

Cyanobacterial crowding-out effects on metabolite partitioning: modeling 2-methylisoborneol (MIB) release dynamics and implications

Supplementary Information

Yufan Ai^{a,d,#}

Yongnian Wu^{b,#}

Ming Su^{a,d,*}

Yuying Gui^{a,c}

Yifan Du^a

Jiao Fang^a

Tengxin Cao^{a,d}

Min Yang^{a,d}

These authors contributed equally to this work.

^a Key Laboratory of Environmental Aquatic Chemistry, State Key Laboratory of Regional Environment and Sustainability, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences., Beijing 100085, China.

^b Bureau of Hydrology Information Center of Taihu Basin Authority, Shanghai 200434, China.

^c College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China.

^d University of Chinese Academy of Sciences., Beijing 100049, China.

* Corresponding to: [Ming Su \(mingsu@rcees.ac.cn\)](mailto:mingsu@rcees.ac.cn)

Table of contents

Fig. S1	1
Fig. S2	2
Fig. S3	3
Fig. S4	4
Fig. S5	5
Fig. S6	6
Fig. S7	7
Fig. S8	8
Fig. S9	9
Fig. S10	10
Fig. S11	11
Fig. S12	12
Table S1	13
Table S2	14
Table S3	15
Code	16
References	27

12 figures and 3 tables provided below for the evidence of main text.

Fig. S1

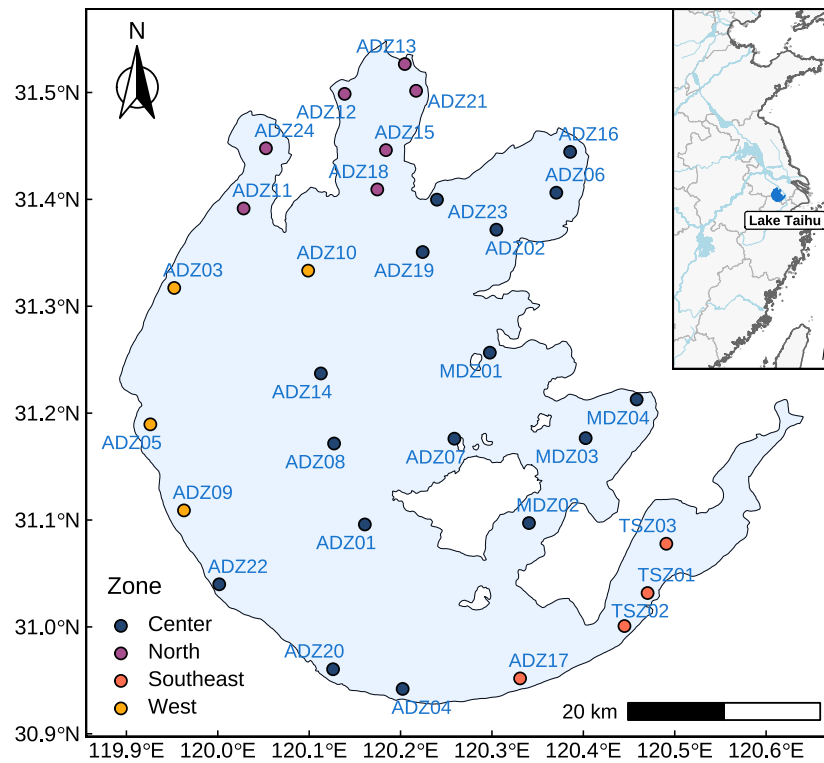


Fig. S1: Spatial clustering map of sampling sites in Lake Taihu used in this study.

Fig. S2

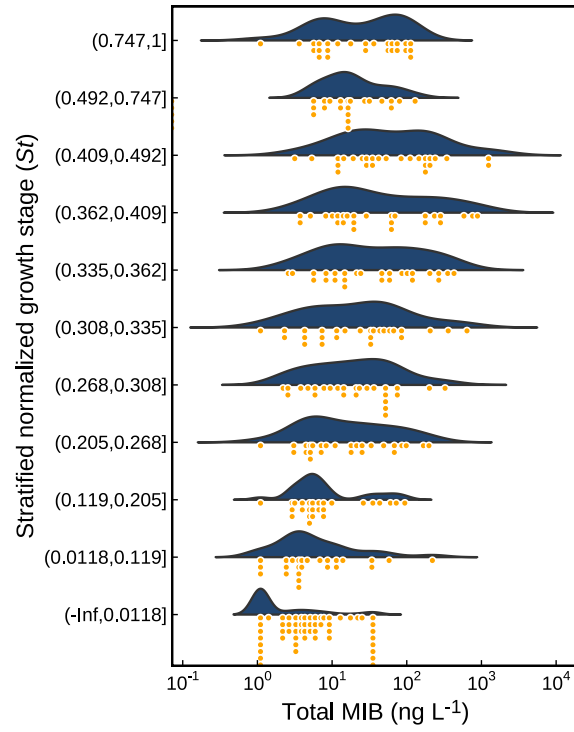


Fig. S2: Frequency distribution of modeled St and t_{MIB} values for odor-producing cyanobacteria in Lake Taihu.

Fig. S3

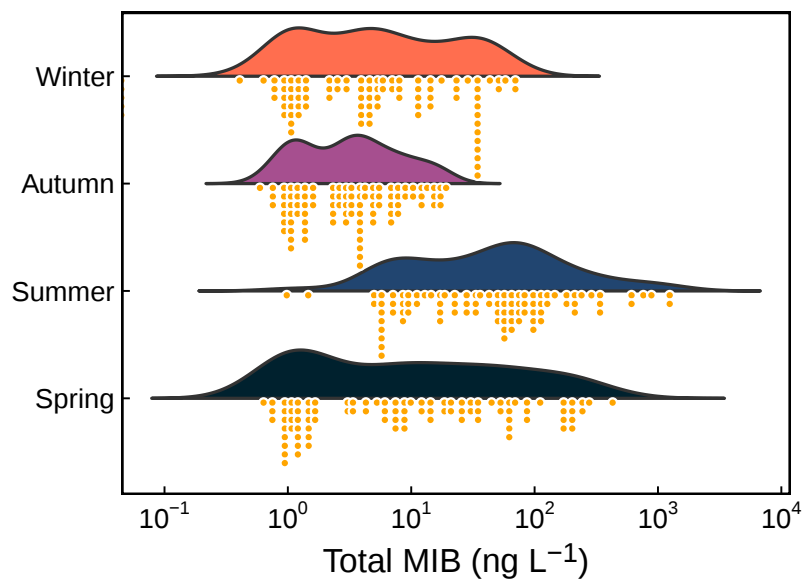


Fig. S3: Seasonal distribution of total MIB concentration in Lake Taihu.

Fig. S4

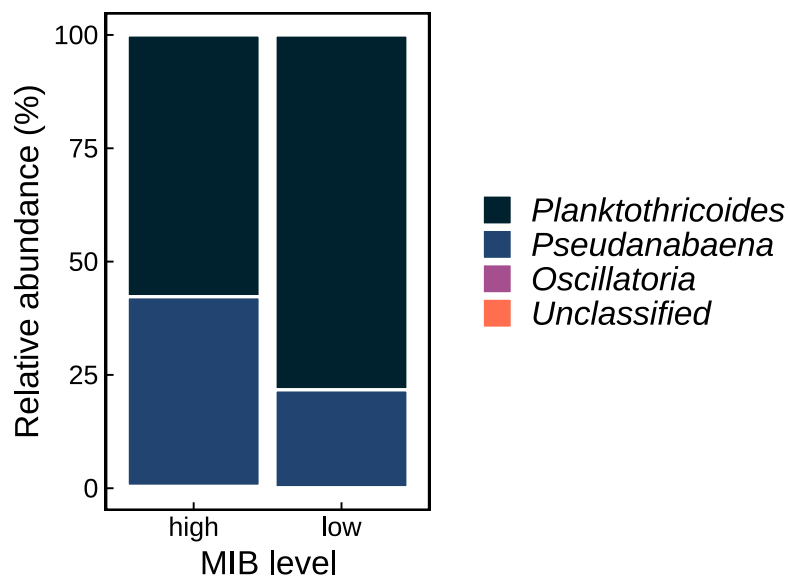


Fig. S4: Concurrent 16S RNA gene sequencing identified *Pseudanabaena* and *Planktothricoides* as the dominant MIB-producing genera.

Fig. S5

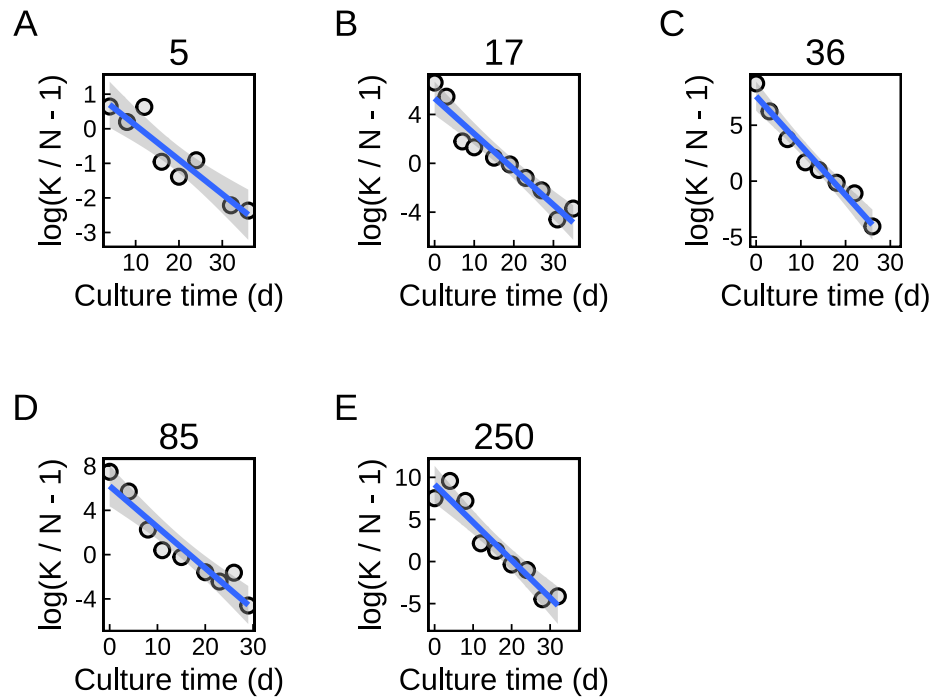


Fig. S5: Logistic fit of *Pseudanabaena* growth under various light conditions (A: 5 $\text{mol m}^{-2} \text{s}^{-1}$; B: 17 $\text{mol m}^{-2} \text{s}^{-1}$; C: 36 $\text{mol m}^{-2} \text{s}^{-1}$; D: 85 $\text{mol m}^{-2} \text{s}^{-1}$; E: 250 $\text{mol m}^{-2} \text{s}^{-1}$).

Fig. S6

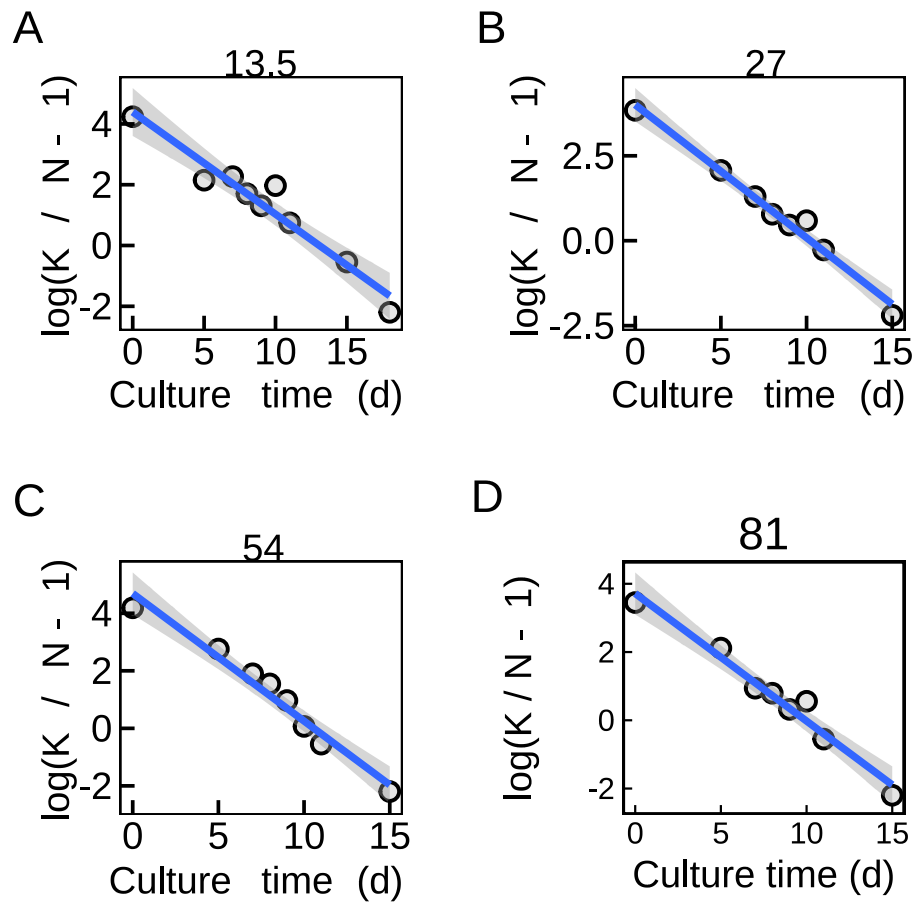


Fig. S6: Logistic fit of *Planktothricoides* growth under various light conditions (A: 13.5 mol m⁻² s⁻¹; B: 27 mol m⁻² s⁻¹; C: 54 mol m⁻² s⁻¹; D: 81 mol m⁻² s⁻¹).

Fig. S7

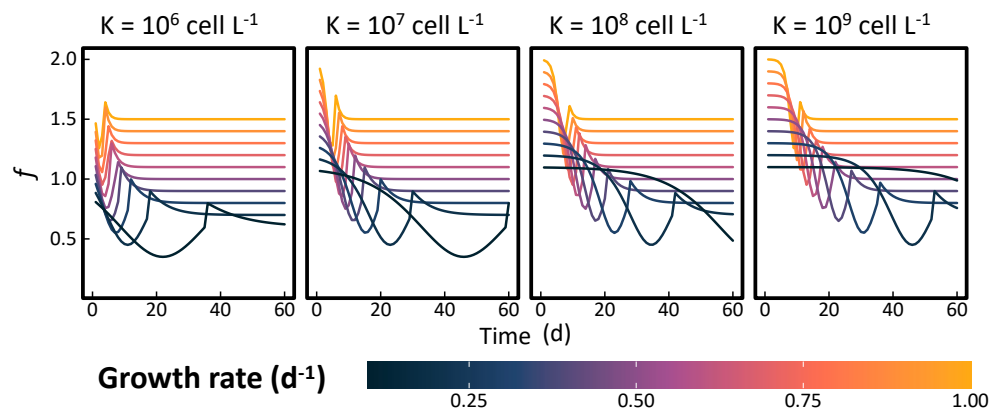


Fig. S7: Fitted curves of the extracellular-to-total MIB ratio (f) under low, medium, and high cyanobacterial growth rates. Note that the y-axis values (f) represent ridge line means vertically offset for clarity.

Fig. S8

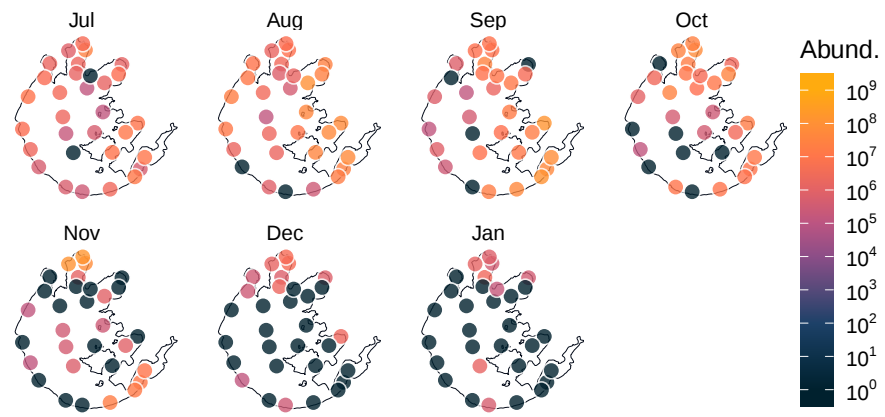


Fig. S8: Temporal and spatial distribution of *Pseudanbaena* in Lake Taihu.

Fig. S9

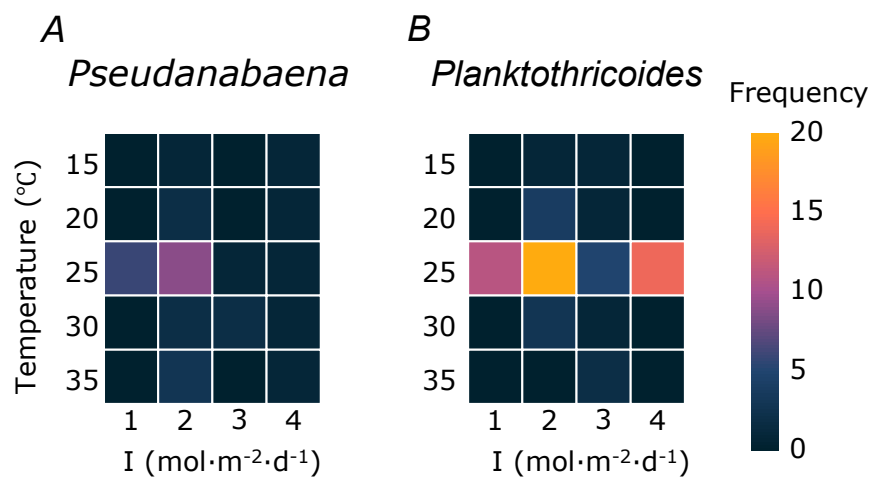


Fig. S9: Studies in other literature involving the cultivation of *Pseudanabaena* and *Planktothricoides*.

Fig. S10

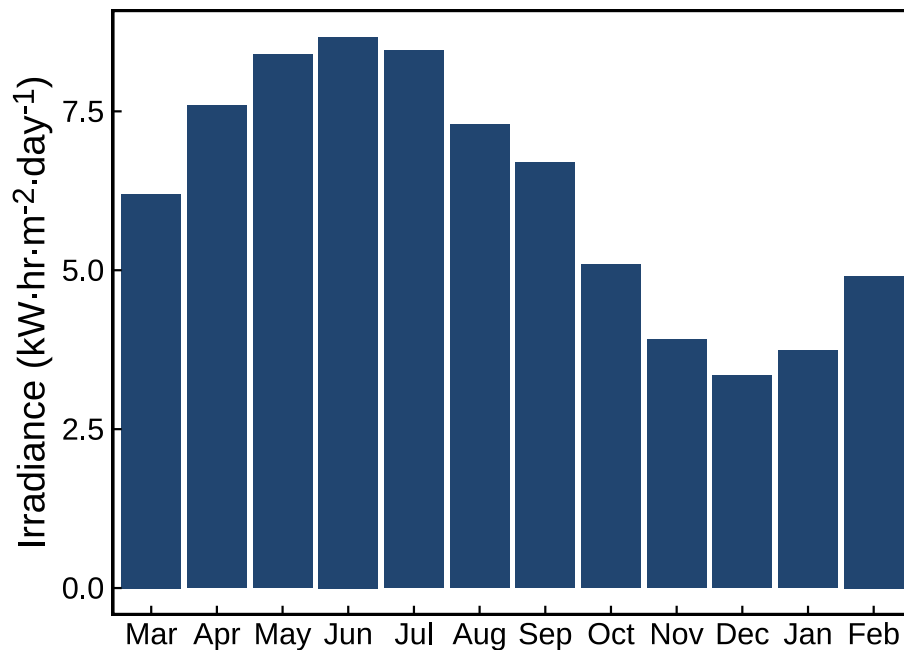


Fig. S10: Surface Shortwave Downward Irradiance in the Lake Taihu region from March 2022 to February 2023 (Data source: NASA POWER, <https://power.larc.nasa.gov/>).

Fig. S11

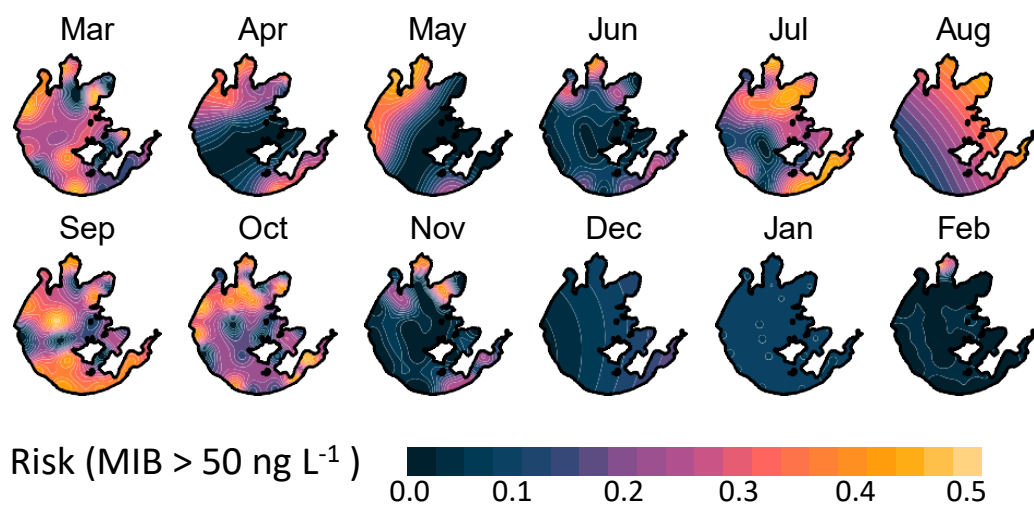


Fig. S11: Odor risk assessment across the entire Lake Taihu (probability of $t_{\text{MIB}} > 50 \text{ ng L}^{-1}$).

Fig. S12

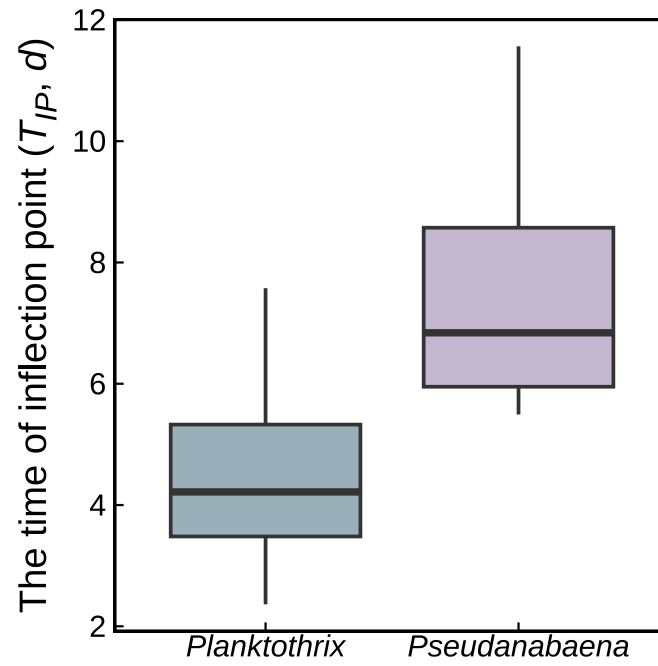


Fig. S12: Boxplot comparison of inflection point times (T_{IP}) for *Pseudanabaena* and *Planktothricoides*.

Table S1

Genus	Strain	Ref.
<i>Planktothricoides</i>	<i>Planktothricoides raciborskii</i>	(Chang et al. 2012)
<i>Planktothricoides</i>	<i>Planktothricoides raciborskii</i>	(Duong et al. 2021)
<i>Planktothricoides</i>	<i>Planktothricoides raciborskii</i>	(Lu et al. 2022)
<i>Planktothricoides</i>	<i>Planktothricoides raciborskii</i>	(Tan et al. 2022)
<i>Planktothricoides</i>	<i>Planktothricoides raciborskii</i> 1372	(Jianguo et al. 2022)
<i>Planktothricoides</i>	<i>Planktothricoides</i> sp.	(Mohanty et al. 2022)
<i>Planktothricoides</i>	<i>Planktothricoides</i> sp. SR001	(Te et al. 2017)
<i>Pseudanabaena</i>	<i>Pseudanabaena cinerea</i> FACHB1277	(Cao et al. 2023)
<i>Pseudanabaena</i>	<i>Pseudanabaena cinerea</i> FACHB1278	(Su et al. 2022)
<i>Pseudanabaena</i>	<i>Pseudanabaena cinerea</i> FACHB1279	(Su et al. 2023)
<i>Pseudanabaena</i>	<i>Pseudanabaena cinerea</i> FACHB1280	(Zhang et al. 2016)
<i>Pseudanabaena</i>	<i>Pseudanabaena galeata</i>	(Muhetaer et al. 2020)
<i>Pseudanabaena</i>	<i>Pseudanabaena</i> sp.	(Xiao-Qin Wang and Qiu 2015)
<i>Pseudanabaena</i>	<i>Pseudanabaena</i> sp.	(Mishra et al. 2012)
<i>Pseudanabaena</i>	<i>Pseudanabaena</i> sp. dqh15	(Wang and Li 2015)
<i>Pseudanabaena</i>	<i>Pseudanabaena</i> sp. TH-1	(Hu et al. 2023)
<i>Pseudanabaena</i>	<i>Pseudanabaena</i> sp. Xili	(Gao et al. 2018)

Table S1: Studies in other literature involving the cultivation of *Pseudanabaena* and *Planktothricoides*.

Table S2

Comparison	t_{MIB}	e_{MIB}	i_{MIB}	f
Autumn vs Spring	1.0000	0.0879	1.0000	1.0000
Autumn vs Summer	0.0003 ^{***}	<0.0001 ^{***}	0.0104 [*]	1.0000
Spring vs Summer	0.0081 ^{**}	0.0398 [*]	0.0599	1.0000
Autumn vs Winter	0.0022 ^{**}	0.7217	<0.0001 ^{***}	0.0000 ^{***}
Spring vs Winter	<0.0001 ^{***}	0.0024 ^{**}	<0.0001 ^{***}	0.0000 ^{***}
Summer vs Winter	<0.0001 ^{***}	<0.0001 ^{***}	<0.0001 ^{***}	0.0000 ^{***}

Table S2: Statistical comparisons between seasons for various metrics. t_{MIB} : total MIB; e_{MIB} : extracellular MIB; i_{MIB} : intracellular MIB; f : ratio of e_{MIB} and t_{MIB} ($f = e_{\text{MIB}}/t_{\text{MIB}}$).

Table S3

Season	Mean	SD	First quartile	Median	Third quartile
Spring	43.0	77.5	3.16	8.23	33.5
Summer	107	230	6.78	25.9	80.8
Autumn	23.7	47.7	2.80	6.54	17.57
Winter	9.93	16.6	1.10	2.42	5.34

Table S3: Statistical Summary of total MIB in Different Seasons (Total n = 372). t_{MIB} : total MIB.

Code

```
require(tidyverse)
require(lubridate)
require(patchwork)
require(drwaterR)
require(grid)
require(ggsci)
require(sf)

logistic_model <- function(
  culturedf,
  main = NULL,
  xlab = "Culture time (d)",
  ylab = "Cell density (cell L<sup>-1</sup>)",
  Kcoef = 0.99,
  N00 = 5e5,
  check = FALSE,
  plot = FALSE,
  returnfitdf = TRUE,
  fitdf_x = seq(
    min(culturedf$julian),
    1.2 * max(culturedf$julian),
    length.out = 100
  )
) {
  K <- max(culturedf$n) / Kcoef
```

```

culturedf <- culturedf |>
  dplyr::mutate(z = log(K / n - 1))
out <- list()
out[["culturedf"]] <- culturedf
out[["K"]] <- K
m <- lm(z ~ julian, data = culturedf)
r <- -unname(coef(m)[2])
tstar <- unname(coef(m)[1] / r) #tstar = log(a)/r
# N0 <- K - K / (1 + exp(-r * tstar))
N0 <- K / (1 + exp(r * tstar))
t00 <- tstar - log(K / N00 - 1) / r
r2 <- summary(m)$r.squared
pval <- summary(m)$coefficients[8]
out[["m"]] <- m
out[["r"]] <- r
out[["tstar"]] <- tstar
out[["N0"]] <- N0
out[["r2"]] <- r2
out[["pval"]] <- pval
out[["t00"]] <- t00
out[["N00"]] <- N00
if (isTRUE(check)) {
  p1 <- culturedf |>
    ggplot(aes(julian, z)) +
      geom_point() +
      geom_smooth(method = "lm") +
      labs(x = xlab, y = "log(K / N - 1)", fill = NULL, title = main) +

```

```

      dwfun::theme_sci()
    out[["p1"]] <- p1
  }
  if (isTRUE(returnfitdf)) {
    fitdf <- tibble::tibble(julian = fitdf_x) |>
      dplyr::mutate(n = K / (1 + exp(-r * (julian - tstar))))
    out[["fitdf"]] <- fitdf
  }
  if (isTRUE(plot)) {
    p2 <- fitdf |>
      ggplot(aes(julian, n)) +
      geom_point(data = culturedf, aes(y = n)) +
      geom_line() +
      labs(x = xlab, y = ylab, fill = NULL, title = main) +
      dwfun::theme_sci()
    out[["p2"]] <- p2
  }
  return(out)
}

culturemdf <- readRDS("culturedf.rds") |>
  tidyr::nest(idf = -c(strain, ppf)) |>
  tidyr::expand_grid(Kcoef = c(0.90, 0.95, 0.99, 1.05, 1.1)) |>
  dplyr::mutate(
    model = purrr::pmap(
      list(idf, ppf, Kcoef),
      \(x, I, Kcoef) {

```

```

logistic_model(
  culturedf = x |>
    dplyr::filter(valid == "GOOD") |>
    dplyr::filter(!is.na(n)),
  Kcoef = Kcoef,
  main = I,
  check = TRUE,
  plot = TRUE,
  returnfitdf = TRUE,
  fitdf_x = 0:40
)
}
)
) |>
dplyr::mutate(culturedf = purrr::map(model, \(x) x[["culturedf"]])) |>
dplyr::mutate(m = purrr::map(model, \(x) x[["m"]])) |>
dplyr::mutate(K = purrr::map_dbl(model, \(x) x[["K"]])) |>
dplyr::mutate(r = purrr::map_dbl(model, \(x) x[["r"]])) |>
dplyr::mutate(N0 = purrr::map_dbl(model, \(x) x[["N0"]])) |>
dplyr::mutate(r2 = purrr::map_dbl(model, \(x) x[["r2"]])) |>
dplyr::mutate(pval = purrr::map_dbl(model, \(x) x[["pval"]])) |>
dplyr::mutate(tstar = purrr::map_dbl(model, \(x) x[["tstar"]])) |>
dplyr::mutate(p1 = purrr::map(model, \(x) x[["p1"]])) |>
dplyr::mutate(p2 = purrr::map(model, \(x) x[["p2"]])) |>
dplyr::mutate(fitdf = purrr::map(model, \(x) x[["fitdf"]])) |>
dplyr::group_by(strain, ppf) |>
dplyr::slice_max(r2, n = 1) |>

```

```

dplyr::arrange(strain, ppf)

Kprop <- 0.8
mdf <- culturemdf |>
  unnest(culturedf) |>
  dplyr::mutate(a = (K - N0) / N0) |>
  dplyr::mutate(
    x1 = exp(r * julian) *
      a *
      log(2) /
      (exp(2 * r * julian) + exp(r * julian) * 2 * a + a^2)
  ) |>
  dplyr::mutate(x2 = (K - n) / K) |>
  dplyr::mutate(
    stage = factor(
      n < K * Kprop,
      levels = c("TRUE", "FALSE"),
      labels = c("<80%K", "≥80%K")
    )
  ) |>
  tidyr::nest(
    idf = -c(
      strain,
      ppf,
      stage,
      Kcoef,
      model,

```

```

      m,
      r,
      tstar,
      N0,
      r2,
      pval,
      p1,
      p2,
      fitdf
    )
  ) |>
tidyr::pivot_wider(names_from = stage, values_from = idf) |>
dplyr::mutate(
  mb = purrr::map(`<80%K`, \(x) {
    x |>
      dplyr::mutate(y = d2t - 1) |>
      lm(formula = y ~ x1 + 0, data = _)
  })
) |>
dplyr::mutate(
  mc = purrr::map(`≥80%K`, \(x) {
    x |>
      lm(formula = d2t ~ x2, data = _)
  })
) |>
dplyr::mutate(b = -purrr::map_dbl(mb, \(x) coef(x)[1])) |>
dplyr::mutate(c = purrr::map_dbl(mc, \(x) coef(x)[2])) |>

```

```
dplyr::mutate(d = purrr::map_dbl(mc, \(x) coef(x)[1] - 0.5)) |>
dplyr::relocate(strain, ppf, Kcoef, r, N0, r2, pval, tstar, b, c, d, p1, p2)
```

```
Kprop <- 0.8
mdf <- culturemdf |>
tidyr::unnest(culturedf) |>
dplyr::mutate(a = (K - N0) / N0) |>
dplyr::mutate(
  x1 = exp(r * julian) *
    a *
    log(2) /
    (exp(2 * r * julian) +
      exp(r * julian) * 2 * a +
      a^2)
) |>
dplyr::mutate(x2 = (K - n) / K) |>
dplyr::mutate(
  stage = factor(
    n < K * Kprop,
    levels = c("TRUE", "FALSE"),
    labels = c("<80%K", "≥80%K")
  )
) |>
tidyr::nest(
  idf = -c(
    strain,
```

```

    ppf,
    stage,
    Kcoef,
    model,
    K,
    m,
    r,
    tstar,
    N0,
    deltaT,
    r2,
    pval,
    p1,
    p2,
    fitdf
  )
) |>
tidyr::pivot_wider(names_from = stage, values_from = idf) |>
dplyr::mutate(
  mb = purrr::map(`<80%K`, \(x) {
    x |>
      dplyr::mutate(y = d2t - 1) |>
      lm(formula = y ~ x1 + 0, data = _)
  })
) |>
dplyr::mutate(
  mc = purrr::map(`≥80%K`, \(x) {

```

```

    x |>
      lm(formula = d2t ~ x2, data = _)
    })
  ) |>
  dplyr::mutate(b = -purrr::map_dbl(mb, \(x) coef(x)[1])) |>
  dplyr::mutate(c = purrr::map_dbl(mc, \(x) coef(x)[2])) |>
  dplyr::mutate(d = purrr::map_dbl(mc, \(x) coef(x)[1] - 0.5)) |>
  dplyr::relocate(
    strain,
    ppf,
    Kcoef,
    K,
    r,
    N0,
    deltaT,
    r2,
    pval,
    tstar,
    b,
    c,
    d,
    p1,
    p2
  ) |>
  dplyr::left_join(
    culturemdf_adj |>
      dplyr::select(strain, ppf, culturedf, fitdf)

```

```

) |>
dplyr::mutate(theta = (K - N0) / N0) |>
dplyr::mutate(
  fpreddf = purrr::pmap(
    list(fitdf, K, r, N0, deltaT, theta, b, c, d),
    \(df, K, r, N0, deltaT, theta, alpha1, alpha2, beta) {
      df |>
        dplyr::filter(n < 0.8 * K) |>
        dplyr::mutate(
          f = 1 -
            alpha1 *
              log(2) *
                theta *
                  exp(r * julian) /
                    (exp(2 * r * julian) + 2 * theta * exp(r * julian) + theta^2)
        ) |>
        bind_rows(
          df |>
            dplyr::filter(n ≥ 0.8 * K) |>
            dplyr::mutate(f = 0.5 + (K - n) / K * alpha2 + beta)
        )
      }
    )
  )
)

culturemdf |>
unnest(culturedf) |>

```

```
ggplot(aes(julian, d2t)) +
  geom_point() +
  facet_wrap(~ paste(strain, ppf), ncol = 3, scale = "free") +
  theme_sci(9, 6)
```

mdf |>

```
unnest(` $<80\%K$ ` ) |>
dplyr::filter(julian > 0) |>
ggplot(aes(x1 * log(2) / r, d2t)) +
  geom_point() +
  scale_x_log10(
    breaks = scales::trans_breaks("log10", function(x) 10^x),
    labels = scales::trans_format("log10", scales::math_format(10^.x))
  ) +
  facet_wrap(~ paste0(strain, ppf), ncol = 3, scale = "free") +
  theme_sci(9, 5)
```

mdf |>

```
tidyr::unnest(fpreddf) |>
ggplot(aes(julian, f)) +
  geom_point() +
  geom_line(color = "red") +
  facet_wrap(~ paste(strain, ppf), ncol = 3, scale = "fixed") +
  theme_sci(9, 5)
```

References

- Cao, Tengxin, Jiao Fang, Zeyu Jia, Yiping Zhu, Ming Su, Qi Zhang, Yichao Song, Jianwei Yu, and Min Yang. 2023. "Early Warning of MIB Episode Based on Gene Abundance and Expression in Drinking Water Reservoirs." *Water Research* 231: 119667. <https://doi.org/10.1016/j.watres.2023.119667>.
- Chang, De-Wei, Peter Hobson, Michael Burch, and Tsair-Fuh Lin. 2012. "Measurement of Cyanobacteria Using in-Vivo Fluoroscopy - Effect of Cyanobacterial Species, Pigments, and Colonies." *Water Research* 46 (16): 5037–48. <https://doi.org/10.1016/j.watres.2012.06.050>.
- Duong, Thi Thuy, Thi Thu Lien Nguyen, Thi Hai Van Dinh, Thi Quynh Hoang, Thi Nguyet Vu, Thi Oanh Doan, Thi Mai Anh Dang, et al. 2021. "Auxin Production of the Filamentous Cyanobacterial *Planktothricoides* Strain Isolated from a Polluted River in Vietnam." *Chemosphere* 284: 131242. <https://doi.org/10.1016/j.chemosphere.2021.131242>.
- Gao, Jingsi, Jia Zhu, Maowei Wang, and Wenyi Dong. 2018. "Dominance and Growth Factors of *Pseudanabaena* sp. In Drinking Water Source Reservoirs, Southern China." *Sustainability* 10 (11): 3936. <https://doi.org/10.3390/su10113936>.
- Hu, Lili, Haiyan Wang, Jingzhen Cui, Wansheng Zou, Jie Li, and Kun Shan. 2023. "Temperature-Dependent Growth Characteristics and Competition of *Pseudanabaena* and *Microcystis*." *Water* 15 (13): 2404. <https://doi.org/10.3390/w15132404>.
- Jianguo, Cheng, Ma Litong, Zhao Wenyan, and Liu Yunying. 2022. "Effects of Environmental Factors on the Growth and 2-MIB Production of *Planktothrix* Sp." *Journal of Hydroecology* 43 (1): 70–76. <https://doi.org/10.15928/j.1674-3075.202008180236>.
- Lu, Jinping, Ming Su, Yuliang Su, Bin Wu, Tengxin Cao, Jiao Fang, Jianwei Yu, Honggang Zhang, and Min Yang. 2022. "Driving Forces for the Growth of MIB-Producing *Planktothricoides Raciborskii* in a Low-Latitude Reservoir." *Water Research*, 118670. <https://doi.org/10.1016/j.watres.2022.118670>.
- Mishra, Sanjiv K., Anupama Shrivastav, Rahulkumar R. Maurya, Shailesh K. Patidar, Soumya Hal-

- dar, and Sandhya Mishra. 2012. "Effect of Light Quality on the c-Phycoerythrin Production in Marine Cyanobacteria *Pseudanabaena* Sp. Isolated from Gujarat Coast, India." *Protein Expression and Purification* 81 (1): 5–10. <https://doi.org/10.1016/j.pep.2011.08.011>.
- Mohanty, Bijayalaxmi, Seyed Mohammad Majedi, Shruti Pavagadhi, Shu Harn Te, Chek Yin Boo, Karina Yew-Hoong Gin, and Sanjay Swarup. 2022. "Effects of Light and Temperature on the Metabolic Profiling of Two Habitat-Dependent Bloom-Forming Cyanobacteria." *Metabolites* 12 (5): 406. <https://doi.org/10.3390/metabo12050406>.
- Muhetaer, Guligena, Takashi Asaeda, Senavirathna M. D. H. Jayasanka, Mahendra B. Baniya, Helayaye D. L. Abeynayaka, M. Harun Rashid, and HongYu Yan. 2020. "Effects of Light Intensity and Exposure Period on the Growth and Stress Responses of Two Cyanobacteria Species: *Pseudanabaena Galeata* and *Microcystis Aeruginosa*." *Water* 12 (2). <https://doi.org/10.3390/w12020407>.
- Su, Ming, Jiao Fang, Zeyu Jia, Yuliang Su, Yiping Zhu, Bin Wu, John C. Little, Jianwei Yu, and Min Yang. 2023. "Biosynthesis of 2-Methylisoborneol Is Regulated by Chromatic Acclimation of *Pseudanabaena*." *Environmental Research* 221: 115260. <https://doi.org/10.1016/j.envres.2023.115260>.
- Su, Ming, Yiping Zhu, Tom Andersen, Xianyun Wang, Zhiyong Yu, Jinping Lu, Yichao Song, et al. 2022. "Light-Dominated Selection Shaping Filamentous Cyanobacterial Assemblages Drives Odor Problem in a Drinking Water Reservoir." *Npj Clean Water* 5 (1): 37. <https://doi.org/10.1038/s41545-022-00181-2>.
- Tan, Hui Teng, Fatimah Md. Yusoff, Yam Sim Khaw, Muhammad Farhan Nazarudin, Nur Amirah Izyan Noor Mazli, Siti Aqlima Ahmad, Noor Azmi Shaharuddin, and Tatsuki Toda. 2022. "Characterisation and Selection of Freshwater Cyanobacteria for Phycobiliprotein Contents." *Aquaculture International*. <https://doi.org/10.1007/s10499-022-00985-6>.
- Te, Shu Harn, Boon Fei Tan, Chek Yin Boo, Janelle Renee Thompson, and Karina Yew-hoong Gin. 2017. "Genomics Insights into Production of 2-Methylisoborneol and a Putative Cyanobactin by *Planktothricoides* Sp. sr001." *Standards in Genomic Sciences* 12 (1): 35.

<https://standardsingenomics.biomedcentral.com/articles/10.1186/s40793-017-0247-1>.

- Wang, Zhongjie, and Renhui Li. 2015. "Effects of Light and Temperature on the Odor Production of 2-Methylisoborneol-Producing *Pseudanabaena* Sp. and Geosmin-Producing *Anabaena Ucrainica* (Cyanobacteria)." *Biochemical Systematics and Ecology* 58: 219–26. <https://doi.org/10.1016/j.bse.2014.12.013>.
- Xiao-Qin Wang, Hai-Bo Jiang, and Bao-Sheng Qiu. 2015. "Effects of Iron Availability on Competition Between Microcystis and Pseudanabaena or Chlorella Species." *European Journal of Phycology* 50 (3): 260–70. <https://doi.org/10.1080/09670262.2015.1020516>.
- Zhang, Ting, Lingling Zheng, Lin Li, and Lirong Song. 2016. "2-Methylisoborneol Production Characteristics of *Pseudanabaena* sp. FACHB 1277 Isolated from Xionghe Reservoir, China." *Journal of Applied Phycology*, 1–10. <https://doi.org/10.1007/s10811-016-0864-x>.