$species_name) == 294)
assert("GetDataCombined: needs to be 36 Nereis diversicolor",length(subset(
     GetDataCombined(),species_name == "Nereis_diversicolor")$species_name) == 9)
assert("GetRedoxesHydrobia: must be 294 values",length(GetRedoxesHydrobia()
     $redox_calib) == 294)
assert("GetRedoxesNereis: must be 9 values",length(GetRedoxesNereis()$redox_calib)
     == 9)
assert("TallySpeciesPerRedox: must be 9 columns (redoxes and each species its
     frequency",length(TallySpeciesPerRedox()) == 9)
assert("TallySelectedSpeciesPerRedox: must be 3 columns (redoxes, Hydrobia and
     Nereis",length(TallySelectedSpeciesPerRedox()) == 3)
assert("CalcOrderednessPerDistance: must have 16 distances",length(
     CalcOrderednessPerDistance()$dist_m) == 16)

write.csv(GetSpeciesCountAtDepths(),file="table_species_count_at_depth.csv")
write.csv(GetSelectedSpecies(),file="table_selected_species.csv")
write.csv(CalcOrderednessPerDistance(),file="table_orderedness_per_distance.csv")

# Do statistics per species
shapiro.test(GetRedoxesHydrobia()$redox_calib)
# Shapiro-Wilk normality test
#
# data:  redoxes_hydrobia$redox_calib
# W = 0.7567, p-value < 2.2e-16

shapiro.test(GetRedoxesNereis()$redox_calib)
# Shapiro-Wilk normality test
#
# data:  redoxes_nereis$redox_calib
# W = 0.8341, p-value = 0.04965

write.csv(TallySpeciesPerRedox(),file="table_redox_to_species.csv")
write.csv(TallySelectedSpeciesPerRedox(),file="table_redox_to_selected_species.csv")

# Generate figure for species abundances for the range of redox potentials
#   in two vertically aligned plots
par(mfrow=c(2,1))
plot(
  Hydrobia_ulvae ~ redox_calib,
  data = TallySpeciesPerRedox(),
  t = "b",
  pch = 19,
```

```
  col = "black",
  main = "Hydrobia ulvae abundance",
  xlab = "Redox potential (mV)",
  ylab = "Number of individuals"
)
plot(
  Nereis_diversicolor ~ redox_calib,
  data = TallySpeciesPerRedox(),
  t = "b",
  pch = 19,
  col = "black",
  main = "Nereis diversicolor abundance",
  xlab = "Redox potential (mV)",
  ylab = "Number of individuals",
  ylim = c(0,4)
)
par(mfrow=c(1,1))

# Generate figure for species abundances for the range of redox potentials
#   in the same plot
par(mar = c(5, 4, 4, 4) + 0.3)  # Leave space for z axis
plot(Hydrobia_ulvae ~ redox_calib, data = TallySpeciesPerRedox(), pch=19, axes=FALSE
     , xlab="Redox potential (mV)", ylab="",
   type="b",col="blue", main="Species abundandances\nfor different redox potentials"
)
axis(1, col="black",las=1) #'las=1' align labels horizontally
axis(2, col="blue",las=1) #'las=1' align labels horizontally
mtext("# Hydrobia",side=2,line=2.5,col="blue")
box()
par(new = TRUE) # Prevents R from clearing the area
plot(
  Nereis_diversicolor ~ redox_calib, axes = FALSE, bty = "n", xlab = "", ylab = "",
       data = TallySpeciesPerRedox(),
  type="b",
  col = "red",
  yaxt="n",
  pch = 19
)
axis(
  side=4,
  at=seq(0,3,1), #Otherwise, 0.5 would be shown as a tick mark
  col="red",
  las=1 #Align labels horizontally
)
mtext("# Nereis", side=4, line=3,col="red")
legend("topright",
  inset=0.05,title = "Species name", c("Hydrobia ulvae", "Nereis diversicolor"),
       horiz=TRUE,
  fill=c("blue","red"),
  cex = 0.75
)

# Redox potentials along the transect for the two depths
dist_to_redox <- subset(CreateDataRedox(), CreateDataRedox()$dist_m %in%
     GetDistances())
y_min <- min(dist_to_redox$redox_calib) - 100
y_max <- max(dist_to_redox$redox_calib) + 1000
y_text <- y_max - 200
plot(
  dist_to_redox$redox_calib ~ dist_to_redox$dist_m,
  col = as.factor(dist_to_redox$depth_cm),
  pch = 19,
  main="Redox potentials along the transect\nfor the two depths",
  xlab="Distance along transect (m)",
  ylab="Redox potential (mV)",
  ylim=c(y_min,y_max)
)
segments(1125,y_min,1125,y_max,col="black",lty=3)
segments(1900,y_min,1900,y_max,col="black",lty=3)
segments(2225,y_min,2225,y_max,col="black",lty=3)
text((1125 + 150) / 2,y_text,"Salt\nmarsh")
text((1900 + 1125) / 2,y_text,"Mud\nflat")
text((2225 + 1900) / 2,y_text,"Mussel\nbed")
text(-50 + ((2600 + 2225) / 2),y_text,"Mud\nflat")
legend("bottomleft",
  inset=0.05,title = "Sampling depth", c("2 cm", "10 cm"),horiz=TRUE,
  fill=c(1,2,3), cex = 1.0
)
rm(dist_to_redox)
rm(y_min)
rm(y_max)
rm(y_text)
```