

## **Marine diatoms change their gene expression profile when exposed to microscale turbulence under nutrient replete conditions**

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### **Supplementary Methods**

**Cultivation details and medium preparation.** The non-axenic *Chaetoceros decipiens* SZN-Cdec and *Thalassiosira rotula* SZN-Trot cultures used in this paper were obtained by single cell or single chain isolation from net samples collected at the long term monitoring station MareChiara (LTMS MC<sup>1</sup>). The non-axenic *Skeletonema marinoi* strain V32 was a kind gift of Dr. Anna Gohde (University of Gothenburg, Sweden). Isolation was performed using a flame-capillarised glass Pasteur pipette using a Leica light microscope at a 100x magnification. Temperature was set at  $18 \pm 1^\circ\text{C}$ , photoperiod at 12L:12D and irradiance at  $80 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . F2 medium<sup>2</sup> was prepared by adding the adequate quantity of nutrients from single solutions following Guillard's recipe<sup>2</sup> to ultrafiltered, autoclaved sea water collected at the LTMS MC. Superficial sea water samples were first filtered through Millipore 0.22  $\mu\text{m}$  membrane filters (Millipore nitrocellulose filter, GSWP09000, Merck Millipore Darmstadt, Germany) then all the nutrients but vitamins were added. The medium was then left stand for 24 hours to let the chemical species reach equilibrium. The medium was filtered through 0.45  $\mu\text{m}$  Millipore membranes (Millipore, HAWP09000) to remove flocculates that form after nutrients (especially  $\text{Na}_2\text{SiO}_3\cdot 9\text{H}_2\text{O}$ ) addition. Autoclave sterilization for 1 hour at  $121^\circ\text{C}$  in 10L-Nalgene polycarbonate bottles was performed. Once the temperature reduced to less than  $37^\circ\text{C}$ , vitamins were added under sterility conditions. The medium needed for the experiments was stored for at least 48 hours in the growth chamber where the experiments were carried out in order to have the medium at the same temperature of the chamber and thus avoid thermal shock to the cells at the moment of the inoculum. The sea water to prepare culture medium for *S. marinoi* was lowered in salinity to 26 psu by adding MilliQ water because the strain was isolated from the Northern Sea where the salinity is lower compared to LTMS MC.

**Experimental set-up.** TURBOGEN<sup>3</sup> is a prototypic instrument designed and produced in collaboration with M2M Engineering. It is composed of six 13L Plexiglass<sup>®</sup> cylinders. A fully digitally controlled high performance engine moves circular grids into the cylinders in order to produce turbulence. At the conditions imposed for our experiments (stroke 240 mm, grid speed  $100 \text{ mm}\cdot\text{s}^{-1}$ , acceleration  $1000 \text{ mm}\cdot\text{s}^{-2}$ ), the turbulent kinetic energy dissipation rate,  $\epsilon$ , was in the order of  $10^{-4} \text{ m}^2\cdot\text{s}^{-3}$ , i.e. in a range where for cells of the size of those used here the flux of nutrients toward the cell is not enhanced by turbulent motion. For an extensive technical overview of the instrument and the calibration procedure and results see ref. <sup>3</sup>.

**RNA isolation, reverse transcription and qPCR.** For experiments 1 and 2,  $1$  to  $1.5 \times 10^7$  cells were filtered onto 1.2  $\mu\text{m}$ -porosity Millipore RAWP membrane filters (Mf-Millipore RAWP04700). Membrane filters were mounted onto filter holders then washed with sterile

MilliQ water. After filtration, 50 ml of PBS were used to wash the filters. Filters were cut into two halves with a razor blade and put into 2 ml Eppendorf tubes previously filled with 1.5 ml of Roche TriPure® (Sigma - Aldrich s.r.l. Milan, Italy, cat. n. 011667157001) isolation reagent. Eppendorf tubes were snap-frozen in liquid nitrogen. Samples were stored at -80°C until use. RNA was isolated from TriPure® frozen filters with the following protocol. TriPure®-soaked half filters were thawed at room temperature. A dash of Sigma 212-300 µm acid-washed glass beads (Sigma, cat. n. G1277) were added and incubated for 10 minutes at 60°C on a thermoshaker at 1,400 rpm. Tubes were spun to get rid of the beads and the filter. The supernatant was transferred to a new 2-ml Eppendorf tube and 200 µl of chloroform per ml of TriPure® were added. Tubes were vigorously shaken for 15 s and incubated for 15 minutes at room temperature. Tubes were centrifuged at 10,600 rpm (12,000 g) for 15 minutes at 4°C. The upper aqueous phase containing RNA was transferred to a new 2 ml Eppendorf tube and an equal volume of isopropanol added to precipitate RNA. Tubes were gently mixed by inversion and incubated at room temperature for 10 minutes. Samples were centrifuged at 10,600 rpm for 10 minutes at 4°C to pellet down RNA salts. One ml of 75% ethanol prepared with DEPC-treated water was used to clean the RNA pellet. Tubes were centrifuged at 8,500 rpm (7,500 g) for 10 minutes at 4°C, the ethanol discarded and let the pellet air-dry for 20-30 minutes. The RNA pellet was resuspended in 20-30 µl of RNase-free water and incubated for 5-10 minutes at 55°C to facilitate elution. RNA samples were DNase-treated (Roche DNase I recombinant, RNase-free, cat. n. 04716728001) to get rid of genomic DNA contamination and cleaned up by Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany, cat. n. 74106). Samples were stored at -80°C until use.

RNA samples were analysed at an Agilent 2100 Bioanalyzer platform (Agilent Technologies 5301 Stevens Creek Blvd. Santa Clara, California 95051 USA) to assess integrity, at a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) to assess purity, and quantified at a Qubit fluorometer (Thermo Fisher Scientific Inc.) following manufacturer's instructions. 1.5 µg RNA from two still and two turbulent samples from time points T2 (experiment 1) and T3 (experiment 2) were sent to EMBL Gencore Facility for sequencing at the Illumina HiSeq2000 platform, producing non paired-end 50 bp reads.

1 µg RNA from three turbulent and three still samples from time point T2 (experiment 1) and T3 (experiment 2) was reverse transcribed using Qiagen QuantiTect Reverse Transcription Kit (cat. n. 205313, Qiagen) following manufacturer's instructions for qPCR validation of RNA-seq results. Primers used in this paper are listed in Supplementary Table 4. For qPCR 1 µl of a 1:5 dilution was used in each 10 µl reaction. The reaction contained: 5 µl Fast SYBR® Green Master Mix (Thermo Fisher Scientific Inc., cat. n. 4385612), 7 pmols of each primer and 1 µl of 1:5 cDNA dilution. An Applied Biosystems ViiA™ 7 Real-Time PCR System (Thermo Fisher Scientific Inc.) was used with the following reaction programme: a first denaturation step at 95°C for 20 s then 40 cycles at 95°C for 1 s, 60°C for 20 s. For melting curve production the following cycle was run after the amplification: 95°C for 15s, 60°C for 60s and 95°C for 15 s.

**Cell counts and data analyses.** Cell counts were used both to define chain distribution over time and to draw growth curves. On average 427 chains per sample (16,817 total chains for *C. decipiens* over a total of 42 samples counted; 10,538 for *T. rotula* and 10656 for *S. marinoi* over a total of 24 samples per species) were counted in Sedgwick-Rafter counting chambers in a light microscope at a magnification of 100x. For *S. marinoi*, Utermöhl<sup>4</sup> counting chambers were used instead, at an inverted microscope (magnification 200x). For chain distribution every chain class was weighed on the total number of chains counted and expressed in percentage. The division rate was calculated as the slope of the growth curve

built with  $\log_2$ -transformed cell density values between every time point both in single cylinders and on the mean values. The maximum division rate was calculated on the steepest portion of the growth curve. For *C. decipiens* the number of separating chains (chains presenting at least one separation point identified by two adjacent separating cells within the chain itself, see Fig. 3, inlet) was recorded as well. The chain length of the separated subchains was recorded and compared among replicates and between treatments and controls. The number of mechanically broken chains was recorded as well. Broken chains can be recognised because at one or both apices they lack the terminal cells, identifiable by the presence of terminal setae (see main text and Fig. 2, inlet).

Statistical relevance of results was estimated by applying Wilcoxon and Kolmogorov-Smirnov two-sample (KS2) tests (alpha 0.001 i.e. 99.9% accuracy) in MatLab. If the null hypothesis (samples are identical) can be rejected, the test result equals 1, otherwise the outcome equals 0.

Both tests were carried out between replicates (turbulent vs. turbulent or still vs. still) in order to verify congruence of replicates and between experimental and control samples (turbulent vs. still). All possible pairwise combinations were tested.

**Generating a reference transcriptome.** Sequencing of the cDNA from the *Chaetoceros decipiens* samples was performed using Illumina technology. The resulting fastq reads files were inspected using FastQC tool (<http://bit.ly/1aNGclw>) and further cleaned from adapters and trimmed for quality using Trimmomatic with the following parameters: ILLUMINACLIP: TruSeq3-SE.fa:2:30:10:1:true SLIDINGWINDOW:3:28 MINLEN:40<sup>5</sup>. Trinity software (ver. trinity\_201407) was used for the assembling of the reads using these parameters: --normalize\_reads --inchworm\_cpu 24 --bflyHeapSpaceInit 24G --bflyHeapSpaceMax 240G --bflyCalculateCPU --CPU 24 --jaccard\_clip --min\_kmer\_cov 2. Here Jaccard clip was used to mitigate false fusion of adjacent transcripts<sup>6</sup>. The assembling resulted in 27,923 contigs with a N50 value of 1,912 bp and average contigs length 1,211 bp. We chose to filter out transcripts showing no relevant expression. To this aim, we quantified transcript expression levels by mapping reads against the assembled transcriptome using Bowtie (ver. 1.1) with parameters: --p 20 --chunkmbs 10240 --maxins 500 --seedlen 20 --tryhard -a -S<sup>7</sup>. To count the reads mapped we used the idxstats program in Samtools (ver. 0.1.19-44428cd). Only transcripts with count per million (CPM) greater than one for at least 2 out of 8 samples were considered expressed beyond background noise and retained.

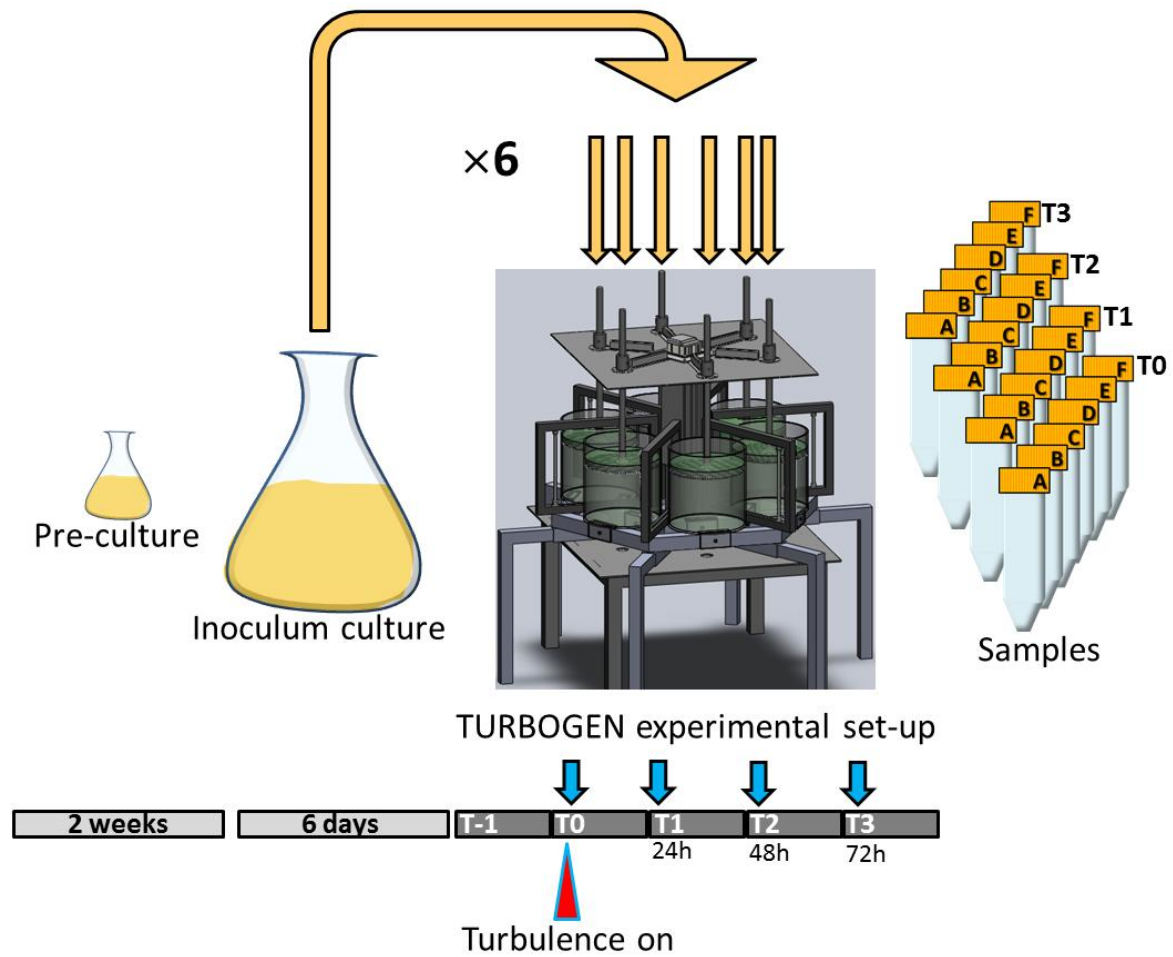
**Transcriptome annotation.** The resulting reference transcriptome contains 21,224 transcripts which were annotated using the software Annocript<sup>8</sup> (Supplementary Table 12). This pipeline permitted to align the transcripts against known proteins, domains and non-coding RNAs. A blastx<sup>9</sup> against SwissProt<sup>10</sup> and UniRef90<sup>11</sup> (version 2014\_08) was performed using the following parameters (-word\_size 4 -evalue 1E-5 -num\_threads 20 -num\_descriptions 5 -num\_alignments 5 -threshold 18). The rpstblastn against the Conserved Domains Database (CDD) returned the domains composition of the putative proteins using these parameters: -evalue 0.00001 -num\_descriptions 20 -num\_alignments 20. The CDD database contains domains models from Pfam, SMART, COG, PRK and TIGRFAM<sup>12</sup>. Blastn against Rfam (performed with parameters: evalue 0.00001 -num\_threads 4 -num\_descriptions 1 -num\_alignments 1) permitted the annotation of putative non-coding ribosomal RNAs<sup>13</sup>. Moreover, the best scored proteins in Annocript are associated with Gene Ontology (GO) terms<sup>14</sup>, enzymes from the ExpASy database<sup>15</sup> and UniPathways<sup>16</sup>. The specific databases used can be found at the Annocript GitHub reference page (<http://bit.ly/1y6Ixxo>).

**Refinement of domain annotation by Meta-CLADE.** The importance of a fine domain annotation is crucial to estimate the abundance of certain functional classes of domains within the community and the absence of others, and to highlight enzymatic biochemical functions associated to specific environmental substrates. Probabilistic models generated from the consensus of a set of homologous sequences are often used as representations of protein domains. When sequences are well conserved, the resulting probabilistic model captures the most conserved features in the domain sequences and these latter can be successfully used to find new domains in databases of sequences, sharing the same features of the original sequence. However, when sequences have highly diverged, consensus signals become too weak to generate a useful probabilistic representation, and models constructed by global consensus do not properly characterize domain features. To overcome this fundamental bottleneck, we introduced CLADE-centered models<sup>19</sup>, defined by considering a set of homologous sequences, and we constructed a model for each sequence  $S_i$  from a set of conserved sequences that are similar to  $S_i$ . By so doing, the probabilistic models generated to represent a domain, are displaying features that are characteristic of each sequence  $S_i$ , and they might be very different from  $S_i$  to  $S_j$ . More divergent the homologous domain sequences  $S_i$  and  $S_j$  are, more the models constructed from these sequences are expected to display different features. It is therefore important for a domain to be represented by several models that can characterise its different paths of evolution within different clades. The multi-source representation has been demonstrated to be more efficient than a single source strategy for domain identification in Bernardes et al.<sup>19</sup>. Based on multiple models associated to each domain, we constructed a large probabilistic model library for all Pfam domains. For each domain, the library includes the Pfam consensus models and about 350 clade-centered models. This amounts to more than 2.5 million probabilistic models. Such models have been previously used in CLADE<sup>19</sup>, a tool for domain identification designed for improving genome annotation. META-CLADE (Ugarte et al, in preparation) has been designed based on CLADE with the purpose to annotate metagenomics and metatranscriptomics reads. Due to the read features described above, these data demand a special treatment and a specific algorithmic design. META-CLADE is based on two main steps. The META-CLADE first step takes as input a set of metaG/metaT reads or contigs where CDS/ORFs have been previously annotated, together with all probabilistic CLADE-centered models and Pfam pHMM models. For each sequence, the CDS region is scanned with the probabilistic models and all domain hits are identified. For each hit, two scores are assigned, the bit-score for the entire hit and the mean-bit-score for each hit residue (obtained as the bit-score of the hit divided by the length of the hit). The META-CLADE second step estimates domain specific thresholds for bit-scores and mean-bit-scores, that best separate true hits from false hits, for each domain. Hence, each CDS sequence is represented by a set of domain hits, where each domain hit is described by its bit-score, mean-bit-score, predicted state (positive or negative) and probability of being a positive/negative prediction. The bit-score and mean-bit-score are provided by the matching of a probabilistic model, and the predictive state of the hit together with its probability are provided by a learning step. Then, the set of hits is filtered in several steps: 1. all pairs of overlapping hits that are associated to the same domain and that cover at least 85% of the length of at least one of the two hits, are filtered from the list of CDSs. For such pair of hits, we eliminate the hit that has the lower bit-score. 2. Second, META-CLADE filters out all hits having a bit-score which is smaller than the threshold provided by positive sequences, as determined in the analysis of fragmented segments in read sequences. 3. Based on the parameter estimation obtained with the BayesNaiveBayes method applied to each Pfam domain, the filter accepts only those domain hits that are positive hits and whose probability  $p$  for being a positive hit is  $p > .9$ . 4. Hits are filtered by best E-value score, associated to each CLADE-centered model and pHMM model. Namely, domain hits are

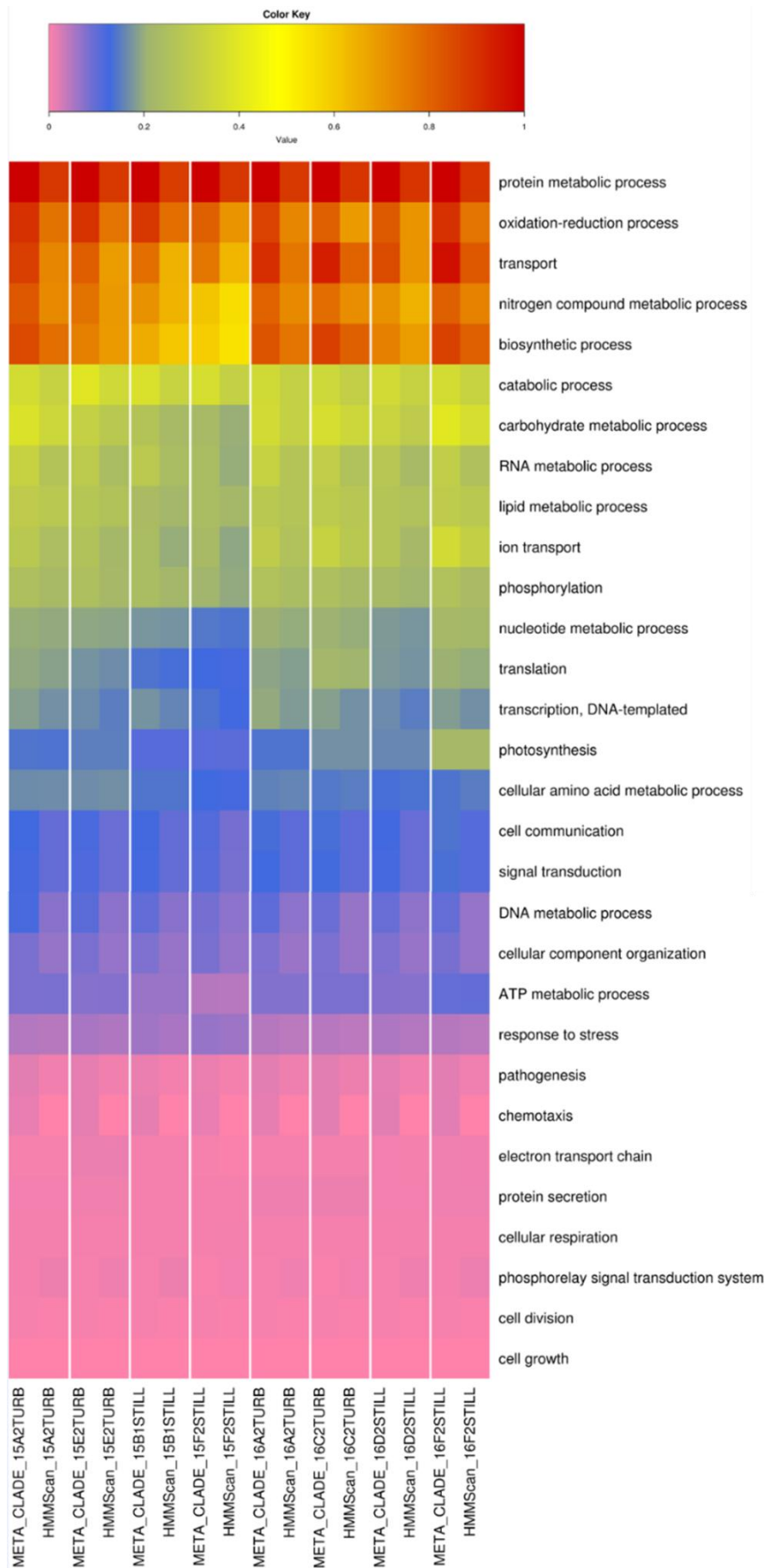
ordered by considering lowest E-value scores and iteratively eliminated hits whether they overlap some domain at least 15% of the length of the hit sequence having best E-value. The output of this filtering pipeline is the contig annotated with non-overlapping domain hits.

**Differential expression analysis.** A plugin, using EdgeR libraries<sup>17</sup> is included in Annocript and is used to select transcripts that are differentially expressed between still and turbulent conditions. Transcripts were considered differentially expressed if the false discovery rate (FDR) was smaller or equal to 0.05 and the fold change greater than 2. Another included plugin was used to perform enrichment analysis of GO terms and Pathways exploiting the Fisher exact test and the Benjamini and Hochberg correction of the p-values. GO terms and Pathways were considered enriched when associated to at least 10 differentially expressed transcripts with an adjusted p-value smaller than 0.1.

## Supplementary Figures



**Supplementary Figure 1. Workflow of the experiments run in this investigation.** Vertical golden arrows indicate diatom inoculum into the TURBOGEN<sup>3</sup>. Vertical blue arrows indicate sampling time points. The red arrowhead indicates the moment at which turbulence was applied in three out of the six cylinders. For more details about the experimental plan see Materials and Methods and Supplementary Methods.

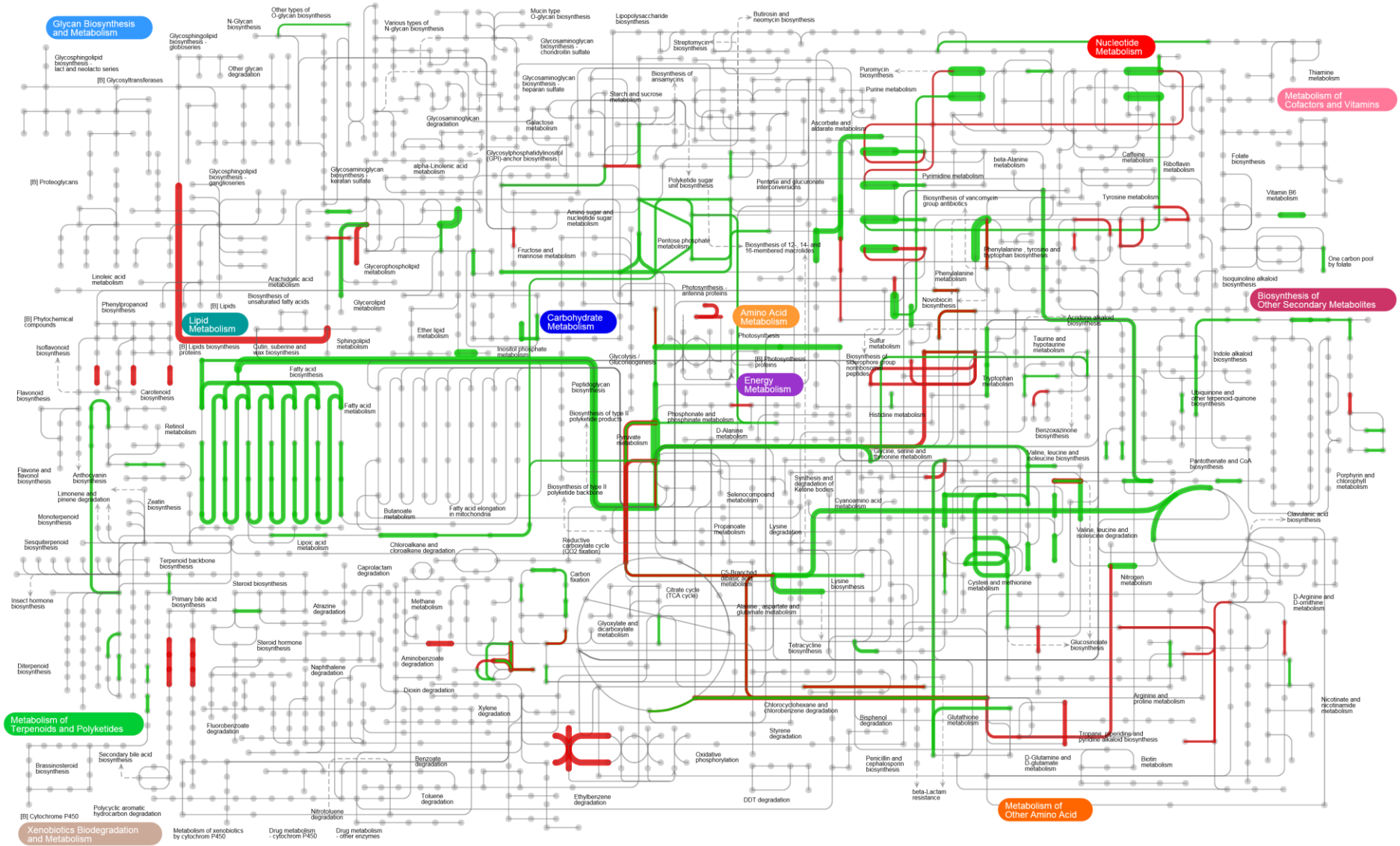


**Supplementary Figure 2. Comparative table of domain abundance organized with respect to the domain classification based on GO-slim functional classes.**

The level of domain abundance (entries of the table) for each sample and each GO-slim functional class are described. Samples are arranged in eight columns reporting, for each functional class, the level of abundance obtained with META-CLADE and HMMScan. The color bar ranks high levels of abundance in dark red and very low levels in pink. The ranking of GO-slim functional classes (from top to bottom in the table) is fixed by the average abundance of domains in the eight samples detected by META-CLADE. Six GO-slim classes are not reported in the figure because they have no signal.

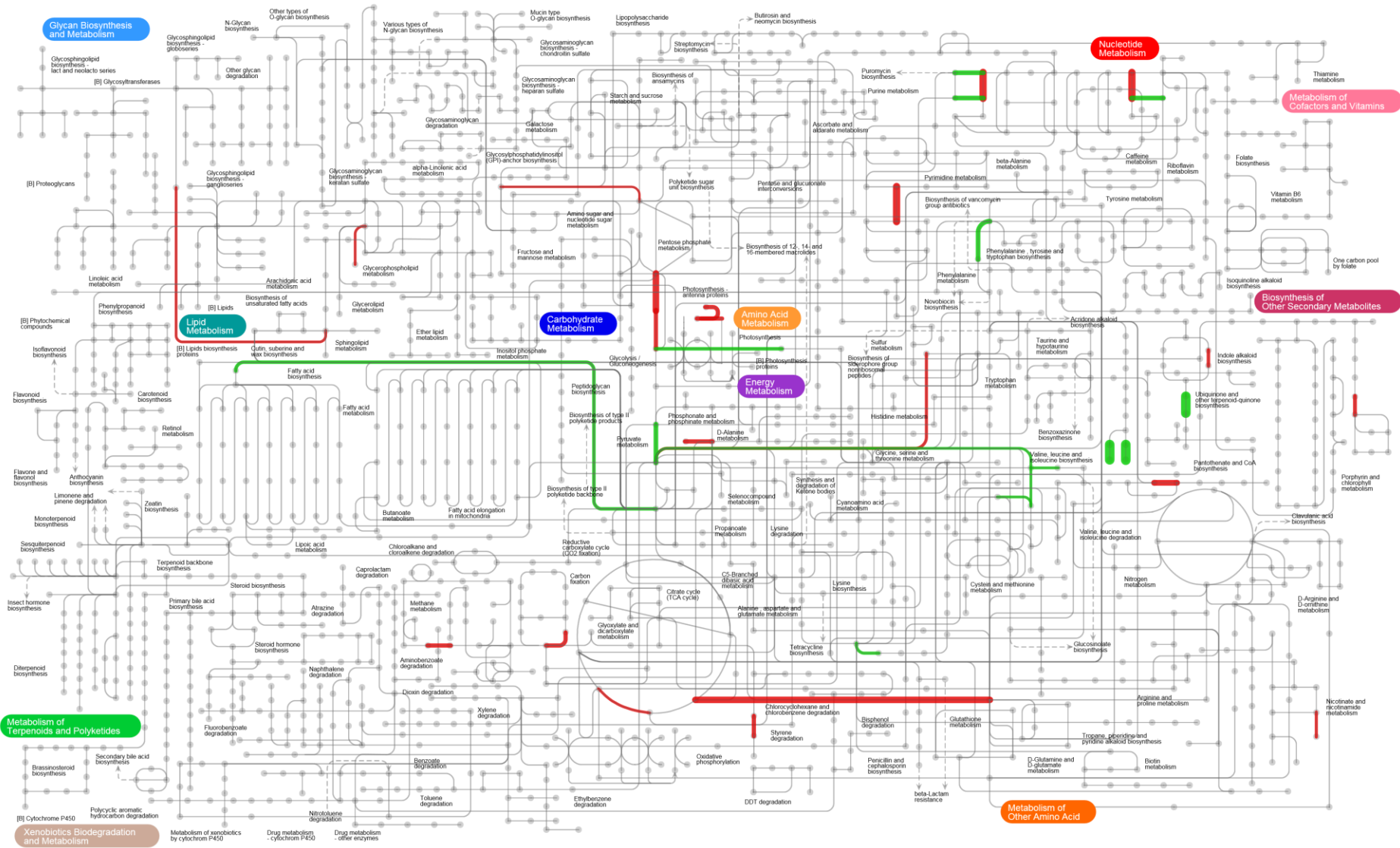


a





b



**Supplementary Figure 3. iPath representations of DE transcripts a) at time point T2 b) and time point T3.** Green lines indicate up-regulation in turbulence-exposed cells compared to still cultures. Red lines indicate down-regulation. The thickness of the lines is proportional to the extent of the change.

## Supplementary Tables

### Legend to the Supplementary Table 1

Supplementary Table 1 contains the output of the differential expression analyses performed at time point T2. The table is arranged as follows: each row is a transcript and columns contain transcript features.

TranscriptName: is the name of the transcript as given in the FASTA file

logFC: Fold Change in log scale, a positive FC means higher expression in turbulence, a negative FC higher expression in still

PValue: P value for significance

FDR: False Discovery Rate

cpm\_T2\_B1\_STILL, cpm\_T2\_F2\_STILL, cpm\_T2\_A2\_TURB, cpm\_T2\_E2\_TURB: counts per million for the two still and two turbulent samples respectively

HSPNameSP: this is the first HSP result (lowest e-value) as given from the BLASTx output against Swiss-Prot

HSPEvalueSP: is the corresponding e-value assigned to the HSP

DescriptionSP: description of the HSP

EnzymeDescs: enzyme descriptions of the EnzymeIDs

Pathways: Pathways of first level associated to the transcripts as they are found in UniPathway

DescriptionUf: description of the HSP against UniRef

HSPEvalueUf: the corresponding e-value assigned to the HSP

Taxonomy: taxonomy corresponding to the result of UniRef

BPDesc: Biological processes descriptions

MFDesc: molecular descriptions

CCDesc: cellular components descriptions

AC\_pfamID: pfam IDs

AC\_GOid: Gene Ontology (GO) IDs

AC\_GOdesc: GO descriptions

CDDesc: domains descriptions

### Legend to the Supplementary Table 2

Supplementary Table 2 contains the output of the differential expression analyses performed at time point T3. The table is arranged as described for Supplementary Table 1.

**Supplementary Table 3. Selection of regulated transcripts at T2 and T3** grouped by the most represented KO pathways. Supplementary Tables 1 and 2 show the complete dataset. Differential expression (log fold change) and False Discovery Rate are indicated for each experimental point. Annotation and best hit of a tblastn search in NCBI are reported. Green shades indicate up-regulation, red-shades indicate down-regulation. Darker nuances indicate up-regulation above 1.5 or down-regulation below -1.5 (arbitrarily set cut-off values). For all the transcripts reported here a tblastn search in MMETSP database was performed and the first 50 hits are listed in Supplementary Table 11.

| Level 2 KO pathways     | Transcript | Annotation and best hit                                              | T2    |          | T3    |          |
|-------------------------|------------|----------------------------------------------------------------------|-------|----------|-------|----------|
|                         |            |                                                                      | logFC | FDR      | logFC | FDR      |
| Calvin cycle            | c8596      | SBPase <a href="#">XM_002176982.1</a>                                | 1.30  | 1.76E-03 |       |          |
|                         | c10813     | PRK <a href="#">Y08610.2</a>                                         | 1.30  | 8.77E-04 |       |          |
|                         | c14492     | chl-PGK<br><a href="#">XM_002288305.1</a>                            | 1.24  | 3.95E-04 |       |          |
|                         | c6859      | PGK precursor<br><a href="#">AF108451.1</a>                          | -1.31 | 3.27E-04 | -1.08 | 2.12E-02 |
| fatty acid biosynthesis | c4257      | FabF <a href="#">XM_002181417.1</a>                                  | 2.23  | 4.81E-07 |       |          |
|                         | c9683      | FabI <a href="#">XM_002295703.1</a>                                  | 2.15  | 4.55E-07 |       |          |
|                         | c6844      | (3R)-3-hydroxyacyl-CoA dehydratase<br><a href="#">XM_002295703.1</a> | 2.15  | 2.95E-07 |       |          |
|                         | c14925     | FAS2/FabB <a href="#">AY099587.1</a>                                 | 1.98  | 2.75E-07 |       |          |
|                         | c5520      | FabG <a href="#">XM_002180866.1</a>                                  | 1.83  | 3.94E-06 |       |          |
|                         | c5253      | FabH <a href="#">XM_002295284.1</a>                                  | 1.47  | 3.37E-05 |       |          |
|                         | c1415      | FabD <a href="#">XM_002181731.1</a>                                  | 1.41  | 3.38E-04 |       |          |
|                         | c3263      | Enoyl-ACP reductase<br><a href="#">AY826870.1</a>                    | 1.28  | 1.69E-03 |       |          |

|                                         |        |                                                                 |      |          |      |          |
|-----------------------------------------|--------|-----------------------------------------------------------------|------|----------|------|----------|
|                                         | c1981  | PTER <a href="#">XM_005855553.1</a>                             | 1.05 | 2.72E-02 |      |          |
| polyunsaturated fatty acid biosynthesis | c9333  | Elongase<br><a href="#">XM_002288445.1</a>                      | 1.56 | 6.89E-05 |      |          |
|                                         | c9138  | Omega-6 fatty acid desaturase<br><a href="#">XM_001569292.1</a> | 1.48 | 3.80E-05 |      |          |
|                                         | c10316 | Omega-6 fatty acid desaturase <a href="#">AY165024.1</a>        | 1.38 | 2.35E-04 |      |          |
|                                         | c9857  | Delta(12) fatty acid desaturase <a href="#">AB858398.1</a>      | 1.05 | 6.98E-03 |      |          |
| malonyl-CoA biosynthesis                | c10543 | Acetyl-CoA carboxylase<br><a href="#">KF673101.1</a>            | 3.84 | 2.12E-14 | 1.53 | 1.34E-02 |
|                                         | c10935 | Acetyl-CoA carboxylase<br><a href="#">XM_002296047.1</a>        | 1.96 | 4.90E-06 |      |          |
| glycolysis                              | c13267 | Pyruvate kinase<br><a href="#">XM_002289369.1</a>               | 4.37 | 1.45E-10 | 2.19 | 2.09E-03 |
|                                         | c7832  | Pyruvate kinase<br><a href="#">XM_002289831.1</a>               | 3.47 | 1.02E-12 | 1.87 | 5.82E-04 |
|                                         | c7622  | Enolase<br><a href="#">XM_002295703.1</a>                       | 2.62 | 1.78E-08 |      |          |
|                                         | c10371 | GPI 3 <a href="#">XM_002185239.1</a>                            | 2.17 | 9.73E-06 |      |          |
|                                         | c7193  | Fba I <a href="#">XM_005840665.1</a>                            | 1.75 | 2.57E-06 |      |          |
|                                         | c10844 | Fba C1 <a href="#">XM_002176146.1</a>                           | 1.68 | 5.68E-05 |      |          |
|                                         | c5521  | ENO1 <a href="#">XM_002289830.1</a>                             | 1.63 | 9.76E-06 |      |          |
|                                         | c5369  | PGAM6<br><a href="#">XM_002178483.1</a>                         | 1.48 | 4.48E-05 |      |          |
|                                         | c5850  | Fba C2 <a href="#">XM_002183735.1</a>                           | 1.18 | 4.54E-03 |      |          |
|                                         | c10803 | PK1 <a href="#">XM_002182782.1</a>                              | 1.09 | 4.30E-03 |      |          |

|                                                                       |         |                                                             |       |          |       |          |
|-----------------------------------------------------------------------|---------|-------------------------------------------------------------|-------|----------|-------|----------|
|                                                                       | c16505  | Phosphoglycerate kinase<br><a href="#">XM_004532595.1</a>   | -1.20 | 3.09E-02 |       |          |
|                                                                       | c9688   | GAPDH <a href="#">AF063801.1</a>                            | -2.13 | 4.00E-03 |       |          |
|                                                                       | c4834   | GapC3 precursor<br><a href="#">AF063802.1</a>               | 1.18  | 8.03E-03 | 1.28  | 1.90E-02 |
|                                                                       | c17273* | GAPDH <a href="#">CP004349.1</a>                            |       |          | -3.72 | 7.85E-04 |
| pentose phosphate pathway                                             | c8818   | 6-phosphogluconolactonase<br><a href="#">XM_002182523.1</a> | 2.20  | 3.35E-07 |       |          |
|                                                                       | c3274   | TAL <a href="#">XM_002185209.1</a>                          | 1.34  | 1.33E-03 |       |          |
|                                                                       | c10651  | Transketolase<br>XM_002287164.1                             | 1.25  | 7.13E-03 |       |          |
| L-tryptophan biosynthesis                                             | c16748  | TrpAB <a href="#">XM_002294670.1</a>                        | 1.08  | 3.89E-03 |       |          |
|                                                                       | c3306   | TRP1 <a href="#">AY030294.1</a>                             | 1.44  | 1.31E-03 |       |          |
| isopentenyl diphosphate biosynthesis via DXP pathway                  | c7546   | CMK <a href="#">XM_002287613.1</a>                          | 2.26  | 2.23E-06 |       |          |
|                                                                       | c13504  | DXR <a href="#">XM_002176918.1</a>                          | 1.72  | 9.34E-06 |       |          |
|                                                                       | c7234   | HDS <a href="#">FJ175678.1</a>                              | 1.48  | 3.17E-05 |       |          |
|                                                                       | c2948   | <a href="#">XM_005820425.1</a>                              | 1.35  | 3.02E-04 |       |          |
| farnesyl diphosphate biosynthesis<br>geranyl diphosphate biosynthesis | c10583  | <a href="#">KM360174.1</a>                                  | 1.44  | 2.87E-04 |       |          |
|                                                                       | c7924   | <a href="#">XM_002288303.1</a>                              | 1.35  | 1.59E-   |       |          |

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|  |  |  |  | 04 |  |  |
|--|--|--|--|----|--|--|

\* tblastn hits are all from bacteria



**Supplementary Table 4. Primers for qPCR validations.** Transcript ID, primer sequence, primer length (bp) and amplicon length are reported.

| <b>Transcript</b> | <b>Forward primer</b> | <b>bp</b> | <b>Reverse primer</b>  | <b>bp</b> | <b>Amplicon length (bp)</b> |
|-------------------|-----------------------|-----------|------------------------|-----------|-----------------------------|
| c2730             | CCATCTCAGCACTTGGAAACA | 20        | ACATGACGAGCAAGCATGAG   | 20        | 199                         |
| c13267            | AAACAAGTCACCCTCGTTGG  | 20        | CCAAATGTTCCAATCGCAGT   | 20        | 177                         |
| c2115             | AGGAAGCACAAGCAAAGCTC  | 20        | CAAGGACTTCCCATCCAATC   | 20        | 189                         |
| c14633            | CCTGGTGCAGCTCGGATA    | 18        | TGATTCAAGAAGGTCAGCAATG | 22        | 105                         |
| c71               | GTGAGTTTGCCATCCTTGTC  | 20        | CAATGGAATGGTTTCTGGTT   | 20        | 168                         |
| c16363            | GCAATTCCTTCTCTGCATT   | 20        | TGCTTTCTGGACCTGTTGTT   | 20        | 190                         |
| c2210             | AAAGCAGGACATGAACCAGA  | 20        | GCGCTATACCCATCTTTCCT   | 20        | 169                         |
| c17476            | CGCCTTAATCCAACACAAAC  | 20        | TATTTCCACCCAAACGAACA   | 20        | 202                         |
| c5660             | TAGGAATCCGAGGCACTACA  | 20        | GCACAAGTCATGGCAACTC    | 19        | 175                         |
| c7202             | AATCCTGAAACCCAGAGTGA  | 20        | GAGCCTATCCATTCCTCCA    | 19        | 207                         |
| c17770            | ATCAGTTGCTTGCTGTTGGT  | 20        | CCTCAGATGGAGAAGGTGTC   | 20        | 248                         |
| c5179             | GATGTTCCAGGGTGTCTTG   | 20        | AACTGCTTCAAATCCGCTAC   | 20        | 219                         |
| c991              | ACCACTTCATGTTGCTGCAC  | 20        | AGCGGAAGACATGAAGAAGC   | 20        | 207                         |
| c11391            | CCAAGTCCATCCTCTTTGGA  | 20        | AGCGGAAGACATGAAGAAGC   | 20        | 181                         |
| c17437            | ACGTAGTGGTGATGCCAAACT | 21        | CATCTTACCCGCAGCTCTCT   | 20        | 193                         |
| c12330            | CCGTAACATTTGCGTTAGAAG | 21        | GCGTCATCAAGTGTGGTAAA   | 20        | 235                         |
| c10008<br>(CDK-A) | GAATGGCTGTACTTGGGATG  | 20        | CAACAATGGAACGGTATCAAA  | 21        | 153                         |
| c6390<br>(TBP)    | TGCACTCAAATGCCGAAATA  | 20        | TCACTGGTTGTGTCTTGAATCC | 22        | 206                         |
| c5402<br>(TUBb)   | AGTTCAGCTCCCTCGGTGTA  | 20        | TTCCAAGGGCAATCCTAATG   | 20        | 157                         |

**Supplementary Table 5. qPCR validation of a selection of transcripts either up or down regulated at time points T2 and T3.** For qPCR RNA samples from all the cylinders were reverse transcribed and amplified with primer pairs listed in Supplementary Table 4.








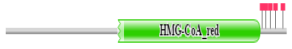
Transcript ID, Time point, Gene function (where available), qPCR and RNA-seq differential expression values (expressed as log2 fold change) are reported. For qPCR the differential expression is reported both as mean over the three cylinders and by cylinder.








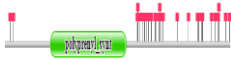

| Transcript ID | Time point | Gene Function                                  | Log2 average relative expression                |         |
|---------------|------------|------------------------------------------------|-------------------------------------------------|---------|
|               |            |                                                | qPCR (±SD)                                      | RNA-seq |
| C2730         | T2         | Uncharacterized protein                        | 3.85 (0.64)<br>A = 4.37<br>C = 4.05<br>E = 3.13 | 4.37    |
| C13267        | T2         | Pyruvate kinase                                | 3.31 (0.57)<br>A = 3.72<br>C = 3.54<br>E = 2.66 | 4.41    |
| C2115         | T2         | Predicted protein ion binding                  | 3.16 (0.84)<br>A = 4.13<br>C = 2.72<br>E = 2.64 | 4.43    |
| C14633        | T2         | -                                              | 3.07 (1.53)<br>A = 4.09<br>C = 3.81<br>E = 1.31 | 5.91    |
| C71           | T2         | Tricarboxylate transport protein mitochondrial | 3.53 (0.70)<br>A = 3.97<br>C = 3.90<br>E = 2.72 | 4.12    |
| C16363        | T2         | Nucleoside diphosphate kinase                  | 3.03 (0.95)<br>A = 3.46<br>C = 3.68<br>E = 1.94 | 4.10    |
| C2210         | T2         | Predicted protein GTP binding protein          | 3.22 (0.80)<br>A = 3.86<br>C = 3.47<br>E = 2.33 | 4.08    |
| C17476        | T2         | Hairpin binding protein 1                      | 3.55 (0.66)<br>A = 3.99<br>C = 3.88             | 3.36    |

|        |    |                                                      |                                                        |       |
|--------|----|------------------------------------------------------|--------------------------------------------------------|-------|
|        |    |                                                      | E = 2.79                                               |       |
| C5660  | T3 | Uncharacterized protein triglyceride lipase activity | 3.88 (1.05)<br>A = 2.96<br>C = 3.66<br>E = 5.03        | 4.97  |
| C7202  | T2 | Predicted protein Glucosylceramidase                 | -2.90<br>(0.28)<br>A = -3.22<br>C = -2.75<br>E = -2.72 | -3.18 |
| C19910 | T2 | Uncharacterized protein transmembrane transporter    | -3.97<br>(0.40)<br>A = -4.30<br>C = -3.53<br>E = -4.08 | -4.25 |
| C17770 | T2 | Uncharacterized protein                              | -3.79<br>(1.49)<br>A = -4.01<br>C = -2.20<br>E = -5.15 | -3.00 |
| C17770 | T3 | Uncharacterized protein                              | -6.04<br>(3.27)<br>A = -5.94<br>C = -9.36<br>E = -2.83 | -7.44 |
| C5179  | T3 | Frustulin 5                                          | -4.71<br>(1.31)<br>A = -4.65<br>C = -6.05<br>E = -3.42 | -3.76 |
| c991   | T3 | Uncharacterized protein                              | 1.17 (1.23)<br>A = 0.33<br>C = 0.61<br>E = 2.59        | 2.94  |
| c11391 | T3 | Uncharacterised protein                              | 0.35 (1.01)<br>A = -0.50<br>C = 0.07<br>E = 1.47       | 3.00  |
| c17437 | T3 | Predicted protein                                    | 0.78 (0.93)<br>A = 0.07<br>C = 0.43<br>E = 1.84        | 3.69  |

|        |    |   |                                                       |      |
|--------|----|---|-------------------------------------------------------|------|
| c12330 | T3 | - | -0.47<br>(1.65)<br>A = -0.88<br>C = -1.88<br>E = 1.34 | 2.84 |
|--------|----|---|-------------------------------------------------------|------|







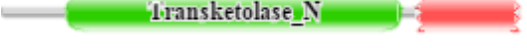


**Supplementary Table 6. List of transcripts involved in isoprenoid biosynthesis, fatty acid  $\beta$ -oxidation and pentose phosphate and glycolysis pathways.** Desaturases and elongase as reported by Dolch and Maréchal<sup>18</sup> were also added. Transcripts involved in fatty acid biosynthesis are listed in Supplementary Table 3. Green shades indicate up-regulation, red-shades indicate down-regulation. Darker nuances indicate up-regulation above 1.5 or down-regulation below -1.5 (arbitrary cut-off values). For all the transcripts reported here, except those marked with the symbol +, a tBLASTn search in MMETSP database was performed and the first 50 hits are listed in Supplementary Table 11.



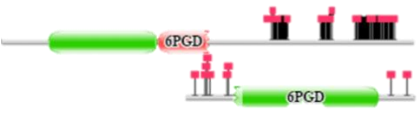






| Transcript                               | Annotation                    | Best hit       | logFC | pfam                                                                                               |
|------------------------------------------|-------------------------------|----------------|-------|----------------------------------------------------------------------------------------------------|
| Inositol pathway and signal transduction |                               |                |       |                                                                                                    |
| c19854 <sup>+</sup>                      | Inositol monophosphatase 3    | XM_002502522.1 | 4.2   |                 |
| c12037 <sup>+</sup>                      | Phosphatidylinositol 4-kinase | XM_002286092.1 | 1.9   |  PI3_PI4_kinase |
| c13992 <sup>+</sup>                      | Phosphatidylinositol 4-kinase | XM_013307010.1 | 1.8   |  PI3_PI4_kinase |
| c16363 <sup>+</sup>                      | NDP kinase                    |                | 4.06  |                 |
| c1791 <sup>+</sup>                       | NDP kinase                    |                | 1.70  |                 |
| c787 <sup>+</sup>                        | NDP kinase                    |                | 1.30  |               |
| MVA pathway (isoprenoid biosynthesis)    |                               |                |       |                                                                                                    |
| c2017                                    | HMGS                          | XM_002286531.1 | -     |               |
| c8934                                    | HMGR                          | XM_004337355.1 | -     |               |




|                                       |                                                        |                              |      |                                                                                                                    |
|---------------------------------------|--------------------------------------------------------|------------------------------|------|--------------------------------------------------------------------------------------------------------------------|
| c12                                   | MPDC                                                   | XM_002286995.1               | -    |  GHMP kinases N terminal domain |
| c7602                                 | DXS                                                    | XM_002176350.1               | 1.01 |                                 |
| MPE pathway (isoprenoid biosynthesis) |                                                        |                              |      |                                                                                                                    |
| c13504                                | DXR (DXR domains)                                      | XM_002176918.1               | 1.72 |                                 |
| c7546                                 | CMK                                                    | XM_002287613.1               | 2.26 | no domains in Tp                                                                                                   |
| c1881                                 | MDS                                                    | XM_005826781.1               | -    |                                 |
| c7234                                 | HDS (GcpE protein)                                     | FJ175678.1                   | 1.48 |                                 |
| c7419                                 | HDR                                                    | XM_002178581.1               | -    |                                 |
| c2948                                 | 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase | XM_005820425.1               | 1.35 |                                 |
| c10583                                | Polyprenyl synthetase                                  | KM360174.1<br>XM_002185991.1 | 1.44 |                               |
| c7924                                 | Polyprenyl synthetase                                  | XM_002288303.1               | 1.35 |                               |
| Fatty acids $\beta$ -oxidation        |                                                        |                              |      |                                                                                                                    |

|            |                                                                                                                                                                    |                             |      |  |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|------|--|
| c3617      | KCT3<br>Enoyl-CoA<br>hydratase/isomerase<br>3-hydroxyacyl-CoA<br>dehydrogenase, NAD<br>binding domain<br>3-hydroxyacyl-CoA<br>dehydrogenase, C-terminal<br>domain) | Phatr_35240 <sup>18</sup>   | -    |  |
| c1094      | acyl-CoA dehydrogenase                                                                                                                                             | Phatr_11014 <sup>18</sup>   | -    |  |
| c7189      | enoyl-CoA hydratase                                                                                                                                                | Phatr_55192 <sup>18</sup>   | -    |  |
| c3358      | 3-ketoacyl-CoA<br>dehydrogenase                                                                                                                                    | Phatr_54947 <sup>18</sup>   | -    |  |
| Desaturase |                                                                                                                                                                    |                             |      |  |
| c9857      | FAD2                                                                                                                                                               | Phatr_25769 <sup>1</sup>    | 1.05 |  |
| c10316     | FAD6                                                                                                                                                               | Phatr_48423 <sup>1</sup>    | 1.4  |  |
| c10828     | ERΔ5FAD.1/PTD5b                                                                                                                                                    | Phatr_46830 <sup>1</sup>    | 1.4  |  |
| c8233      | PTD6                                                                                                                                                               | XM_002291493.1              | 1.30 |  |
| c9138      | PlastidΔ6FAD                                                                                                                                                       | Phatr_50443 <sup>1</sup>    | 1.48 |  |
| c8389      | PAD/SAD                                                                                                                                                            | Phatraft_9316 <sup>18</sup> | -    |  |
| c10330     | ADS                                                                                                                                                                | Phatr_28797 <sup>18</sup>   | -    |  |
| c10282     | ERΔ5FAD.2*                                                                                                                                                         | Phatr_22459 <sup>18</sup>   | -    |  |



|                                           |                                             |                           |      |                                                                                       |
|-------------------------------------------|---------------------------------------------|---------------------------|------|---------------------------------------------------------------------------------------|
| c862                                      | ERΔ4FAD                                     | Phatr_22510 <sup>18</sup> | -    |                                                                                       |
| c7981                                     | Plastidω3FAD/FAD7/PTD<br>15                 | Phatr_41570 <sup>18</sup> | -    |                                                                                       |
| c5093                                     | FAD4                                        | Phatr_41301 <sup>18</sup> | -    |                                                                                       |
| c9333                                     | Elongase                                    | XM_002288445.1            | 1.56 |    |
| Pentose Phosphate and Glycolysis Pathways |                                             |                           |      |                                                                                       |
| c10371                                    | Glucose-6-phosphate<br>isomerase            | XM_002185239.1            | 2.13 |    |
| c10844                                    | Fructose-1,6 biphosphate<br>aldolase        | XM_002176146.1            | 1.68 |    |
| c5850                                     | Fructose-1,6 biphosphate<br>aldolase        | XM_002288284.1            | 1.1  |    |
| c3274                                     | Transaldolase                               | XM_002185209.1            | 1.33 |    |
| c7602                                     | 1-deoxy-D-xylulose-5-<br>phosphate synthase | XM_002176350.1            | 1.00 |  |
| c10651                                    | Transketolase                               | XM_002287164.1            | 1.00 |  |
| c5034                                     | triosephosphate isomerase                   | XM_002178067.1            | 1.4  |  |
| c9233                                     | triosephosphate isomerase                   | XM_002177666.1            | 1.6  |  |

|                     |                                           |                |      |                                                                                       |
|---------------------|-------------------------------------------|----------------|------|---------------------------------------------------------------------------------------|
| c4834               | Glyceraldehyde-3-phosphate dehydrogenase  | AF063802.1     | 1.18 |    |
| c8818               | 6-phosphogluconolactonase                 | XM_002182523.1 | 2.19 |    |
| c9012 <sup>+</sup>  | 6-phosphogluconate dehydrogenase          | XM_002289784.1 | -    |    |
| c10101 <sup>+</sup> | fructose 6-phosphate 1-phosphotransferase | AF268276.1     | -    |    |
| c18606 <sup>+</sup> | Ribose-phosphate pyrophosphokinase        | XM_002287674.1 | -    |    |
| c5366 <sup>+</sup>  | Ribose-phosphate pyrophosphokinase        | XM_002180827.1 | -    |    |
| c4769 <sup>+</sup>  | ribose-5-phosphate isomerase              | XM_002287269.1 | -    |  |
| c6782 <sup>+</sup>  | ribulose-phosphate 3-epimerase            | XM_002179628.1 | -    |  |
| c19160 <sup>+</sup> | Fructose-1 6-bisphosphatase               | XM_007512729.1 | -    |  |

|                     |                                                    |                |   |                                                                                     |
|---------------------|----------------------------------------------------|----------------|---|-------------------------------------------------------------------------------------|
| c9258 <sup>+</sup>  | FGGY carbohydrate kinase domain-containing protein | XM_003057750.1 | - |  |
| c10668 <sup>+</sup> | Fructose-1 6-bisphosphatase                        | XM_002176491.1 | - |  |
| c19105 <sup>+</sup> | Fructose-1 6-bisphosphatase                        | XM_002176491.1 | - |  |

\* ERΔ5FAD.1 and ERΔ5FAD.2 have a high level of identity between each other and thus it is difficult to tell which is which. ERΔ5FAD.1 was functionally validated<sup>18</sup>

<sup>+</sup> Not blasted in the MMETSP database

**Supplementary Table 7. Cell concentration expressed as cells·ml<sup>-1</sup> estimated by Sedgewick-Rafter counts (Supplementary Methods) for *Chaetoceros decipiens* Experiment 1 and Experiment 2, *Thalassiosira rotula* and *Skeletonema marinoi*.** At the moment of inoculum (T-1), cell concentrations were set at 250, 500, and 2.5×10<sup>3</sup> cell·ml<sup>-1</sup>, respectively (see Materials and Methods). Average (Avg) and standard deviations ( $\sigma$ ) are reported. Cell concentration data were used to build growth curves (Fig. 2a-d) and log<sub>2</sub>-transformed to calculate division rates.

| Time point | <i>C. decipiens</i> experiment 1 |           |           |       |            |           |           |           |       |            |
|------------|----------------------------------|-----------|-----------|-------|------------|-----------|-----------|-----------|-------|------------|
|            | Turbulent                        |           |           |       |            | Still     |           |           |       |            |
|            | A                                | C         | E         | Avg   | $\sigma$   | B         | D         | F         | Avg   | $\sigma$   |
| <b>T0</b>  | 469                              | 609       | 518       | 532   | 71.3       | 507       | 512       | 429       | 483   | 46.4       |
| <b>T1</b>  | 518                              | 583       | 594       | 565   | 41.3       | 730       | 1215      | 977       | 974   | 242.7      |
| <b>T2</b>  | 1157                             | 1218      | 1628      | 1334  | 256.0      | 2430      | 1908      | 2217      | 2185  | 262.5      |
| Time point | <i>C. decipiens</i> experiment 2 |           |           |       |            |           |           |           |       |            |
|            | Turbulent                        |           |           |       |            | Still     |           |           |       |            |
|            | A                                | C         | E         | Avg   | $\sigma$   | B         | D         | F         | Avg   | $\sigma$   |
| <b>T0</b>  | 495                              | 492       | 472       | 486   | 12.8       | 518       | 795       | 544       | 619   | 153.0      |
| <b>T1</b>  | 600                              | 676       | 408       | 561   | 138.4      | 841       | 538       | 676       | 685   | 152.0      |
| <b>T2</b>  | 1033                             | 1059      | 917       | 1003  | 75.9       | 1725      | 1158      | 1672      | 1518  | 313.4      |
| <b>T3</b>  | 1478                             | 1818      | 1445      | 1581  | 206.3      | 3053      | 2538      | 2226      | 2606  | 417.6      |
| Time point | <i>Thalassiosira rotula</i>      |           |           |       |            |           |           |           |       |            |
|            | Turbulent                        |           |           |       |            | Still     |           |           |       |            |
|            | A                                | C         | E         | Avg   | $\sigma$   | B         | D         | F         | Avg   | $\sigma$   |
| <b>T0</b>  | 718                              | 546       | 743       | 669   | 107.2      | 670       | 599       | 577       | 615   | 48.8       |
| <b>T1</b>  | 883                              | 1171      | 810       | 954   | 191.0      | 1987      | 1521      | 1508      | 1672  | 272.8      |
| <b>T2</b>  | 3134                             | 2245      | 1784      | 2388  | 686.5      | 5829      | 3722      | 3149      | 4233  | 1411.2     |
| <b>T3</b>  | 4215                             | 3471      | 3284      | 3657  | 492.6      | 8580      | 5225      | 5868      | 6558  | 1780.8     |
| Time point | <i>Skeletonema marinoi</i>       |           |           |       |            |           |           |           |       |            |
|            | Turbulent                        |           |           |       |            | Still     |           |           |       |            |
|            | A                                | C         | E         | Avg   | $\sigma$   | B         | D         | F         | Avg   | $\sigma$   |
| <b>T0</b>  | 5350                             | 5500      | 5050      | 5300  | 229.1      | 5150      | 5100      | 5100      | 5117  | 28.9       |
| <b>T1</b>  | 2200<br>0                        | 1900<br>0 | 2000<br>0 | 20333 | 1527.<br>5 | 2100<br>0 | 1800<br>0 | 1850<br>0 | 19167 | 1607.<br>3 |
| <b>T2</b>  | 2490<br>0                        | 2670<br>0 | 3120<br>0 | 27600 | 3245.<br>0 | 2790<br>0 | 2660<br>0 | 2720<br>0 | 27233 | 650.6      |
| <b>T3</b>  | 3270<br>0                        | 3150<br>0 | 2810<br>0 | 30767 | 2386.<br>0 | 2810<br>0 | 3420<br>0 | 4310<br>0 | 35133 | 7543.<br>4 |

**Supplementary Table 8. Non-parametric statistic tests (Kolmogorov-Smirnov and Wilcoxon).** The letters indicate TURBOGEN cylinders: A, C, and E turbulent conditions; B, D, and F still conditions. First six columns (Controls, in red) show internal controls, i.e. turbulent vs turbulent replicates (A-C, A-E, and C-E) and still vs still replicates (B-D, B-F, and D-F). In red bold-face underlined digits, the internal controls that reject null hypothesis. TX ( $0 \leq X \leq 3$ ) indicates time points. ‘h’ indicates the test answer: 0 = null hypothesis accepted; 1 = null hypothesis consistently rejected. ‘p’ indicates the p-value associated to the test outcome. Accuracy set at 99.9% ( $\alpha = 10^{-3}$ ).

| <i>Chaetoceros decipiens</i> Experiment 1 Kolmogorov-Smirnov |          |      |             |      |      |      |                      |                      |                      |                      |                      |                      |                      |                      |                      |
|--------------------------------------------------------------|----------|------|-------------|------|------|------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                                                              | Controls |      |             |      |      |      | Turbulent vs still   |                      |                      |                      |                      |                      |                      |                      |                      |
| T0                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | 0           | 0    | 0    | 0    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    |
| p                                                            | 1        | 0.74 | 0.89        | 0.93 | 0.70 | 0.47 | 0.98                 | 1                    | 0.93                 | 1                    | 0.97                 | 0.97                 | 0.5598               | 0.2805               | 0.9762               |
| T1                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | 0           | 0    | 0    | 0    | 0                    | 0                    | 0                    | 0                    | 0                    | 1                    | 0                    | 0                    | 0                    |
| p                                                            | 0.25     | 0.57 | 0.20        | 0.41 | 0.37 | 0.37 | 0.21                 | 0.21                 | $1.3 \cdot 10^{-3}$  | 0.02                 | 0.09                 | $2.47 \cdot 10^{-5}$ | 0.83                 | 0.08                 | 0.02                 |
| T2                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | 0           | 0    | 0    | 0    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    |
| p                                                            | 0.04     | 0.32 | 0.02        | 0.60 | 0.12 | 0.75 | $4.16 \cdot 10^{-5}$ | $4.91 \cdot 10^{-6}$ | $6.16 \cdot 10^{-4}$ | $1.67 \cdot 10^{-9}$ | $6.81 \cdot 10^{-9}$ | $2.26 \cdot 10^{-6}$ | $8.26 \cdot 10^{-4}$ | $6.39 \cdot 10^{-6}$ | $6.87 \cdot 10^{-4}$ |
| <i>Chaetoceros decipiens</i> Experiment 1 Wilcoxon           |          |      |             |      |      |      |                      |                      |                      |                      |                      |                      |                      |                      |                      |
| T0                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | 0           | 0    | 0    | 0    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    | 0                    |
| p                                                            | 0.91     | 0.12 | 0.12        | 0.53 | 0.19 | 0.56 | 0.75                 | 0.78                 | 0.38                 | 0.63                 | 0.85                 | 0.41                 | 0.04                 | 0.22                 | 0.45                 |
| T1                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | 0           | 0    | 0    | 0    | 0                    | 0                    | 0                    | 0                    | 0                    | 1                    | 0                    | 0                    | 0                    |
| p                                                            | 0.21     | 0.29 | 0.01        | 0.33 | 0.13 | 0.02 | 0.12                 | 0.70                 | $3.4 \cdot 10^{-3}$  | $5.9 \cdot 10^{-3}$  | 0.15                 | $5.13 \cdot 10^{-5}$ | 0.60                 | 0.50                 | 0.04                 |
| T2                                                           | AC       | AE   | CE          | BD   | BF   | DF   | AB                   | AD                   | AF                   | CB                   | CD                   | CF                   | EB                   | ED                   | EF                   |
| h                                                            | 0        | 0    | <u>1</u>    | 0    | 0    | 0    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 1                    | 0                    |
| p                                                            | 4.8      | 0.36 | <u>3.54</u> | 0.59 | 0.09 | 0.28 | 1.10                 | 7.76                 | 6.05                 | 1.04                 | 5.77                 | 1.31                 | 2.27                 | 3.62                 | 0.01                 |

|                                                                     | $\cdot 10^{-3}$                        |                                        | <u><math>\cdot 10^{-4}</math></u> |           |           |           | $\cdot 10^{-7}$       | $\cdot 10^{-6}$       | $\cdot 10^{-4}$       | $\cdot 10^{-13}$      | $\cdot 10^{-11}$      | $\cdot 10^{-8}$       | $\cdot 10^{-5}$       | $\cdot 10^{-4}$       |                       |
|---------------------------------------------------------------------|----------------------------------------|----------------------------------------|-----------------------------------|-----------|-----------|-----------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| <b><i>Chaetoceros decipiens</i> Experiment 2 Kolmogorov-Smirnov</b> |                                        |                                        |                                   |           |           |           |                       |                       |                       |                       |                       |                       |                       |                       |                       |
| <b>T0</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | 0                                      | 0                                      | 0                                 | 0         | 0         | 0         | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| p                                                                   | 0.99                                   | 0.98                                   | 0.47                              | 1         | 0.45      | 0.73      | 0.76                  | 0.98                  | 0.72                  | 0.91                  | 1                     | 0.99                  | 0.14                  | 0.32                  | 0.12                  |
| <b>T1</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | 0                                      | 0                                      | 0                                 | 0         | 0         | 0         | 1                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| p                                                                   | 0.39                                   | 0.03                                   | 0.91                              | 0.12      | 0.95      | 0.44      | $2.41 \cdot 10^{-4}$  | 0.30                  | $3.7 \cdot 10^{-3}$   | 0.10                  | 0.99                  | 0.42                  | 0.45                  | 0.90                  | 0.78                  |
| <b>T2</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | 0                                      | <u>1</u>                               | 0                                 | 0         | 0         | 0         | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 0                     | 0                     |
| p                                                                   | $4.2 \cdot 10^{-3}$                    | <u><math>4.30 \cdot 10^{-6}</math></u> | 0.05                              | 0.33      | 0.52      | 1         | $6.37 \cdot 10^{-21}$ | $9.31 \cdot 10^{-18}$ | $1.24 \cdot 10^{-15}$ | $5.49 \cdot 10^{-9}$  | $9.19 \cdot 10^{-7}$  | $1.28 \cdot 10^{-5}$  | $1.80 \cdot 10^{-4}$  | $3.7 \cdot 10^{-3}$   | 0.02                  |
| <b>T3</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | <u>1</u>                               | 0                                      | 0                                 | 0         | 0         | 0         | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     |
| p                                                                   | <u><math>1.59 \cdot 10^{-6}</math></u> | 0.01                                   | 0.28                              | 0.89      | 0.98      | 1         | $2.48 \cdot 10^{-40}$ | $3.75 \cdot 10^{-31}$ | $2.79 \cdot 10^{-34}$ | $2.46 \cdot 10^{-18}$ | $3.49 \cdot 10^{-19}$ | $6.68 \cdot 10^{-20}$ | $6.40 \cdot 10^{-22}$ | $2.38 \cdot 10^{-22}$ | $2.71 \cdot 10^{-23}$ |
| <b><i>Chaetoceros decipiens</i> Experiment 2 Wilcoxon</b>           |                                        |                                        |                                   |           |           |           |                       |                       |                       |                       |                       |                       |                       |                       |                       |
| <b>T0</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | 0                                      | 0                                      | 0                                 | 0         | 0         | 0         | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| p                                                                   | 0.45                                   | 0.86                                   | 0.35                              | 0.92      | 0.22      | 0.18      | 0.74                  | 0.81                  | 0.11                  | 0.69                  | 0.61                  | 0.39                  | 0.64                  | 0.69                  | 0.07                  |
| <b>T1</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | 0                                      | 0                                      | 0                                 | 0         | 0         | 0         | 1                     | 0                     | 1                     | 0                     | 0                     | 0                     | 0                     | 0                     | 0                     |
| p                                                                   | 0.04                                   | 0.01                                   | 0.68                              | 0.09      | 0.47      | 0.31      | $3.30 \cdot 10^{-6}$  | 0.01                  | $1.34 \cdot 10^{-4}$  | 0.01                  | 0.46                  | 0.07                  | 0.03                  | 0.74                  | 0.16                  |
| <b>T2</b>                                                           | <b>AC</b>                              | <b>AE</b>                              | <b>CE</b>                         | <b>BD</b> | <b>BF</b> | <b>DF</b> | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>             | <b>CF</b>             | <b>EB</b>             | <b>ED</b>             | <b>EF</b>             |
| h                                                                   | <u>1</u>                               | <u>1</u>                               | 0                                 | 0         | 0         | 0         | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 1                     | 0                     | 0                     |
| p                                                                   | <u>8.67</u>                            | <u>2.43</u>                            | 0.01                              | 0.06      | 0.111     | 0.83      | 6.66                  | 1.13                  | 1.69                  | 6.24                  | 5.31                  | 4.81                  | 2.28                  | 0.02                  | 1.25                  |

|                                                       |                                                |                                   |                                                |                             |                                                |                                                |                       |                       |                       |                       |                             |                       |                       |                             |                       |
|-------------------------------------------------------|------------------------------------------------|-----------------------------------|------------------------------------------------|-----------------------------|------------------------------------------------|------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------------|-----------------------|-----------------------|-----------------------------|-----------------------|
|                                                       | <u><math>\cdot 10^{-4}</math></u>              | <u><math>\cdot 10^{-8}</math></u> |                                                |                             |                                                |                                                | $\cdot 10^{-23}$      | $\cdot 10^{-15}$      | $\cdot 10^{-15}$      | $\cdot 10^{-12}$      | $\cdot 10^{-7}$             | $\cdot 10^{-7}$       | $\cdot 10^{-5}$       |                             | $\cdot 10^{-3}$       |
| <b>T3</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | <u>1</u>                                       | 0                                 | 0                                              | 0                           | 0                                              | 0                                              | 1                     | 1                     | 1                     | 1                     | 1                           | 1                     | 1                     | 1                           | 1                     |
| p                                                     | <u><math>\frac{9.32}{\cdot 10^{-6}}</math></u> | 0.01                              | 0.06                                           | 0.63                        | 0.78                                           | 0.83                                           | $1.63 \cdot 10^{-43}$ | $1.35 \cdot 10^{-34}$ | $2.02 \cdot 10^{-38}$ | $1.38 \cdot 10^{-23}$ | $9.82 \cdot 10^{-19}$       | $1.09 \cdot 10^{-20}$ | $3.54 \cdot 10^{-30}$ | $5.69 \cdot 10^{-24}$       | $1.83 \cdot 10^{-26}$ |
| <b><i>Thalassiosira rotula</i> Kolmogorov-Smirnov</b> |                                                |                                   |                                                |                             |                                                |                                                |                       |                       |                       |                       |                             |                       |                       |                             |                       |
| <b>T0</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | 0                                              | 0                                 | 0                                              | 0                           | 0                                              | 0                                              | 0                     | 0                     | 0                     | 0                     | 0                           | 0                     | 0                     | 0                           | 0                     |
| p                                                     | 0.69                                           | 1                                 | 0.91                                           | 0.63                        | 0.86                                           | 0.09                                           | 1                     | 0.49                  | 0.41                  | 0.44                  | 0.02                        | 0.97                  | 1                     | 0.27                        | 0.97                  |
| <b>T1</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | 0                                              | 0                                 | 0                                              | 0                           | <u>1</u>                                       | <u>1</u>                                       | 0                     | 0                     | 1                     | 0                     | 0                           | 1                     | 0                     | 0                           | 1                     |
| p                                                     | 0.10                                           | 0.03                              | 0.99                                           | 0.38                        | <u><math>\frac{3.75}{\cdot 10^{-4}}</math></u> | <u><math>\frac{7.04}{\cdot 10^{-5}}</math></u> | 0.51                  | 0.01                  | $8.14 \cdot 10^{-6}$  | 0.23                  | 1                           | $2.00 \cdot 10^{-5}$  | 0.15                  | 0.91                        | $3.11 \cdot 10^{-7}$  |
| <b>T2</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | 0                                              | 0                                 | <u>1</u>                                       | 0                           | 0                                              | 0                                              | 0                     | 1                     | 0                     | 1                     | 1                           | 0                     | 0                     | 0                           | 0                     |
| p                                                     | $\frac{5.57}{\cdot 10^{-5}}$                   | 0.02                              | <u><math>\frac{7.62}{\cdot 10^{-9}}</math></u> | 0.14                        | $\frac{2.7}{\cdot 10^{-3}}$                    | 0.01                                           | 0.04                  | $7.23 \cdot 10^{-4}$  | 0.94                  | $8.34 \cdot 10^{-10}$ | $1.24 \cdot 10^{-9}$        | $1.3 \cdot 10^{-3}$   | 0.93                  | 0.83                        | 0.01                  |
| <b>T3</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | <u>1</u>                                       | 0                                 | 0                                              | 0                           | <u>1</u>                                       | 0                                              | 1                     | 1                     | 1                     | 0                     | 1                           | 1                     | 1                     | 1                           | 1                     |
| p                                                     | <u><math>\frac{6.85}{\cdot 10^{-4}}</math></u> | 0.50                              | 0.01                                           | $\frac{1.0}{\cdot 10^{-3}}$ | <u><math>\frac{6.47}{\cdot 10^{-6}}</math></u> | 0.71                                           | $3.46 \cdot 10^{-9}$  | $1.48 \cdot 10^{-22}$ | $1.58 \cdot 10^{-26}$ | 0.04                  | $1.50 \cdot 10^{-8}$        | $1.52 \cdot 10^{-10}$ | $2.13 \cdot 10^{-6}$  | $2.07 \cdot 10^{-8}$        | $2.14 \cdot 10^{-20}$ |
| <b><i>Thalassiosira rotula</i> Wilcoxon</b>           |                                                |                                   |                                                |                             |                                                |                                                |                       |                       |                       |                       |                             |                       |                       |                             |                       |
| <b>T0</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | 0                                              | 0                                 | 0                                              | 0                           | 0                                              | 1                                              | 0                     | 0                     | 0                     | 0                     | 0                           | 0                     | 0                     | 0                           | 0                     |
| p                                                     | 0.25                                           | 0.69                              | 0.65                                           | 0.06                        | 0.08                                           | $\frac{4.84}{\cdot 10^{-4}}$                   | 0.89                  | 0.04                  | 0.11                  | 0.19                  | $\frac{1.4}{\cdot 10^{-3}}$ | 0.61                  | 0.40                  | $\frac{7.6}{\cdot 10^{-3}}$ | 0.35                  |
| <b>T1</b>                                             | <b>AC</b>                                      | <b>AE</b>                         | <b>CE</b>                                      | <b>BD</b>                   | <b>BF</b>                                      | <b>DF</b>                                      | <b>AB</b>             | <b>AD</b>             | <b>AF</b>             | <b>CB</b>             | <b>CD</b>                   | <b>CF</b>             | <b>EB</b>             | <b>ED</b>                   | <b>EF</b>             |
| h                                                     | 0                                              | 0                                 | 0                                              | 0                           | 0                                              | 0                                              | 0                     | 0                     | 0                     | 0                     | 0                           | 0                     | 0                     | 0                           | 0                     |



|                                                      |                                                |                                                |                                                 |                                                 |                                                 |                                                 |                              |                               |                               |                               |                               |                               |                               |                               |                               |
|------------------------------------------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| p                                                    | 0.05                                           | 0.03                                           | 0.72                                            | 0.04                                            | 0.97                                            | 0.08                                            | 0.96                         | 0.06                          | 0.74                          | 0.03                          | 0.98                          | 0.07                          | 0.01                          | 0.59                          | 0.03                          |
| <b>T2</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | <u>1</u>                                       | <u>1</u>                                       | <u>1</u>                                        | 0                                               | 0                                               | 1                                               | 0                            | 1                             | 0                             | 1                             | 1                             | 1                             | 0                             | 0                             | 1                             |
| p                                                    | <u><math>\frac{4.90}{\cdot 10^{-5}}</math></u> | <u><math>\frac{3.68}{\cdot 10^{-4}}</math></u> | <u><math>\frac{6.97}{\cdot 10^{-14}}</math></u> | 0.42                                            | $\frac{2.7}{\cdot 10^{-3}}$                     | $\frac{8.34}{\cdot 10^{-4}}$                    | 0.04                         | $\frac{3.73}{\cdot 10^{-4}}$  | 0.82                          | $\frac{9.33}{\cdot 10^{-13}}$ | $\frac{2.44}{\cdot 10^{-13}}$ | $\frac{1.05}{\cdot 10^{-5}}$  | 0.49                          | 0.92                          | $\frac{2.24}{\cdot 10^{-4}}$  |
| <b>T3</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | 0                                              | 0                                               | <u>1</u>                                        | <u>1</u>                                        | 0                                               | 1                            | 1                             | 1                             | 0                             | 1                             | 1                             | 1                             | 1                             | 1                             |
| p                                                    | $\frac{1.2}{\cdot 10^{-3}}$                    | 0.60                                           | 0.01                                            | <u><math>\frac{2.06}{\cdot 10^{-5}}</math></u>  | <u><math>\frac{7.43}{\cdot 10^{-8}}</math></u>  | 0.26                                            | $\frac{1.12}{\cdot 10^{-9}}$ | $\frac{4.57}{\cdot 10^{-23}}$ | $\frac{5.25}{\cdot 10^{-28}}$ | 0.01                          | $\frac{1.53}{\cdot 10^{-11}}$ | $\frac{2.97}{\cdot 10^{-15}}$ | $\frac{2.38}{\cdot 10^{-7}}$  | $\frac{6.98}{\cdot 10^{-19}}$ | $\frac{2.31}{\cdot 10^{-23}}$ |
| <i>Skeletonema marinoi</i> <b>Kolmogorov-Smirnov</b> |                                                |                                                |                                                 |                                                 |                                                 |                                                 |                              |                               |                               |                               |                               |                               |                               |                               |                               |
| <b>T0</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | 0                                              | 0                                               | 0                                               | 0                                               | 0                                               | 0                            | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             |
| p                                                    | 0.72                                           | 1                                              | 0.86                                            | 0.80                                            | 0.39                                            | 0.21                                            | 1                            | 0.81                          | 0.36                          | 0.69                          | 0.98                          | 0.06                          | 1                             | 0.97                          | 0.30                          |
| <b>T1</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | 0                                              | 0                                               | <u>1</u>                                        | <u>1</u>                                        | 0                                               | 0                            | 0                             | 1                             | 0                             | 0                             | 1                             | 0                             | 0                             | 1                             |
| p                                                    | 0.86                                           | 0.59                                           | 0.32                                            | <u><math>\frac{8.79}{\cdot 10^{-4}}</math></u>  | <u><math>\frac{1.08}{\cdot 10^{-4}}</math></u>  | 0.01                                            | 1                            | 0.01                          | $\frac{9.49}{\cdot 10^{-4}}$  | 0.96                          | $\frac{8.6}{\cdot 10^{-3}}$   | $\frac{4.19}{\cdot 10^{-4}}$  | 0.19                          | 0.19                          | $\frac{5.52}{\cdot 10^{-4}}$  |
| <b>T2</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | 0                                              | <u>1</u>                                        | <u>1</u>                                        | <u>1</u>                                        | <u>1</u>                                        | 0                            | 1                             | 1                             | 0                             | 1                             | 0                             | 0                             | 1                             | 1                             |
| p                                                    | 0.01                                           | 0.01                                           | <u><math>\frac{2.61}{\cdot 10^{-6}}</math></u>  | <u><math>\frac{1.48}{\cdot 10^{-19}}</math></u> | <u><math>\frac{3.47}{\cdot 10^{-3}}</math></u>  | <u><math>\frac{2.52}{\cdot 10^{-10}}</math></u> | 1                            | $\frac{5.52}{\cdot 10^{-19}}$ | $\frac{5.49}{\cdot 10^{-7}}$  | $\frac{6.10}{\cdot 10^{-3}}$  | $\frac{9.36}{\cdot 10^{-11}}$ | $\frac{3.70}{\cdot 10^{-3}}$  | 0.02                          | $\frac{5.49}{\cdot 10^{-28}}$ | $\frac{1.53}{\cdot 10^{-16}}$ |
| <b>T3</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | <u>1</u>                                       | <u>1</u>                                        | <u>1</u>                                        | <u>1</u>                                        | 0                                               | 1                            | 1                             | 1                             | 1                             | 1                             | 0                             | 1                             | 1                             | 1                             |
| p                                                    | 0.02                                           | <u><math>\frac{1.46}{\cdot 10^{-6}}</math></u> | <u><math>\frac{1.87}{\cdot 10^{-12}}</math></u> | <u><math>\frac{1.61}{\cdot 10^{-29}}</math></u> | <u><math>\frac{2.18}{\cdot 10^{-23}}</math></u> | 0.02                                            | $\frac{1.53}{\cdot 10^{-9}}$ | $\frac{2.75}{\cdot 10^{-9}}$  | $\frac{4.82}{\cdot 10^{-6}}$  | $\frac{2.18}{\cdot 10^{-15}}$ | $\frac{1.11}{\cdot 10^{-4}}$  | 0.08                          | $\frac{2.67}{\cdot 10^{-11}}$ | $\frac{4.74}{\cdot 10^{-31}}$ | $\frac{1.98}{\cdot 10^{-20}}$ |
| <i>Skeletonema marinoi</i> <b>Wilcoxon</b>           |                                                |                                                |                                                 |                                                 |                                                 |                                                 |                              |                               |                               |                               |                               |                               |                               |                               |                               |
| <b>T0</b>                                            | <b>AC</b>                                      | <b>AE</b>                                      | <b>CE</b>                                       | <b>BD</b>                                       | <b>BF</b>                                       | <b>DF</b>                                       | <b>AB</b>                    | <b>AD</b>                     | <b>AF</b>                     | <b>CB</b>                     | <b>CD</b>                     | <b>CF</b>                     | <b>EB</b>                     | <b>ED</b>                     | <b>EF</b>                     |
| h                                                    | 0                                              | 0                                              | 0                                               | 0                                               | 0                                               | 0                                               | 0                            | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             | 0                             |

|           |           |                                        |                                         |                                         |                                         |                                         |                      |                       |                      |                       |                       |                      |                      |                       |                       |
|-----------|-----------|----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|----------------------|-----------------------|----------------------|-----------------------|-----------------------|----------------------|----------------------|-----------------------|-----------------------|
| p         | 0.68      | 0.95                                   | 0.77                                    | 0.70                                    | 0.20                                    | 0.07                                    | 0.94                 | 0.76                  | 0.18                 | 0.63                  | 0.88                  | 0.06                 | 0.89                 | 0.81                  | 0.15                  |
| <b>T1</b> | <b>AC</b> | <b>AE</b>                              | <b>CE</b>                               | <b>BD</b>                               | <b>BF</b>                               | <b>DF</b>                               | <b>AB</b>            | <b>AD</b>             | <b>AF</b>            | <b>CB</b>             | <b>CD</b>             | <b>CF</b>            | <b>EB</b>            | <b>ED</b>             | <b>EF</b>             |
| h         | 0         | 0                                      | 0                                       | 0                                       | 0                                       | 0                                       | 0                    | 0                     | 0                    | 0                     | 0                     | 1                    | 0                    | 0                     | 0                     |
| p         | 0.60      | 0.98                                   | 0.57                                    | 0.51                                    | $1.10 \cdot 10^{-3}$                    | $1.50 \cdot 10^{-3}$                    | 0.55                 | 0.96                  | $6.90 \cdot 10^{-3}$ | 0.93                  | 0.46                  | $7.22 \cdot 10^{-4}$ | 0.53                 | 0.86                  | $3.00 \cdot 10^{-3}$  |
| <b>T2</b> | <b>AC</b> | <b>AE</b>                              | <b>CE</b>                               | <b>BD</b>                               | <b>BF</b>                               | <b>DF</b>                               | <b>AB</b>            | <b>AD</b>             | <b>AF</b>            | <b>CB</b>             | <b>CD</b>             | <b>CF</b>            | <b>EB</b>            | <b>ED</b>             | <b>EF</b>             |
| h         | 0         | 0                                      | <u>1</u>                                | <u>1</u>                                | <u>1</u>                                | <u>1</u>                                | 0                    | 1                     | 1                    | 0                     | 1                     | 0                    | 0                    | 1                     | 1                     |
| p         | 0.01      | $2.70 \cdot 10^{-3}$                   | <u><math>1.01 \cdot 10^{-7}</math></u>  | <u><math>4.79 \cdot 10^{-27}</math></u> | <u><math>9.11 \cdot 10^{-7}</math></u>  | <u><math>3.01 \cdot 10^{-13}</math></u> | 0.83                 | $9.19 \cdot 10^{-26}$ | $3.25 \cdot 10^{-6}$ | $6.20 \cdot 10^{-3}$  | $4.50 \cdot 10^{-15}$ | 0.09                 | $5.20 \cdot 10^{-3}$ | $2.72 \cdot 10^{-38}$ | $5.00 \cdot 10^{-15}$ |
| <b>T3</b> | <b>AC</b> | <b>AE</b>                              | <b>CE</b>                               | <b>BD</b>                               | <b>BF</b>                               | <b>DF</b>                               | <b>AB</b>            | <b>AD</b>             | <b>AF</b>            | <b>CB</b>             | <b>CD</b>             | <b>CF</b>            | <b>EB</b>            | <b>ED</b>             | <b>EF</b>             |
| h         | 0         | <u>1</u>                               | <u>1</u>                                | <u>1</u>                                | <u>1</u>                                | 0                                       | 1                    | 1                     | 1                    | 1                     | 1                     | 0                    | 0                    | 1                     | 1                     |
| p         | 0.08      | <u><math>1.73 \cdot 10^{-9}</math></u> | <u><math>1.01 \cdot 10^{-17}</math></u> | <u><math>3.70 \cdot 10^{-32}</math></u> | <u><math>4.07 \cdot 10^{-21}</math></u> | $6.30 \cdot 10^{-3}$                    | $2.50 \cdot 10^{-8}$ | $5.13 \cdot 10^{-12}$ | $1.24 \cdot 10^{-5}$ | $2.24 \cdot 10^{-13}$ | $2.33 \cdot 10^{-8}$  | $6.20 \cdot 10^{-3}$ | 0.32                 | $5.31 \cdot 10^{-45}$ | $2.86 \cdot 10^{-29}$ |

**Supplementary Table 9. Non-parametric statistic tests** (Kolmogorov-Smirnov (KS2) and Wilcoxon) run in pairwise among turbulent vs still samples merging results from the three replicates. ‘h’ indicate the test answer: 0 = null hypothesis accepted; 1 = null hypothesis consistently rejected. ‘p’ indicate the p-value associated to the test outcome. Accuracy set at 99.9% ( $\alpha = 10^{-3}$ ).

| <b><i>Chaetoceros decipiens</i> Experiment 1</b> |           |                      |                       |                       |
|--------------------------------------------------|-----------|----------------------|-----------------------|-----------------------|
| <b>KS2</b>                                       | <b>T0</b> | <b>T1</b>            | <b>T2</b>             |                       |
| <b>h</b>                                         | 0         | 1                    | 1                     |                       |
| <b>p</b>                                         | 1         | $1.33 \cdot 10^{-4}$ | $7.81 \cdot 10^{-15}$ |                       |
| <b>Wilcoxon</b>                                  | <b>T0</b> | <b>T1</b>            | <b>T2</b>             |                       |
| <b>h</b>                                         | 0         | 0                    | 1                     |                       |
| <b>p</b>                                         | 0.58      | $3.4 \cdot 10^{-3}$  | $1.69 \cdot 10^{-16}$ |                       |
| <b><i>Chaetoceros decipiens</i> Experiment 2</b> |           |                      |                       |                       |
| <b>KS2</b>                                       | <b>T0</b> | <b>T1</b>            | <b>T2</b>             | <b>T3</b>             |
| <b>h</b>                                         | 0         | 0                    | 1                     | 1                     |
| <b>p</b>                                         | 0.18      | 0.03                 | $1.56 \cdot 10^{-24}$ | $4.24 \cdot 10^{-63}$ |
| <b>Wilcoxon</b>                                  | <b>T0</b> | <b>T1</b>            | <b>T2</b>             | <b>T3</b>             |
| <b>h</b>                                         | 0         | 1                    | 1                     | 1                     |
| <b>p</b>                                         | 0.35      | $5.78 \cdot 10^{-5}$ | $5.78 \cdot 10^{-24}$ | $4.35 \cdot 10^{-83}$ |
| <b><i>Thalassiosira rotula</i></b>               |           |                      |                       |                       |
| <b>KS2</b>                                       | <b>T0</b> | <b>T1</b>            | <b>T2</b>             | <b>T3</b>             |
| <b>h</b>                                         | 0         | 0                    | 0                     | 1                     |
| <b>p</b>                                         | 0.54      | $1.0 \cdot 10^{-3}$  | $3.60 \cdot 10^{-3}$  | $2.69 \cdot 10^{-35}$ |
| <b>Wilcoxon</b>                                  | <b>T0</b> | <b>T1</b>            | <b>T2</b>             | <b>T3</b>             |
| <b>h</b>                                         | 0         | 1                    | 1                     | 1                     |
| <b>p</b>                                         | 0.16      | $4.88 \cdot 10^{-5}$ | $6.28 \cdot 10^{-5}$  | $3.15 \cdot 10^{-40}$ |

**Supplementary Table 10. Incidence of broken chains in *Chaetoceros decipiens* experiments 1 and 2.** The number of broken chains (BC) recorded during cell counts is reported for each TURBOGEN cylinder (turbulent condition A, C, E and still condition B, D, F). These numbers have been weighted over the total number of chains recorded in each sample (Percentage of BC). The overall percentage in turbulent and still conditions (red digits) was calculated as the sum of the BC recorded in each cylinder weighted over the total number of chains. These data were plotted in Fig. 4c (Experiment 1) and Fig. 4d (Experiment 2).

| <b>Experiment 1</b>             |             |          |          |              |          |          |
|---------------------------------|-------------|----------|----------|--------------|----------|----------|
| <b>Number of BC</b>             |             |          |          |              |          |          |
| <b>Time point</b>               | <b>Turb</b> |          |          | <b>Still</b> |          |          |
|                                 | <b>A</b>    | <b>C</b> | <b>E</b> | <b>B</b>     | <b>D</b> | <b>F</b> |
| <b>T0</b>                       | 5           | 1        | 1        | 1            | 2        | 1        |
| <b>T1</b>                       | 11          | 3        | 1        | 11           | 8        | 1        |
| <b>T2</b>                       | 11          | 4        | 6        | 15           | 22       | 14       |
| <b>Percentage of BC</b>         |             |          |          |              |          |          |
| <b>%</b>                        | <b>Turb</b> |          |          | <b>Still</b> |          |          |
|                                 | <b>A</b>    | <b>C</b> | <b>E</b> | <b>B</b>     | <b>D</b> | <b>F</b> |
| <b>T0</b>                       | 1.4         | 0.2      | 0.2      | 0.3          | 0.5      | 0.2      |
| <b>T1</b>                       | 3.4         | 0.8      | 0.2      | 2.9          | 2.2      | 0.2      |
| <b>T2</b>                       | 2.5         | 1.1      | 1.5      | 3.6          | 5.3      | 3.2      |
| <b>Overall percentage of BC</b> |             |          |          |              |          |          |
| <b>%</b>                        | <b>Turb</b> |          |          | <b>Still</b> |          |          |
| <b>T0</b>                       | 0.6         |          |          | 0.3          |          |          |
| <b>T1</b>                       | 1.4         |          |          | 1.8          |          |          |
| <b>T2</b>                       | 1.7         |          |          | 4.0          |          |          |
| <b>Experiment 2</b>             |             |          |          |              |          |          |
| <b>Number of BC</b>             |             |          |          |              |          |          |
|                                 | <b>Turb</b> |          |          | <b>Still</b> |          |          |
|                                 | <b>A</b>    | <b>C</b> | <b>E</b> | <b>B</b>     | <b>D</b> | <b>F</b> |
| <b>T0</b>                       | 2           | 1        | 3        | 1            | 3        | 2        |
| <b>T1</b>                       | 3           | 7        | 4        | 3            | 3        | 6        |
| <b>T2</b>                       | 15          | 17       | 13       | 20           | 19       | 20       |
| <b>T3</b>                       | 17          | 20       | 8        | 103          | 50       | 1        |
| <b>Percentage of BC</b>         |             |          |          |              |          |          |
| <b>%</b>                        | <b>Turb</b> |          |          | <b>Still</b> |          |          |
|                                 | <b>A</b>    | <b>C</b> | <b>E</b> | <b>B</b>     | <b>D</b> | <b>F</b> |
| <b>T0</b>                       | 0.5         | 0.2      | 0.7      | 0.2          | 0.7      | 0.5      |
| <b>T1</b>                       | 0.7         | 1.7      | 1.0      | 0.7          | 0.9      | 1.5      |
| <b>T2</b>                       | 3.7         | 4.2      | 3.5      | 4.7          | 4.4      | 5.0      |
| <b>T3</b>                       | 3.9         | 5.6      | 2.0      | 24.0         | 13.8     | 0.2      |
| <b>Overall percentage of BC</b> |             |          |          |              |          |          |
| <b>%</b>                        | <b>Turb</b> |          |          | <b>Still</b> |          |          |

|           |     |      |
|-----------|-----|------|
| <b>T0</b> | 0.5 | 0.5  |
| <b>T1</b> | 1.2 | 1.0  |
| <b>T2</b> | 3.8 | 4.7  |
| <b>T3</b> | 3.8 | 12.8 |

**Legend to the Supplementary Table 11**

Supplementary Table 11 contains the first 50 hits from a tblastn search in the MMETSP database performed with transcripts listed in Supplementary Table 6.

**Legend to the Supplementary Table 12**

This file contains the annotation for the *Chaetoceros decipiens* transcriptome obtained from Annocript.

File **Supplementary Data 2** contains the nucleotide sequences of the *Chaetoceros decipiens* transcripts.

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