Supplementary Information

A metabolomic snapshot through NMR revealed differences in phase transition during induction of reproduction in *Ulva*

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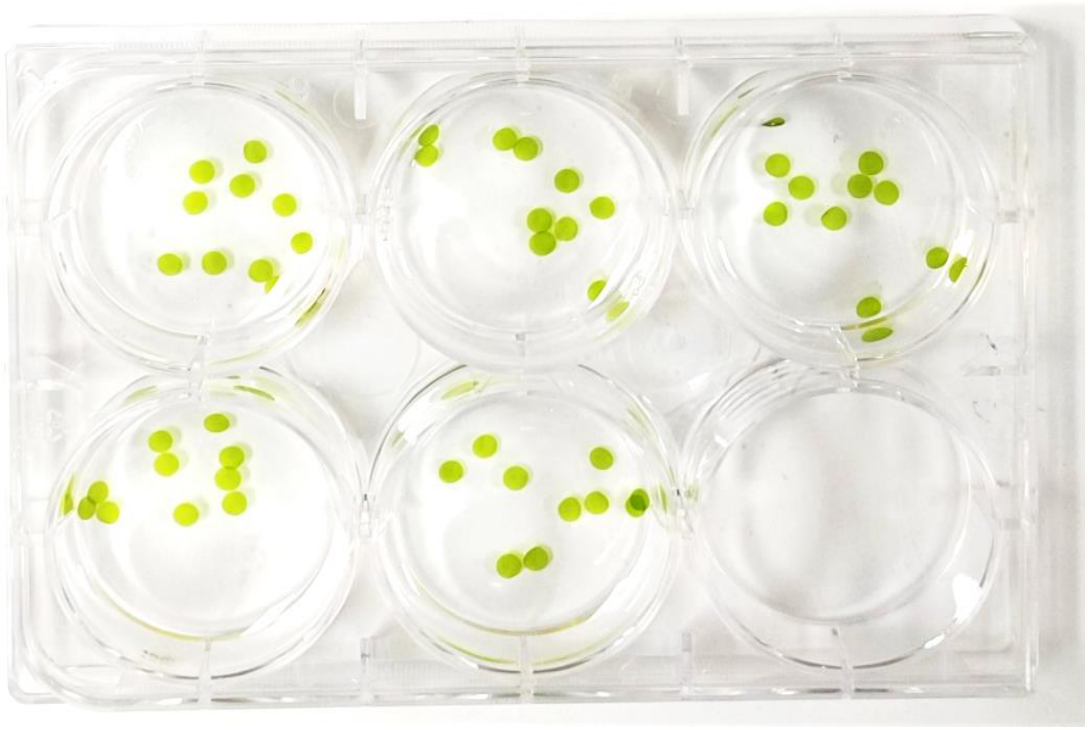

Figure S1. Excised discs (3mm) from the thallus of *Ulva ohnoi*.

Figure S2. Fragmented thallus of *Ulva ohnoi* after artificial induction of reproduction.

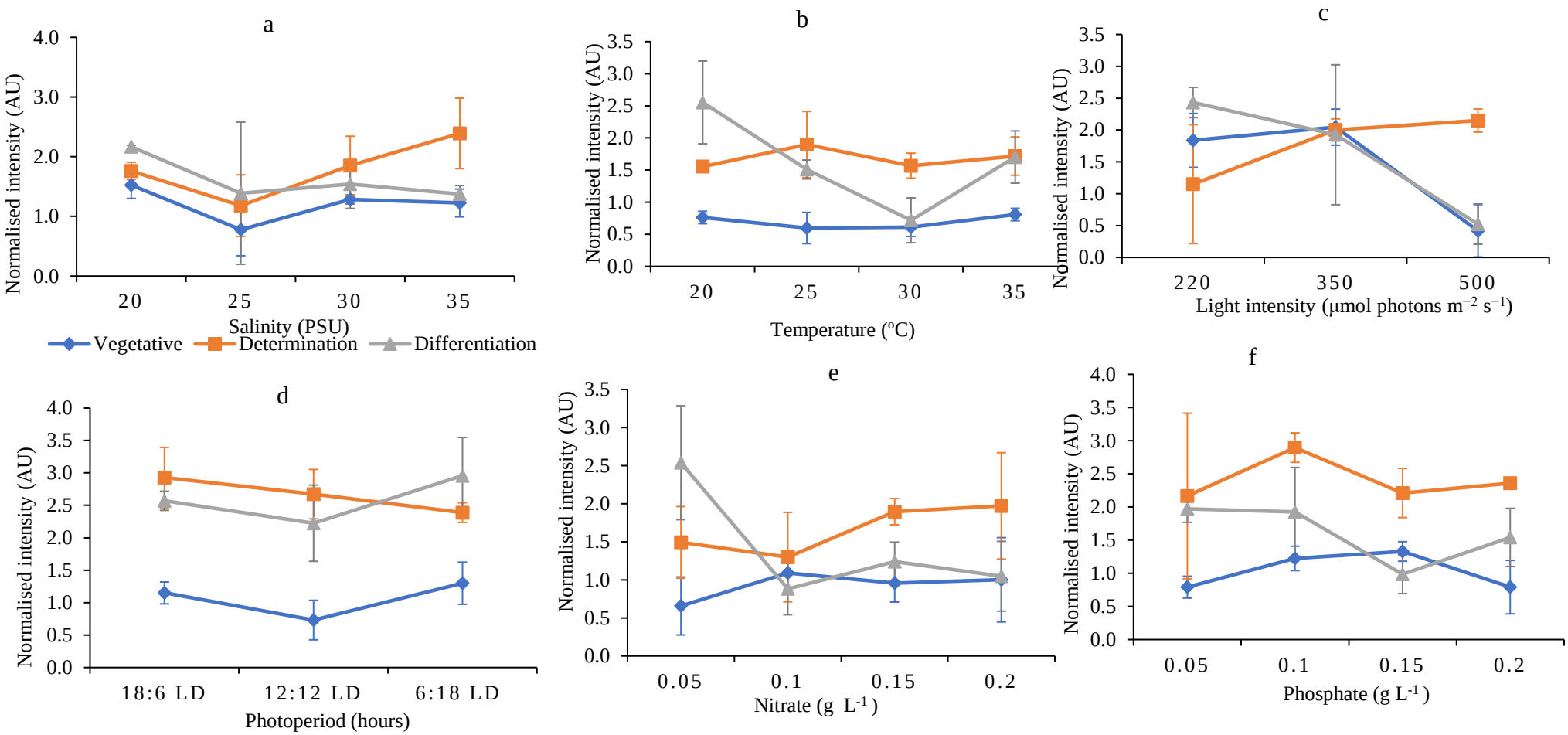

Figure S3. Fluctuation of tryptophan in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

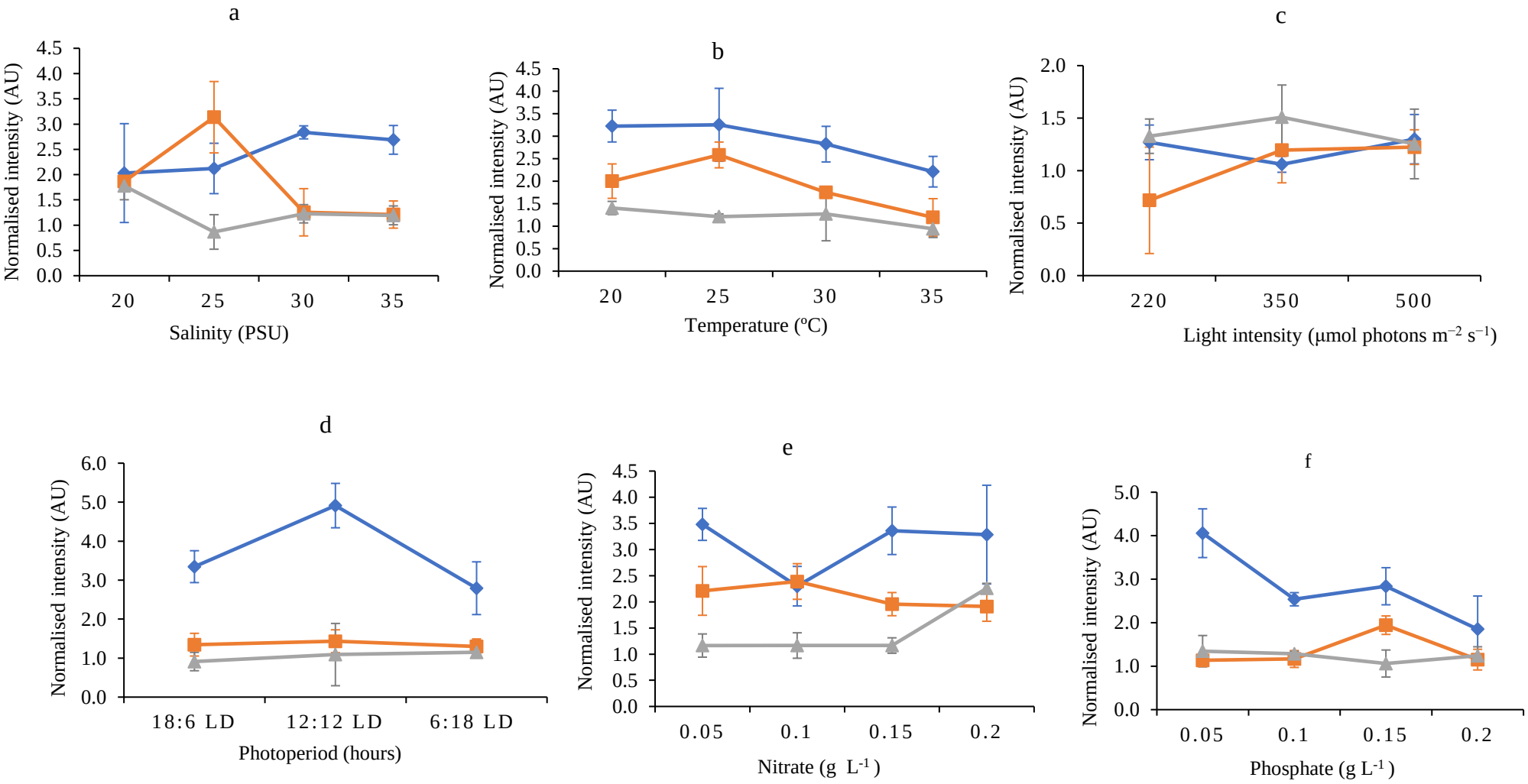

Figure S4. Fluctuation of lactic acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

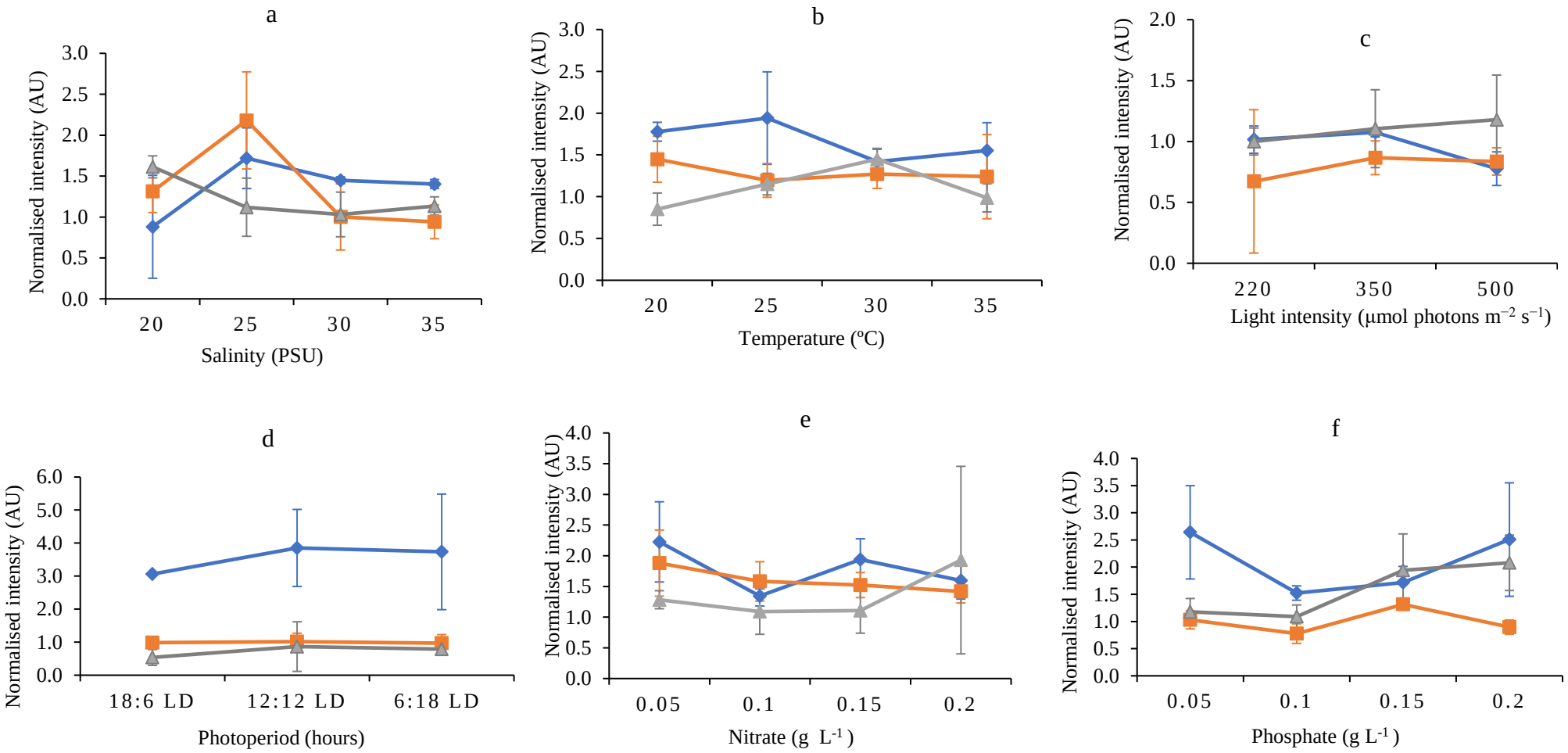

Figure S5. Fluctuation of L- Cysteinesulfinic acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

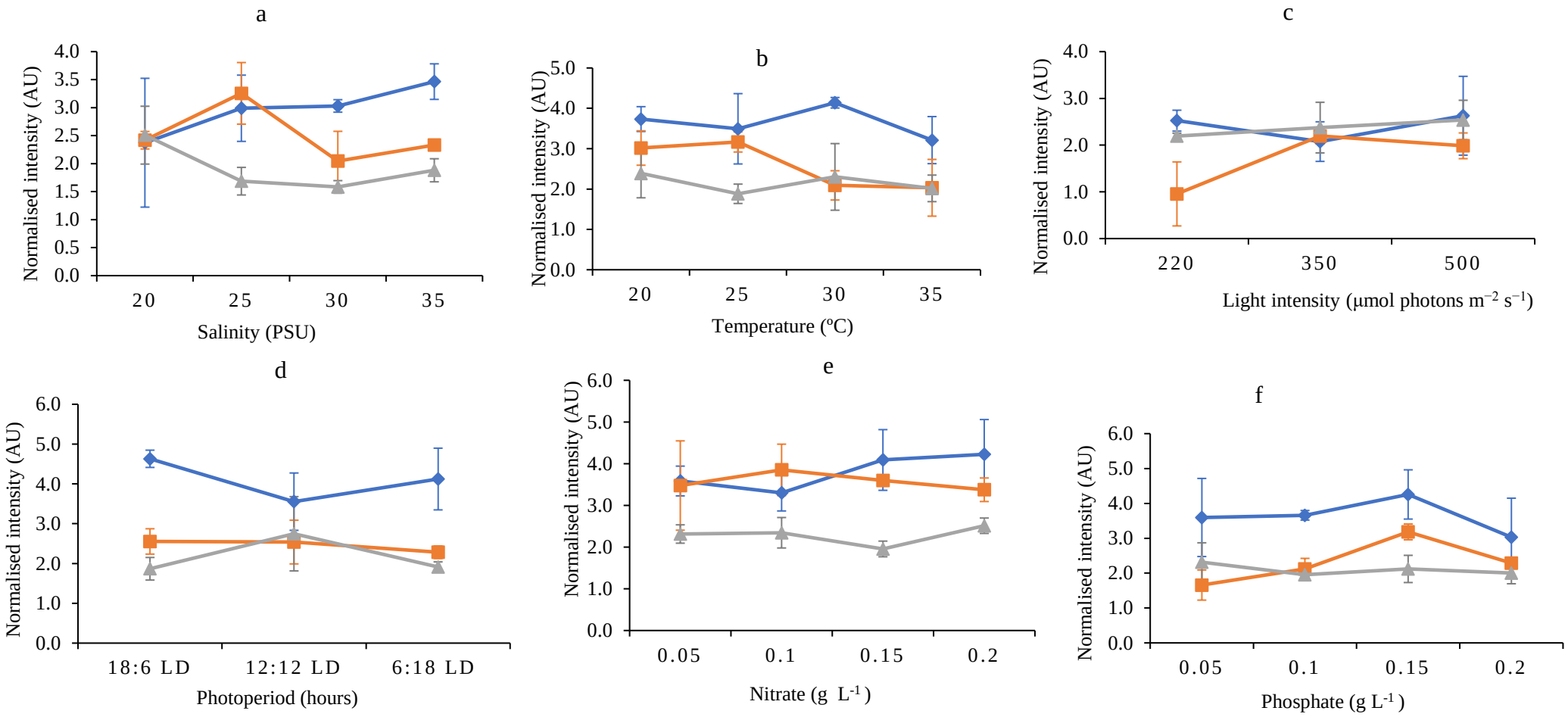

Figure S6. Fluctuation of galactose in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

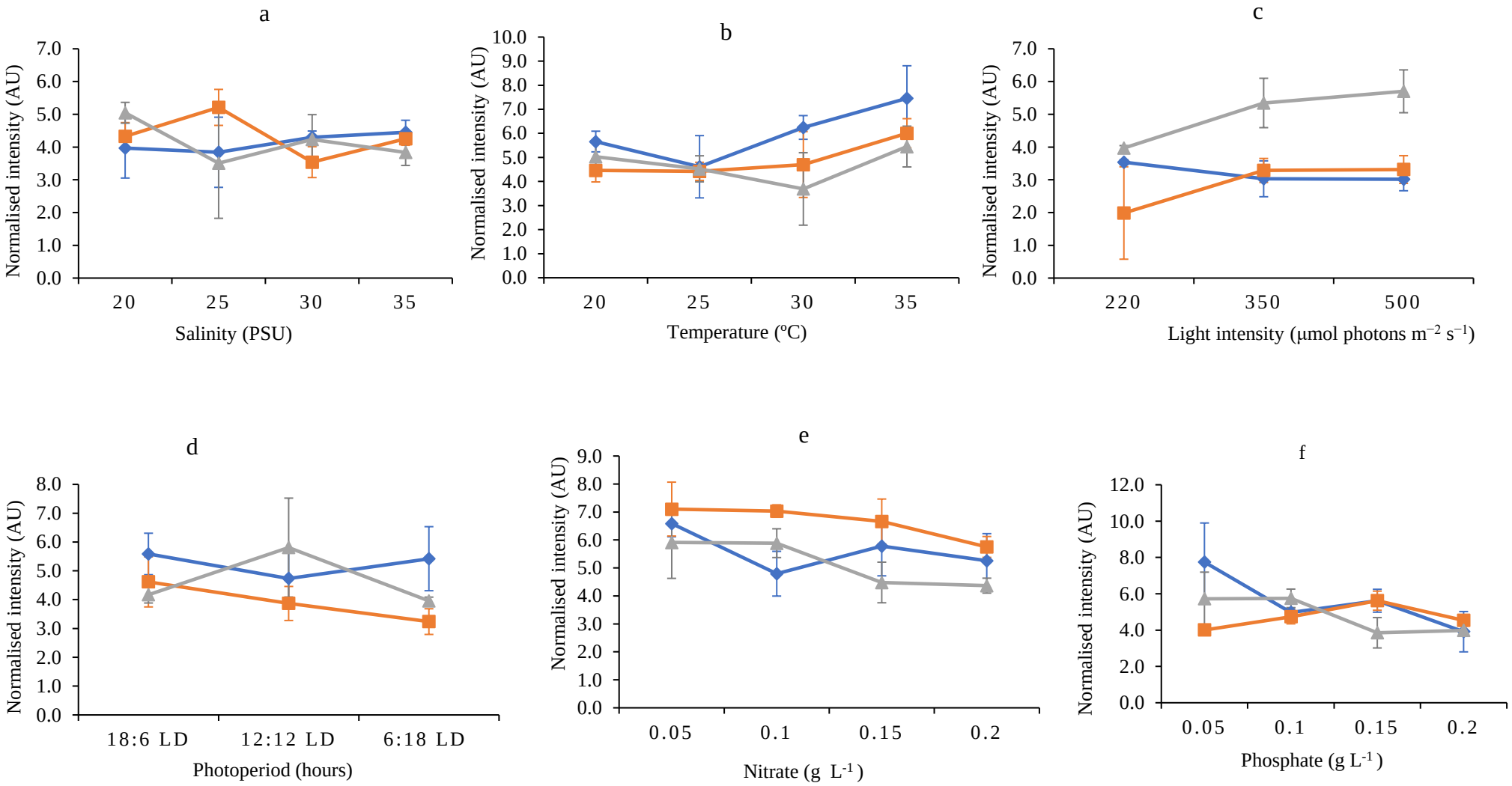

Figure S7. Fluctuation of ethanolamine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

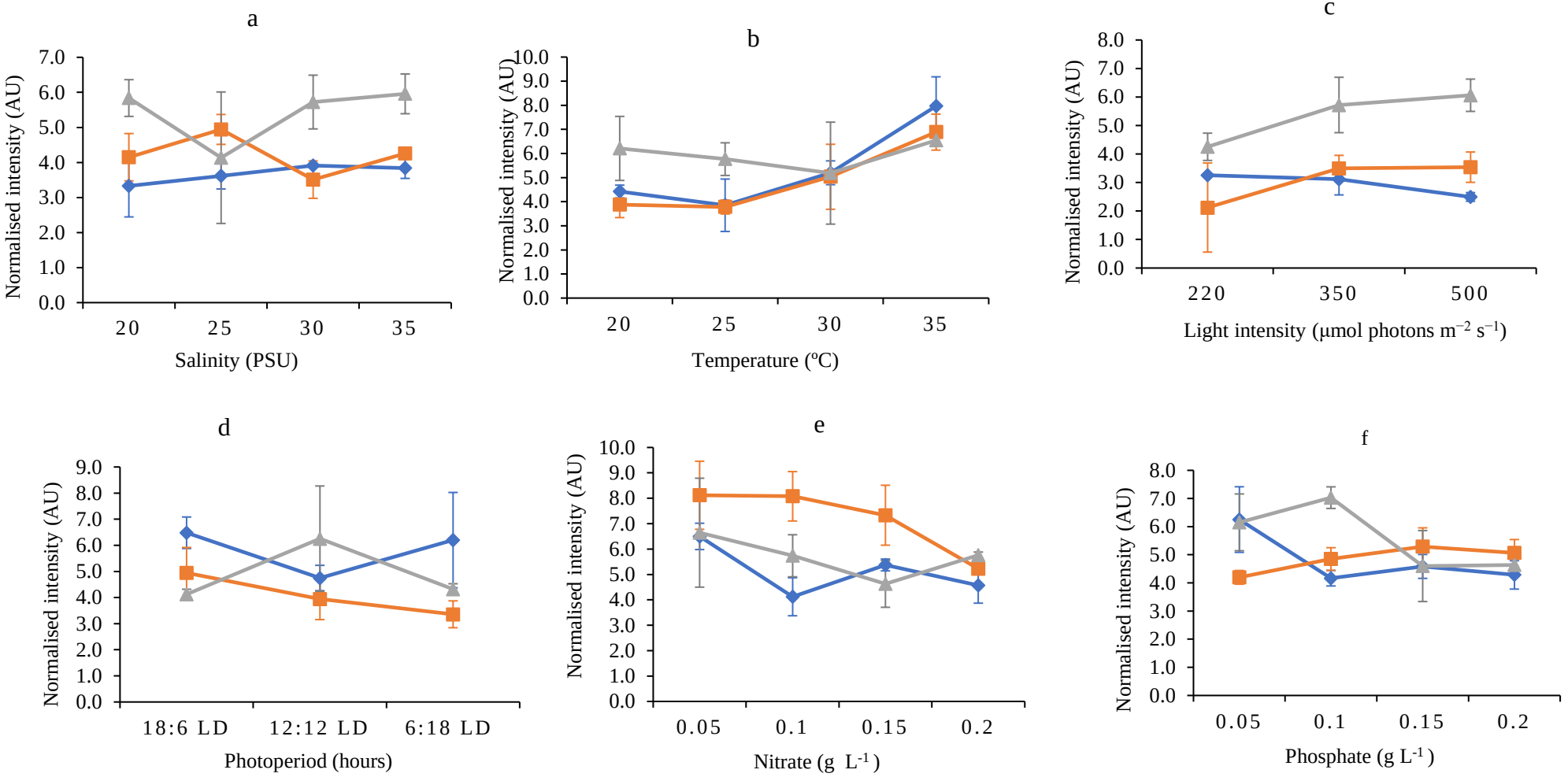

Figure S8. Fluctuation of aspartate in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

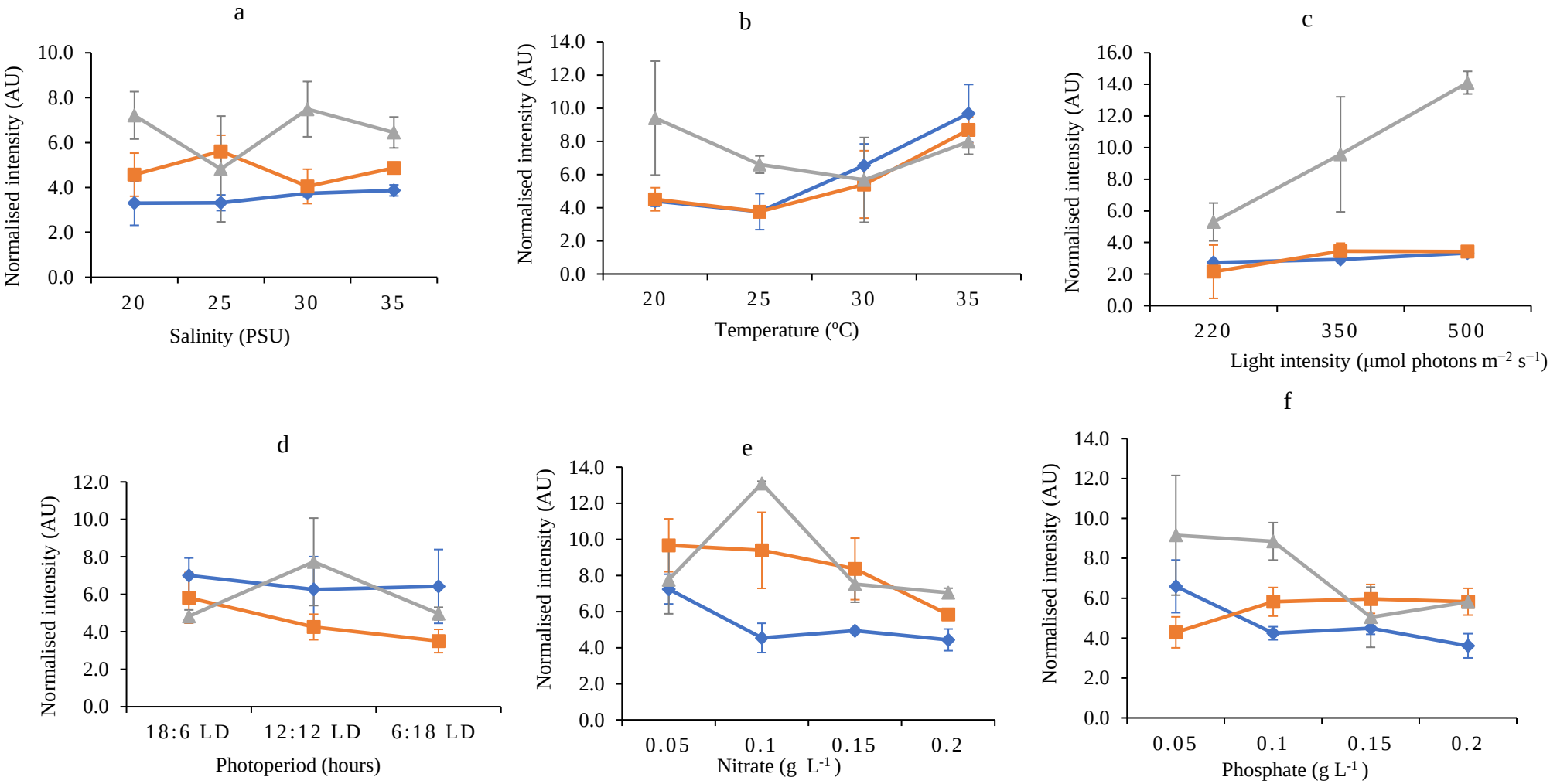

Figure S9. Fluctuation of creatine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

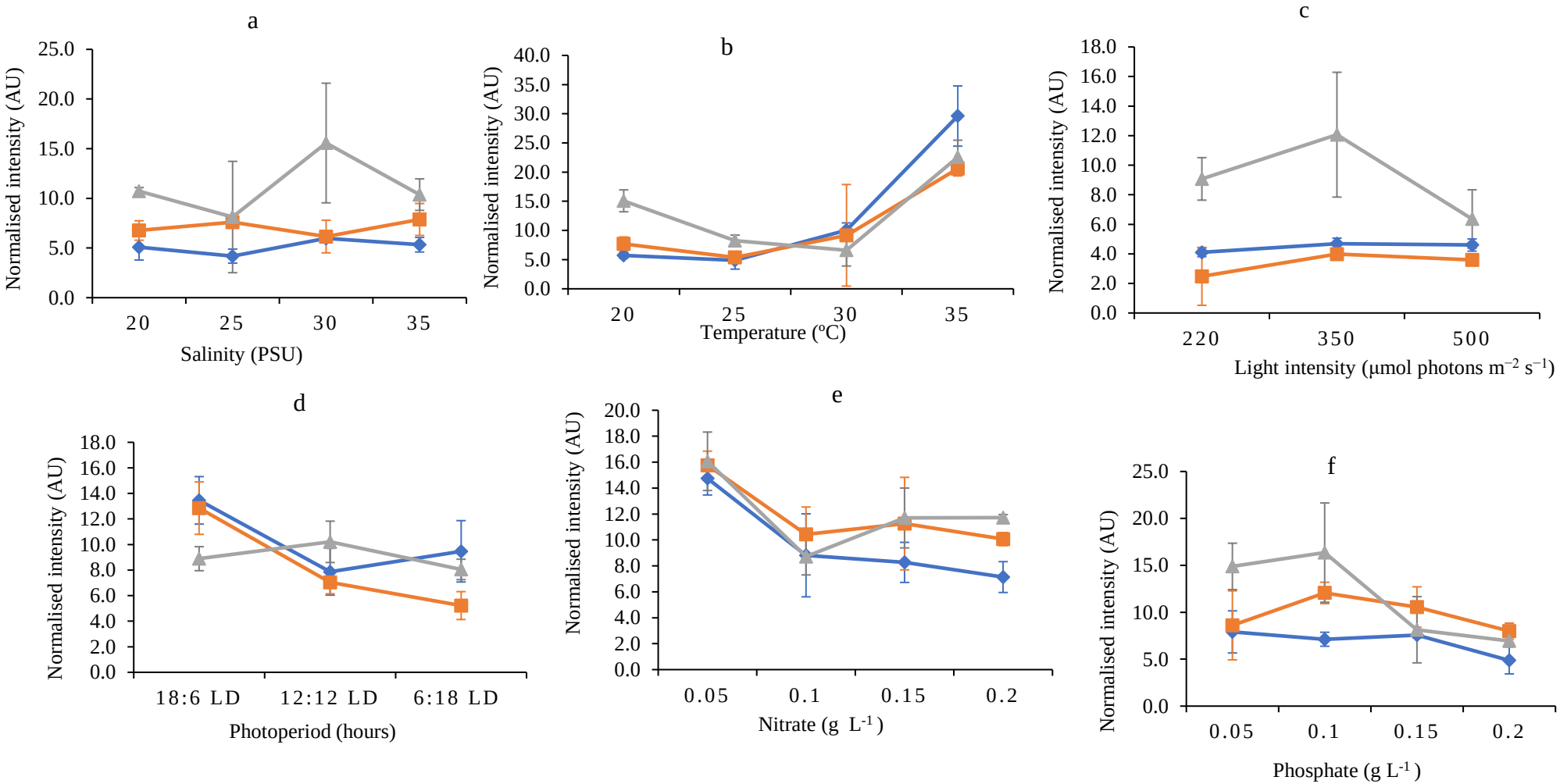

Figure S10. Fluctuation of sucrose in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

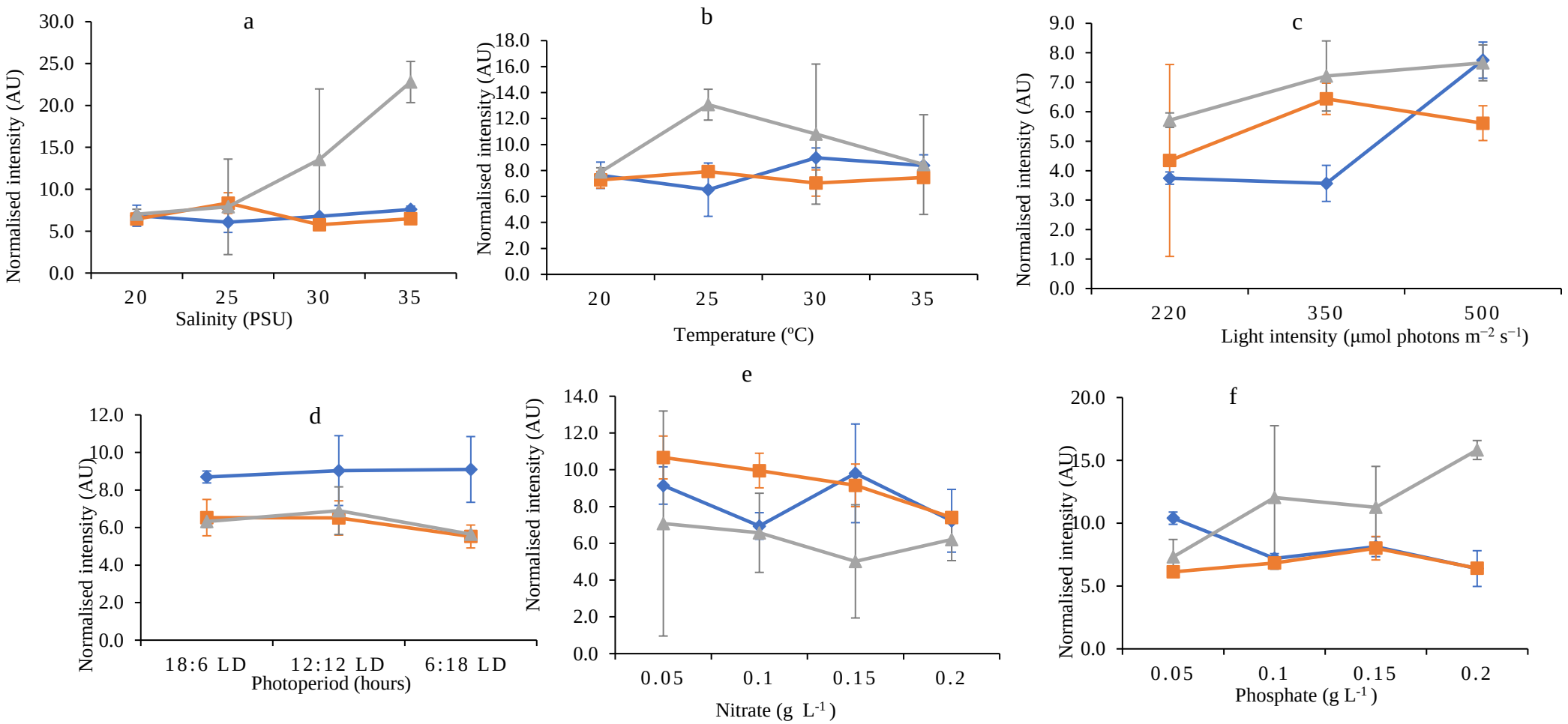

Figure S11. Fluctuation of acetylcholine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

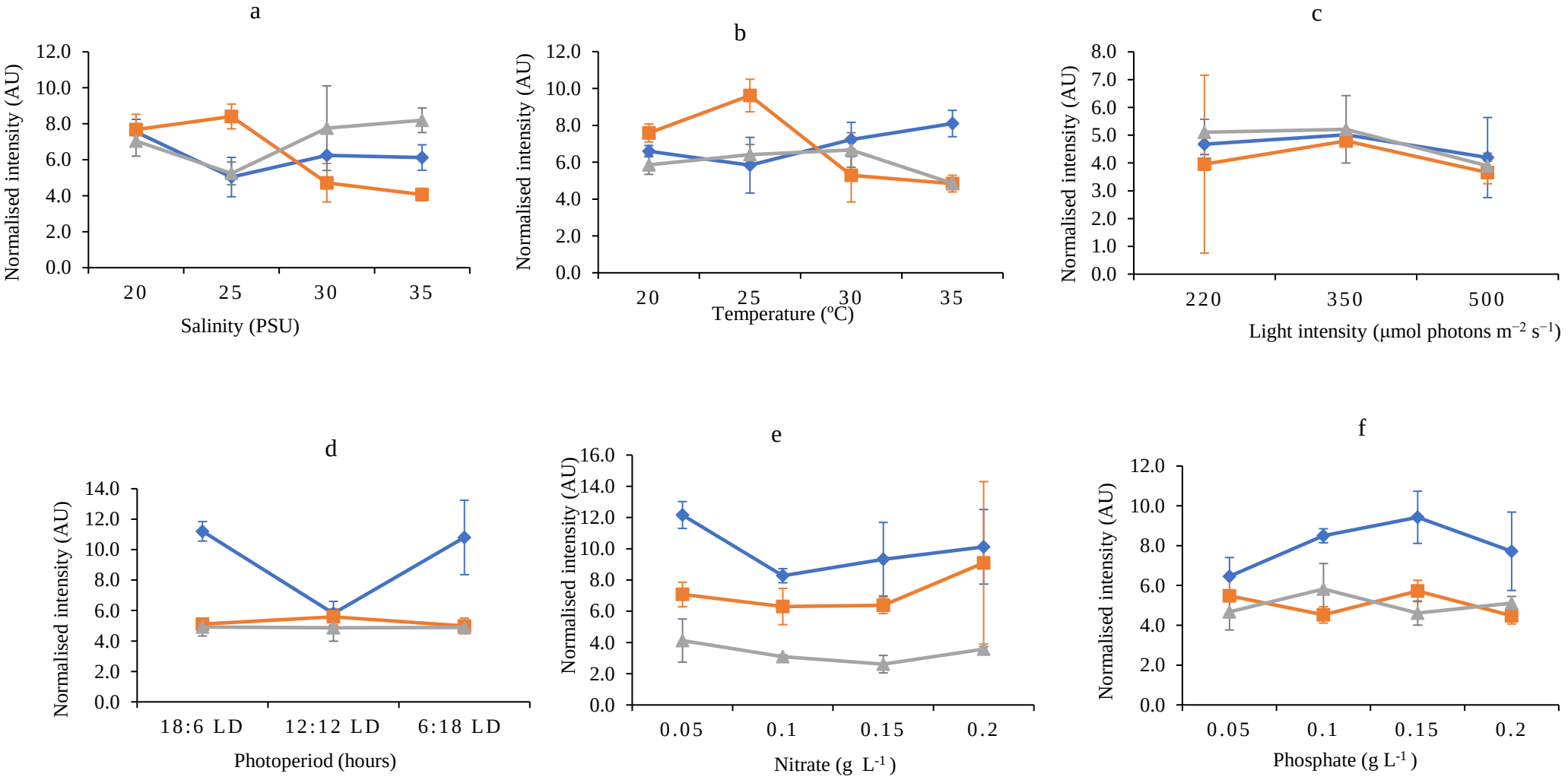

Figure S12. Fluctuation of glucose in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

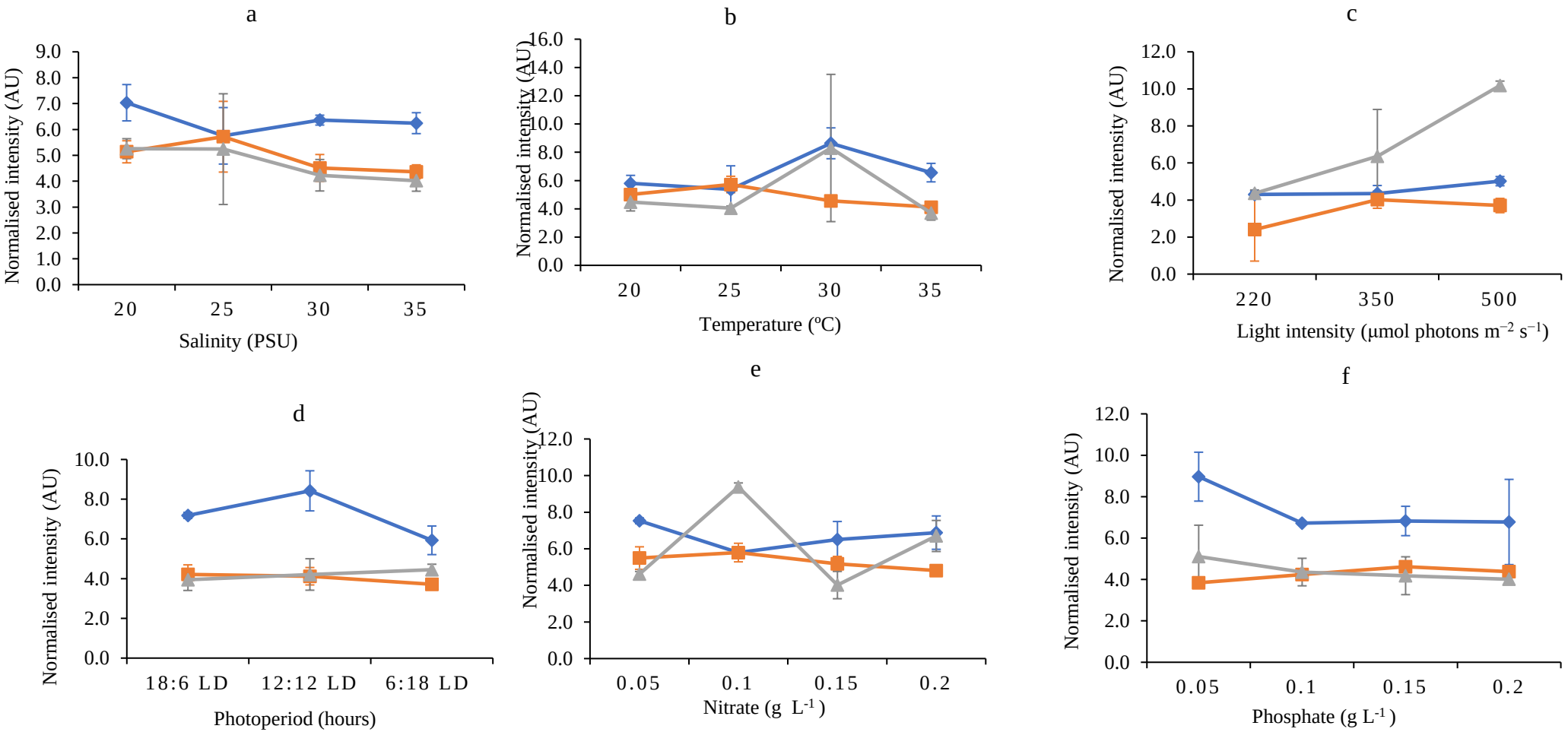

Figure S13. Fluctuation of sorbitol in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

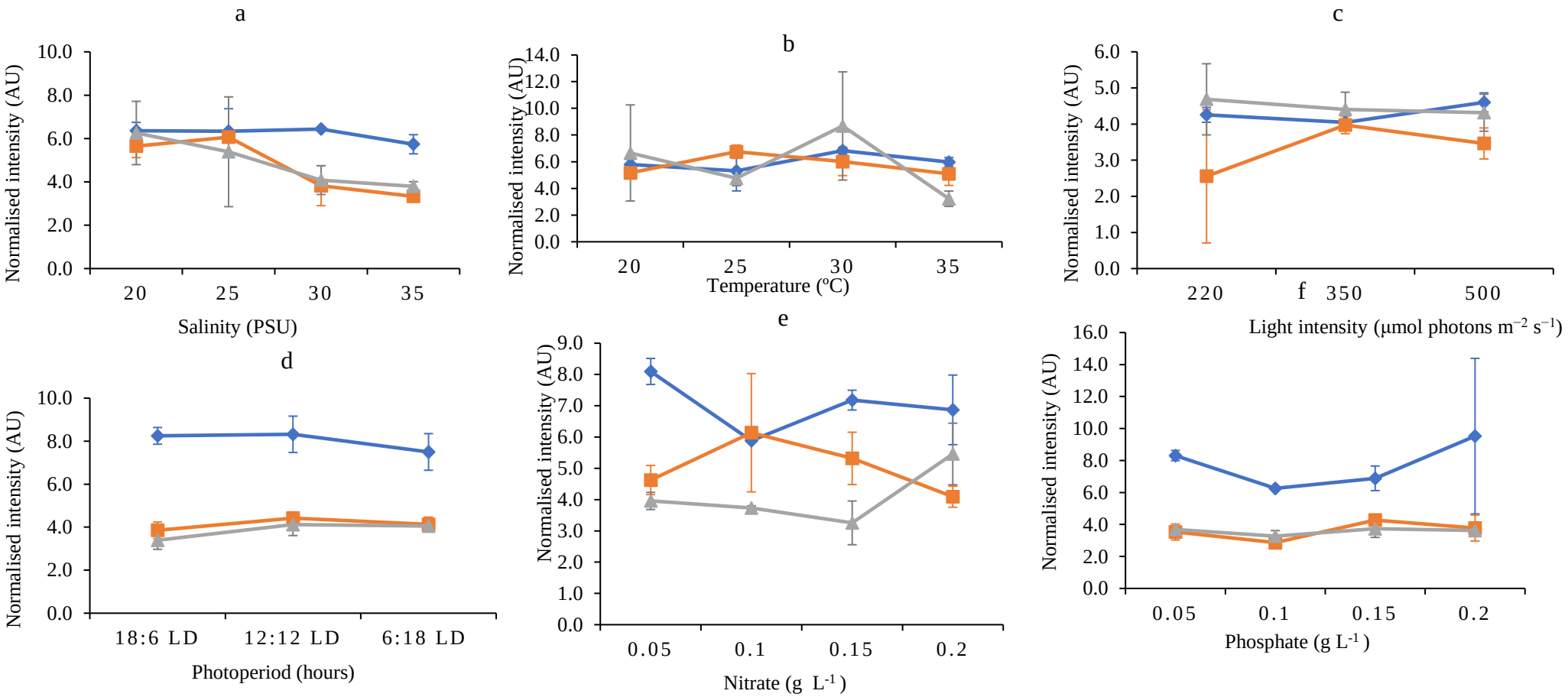

Figure S14. Fluctuation of coniferyl aldehyde in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

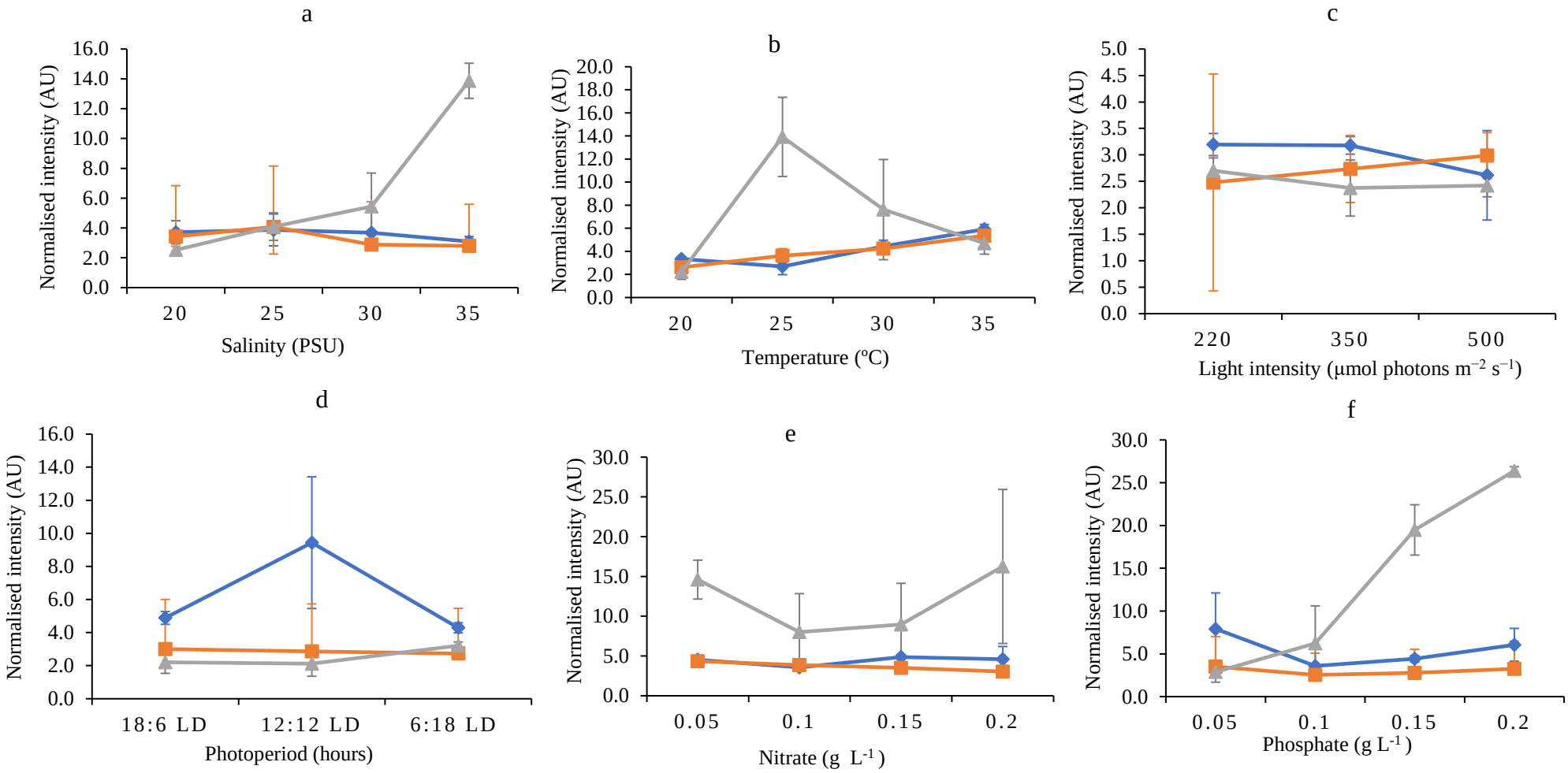

Figure S15. Fluctuation of glycine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

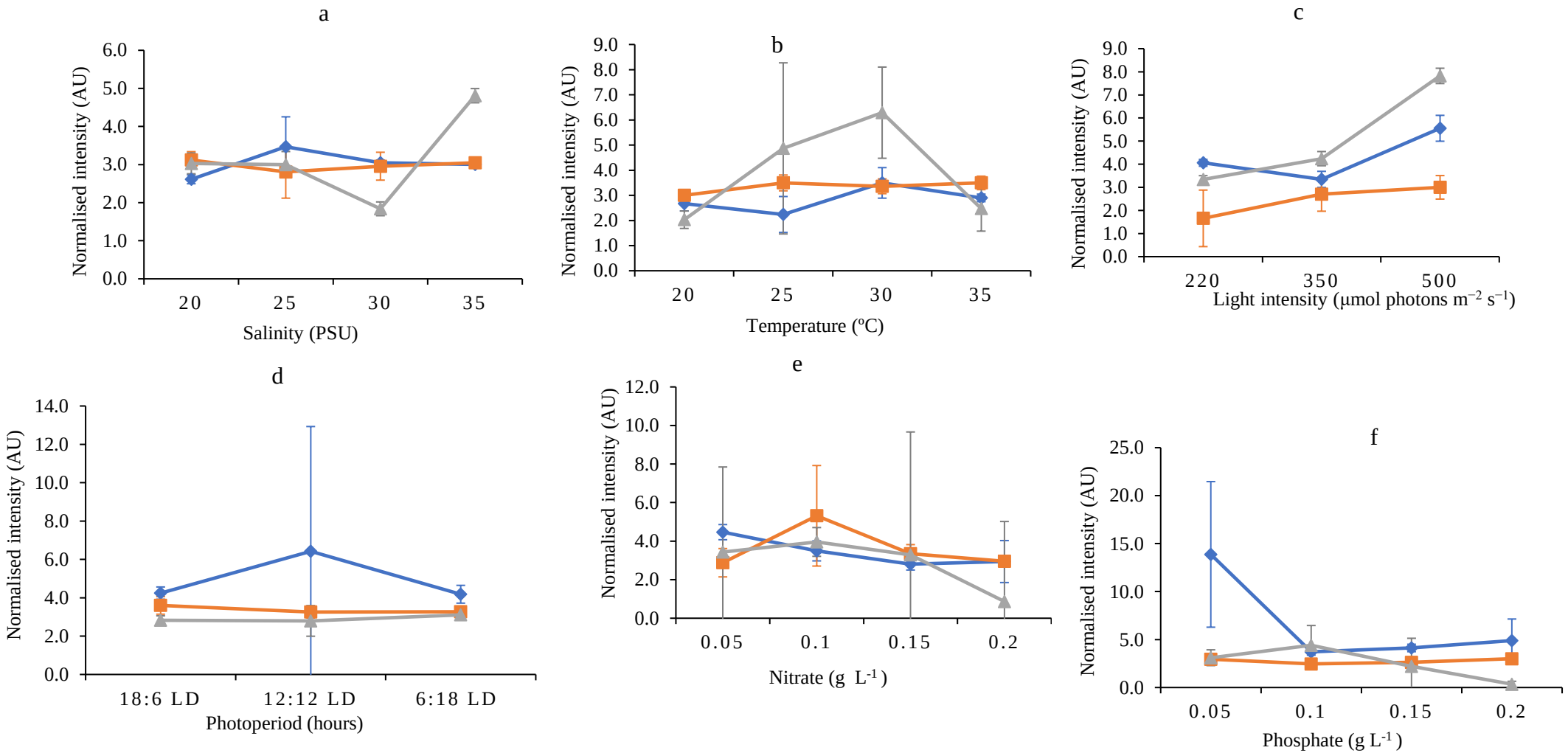

Figure S16. Fluctuation of choline in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

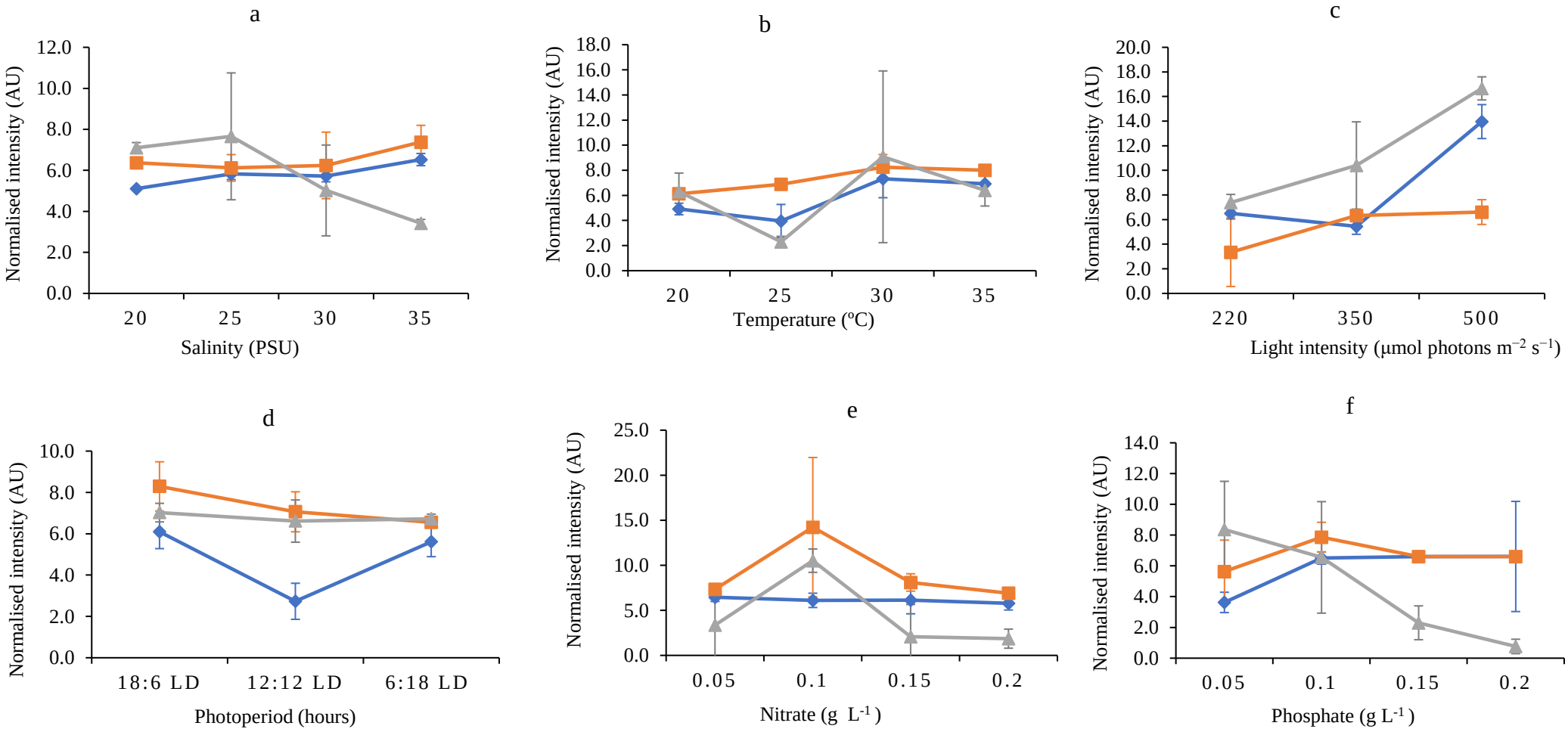

Figure S17. Fluctuation of hypotaurine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

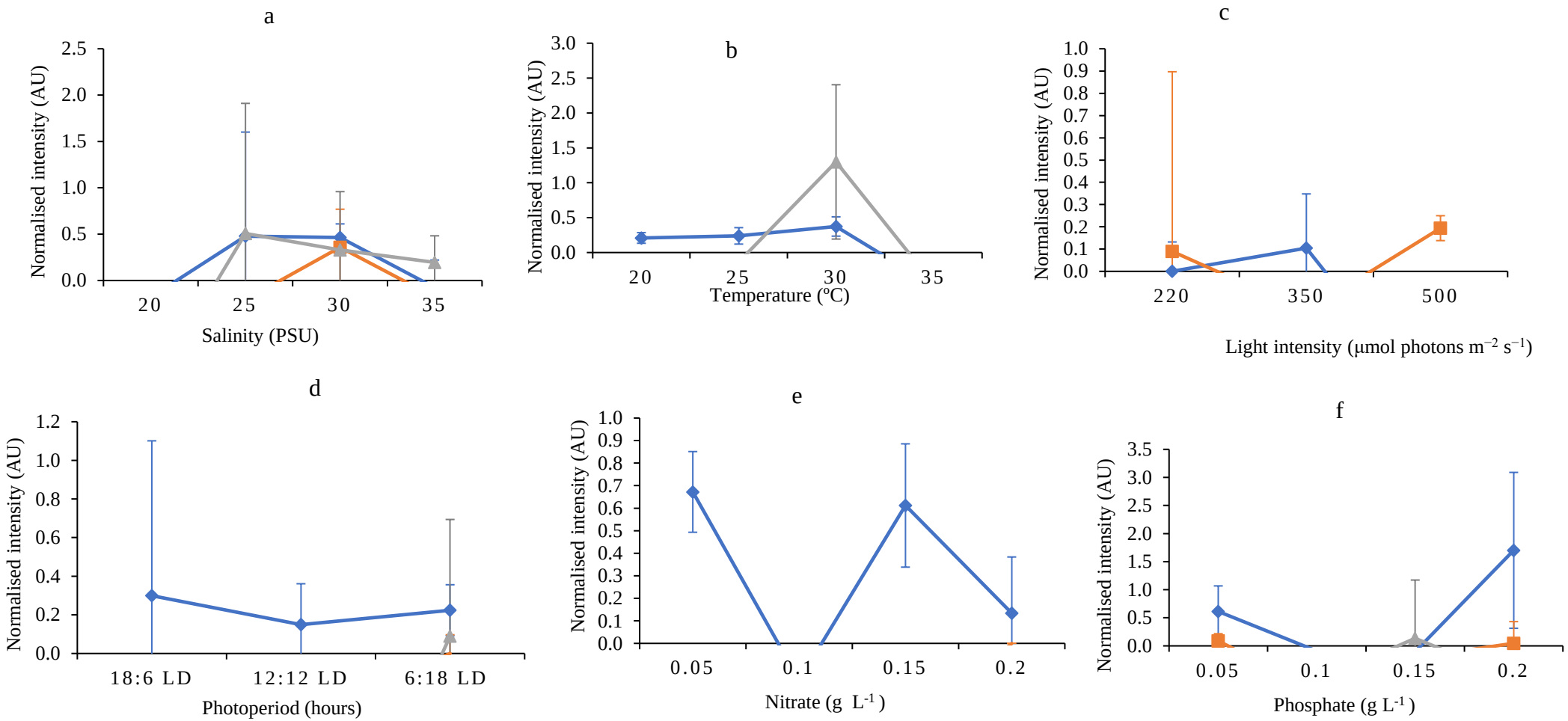

Figure S18. Fluctuation of triethanoalmine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate. (Values of zero implies below detection level)

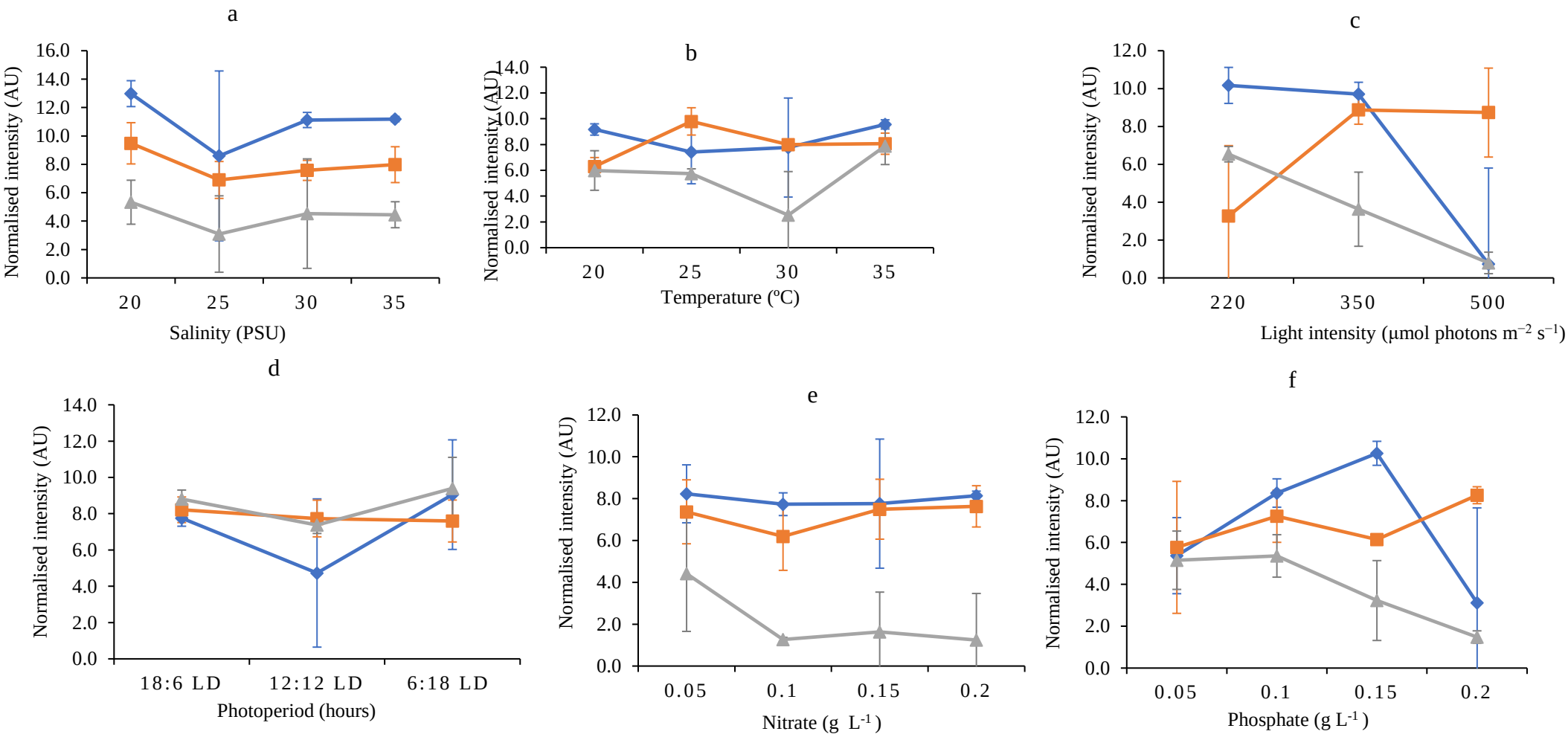

Figure S19. Fluctuation of betain in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

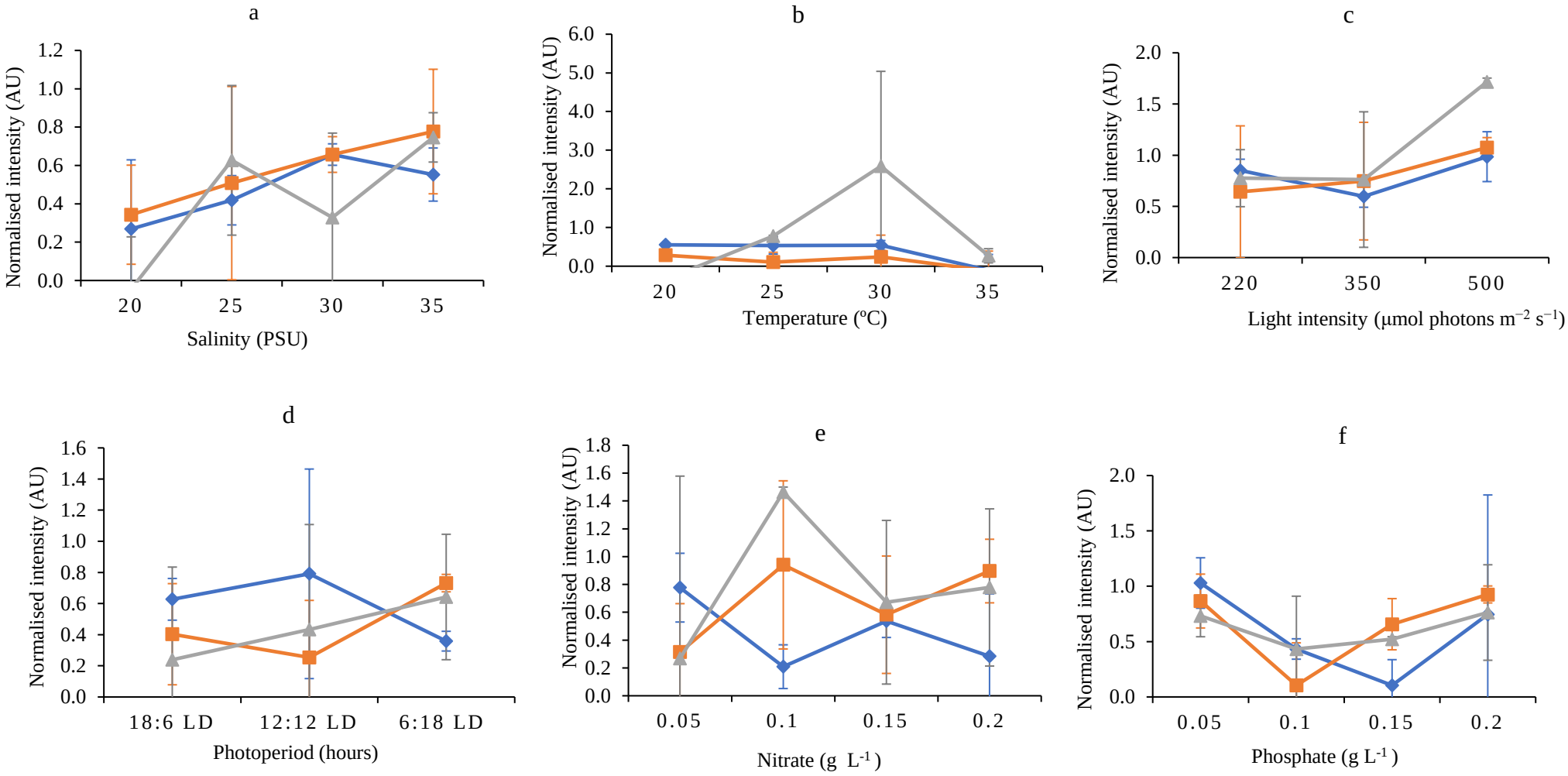

Figure S20. Fluctuation of Isethionic in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

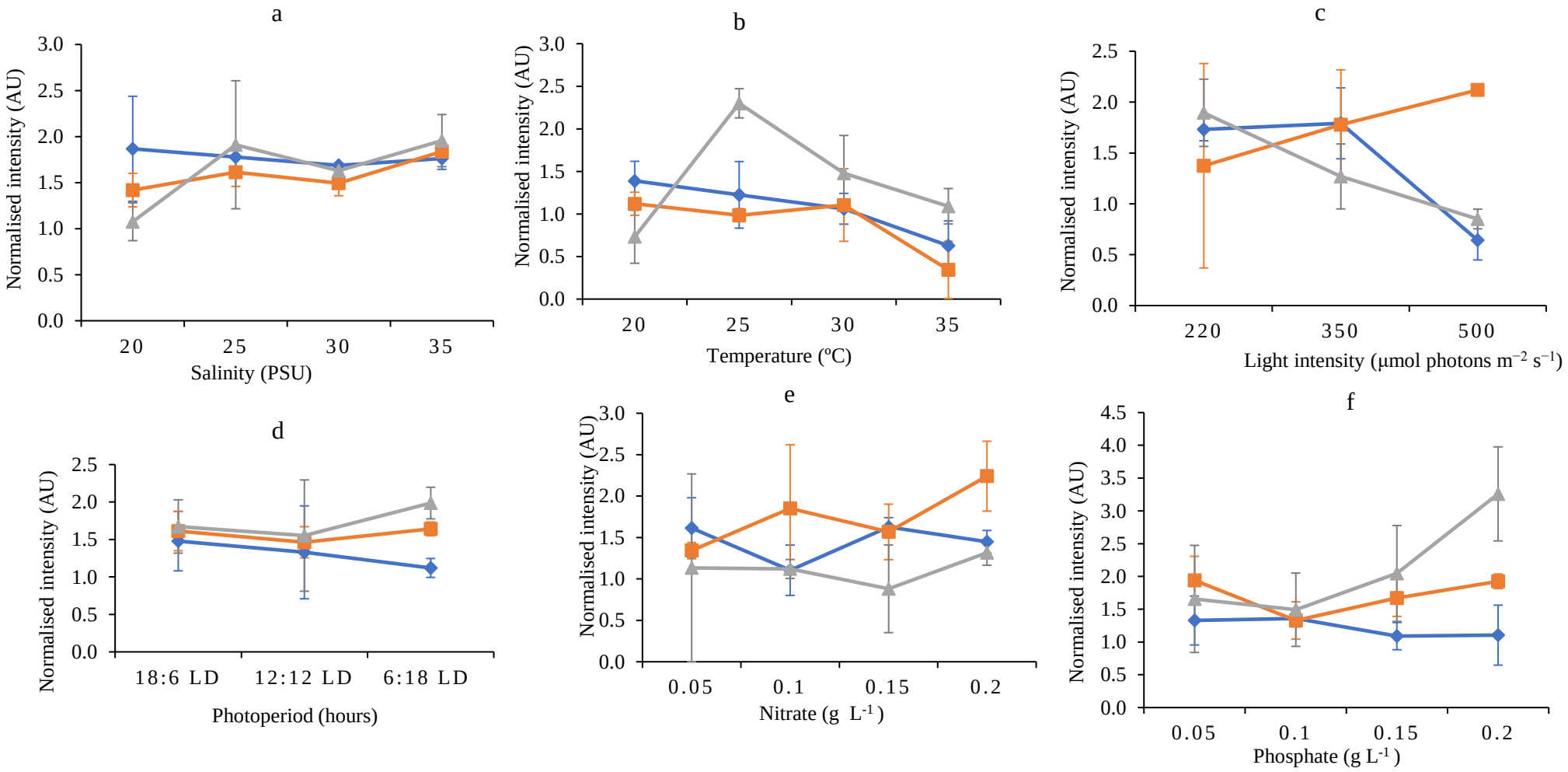

Figure S21. Fluctuation of Creatinine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

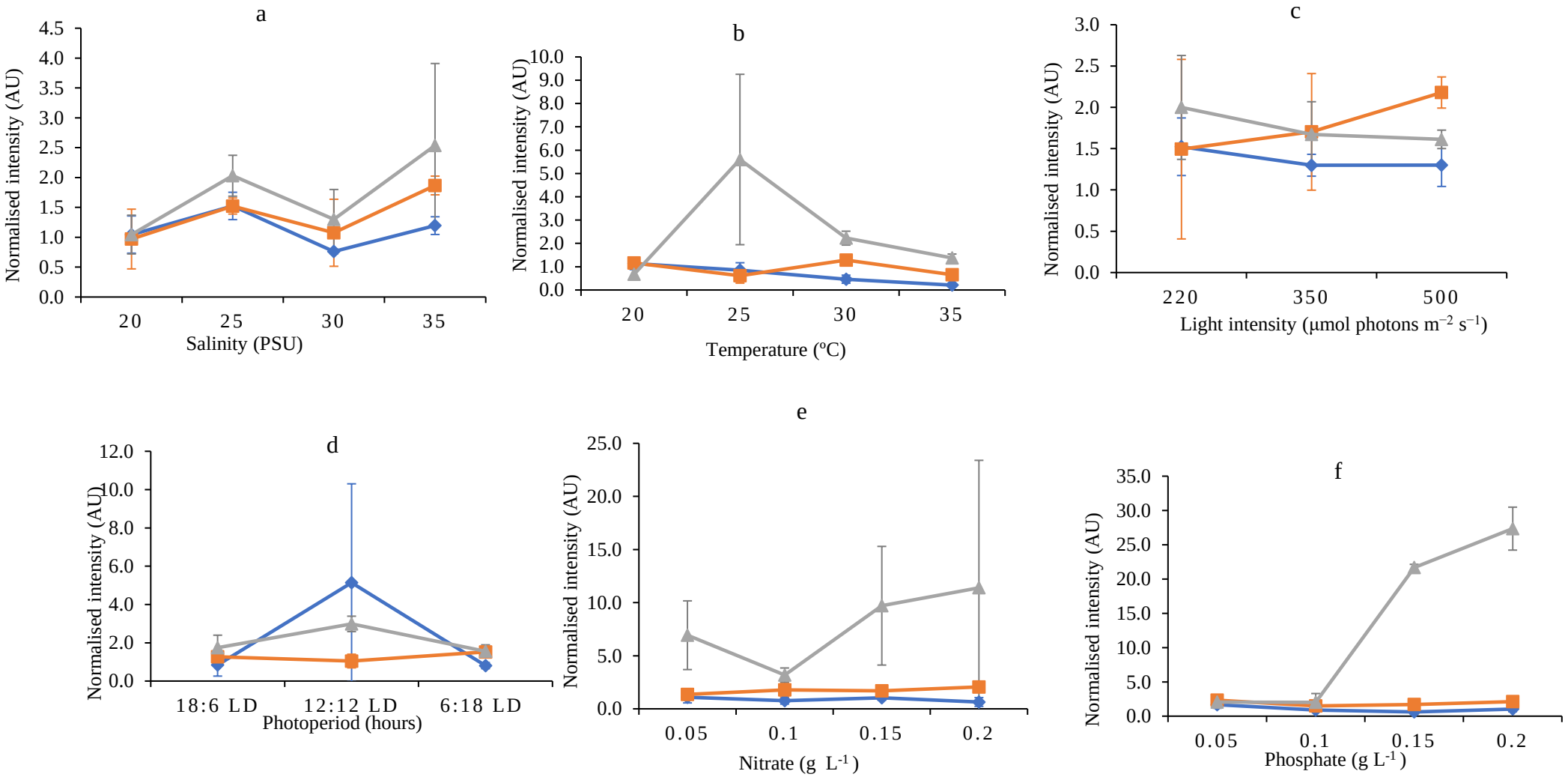

Figure S22. Fluctuation of GABA in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

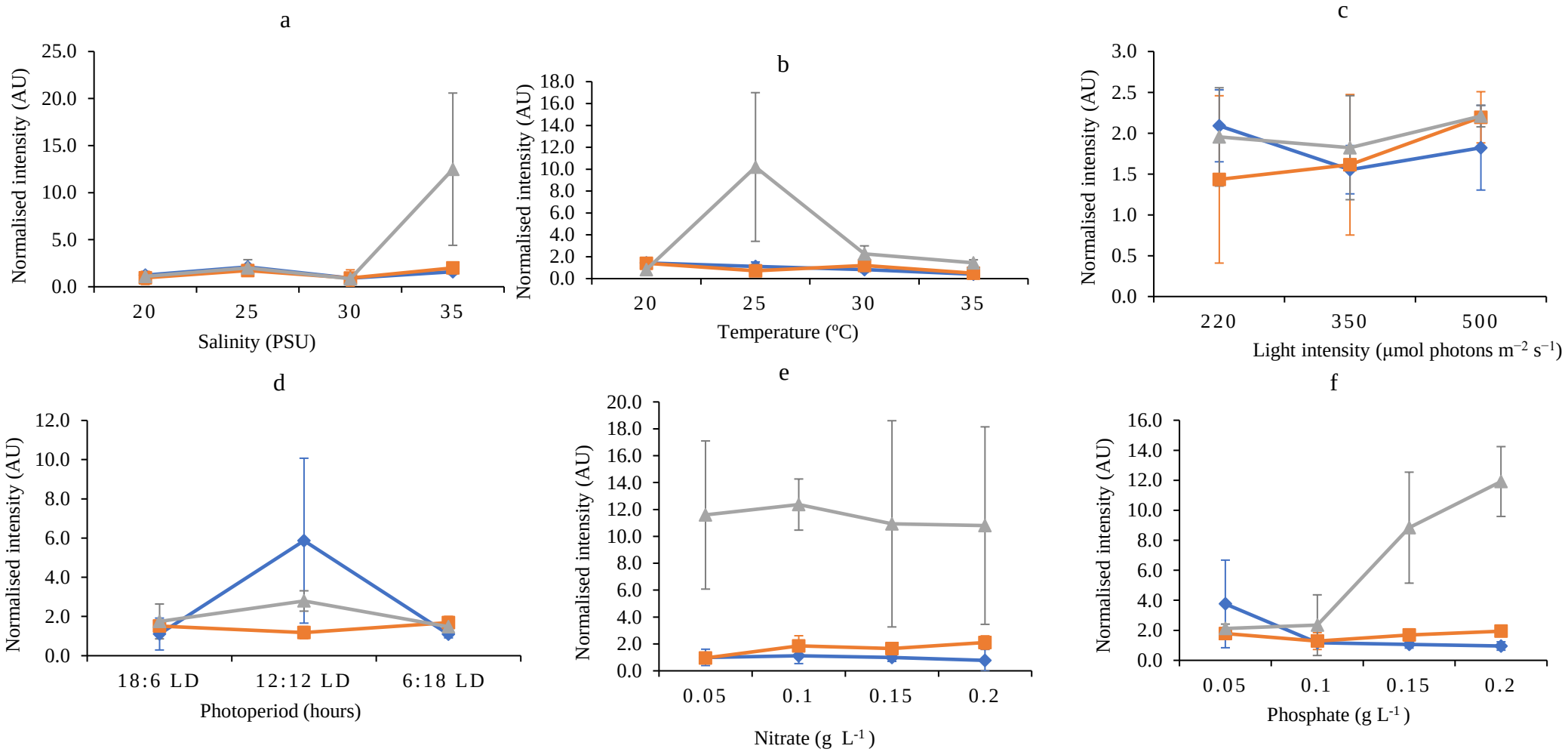

Figure S23. Fluctuation of cysteine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

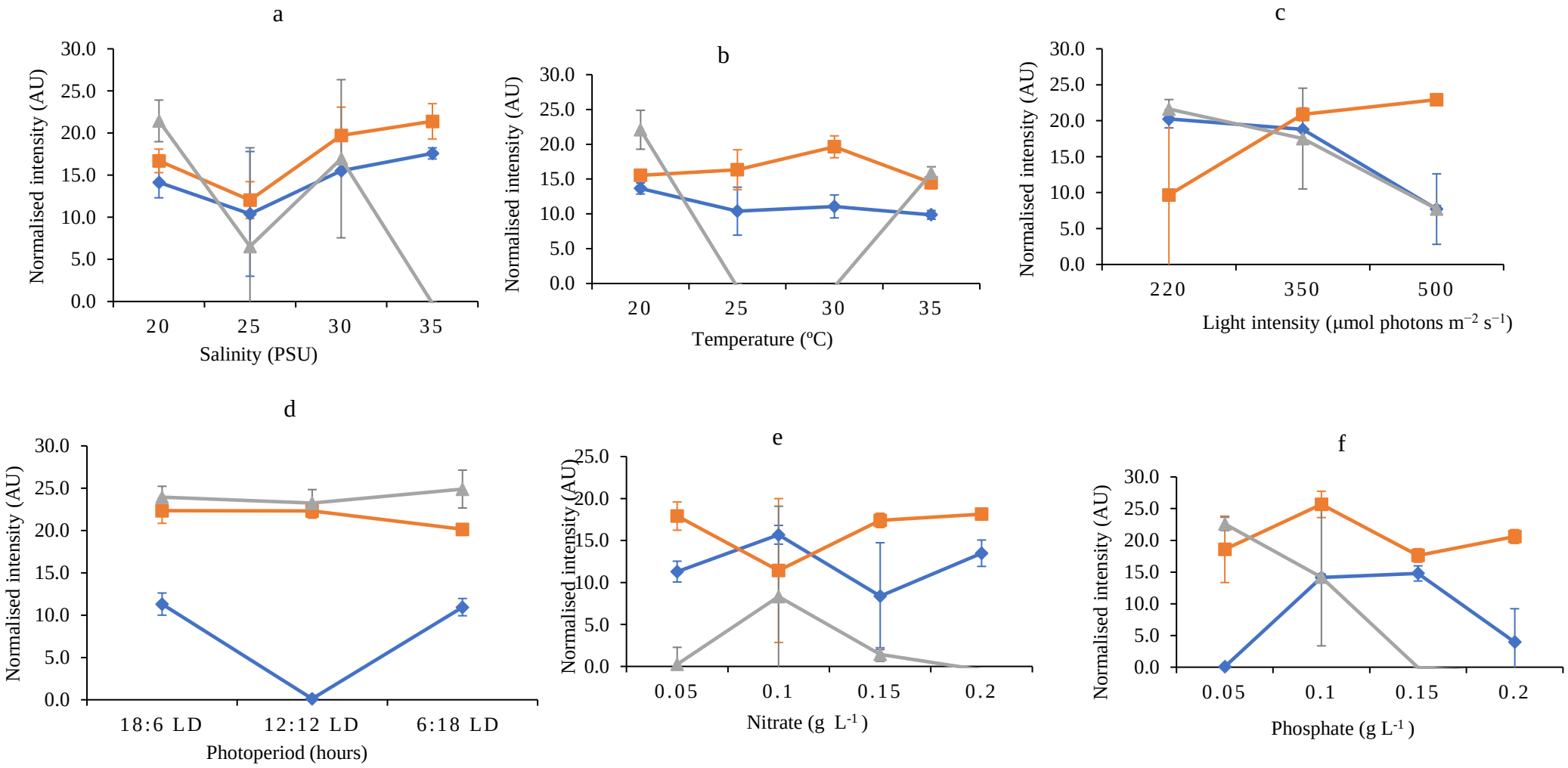

Figure S24. Fluctuation of malic acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate. (Values of zero implies below detection level)

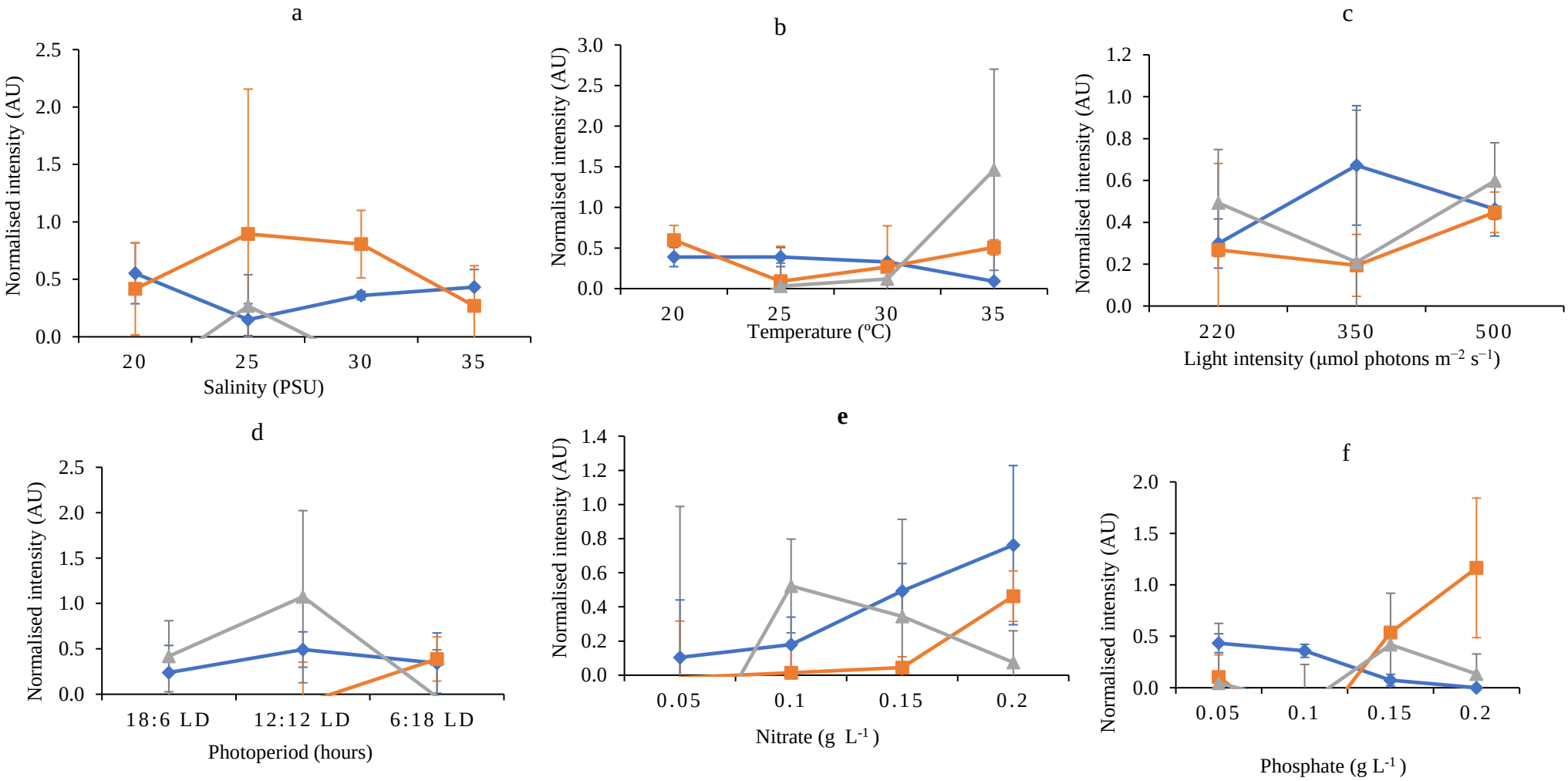

Figure S25. Fluctuation of Succinic acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate. (Values of zero implies below detection level)

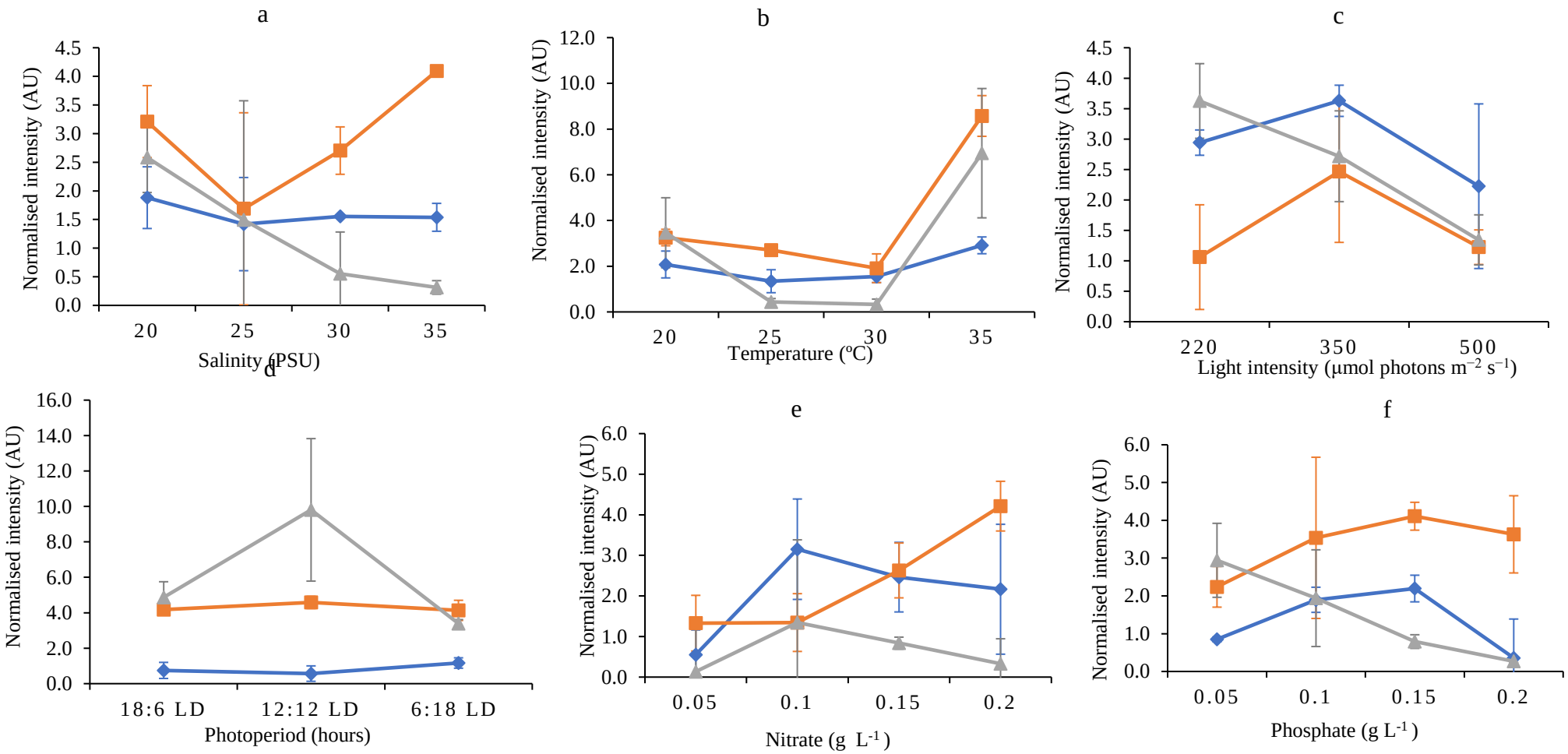

Figure S26. Fluctuation of a-ketoglutaric acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

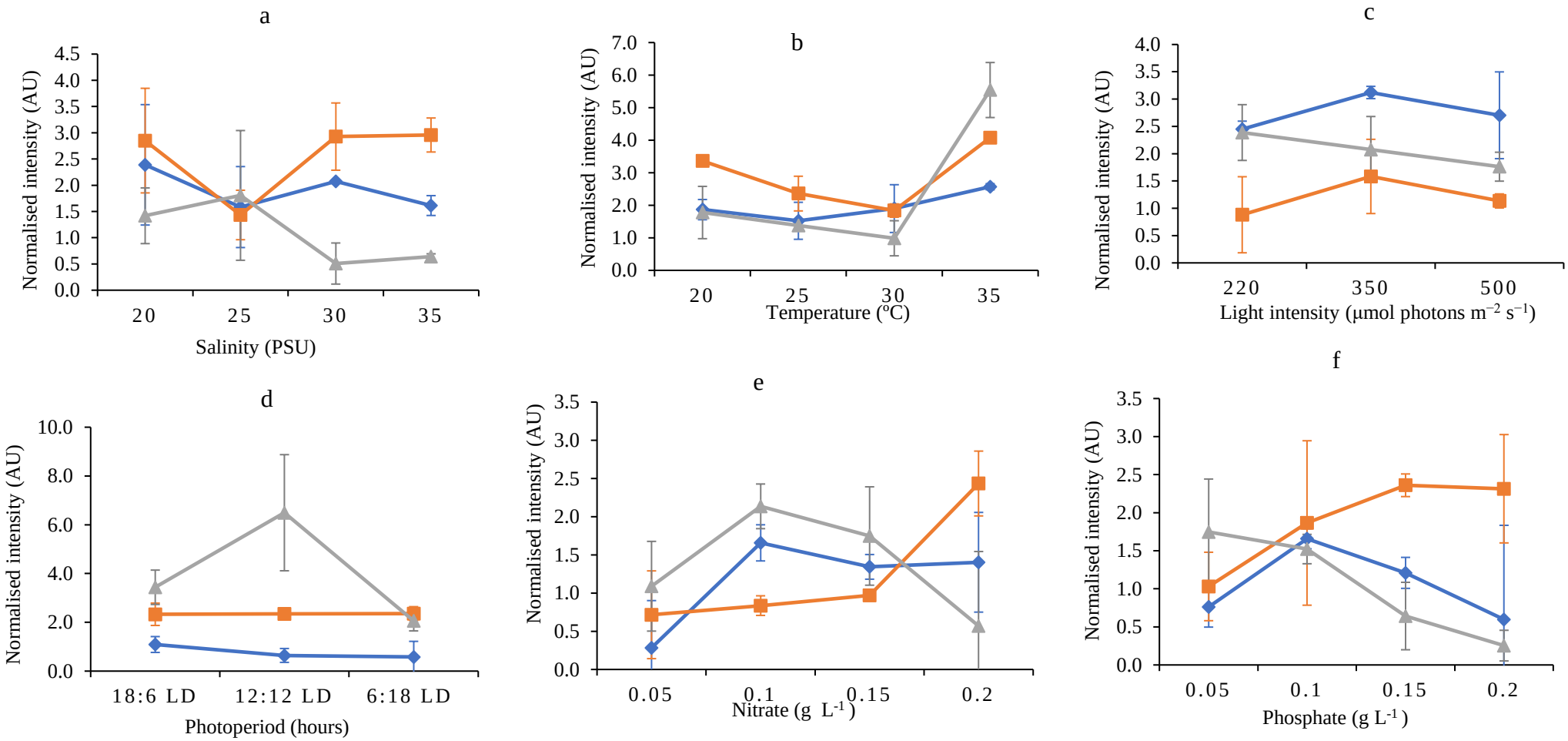

Figure S27. Fluctuation of glutamic acid in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

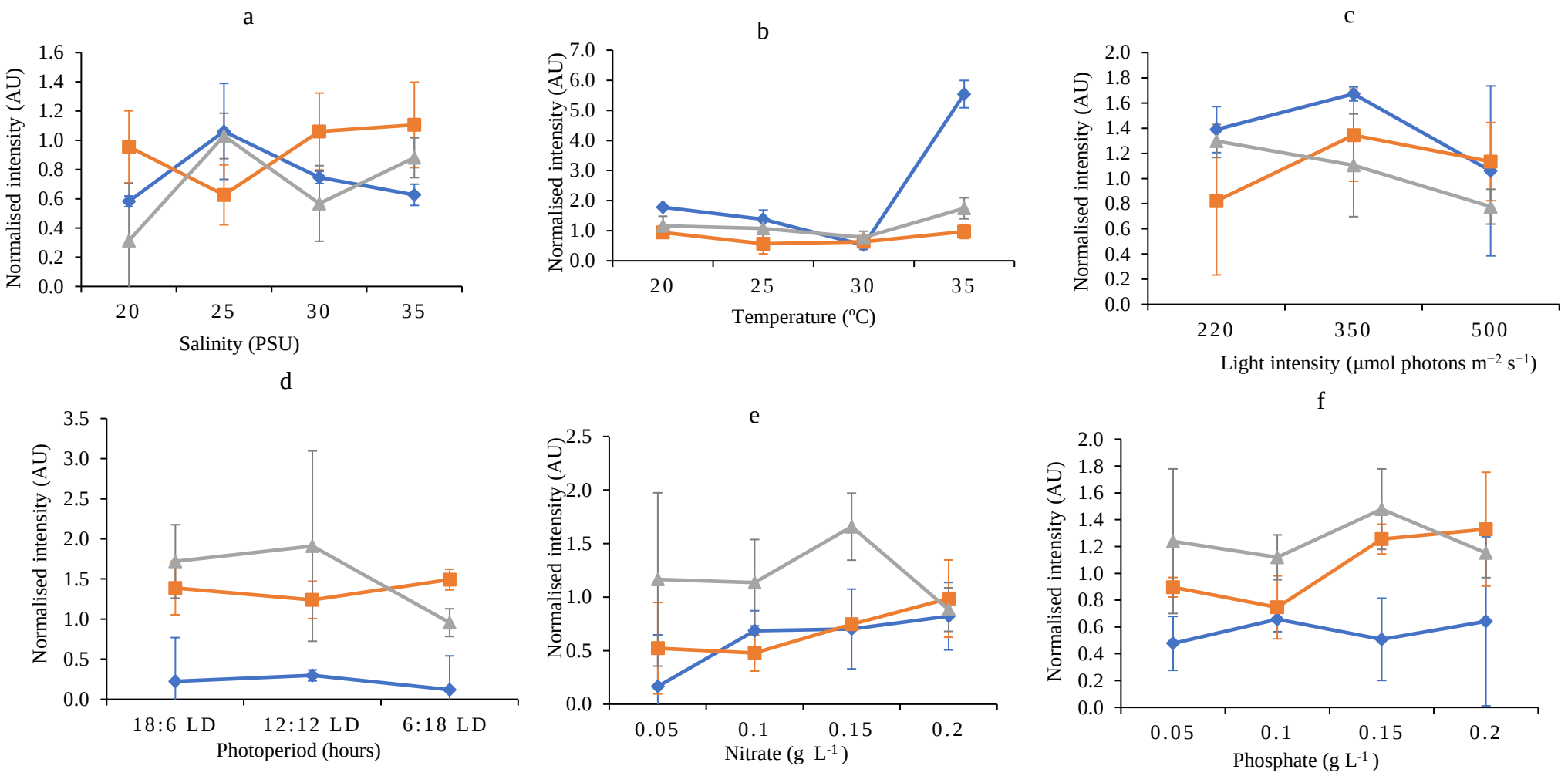

Figure S28. Fluctuation of proline in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate

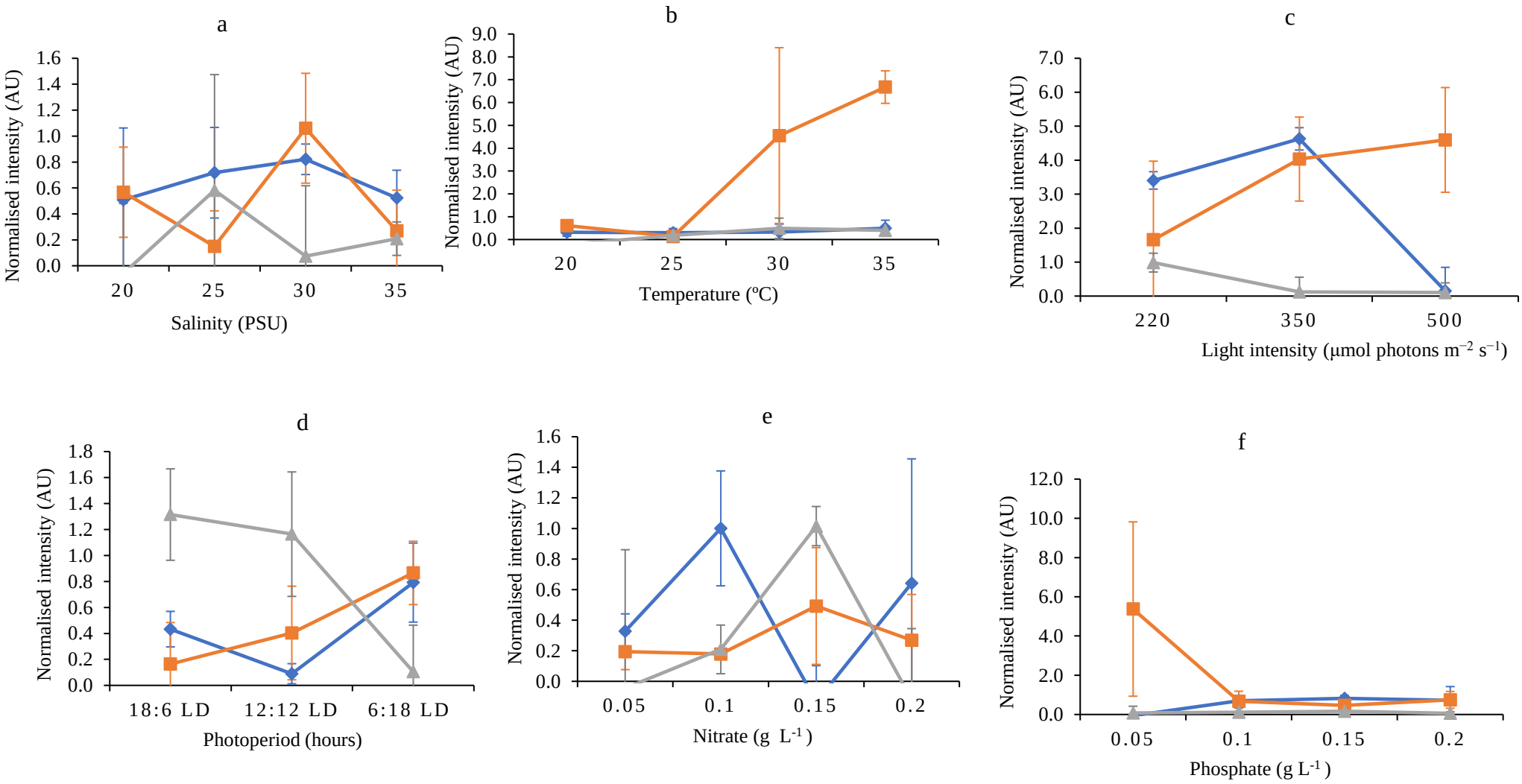

Figure S29. Fluctuation of acetate in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate. (Values of zero implies below detection level)

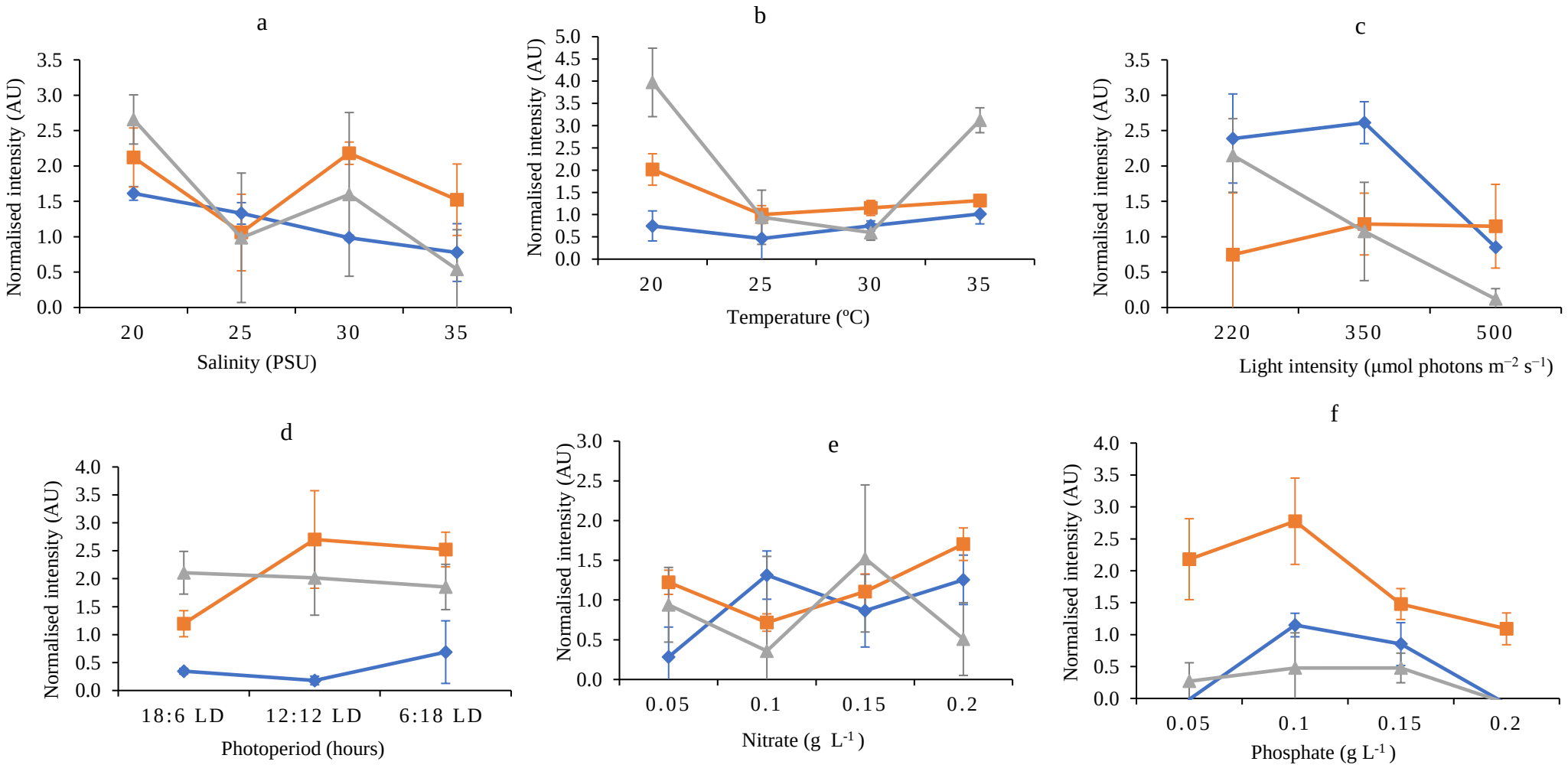

Figure S30. Fluctuation of alanine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate. (Values of zero implies below detection level)

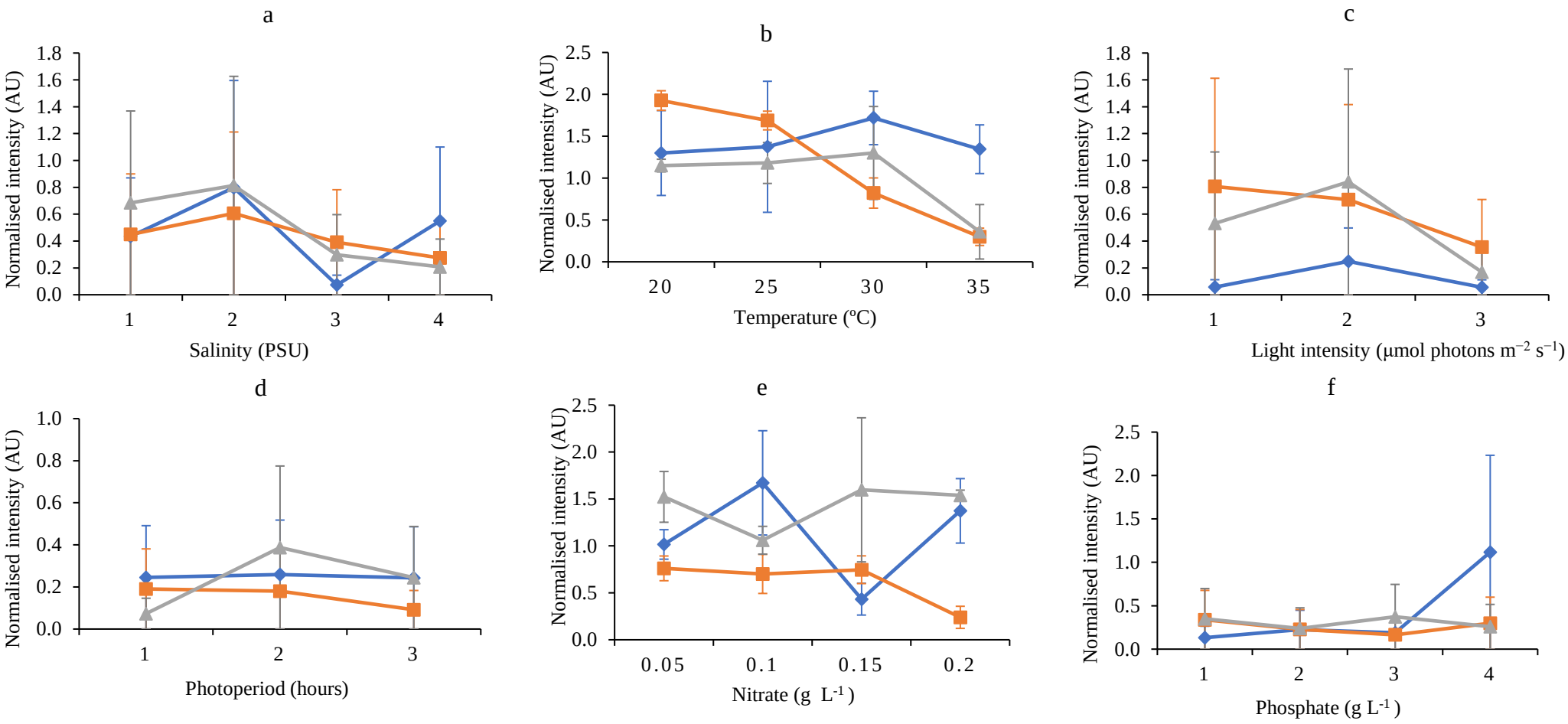

Figure S31. Fluctuation of threonine in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

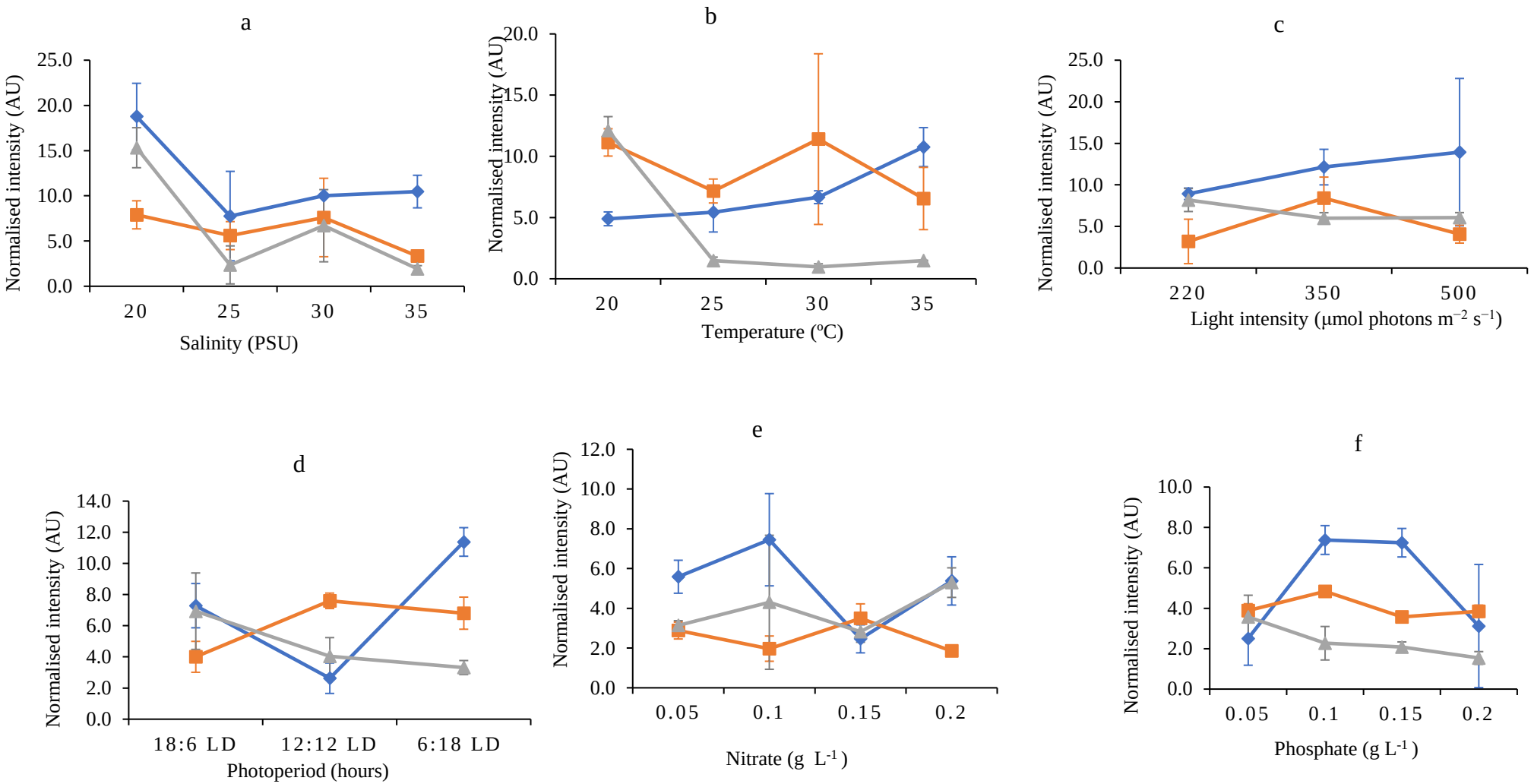

Figure S32. Fluctuation of lactate in vegetative, determination and differentiation phase with respect to variables of a. Salinity, b. Temperature, c. Light Intensity, d. Photoperiod. e. Nitrate f. Phosphate.

Box S2: Spearman correlation between three phases in variable salinity

In case of variable salinity positive correlation was found between metabolites in regions 3, 4, 5, 6 in all three phases whereas a negative correlation was found between metabolites in regions 8-13 and 2-8 in the determination and differentiation phase (Fig. S33, S34, S35). In case of variable salinity in vegetative phase positive correlation found between metabolites in regions 3, 4, 5 wherein 4 (middle) is having no correlation with 3 and 5 regions. Regions 7, and 13-14 showed positive correlation. Negative correlation found between metabolites region 7,8,9 (Fig. S33). In case of variable salinity in determination phase positive correlation found between metabolites from regions 2–7 wherein region 3 is poorly correlated. Region 13–14 is also positively correlated but no correlation found between regions from 8-14 and 1-7(Fig. S34). In the differentiation phase, regions 3-5 and 12-13 are positively correlated whereas regions 3-6 had no correlation with regions 8-12. (Fig. S35).

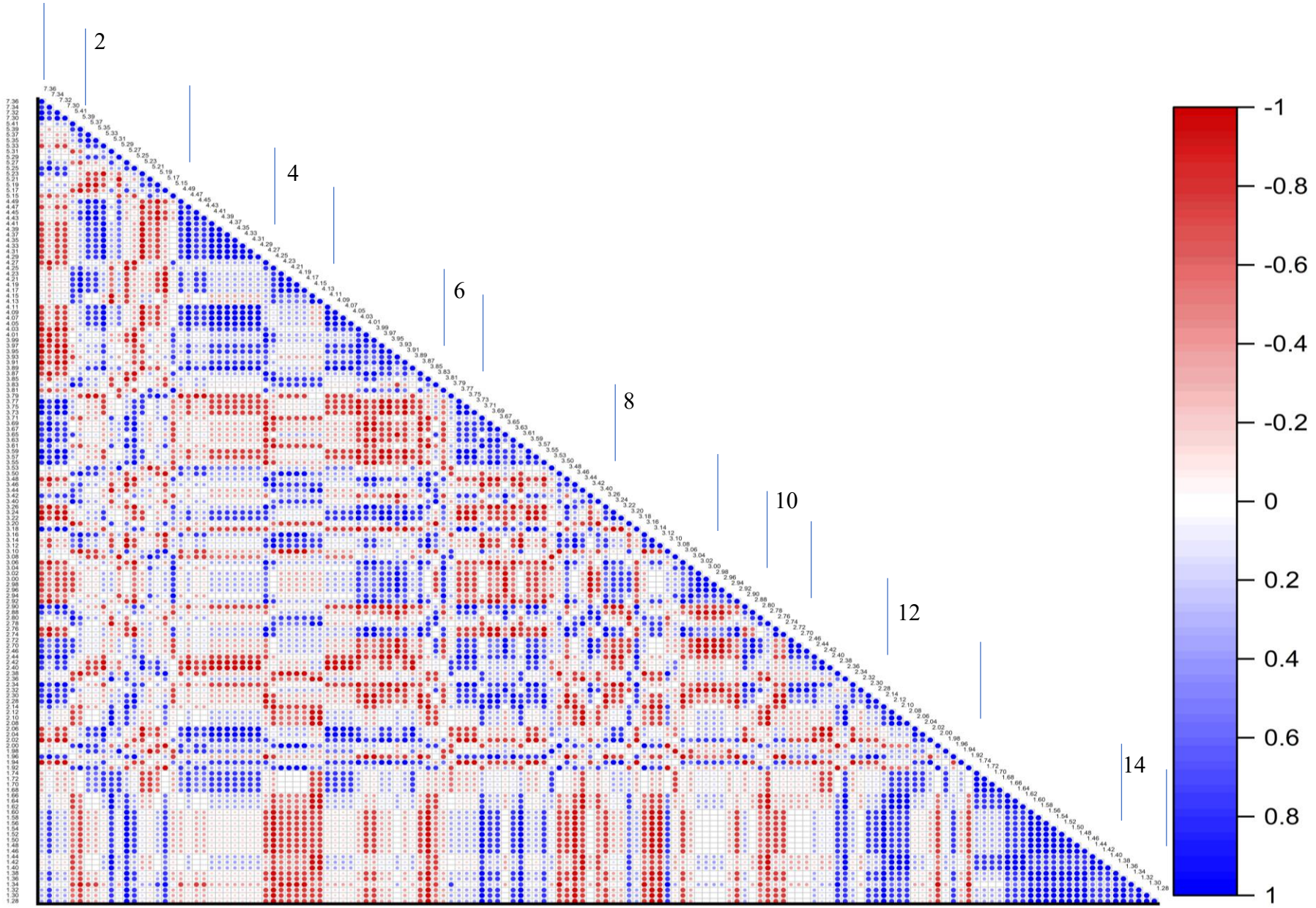

Figure S33. Spearman correlation of group of metabolites present in regions 1-14 of variable salinity (20 -35 psu) in vegetative phase.

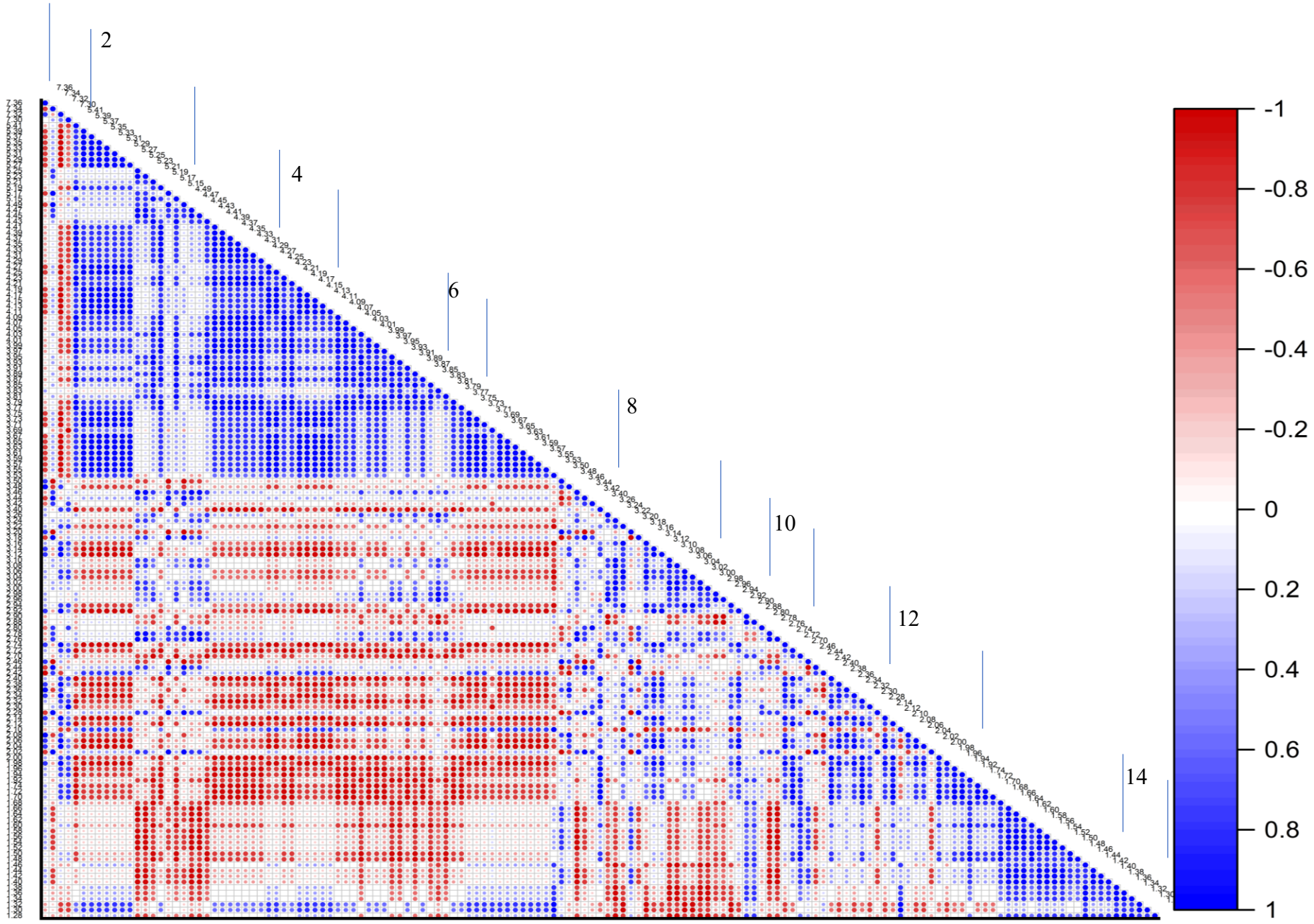

Figure S34. Spearman correlation of group of metabolites present in regions 1-14 of variable salinity (20 -35 psu) in determination phase.

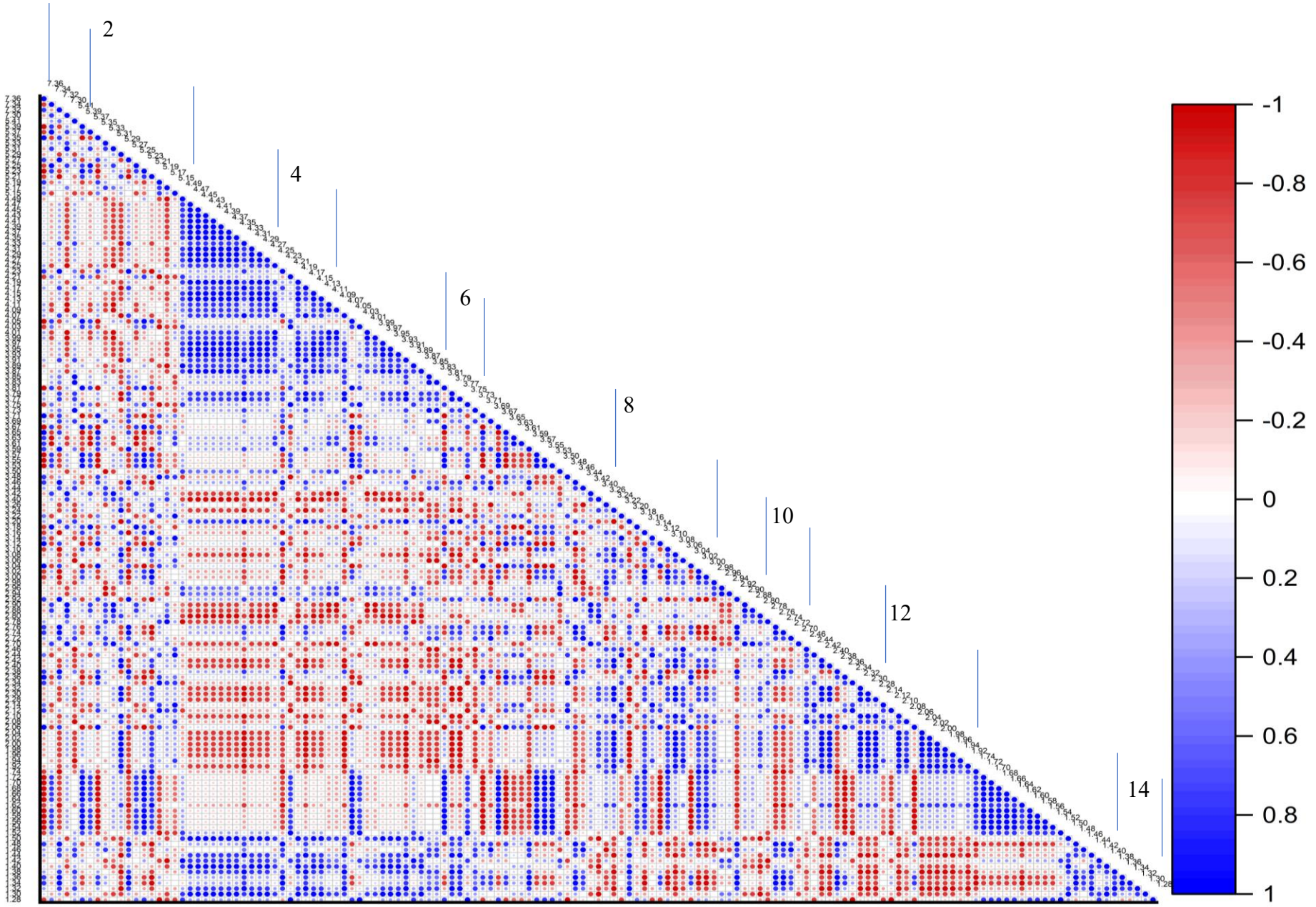

Figure S35. Spearman correlation of group of metabolites present in regions 1-14 of variable salinity (20 -35 psu) in differentiation phase.

Box S3: Spearman correlation between three phases in variable temperature

In variable temperature metabolites in regions 3, 8,9,10 showed a positive correlation in all three phases whereas a negative correlation was found between regions (2-4 and 5-8 and 13) in the vegetative phase (Fig. S36, S37, S38). In case of variable temperature in vegetative phase positive correlation found between metabolites in regions 2-5,6-7 and 13-14. Negative correlation observed between regions 3-5 and 6-8 as well 13-14 (Fig. S36). In case of variable temperature in determination phase positive correlation found between metabolites in regions 3 and 8-10 as well 11-14. Negative correlation found between regions 3-5 and 6 (Fig. S37). In case of variable temperature in differentiation phase positive correlation found between metabolites in regions 4-7 and 11-13. Negative correlation found between region 2 and 3-12(Fig. S38).

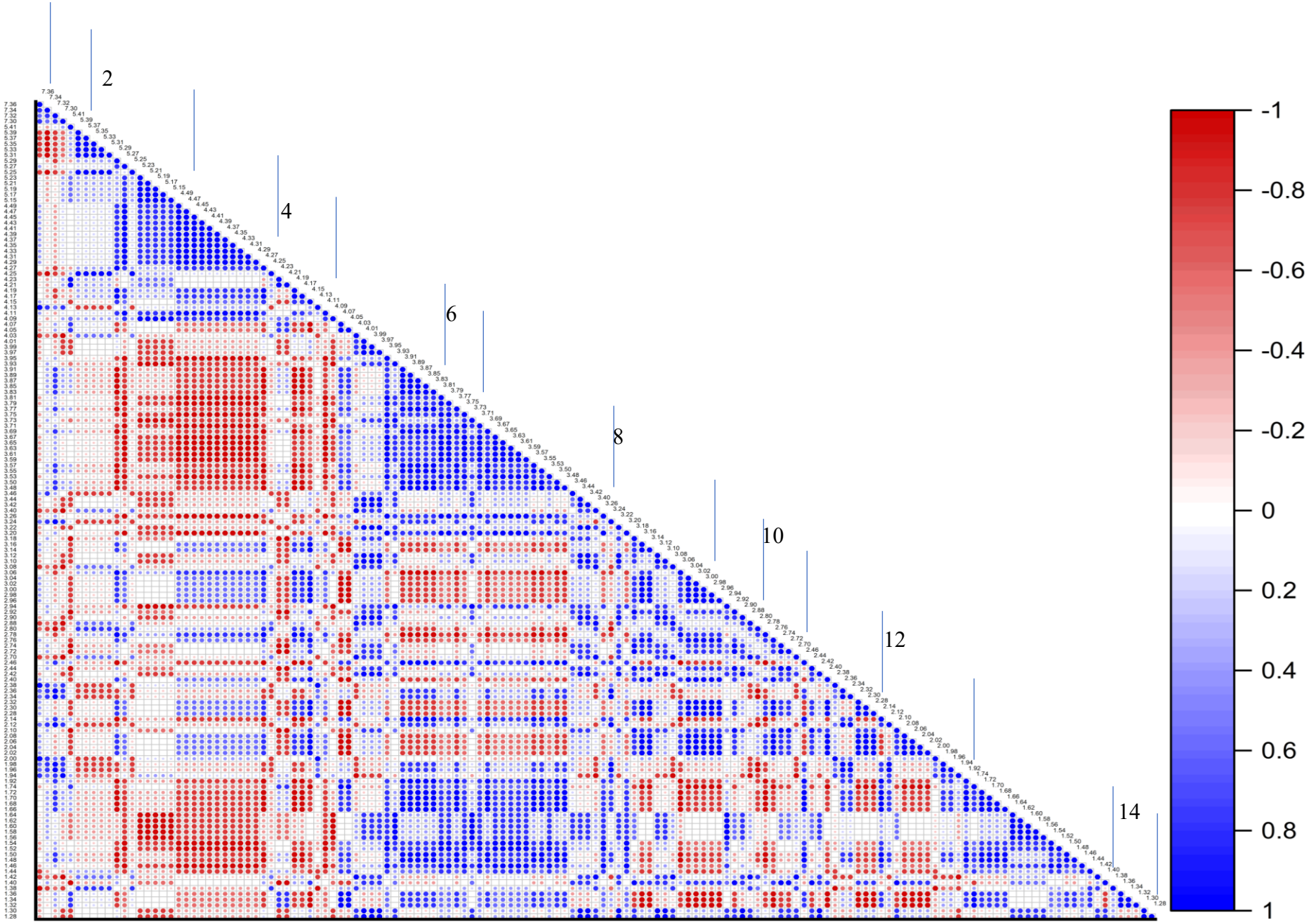

Figure S36. Spearman correlation of group of metabolites present in regions 1-14 of variable temperature (20 -35°C) in vegetative phase.

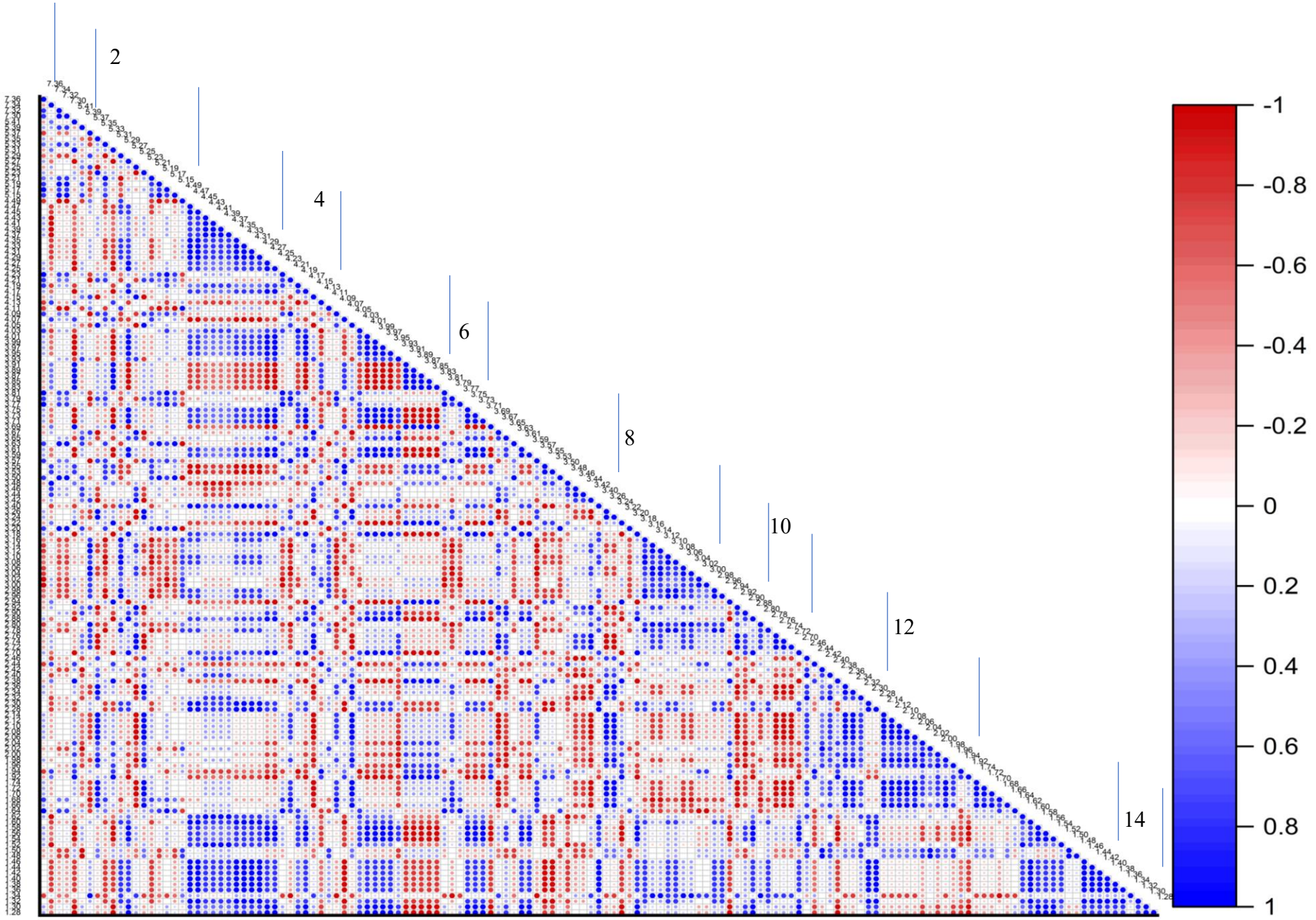

Figure S37. Spearman correlation of group of metabolites present in regions 1-14 of variable temperature (20-35°C) in determination phase.

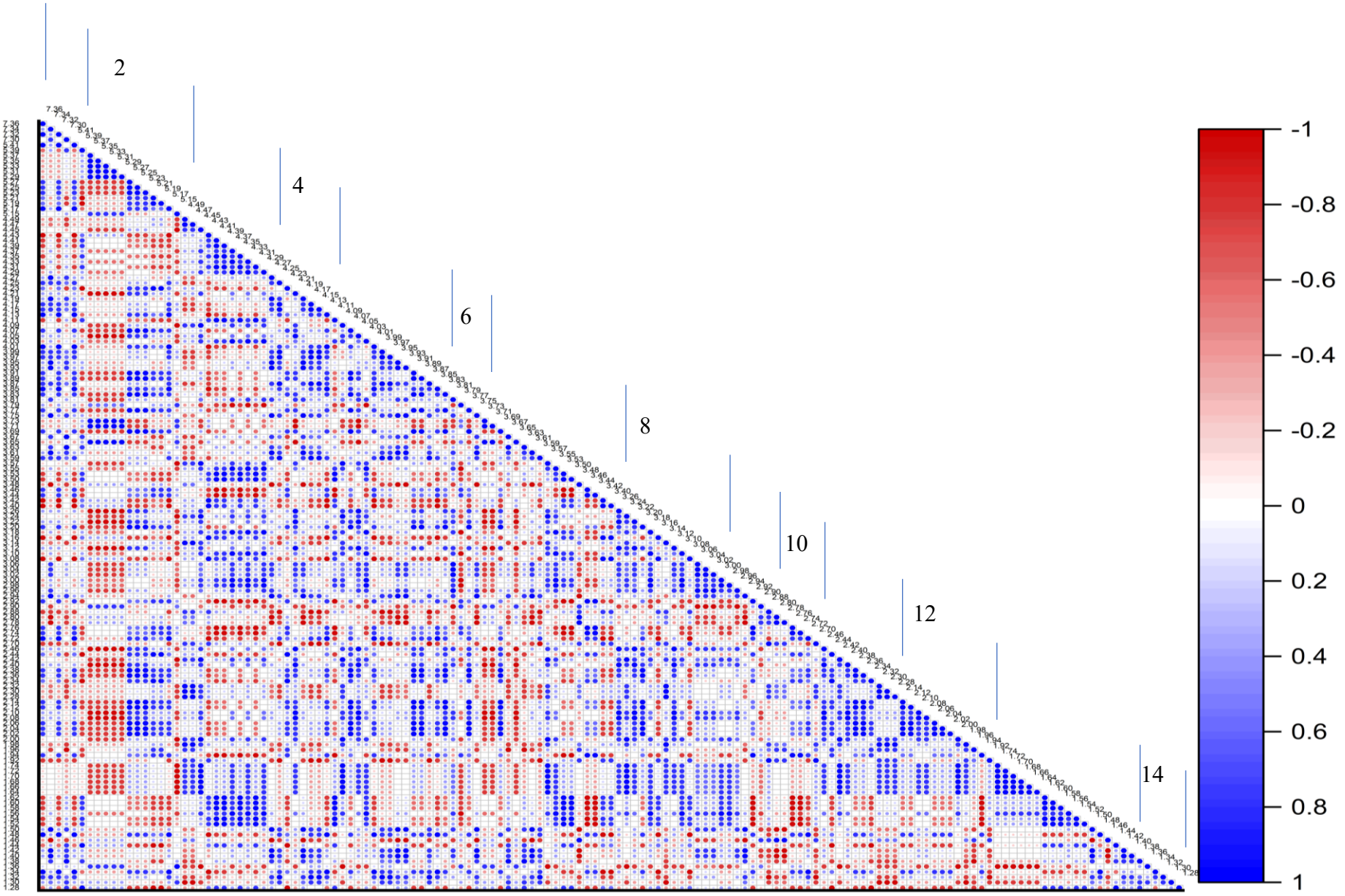

Figure S38. Spearman correlation of group of metabolites present in regions 1-14 of variable temperature (20-35°C) in differentiation phase.

Box S4: Spearman correlation between three phases in variable light intensity

In variable light intensity metabolites in regions 2-7, 8-12 showed a positive correlation in all three phases whereas a negative correlation was found between regions (8-12 and 4-8) in the vegetative and differentiation phase (Fig. S39, S40, S41). In case of variable light intensity in vegetative phase positive correlation found between metabolites in regions 3 and 8-10 but no correlation of region 3 with 13-14. Positive correlation found between regions 4-7 ,9-12 and 13-14 but negative correlation found between regions 4-7 and 8-13, 8-13 and 13-14 (Fig. S39). In case of determination phase positive correlation found between metabolites in regions 3-13 (Fig. S40). In case of differentiation phase positive correlation found between metabolites in regions 3, 4-6, 8-13. Negative correlation observed between regions 4-7 and 8-13 (Fig. S41).

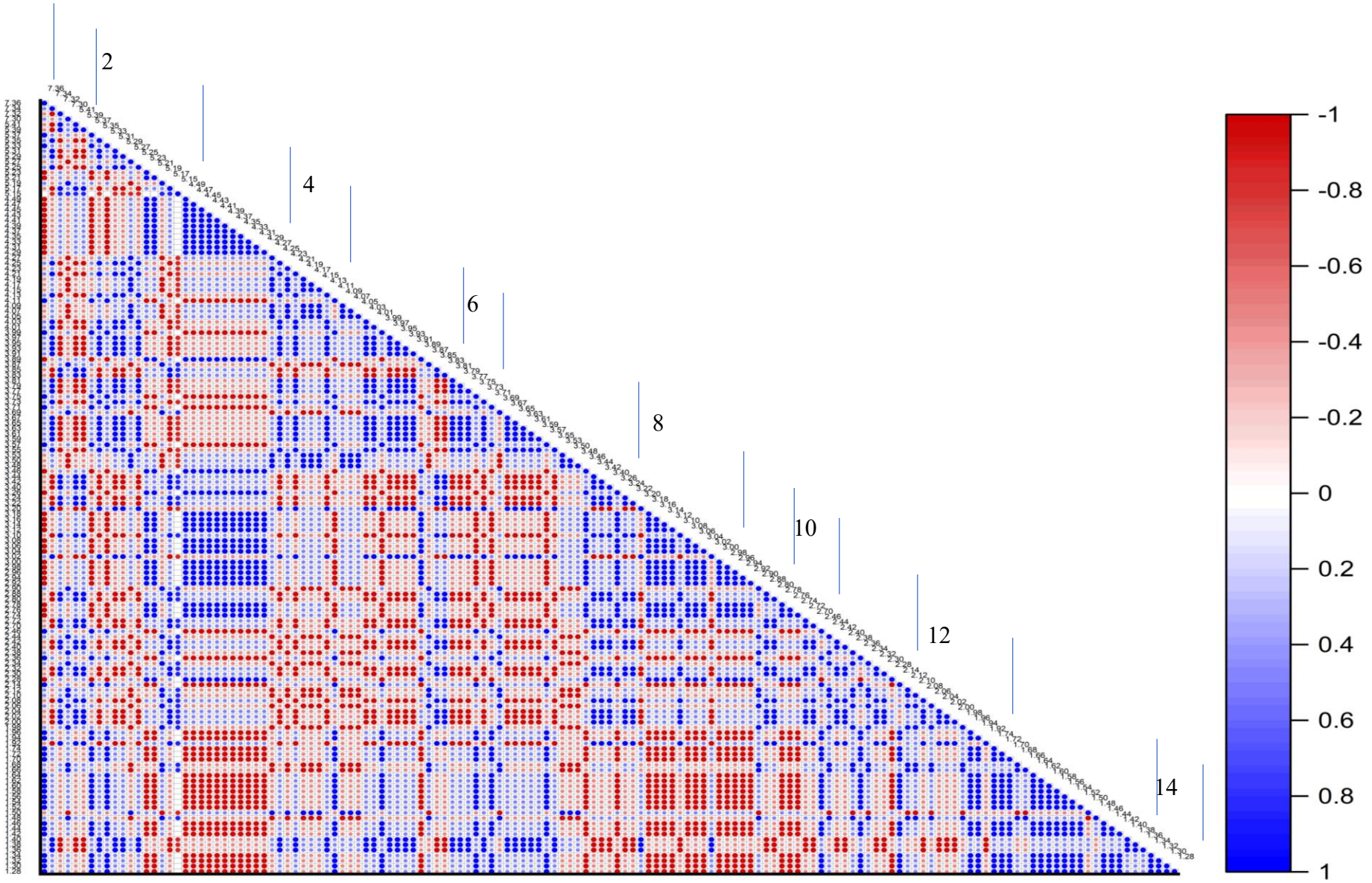

Figure S39. Spearman correlation of group of metabolites present in regions 1-14 of variable light intensity (220-500 µmol photons · $m^{-2} \cdot s^{-1}$) in vegetative phase.

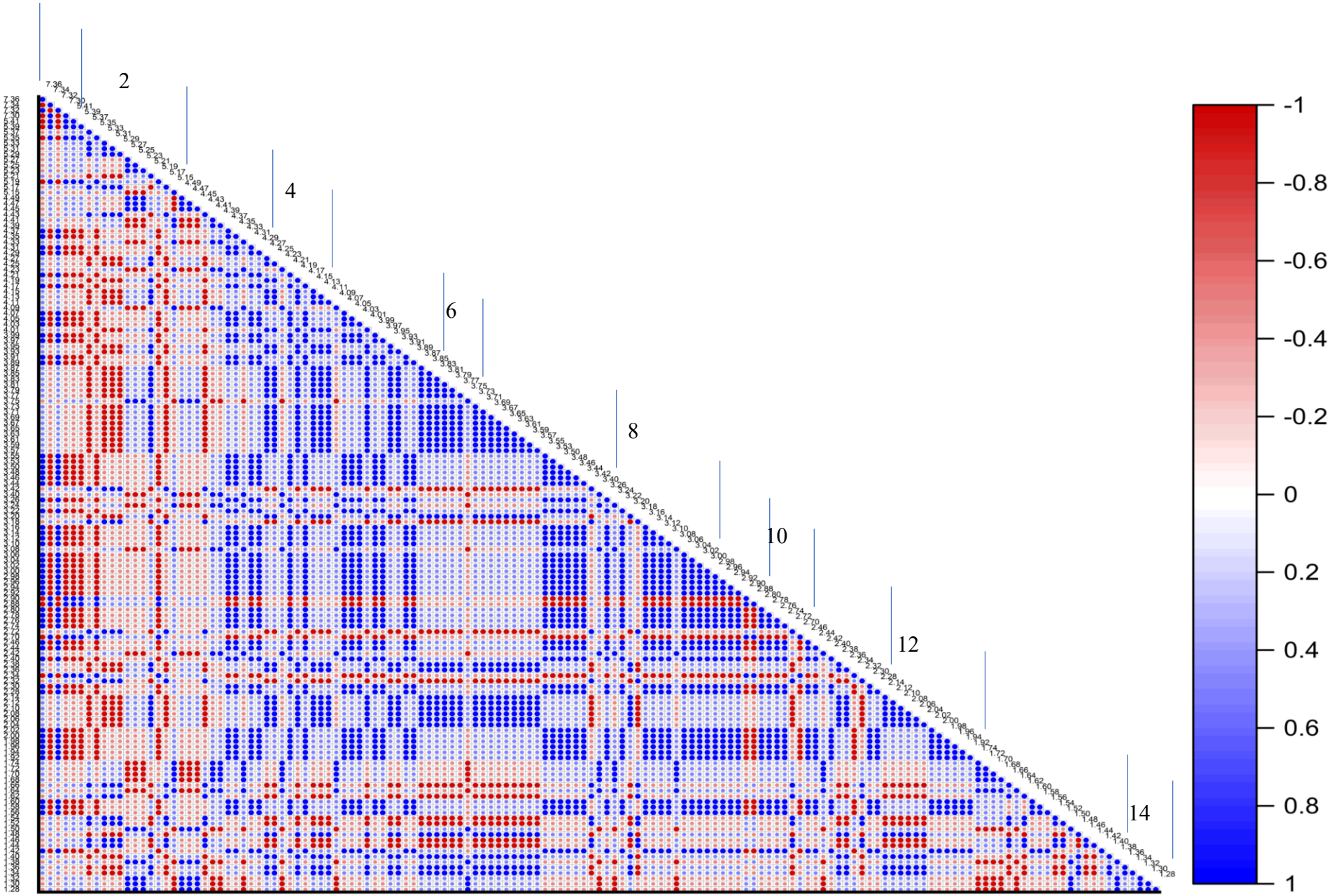

Figure S40. Spearman correlation of group of metabolites present in regions 1-14 of variable light intensity (220-500 µmol photons · $m^{-2} \cdot s^{-1}$) in determination phase.

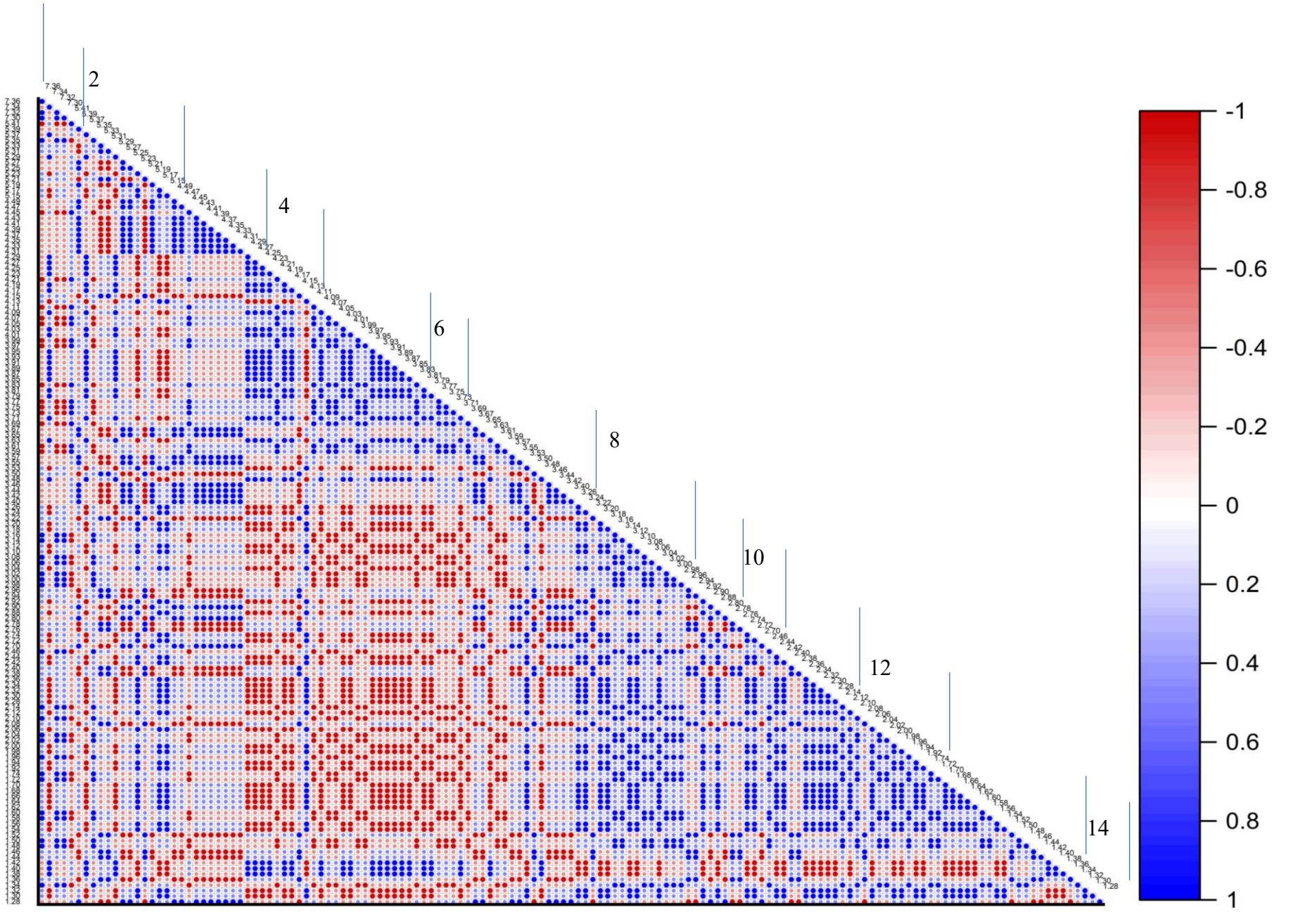

Figure S41. Spearman correlation of group of metabolites present in regions 1-14 of variable light intensity (220-500 µmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) in differentiation phase.

Box S5: Spearman correlation between three phases in variable photoperiod

In variable photoperiod metabolites in regions 4-5 showed a positive correlation in all three phases whereas a negative correlation was found between regions (5-6 and 13) in the vegetative and determination phase (Fig. S42, S43, S44). In case of variable photoperiod in vegetative phase positive correlation found between metabolites in regions 2-5, 6-7, 8-13 whereas negative correlation found between 6-7 and 7-14 as well in regions 3 and 6-7 (Fig. S42). In case of determination phase positive correlation found between metabolites in regions 3-6 and 12-14. Negative correlation found between regions 4-6 and 12-14 (Fig. S43). In case of differentiation phase positive correlation found between metabolites in regions 3-6 but regions 3-4 negatively related with 5-6, and 11-12 (Fig. S44).

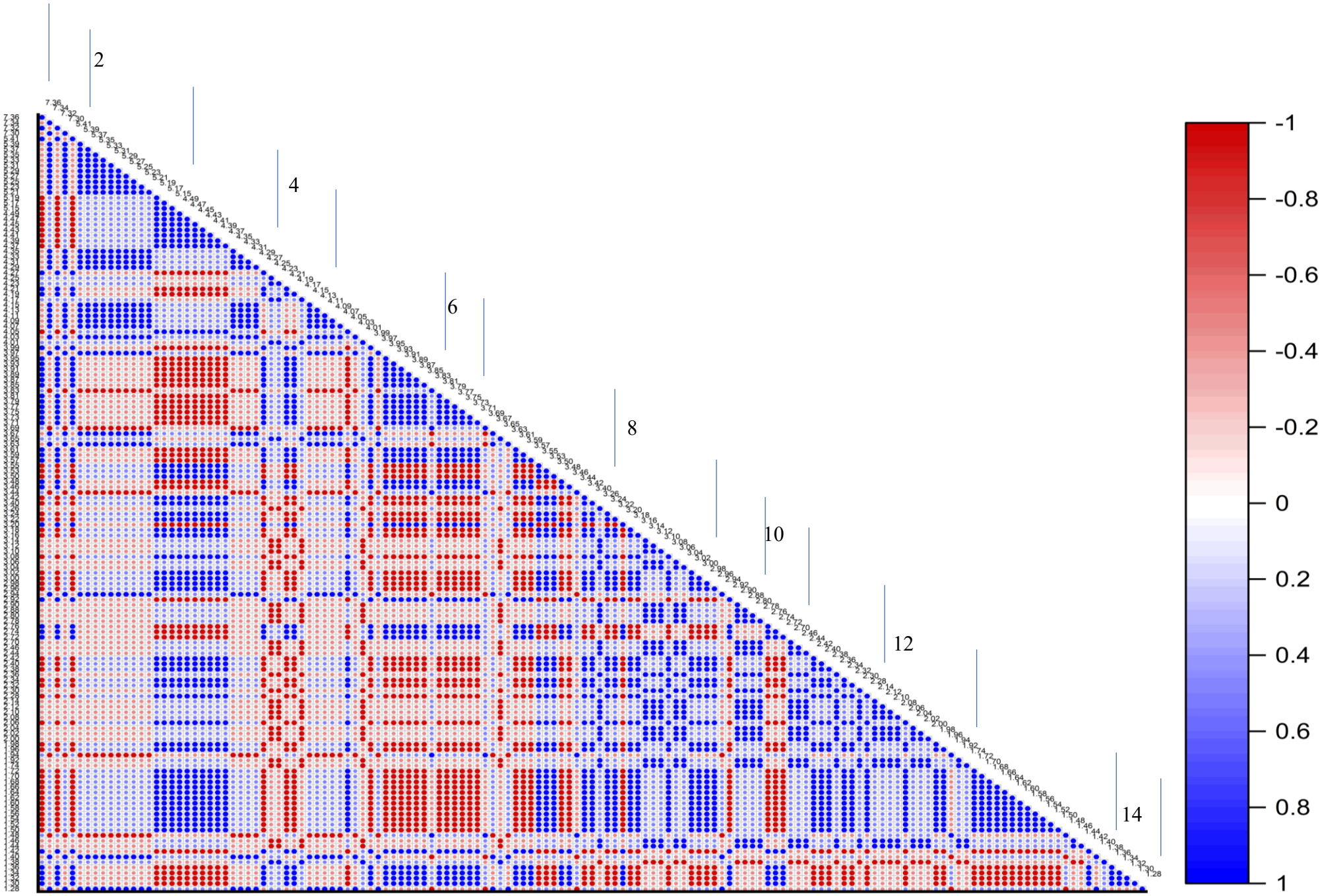

Figure S42. Spearman correlation of group of metabolites present in regions 1-14 of variable photoperiod (16:8, 8:16,12:12 D/N) in vegetative phase.

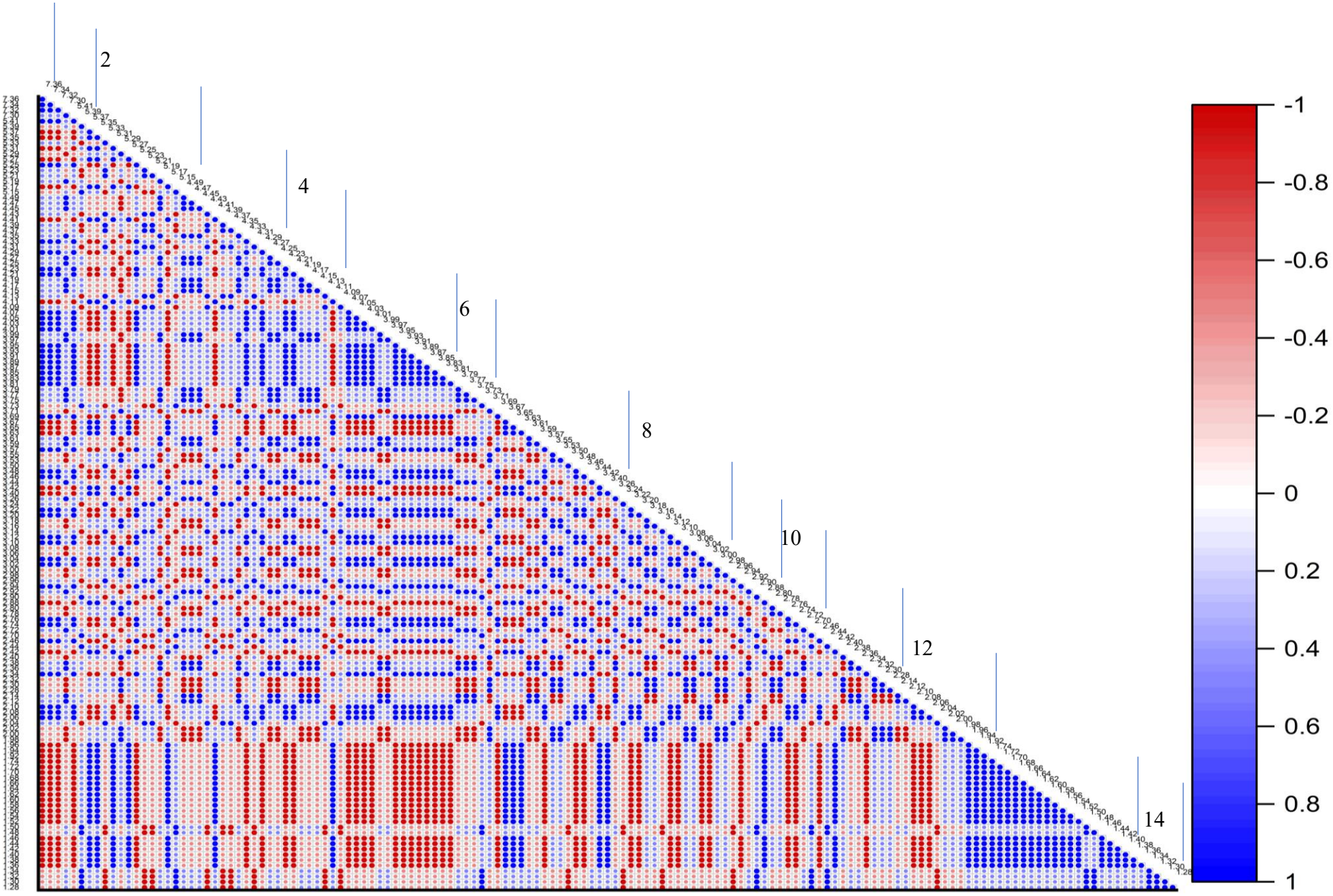

Figure S43. Spearman correlation of group of metabolites present in regions 1-14 of variable photoperiod (16:8, 8:16,12:12 D/N) in determination phase.

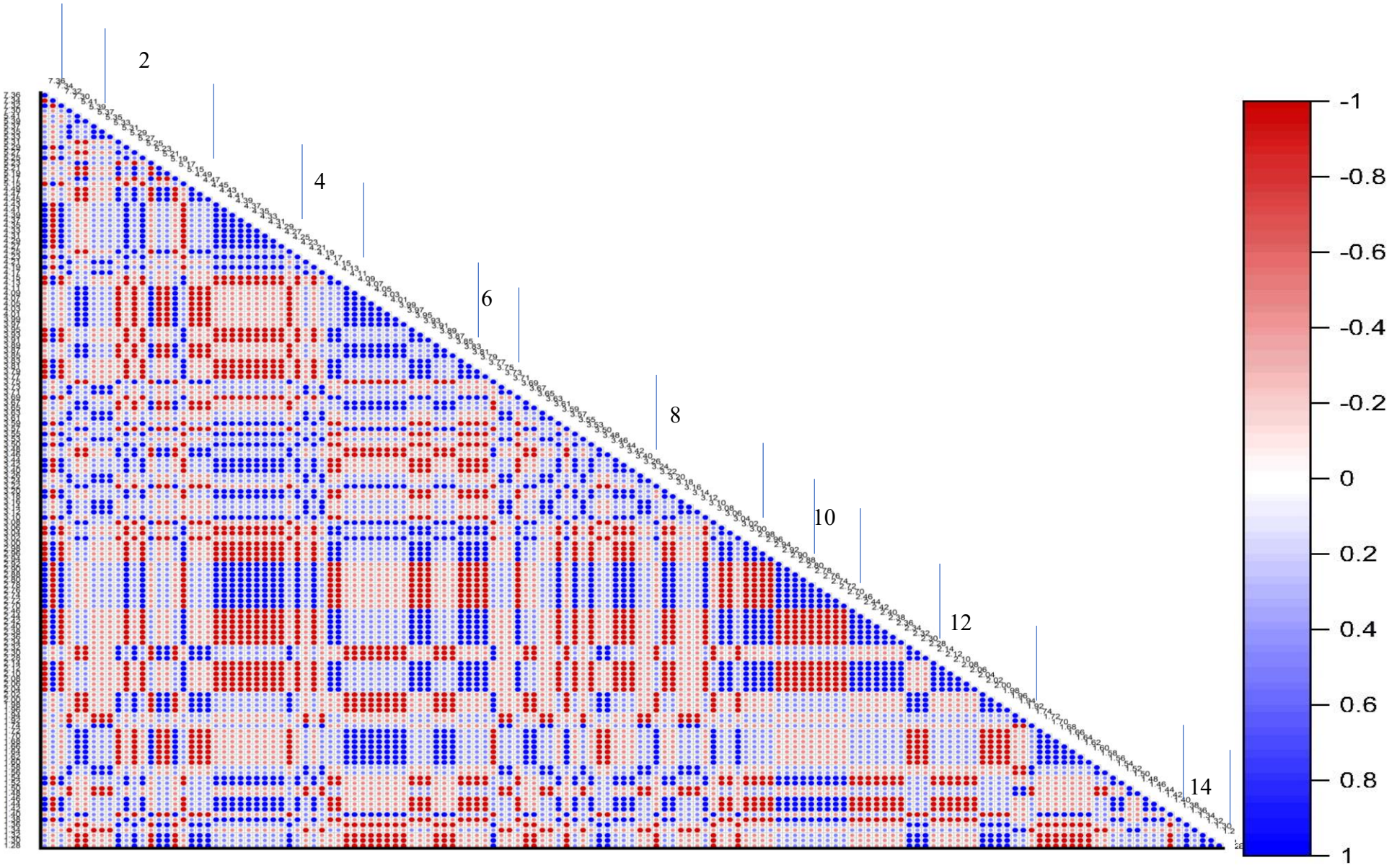

Figure S44. Spearman correlation of group of metabolites present in regions 1-14 of variable photoperiod (16:8, 8:16,12:12 D/N) in differentiation phase.

Box S6: Spearman correlation between three phases in variable nitrate

In variable nitrate metabolites in regions 11-14 showed a positive correlation in all three phases as well regions 4-8 showed a positive correlation in the vegetative and determination phases Negative correlation was found between regions (4-8 and 12) in all three phases (Fig. S45, S46, S47). In case of variable nitrate in vegetative phase positive correlation found between metabolites in regions 4-10, 11-14 but negative correlation found in regions 4-8 and 9-14 (Fig. S45). In case of determination phase positive correlation found between metabolites in regions 4-7, 8-13 but negative correlation found between regions 4-7 and 8-13 (Fig. S46). In case of variable nitrate in differentiation phase positive correlation found between metabolites in regions 4-7,10-11, 12-14 but no relation found

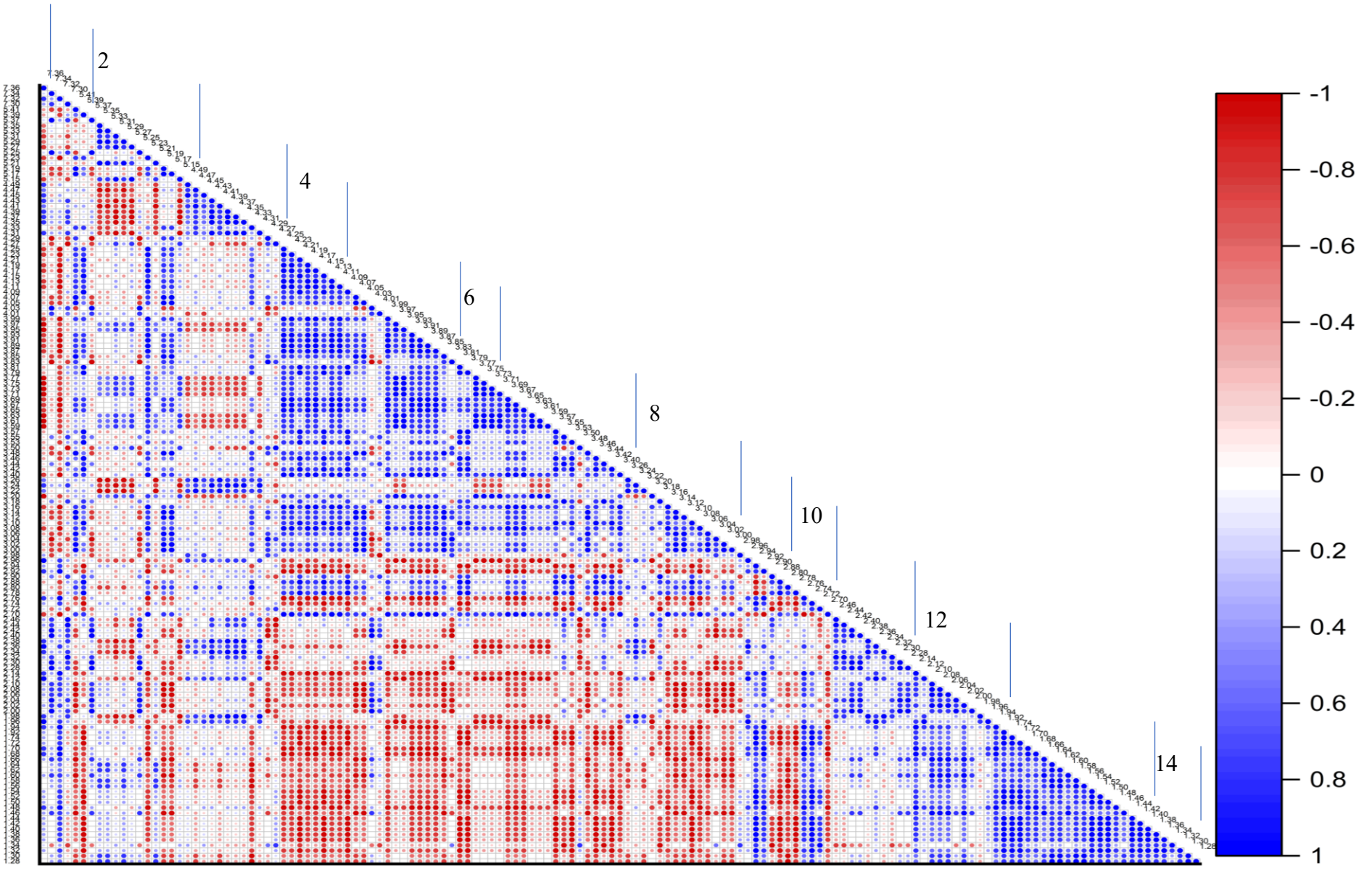

Figure S45. Spearman correlation of group of metabolites present in regions 1-14 of variable nitrate (0.05 - 0.2 g L^{-1}) in vegetative phase.

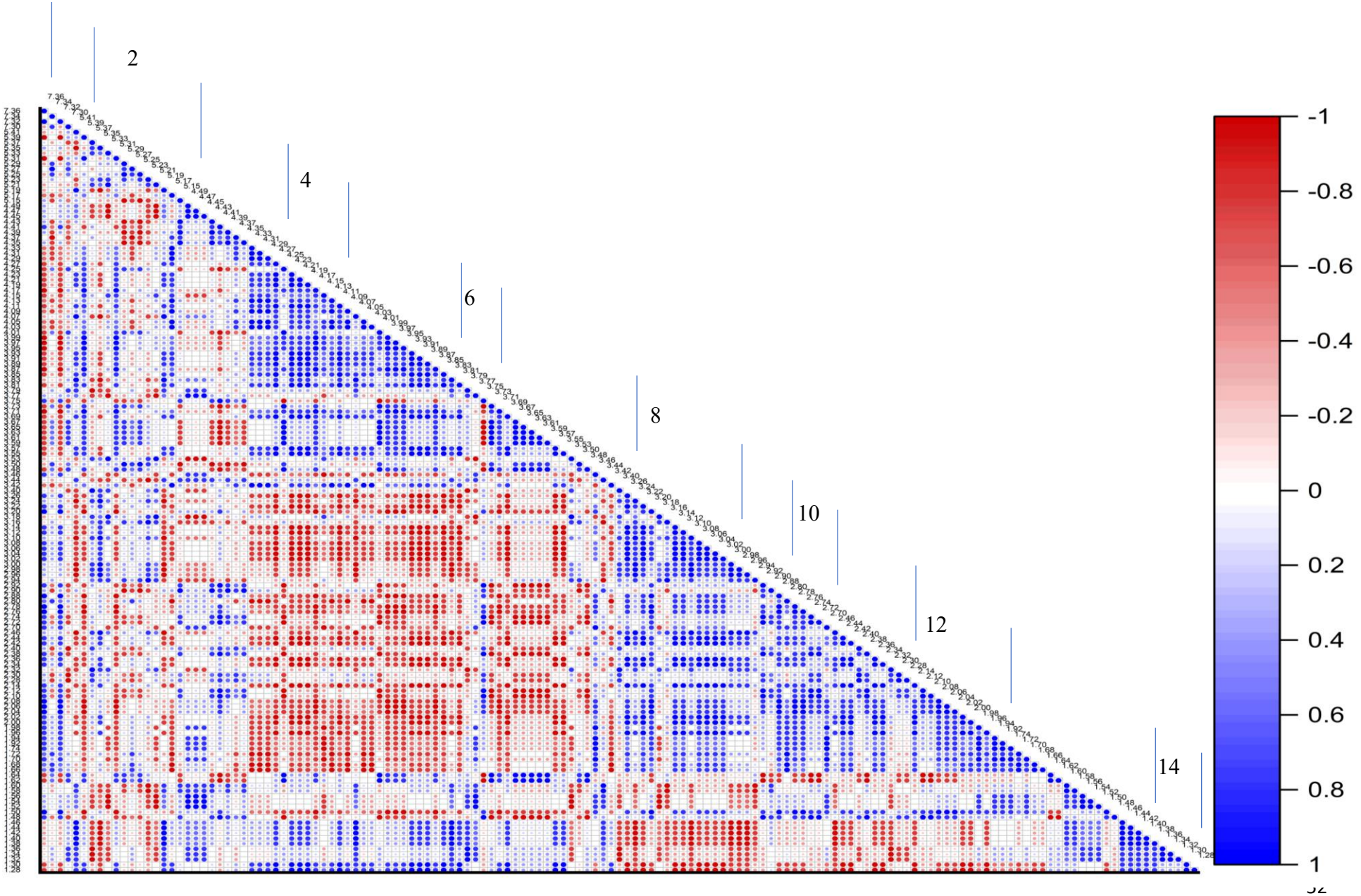

Figure S46. Spearman correlation of group of metabolites present in regions 1-14 of variable nitrate (0.05 - 0.2 g L^{-1})in determination phase.

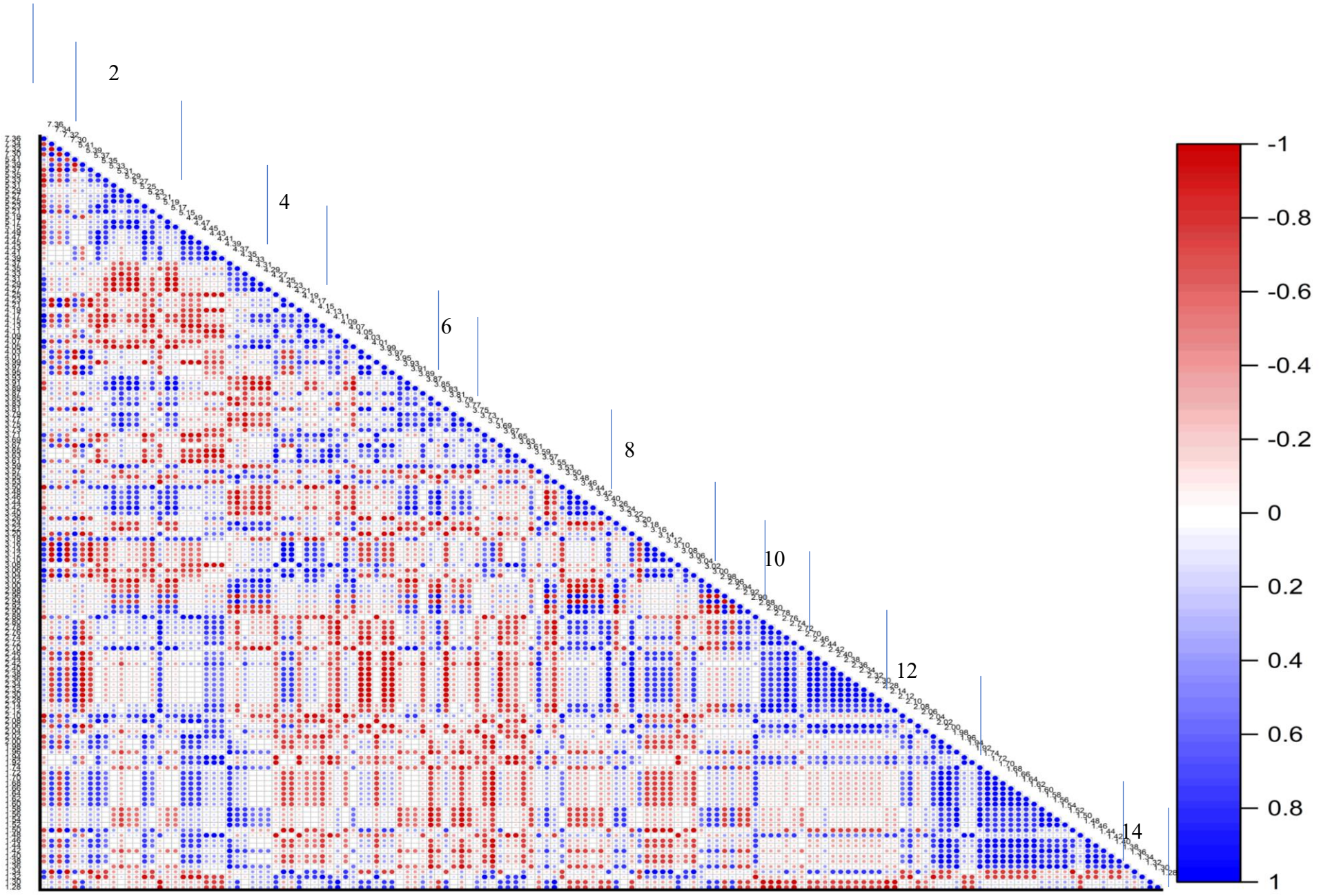

Figure S47. Spearman correlation of group of metabolites present in regions 1-14 of variable nitrate (0.05 - 0.2 g L^{-1})in differentiation phase.

Box S7: Spearman correlation between three phases in variable phosphate

In variable phosphate metabolites in regions 5-8 showed a positive correlation in the vegetative and determination phase as well regions 13-14 showed a positive correlation in all three phases. A negative correlation was found between regions (4-8 and 13-14) in the vegetative and determination phase (Fig. S48, S49, S50). In case of variable phosphate in vegetative phase positive correlation found between metabolites in regions 4-7, 10-14 but negative correlation found between 4-7 and 8-14(Fig. S48). In case of determination phase positive correlation found between metabolites in regions 3-6, 8, 11-12, 12-14. Negative correlation found between regions 11-12 and 13 (Fig. S49). In case of differentiation phase positive correlation found between metabolites in regions 3,5,9-11,12-13 (Fig. S50).

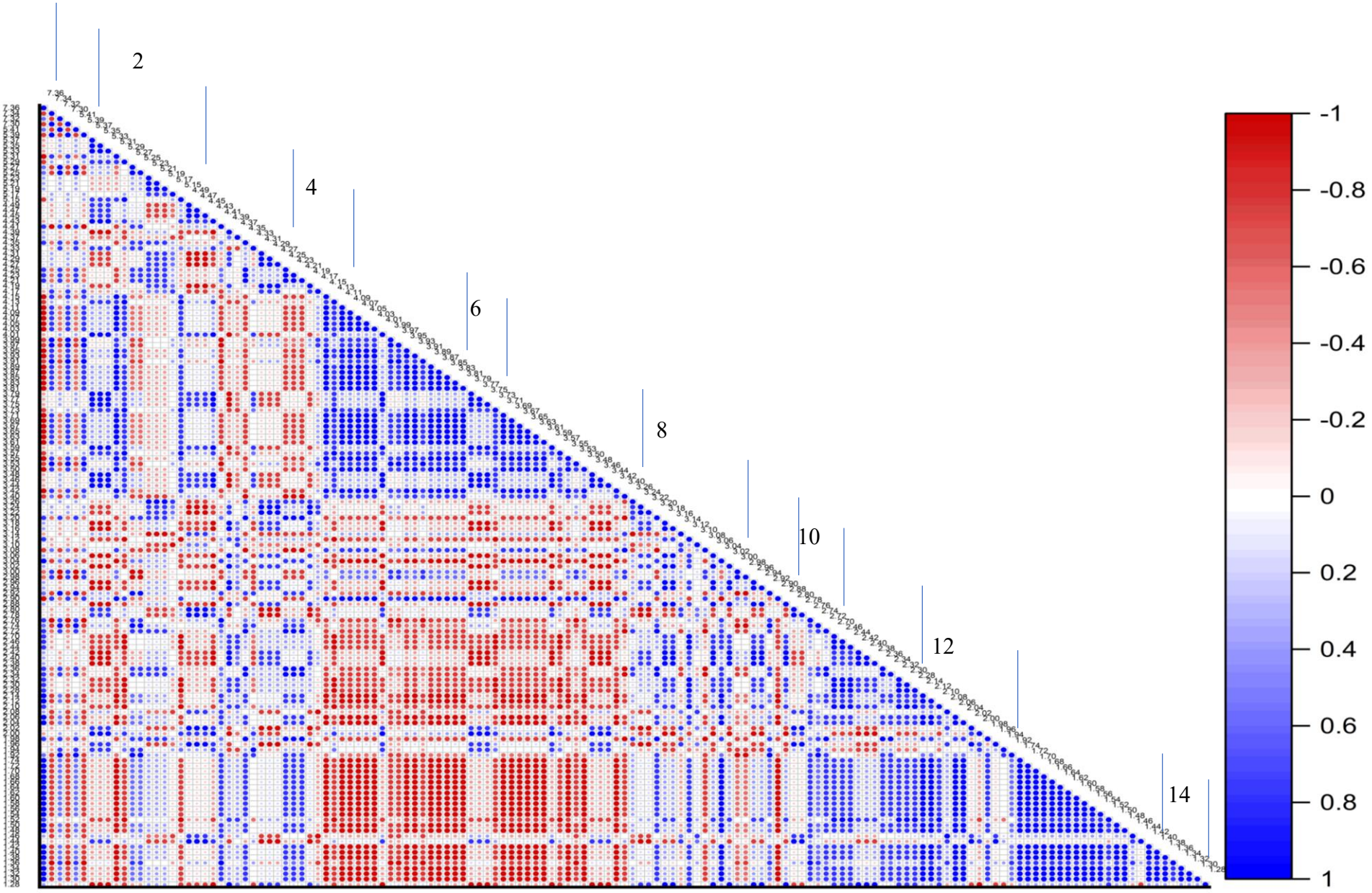

Figure S48. Spearman correlation of group of metabolites present in regions 1-14 of variable phosphate (0.05 - 0.2 g L^{-1}) in vegetative phase.

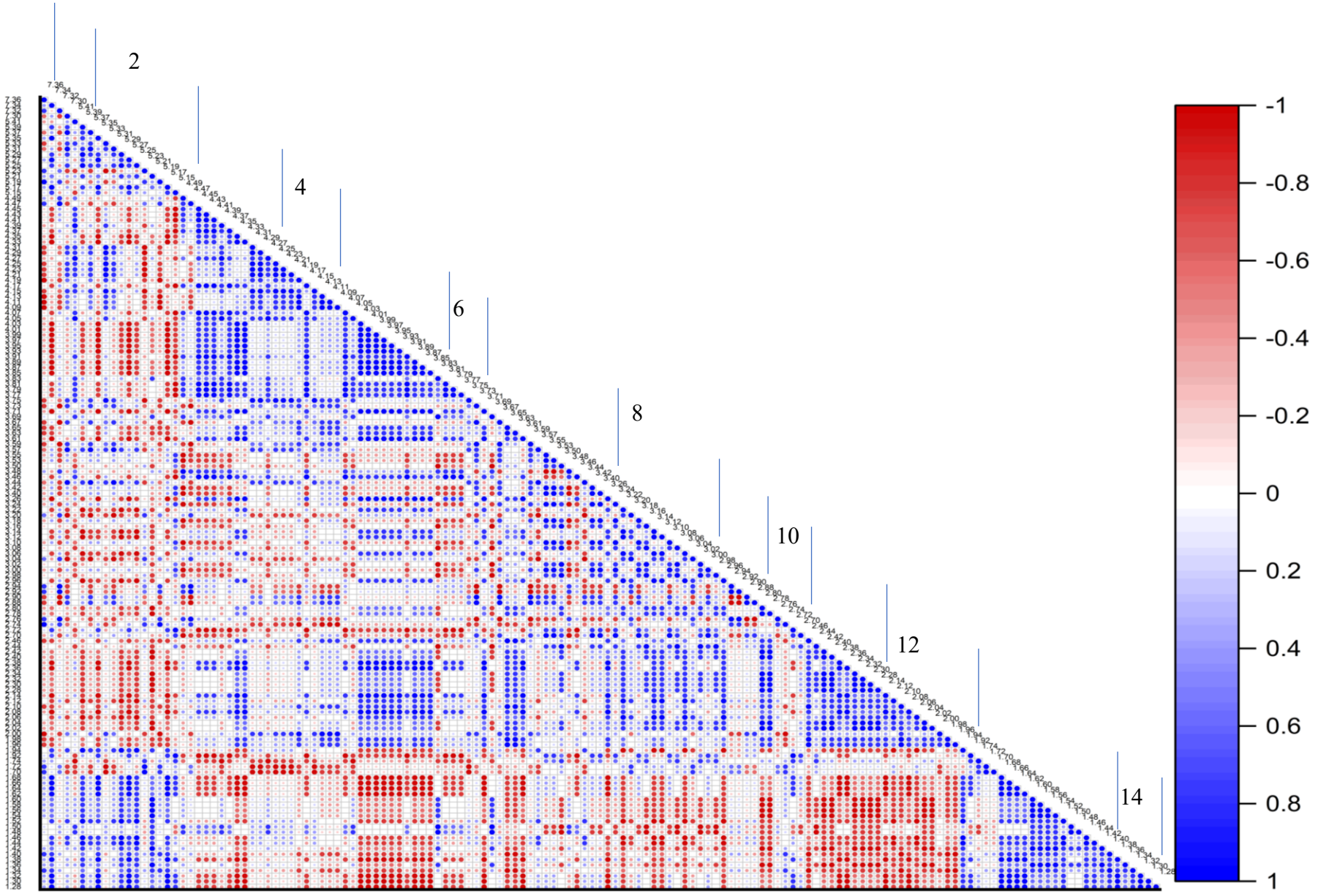

Figure S49. Spearman correlation of group of metabolites present in regions 1-14 of variable phosphate (0.05 - 0.2 g L^{-1}) in determination phase.

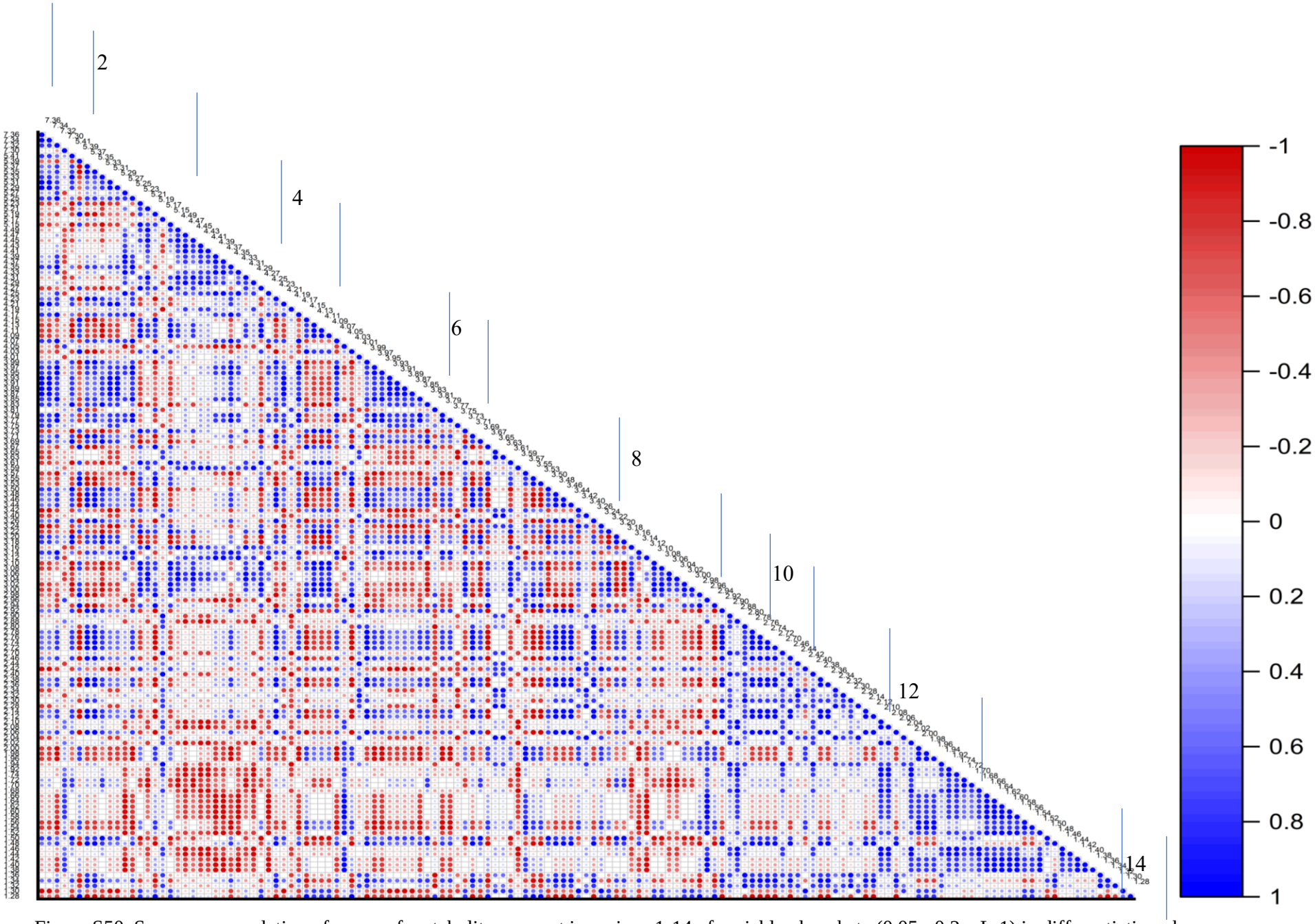

Figure S50. Spearman correlation of group of metabolites present in regions 1-14 of variable phosphate (0.05 - 0.2 g L-1) in differentiation phase

Table S1. Composition of Erdschreiber seawater (ESS) culture medium

1. Na No3: 10g/100ml

2. Na_2HPO_4 : 2g/100 ml

3.PI Metal Stock Stock Solution:

Composition		Mg
EDTA-Na_2	:	0.003
$FeCl_3.6H_2O$ ($FeCl_3$)	:	387
$MnCl_2.4H_2O$	:	232.25
$ZnCl_2$	:	432
$CoCl_2.6\ H_2O$	:	12
$CuSO_4.5\ H_2O$	:	5
$Na_2MoO_4.2H_2$	:	126
H_3PO_4	:	3.53

Volume upto 1 litre made up by adding distilled water

These three stock solutions were autoclaved and stored in amber bottles.

1ml from each three stock solutions added in 997ml filtered autoclaved seawater to make 1 litre of ESS media.

Box S1: PCR reaction mixture (25µl)

Add 1 µl of template DNA, 2.5 mM dNTP's, 10 pM of each primer, 3.2 mM MgCl2, 2.5 µl 10x reaction buffer and 1.25 units of Taq DNA polymerase. The mixture was vortexed and ran in a Biorad thermocycler (M/s. Biorad MyCycler, USA) following the protocol: initial denaturation at 94°C for 3 min, followed by 30 cycles of 1 min at 94°C, 2 min at 45°C and 2 min at 65°C. The amplified product was ran on a 1.2% agarose gel containing EtBr to check quality. The rbcL-forward and rbcL-reverse green seaweed, specific primers sequences were 5′-GCTGGWGTAAAAGATTAYCG-3′ and 5′-TCACGCCAACGCATRAASGG-3′ respectively.

Table S2. Metabolites of interest identified in *Ulva ohnoi* using ^{1}H NMR.

Spectral bin in ^{1}H NMR spectra	Metabolites
1.34	Lactate
1.38	Threonine
1.48	Alanine
1.92	Acetate
2.04	Proline
2.14	Glutamic acid
2.36	a-ketoglutaric acid
2.42	Succinic acid
2.74	Malic acid
2.98	Cysteine
3.00	γ-Amino-butyrate (GABA)
3.10	Creatinine
3.16	Isethionic acid
3.20	Betaine
3.40	Triethanoalmine
3.48	Hypotaurine
3.50	Choline
3.55	Glycine
3.65	Coniferyl aldehyde
3.75	Glucose
3.71	sorbitol
3.81	Acetylcholine
3.83	Sucrose
3.85	Creatine
3.89	Aspartate
3.91	Ethanolamine
4.01	Galactose
4.11	L-Cysteinesulfinic acid
4.17	lactic acid
7.32	Tryptophan

Box S11: Metabolites variation between phases from NMR data

Table S3, S4 and S5 showed that effect of variable abiotic conditions on metabolite variations during the transition of phases showed that amino acids tryptophan and tyrosine in region 1 [7.29 -7.37 ppm (5)] increased (0.41-1.66) in average in all variable conditions from vegetative to differentiation phase. Galactose and sucrose in region 2 [5.15-5.41 ppm (13)] increased (0.02- 0.13) in average in variable salinity, temperature, light intensity and phosphate but decreased (0.46-0.03) in photoperiod and nitrate from vegetative to differentiation phase. Likewise, the amount of glucose in region 3 [4.29 - 4.49 ppm (11)] decreased in average (0.54-0) in all conditions except it increased (0.06-0.16) in light intensity. Lactic acid, 5-hydroxy methyl uracil in region 4 [4.13 - 4.27 (8)] decreased in average (4.10-0.45) in salinity, temperature, photoperiod, nitrate and phosphate but increased in average (1.17-1.31) in light intensity from vegetative to differentiation phase. Sucrose, threonine, lactate, betaine, cysteine, creatinine, isethionic acid, aspartate, creatine, triethanol amine, ethanolamine, l-Cysteinesulfinic acid in region 5 [3.83 - 4.10 ppm (14)] increased in average (2.12-3.43) in variable salinity, light intensity, nitrate and phosphate but decreased (4.72-2.81) in temperature, photoperiod. glucose, sorbitol, acetylcholine in region 6 [3.76 - 3.82 ppm (4)] increased (5.42-9.98) in variable salinity, light intensity, phosphate but decreased (11.30-8.31) in temperature, photoperiod and nitrate. Triethanolamine, glucose, alanine, coniferyl aldehyde, glycine, choline, hypotaurine in region 7 [3.40 -3.74 ppm (18)] increased in average (4.71-5.73) in salinity, light intensity but decreased (5.85-4.64) in rest all conditions. acetylcholine, choline, betaine, isethionic acid, creatinine, aspartate, α-ketoglutaric acid in region 8 [3.00-3.28 ppm (15)] increased in average (1.30-2.79) in variable photoperiod, nitrate, phosphate but decreased (2.04-1.36) in salinity, temperature and light intensity. Cysteine, creatinine in region 9 [2.88-2.98 ppm (6)] increased in average (12.58-22.97) in all conditions. Aspartate, hypotaurine, l-cysteinesulfinic acid in region 10 [2.70-2.80 ppm (6)] increased in average (2.52-7.51) in variables of all conditions except it decreased (4.31-0.64) in nitrate and phosphate. Succinic acid, glutamine, glutamic acid, proline, α-ketoglutaric acid in region 11 [2.28 -2.46 ppm (10)] decreased in average (0.79-0.26) in all conditions but increased (0.07-1.42) in photoperiod. Acetylcholine, glutamic acid, proline, acetate in region 12 [1.91-2.22 ppm (16)] decreased in average (1.52-0.54) in variable salinity, light intensity but increased (0.08-1.42) in rest all conditions. Alanine in region 13 [1.38- 1.73 ppm (18)] decreased in average (1.05-0.29) in variable salinity, light intensity, phosphate but increased (0.15-1.12) in temperature, photoperiod, nitrate. Threonine, lactate in region 14 [1.27-1.38 ppm (6)] decreased in average (6.39-2.37) from vegetative to differentiation phase in all conditions.

Table S3. Combined average and SD of the range of bins in region 1 to 14 in vegetative phase.

Region No.	Bin range	Salinity (20 - 35 PSU)		Temperature (20 - 35°C)		Photoperiod (18:6, 12:12 and 6:18 D/N)		light intensity (220, 350 and 500 µmol photons m^{-2} s^{-1})		Nitrate (0.05 - 0.2 g L^{-1})		Phosphate (0.05 - 0.2 g L^{-1})	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Region 1	7.29 -7.37 (5)	0.76	0.21	0.41	0.15	0.79	0.64	1.22	0.27	0.58	0.30	0.70	0.32
Region 2	5.15 -5.41 (13)	0.02	0.08	0.02	1.16	0.46	0.70	0.14	0.04	0.11	0.11	0.07	0.14
Region 3	4.29 - 4.49 (11)	0.29	0.21	0.46	0.51	0.54	0.65	0.06	0.24	0.28	0.17	0.36	0.17
Region 4	4.13 - 4.27 (8)	2.13	0.50	0.46	0.61	4.10	0.71	1.17	0.19	3.24	0.73	2.89	0.82
Region 5	3.83 - 4.10 (14)	2.85	0.61	3.89	1.06	4.72	1.23	2.12	0.27	3.83	0.79	3.80	1.06
Region 6	3.76 - 3.82 (4)	7.79	0.96	10.24	4.00	11.30	3.16	5.42	0.83	10.48	2.22	8.67	1.52
Region 7	3.40 -3.74(18)	5.57	0.68	5.85	1.58	7.47	2.48	4.71	0.47	6.83	1.18	6.73	2.27
Region 8	3.00 -3.28 (15)	2.04	0.34	1.53	0.49	1.30	1.58	2.04	0.39	1.72	0.59	1.69	1.69
Region 9	2.88 -2.98(6)	16.31	2.69	12.58	3.34	12.72	6.77	19.95	2.53	13.43	3.11	14.93	5.66
Region 10	2.70-2.80 (6)	5.09	0.81	3.90	1.17	2.52	2.65	6.18	0.79	4.31	2.05	3.52	2.51
Region 11	2.28 -2.46 (10)	0.77	0.23	0.79	0.39	0.07	1.02	1.13	0.29	0.67	0.54	0.65	0.33
Region 12	1.91 -2.22 (16)	1.00	0.34	0.96	0.58	0.08	0.74	1.52	0.39	0.74	0.53	0.79	0.38
Region 13	1.39-1.73 (18)	0.71	0.31	0.54	0.37	0.15	0.40	1.05	0.28	0.46	0.36	0.53	0.36
Region 14	1.27-1.38 (6)	6.39	1.87	4.04	1.26	5.48	4.58	5.99	1.84	4.08	1.60	4.30	1.86

Table S4. Combined average and SD of the range of bins in region 1 to 14 in determination phase.

Region No.	Bin range	Salinity (20 - 35 PSU)		Temperature (20 - 35 °C)		Photoperiod (18:6, 12:12 and 6:18 D/N)		light intensity (220, 350 and 500 µmol photons $m^{-2} s^{-1}$)		Nitrate (0.05 - 0.2 g L^{-1})		Phosphate (0.05 - 0.2 g L^{-1})	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Region 1	7.29 -7.37 (5)	1.09	0.41	1.00	0.21	1.42	0.55	1.20	0.68	1.02	0.44	1.53	0.57
Region 2	5.15 -5.41 (13)	0.13	0.31	0.06	0.11	0.03	0.09	0.00	0.08	0.10	0.17	0.03	0.09
Region 3	4.29 - 4.49 (11)	0.37	0.65	0.21	0.17	0.11	0.09	0.05	0.10	0.11	0.24	0.07	0.19
Region 4	4.13 - 4.27 (8)	1.67	0.88	2.01	0.71	1.14	0.37	0.83	0.37	1.77	0.45	0.45	0.36
Region 5	3.84 - 4.10 (14)	2.88	0.61	3.15	0.86	2.50	0.66	1.82	0.77	4.35	0.92	2.90	0.57
Region 6	3.76 - 3.82 (4)	7.80	1.60	9.44	2.64	7.27	2.25	4.98	1.97	10.52	1.80	8.19	1.67
Region 7	3.40 -3.74(18)	5.17	1.06	5.59	1.20	4.96	1.05	4.02	1.91	6.06	1.46	4.99	1.16
Region 8	3.00 -3.28 (15)	1.87	0.47	1.55	0.56	1.72	0.65	1.95	1.07	1.95	0.62	1.93	0.64
Region 9	2.88 -2.98(6)	17.87	3.52	16.51	2.47	19.48	5.31	19.22	10.09	17.33	3.00	21.19	8.84
Region 10	2.70-2.80 (6)	5.89	2.00	5.12	1.02	8.37	8.91	6.39	3.85	5.25	1.83	6.90	2.63
Region 11	2.28 -2.46 (10)	1.02	0.53	1.06	0.76	0.86	0.59	0.67	0.47	0.61	0.63	1.08	0.72
Region 12	1.91 -2.22 (16)	1.06	0.45	1.43	1.05	1.08	0.54	1.48	0.84	0.53	0.48	1.04	0.69
Region 13	1.39-1.73 (18)	0.57	0.40	0.52	0.38	0.51	0.36	0.44	0.48	0.15	0.21	0.29	0.30
Region 14	1.37- 1.27 (6)	4.41	1.62	4.72	2.35	3.56	1.31	3.73	2.25	1.92	0.74	2.77	0.97

Table S5. Combined average and SD of the range of bins in region 1 to 14 in differentiation phase.

Region No.	Bins	Salinity (20 - 35 PSU)		Temperature (20 - 35°C)		Photoperiod (18:6, 12:12 and 6:18 D/N)		light intensity (220, 350 and 500 µmol photons $m^{-2} s^{-1}$)		Nitrate (0.05 - 0.2 g L^{-1})		Phosphate (0.05 - 0.2 g L^{-1})	
		Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Region 1	7.29 -7.37 (5)	1.07	0.54	1.11	0.59	1.66	0.51	1.31	0.42	1.56	0.69	1.13	0.60
Region 2	5.15 -5.41 (13)	0.13	0.15	0.12	0.24	0.06	0.17	0.08	0.09	0.04	0.25	0.08	0.16
Region 3	4.29 - 4.49 (11)	0.14	0.10	0.22	0.25	-0.22	0.67	0.16	0.11	0.08	0.23	0.12	0.12
Region 4	4.13 - 4.27 (8)	1.26	0.46	0.96	0.48	0.79	0.46	1.31	0.32	1.02	0.48	1.20	0.39
Region 5	3.83 - 4.10 (14)	3.07	0.77	3.09	1.40	2.81	0.98	3.20	0.76	3.14	0.84	3.43	0.90
Region 6	3.76 - 3.82 (4)	9.98	3.85	9.07	4.74	8.07	1.60	8.52	1.71	8.31	2.59	9.25	3.31
Region 7	3.40 -3.74(18)	5.73	2.64	5.01	3.16	4.64	0.85	5.16	1.10	4.88	2.63	5.29	2.68
Region 8	3.00 -3.28 (15)	1.36	0.75	1.38	1.17	2.13	0.60	1.80	0.54	2.14	1.84	2.79	1.91
Region 9	2.88 -2.98(6)	20.62	24.73	16.85	22.43	22.97	4.82	21.00	10.77	21.72	19.80	21.77	18.93
Region 10	2.70-2.80 (6)	6.00	10.11	4.23	7.93	7.51	1.24	6.85	2.10	0.64	3.32	2.59	4.08
Region 11	2.28 -2.46 (10)	0.27	0.54	0.76	1.12	1.42	1.22	1.01	0.50	0.26	1.04	0.45	0.60
Region 12	1.91 -2.22 (16)	0.54	0.57	1.15	0.98	1.42	1.08	0.93	0.41	1.26	0.80	0.86	0.54
Region 13	1.39-1.73 (18)	0.34	0.35	0.65	0.71	0.32	0.29	0.29	0.25	1.12	1.00	0.45	0.50
Region 14	1.27-1.38 (6)	4.22	2.82	2.44	2.32	2.49	0.95	3.48	1.28	3.28	1.87	2.37	1.09

Box S8: Spearman correlation among metabolites in vegetative phase with degree of correlation

In the vegetative phase (Fig. 4a, Table S6 in the supportive information) tryptophan displayed a very strong positive correlation with γ-Amino-butyrate (GABA), lactate, cysteine (0.83). Galactose and glycine displayed a very strong positive correlation with glucose and Coniferyl aldehyde (0.94), lactic acid, aspartate and acetylcholine (0.83). The moderate correlation of L-Cysteinesulfinic acid found with creatine (0.77). Lactic acid showed a very strong positive correlation with aspartate (0.83) and creatine (0.94). An ethanolamine showed a moderate correlation with lactic acid (0.77) and aspartate (0.71). Creatine showed a very strong positive correlation with aspartate (0.94), ethanolamine, glucose (0.83) but moderately correlated with L-Cysteinesulfinic acid, galactose (0.77). Sucrose showed a very strong positive correlation with ethanolamine, creatine (0.94), aspartate (0.89), lactic acid, acetylcholine (0.83). An acetylcholine showed a very strong positive correlation with creatine (0.94), aspartate (0.83), galactose (0.83) but showed a moderate correlation with ethanolamine (0.77). Sorbitol showed a very strong positive correlation with lactic acid and acetylcholine (0.83) but a moderate correlation with creatine (0.71). Glucose showed a very strong positive correlation with lactic acid, L-Cysteinesulfinic acid, galactose, acetylcholine (0.94), coniferyl aldehyde (0.89) while moderate correlation with aspartate, sorbitol (0.77). Glycine showed a very strong positive correlation with glucose, Coniferyl aldehyde (0.94), lactic acid (0.83), aspartate (0.83), acetylcholine (0.83) but moderate correlation with creatine (0.77). Betaine displayed a very strong positive correlation with creatinine (0.83), malic acid, glutamic acid (0.89) while a moderate correlation with glutamine, proline (0.77). Creatinine exhibited a very strong positive correlation with alanine, malic acid (0.89), succinic acid (0.94). Malic acid showed a very strong positive correlation with glutamic acid (0.83) but a moderate correlation with glutamine and proline (0.71). Succinic acid showed a very strong positive correlation with malic acid (0.94), acetate (0.83) and a moderate correlation with betaine (0.77). α-ketoglutaric acid showed a very strong positive correlation with malic acid (0.83) and a moderate correlation with succinic acid (0.71). Glutamic acid showed a moderate correlation with succinic acid (0.77), creatinine (0.71). Proline showed a very strong positive correlation with glutamic acid (0.94). Glutamine showed a very strong positive correlation with glutamic acid. Galactose showed a very strong positive correlation with lactic acid (0.83), aspartate (0.83). Acetate showed a moderate correlation with hypotaurine, creatinine, glutamic acid (0.71). Alanine showed a very strong positive correlation with acetate (0.94), succinic acid (0.94), malic acid (0.83), glutamic acid (0.83). Lactate showed a very strong positive correlation with threonine (0.83).

Table S6. Spearman correlation among metabolites in vegetative phase with degree of correlation.

Metabolites	Correlated metabolites	Perfect correlation (1)	Very strong correlation (+0.9)	Very strong correlation (+0.8)	Strong correlation (+0.7)
Tryptophan	γ-Amino-butyrate (GABA), Cysteine, lactate	-	-	0.83	-
lactic acid	L-Cysteinesulfinic acid, galactose	-	-	0.83	-
L-Cysteinesulfinic acid	Galactose, glycine	1	-	-	-
Ethanolamine	Lactic acid	-	-	-	0.77
Ethanolamine	Aspartate	-	-	-	0.71
Aspartate	Lactic acid, L-Cysteinesulfinic acid, galactose, acetylcholine	-	-	0.83	-
Creatine	Lactic acid, aspartate	-	0.94	-	-
Creatine	Ethanolamine, glucose	-	-	0.83	-
Creatine	L-Cysteinesulfinic acid, galactose	-	-	-	0.77
Sucrose	Ethanolamine, creatine	-	0.94	-	-
Sucrose	Lactic acid, acetylcholine	-	-	0.83	-
Sucrose	Aspartate	-	-	0.89	-
Acetylcholine	L-Cysteinesulfinic acid, galactose	-	-	0.83	-
Acetylcholine	Lactic acid	1	-	-	-
Acetylcholine	Creatine	-	0.94	-	-
Acetylcholine	Ethanolamine	-	-	-	0.77
Sorbitol	Lactic acid, acetylcholine	-	-	0.83	-
Sorbitol	Creatine	-	-	-	0.71
Glucose	Coniferyl aldehyde	-	-	0.89	-
Glucose	lactic acid, L-Cysteinesulfinic acid, galactose, acetylcholine	-	0.94	-	-
Glucose	Aspartate, sorbitol	-	-	-	0.77
Coniferyl aldehyde	L-Cysteinesulfinic acid, galactose	-	0.94	-	

Coniferyl aldehyde	Lactic acid, acetylcholine	-	-	-	0.71
Glycine	Galactose	1	-	-	-
Glycine	Glucose, Coniferyl aldehyde	-	0.94	-	-
Glycine	Lactic acid, aspartate, acetylcholine	-	-	0.83	-
Glycine	Creatine	-	-	-	0.77
Betaine	Glutamine, proline	-	-	-	0.77
Isethionic acid	Choline	1	-	-	-
Creatinine	Betaine	-	-	0.83	-
Creatinine	Alanine	-	-	0.89	-
γ-Amino-butyrate (GABA)	Cysteine	1	-	-	-
Malic acid	Betaine, creatinine	-	-	0.89	-
Malic acid	Glutamic acid, alanine	-	-	0.83	-
Malic acid	Glutamine, proline	-	-	-	0.71
Succinic acid	Creatinine, malic acid, alanine	-	0.94	-	-
Succinic acid	Acetate	-	-	0.83	-
Succinic acid	Betaine	-	-	-	0.77
a-ketoglutaric acid	Malic acid	-	-	0.83	
a-ketoglutaric acid	Succinic acid	-	-	-	0.71
Glutamic acid	Betaine	-	-	0.89	-
Glutamic acid	Alanine	-	-	0.83	-
Glutamic acid	Glutamine, proline	-	0.94	-	-
Glutamic acid	Succinic acid	-	-	-	0.77
Glutamic acid	Creatinine	-	-	-	0.71
Glutamine	Proline	1	-	-	-
Acetate	Hypotaurine, creatinine, glutamic acid	-	-	-	0.71
Alanine	Betaine	-	-	-	0.71
Alanine	Acetate	-	0.94	-	-
Lactate	Threonine	-	-	0.83	-

Box S9: Spearman correlation among metabolites in determination phase with degree of correlation

In the determination phase (Fig. 4b, Table S7 in the supportive information) tryptophan showed a very strong positive correlation with malic acid (0.89), proline, alanine (0.83). Lactic acid showed a perfect positive correlation with galactose, ethanolamine, aspartate, creatine, acetylcholine, sorbitol, glucose (0.94), coniferyl aldehyde, glycine, L-Cysteinesulfinic acid (0.89). L-Cysteinesulfinic acid showed a very strong positive correlation with glucose, sorbitol, galactose (0.94), Coniferyl aldehyde, glycine (0.83) but moderate correlation with betaine, acetylcholine, ethanolamine, aspartate, creatine (0.71). Galactose showed a very strong positive correlation with Coniferyl aldehyde, glycine (0.94), ethanolamine, aspartate, creatine, acetylcholine, choline, hypotaurine (0.83) but a moderate correlation with betaine, sucrose (0.77). An ethanolamine showed a very strong positive correlation with sorbitol, glucose, and hypotaurine (0.83) but a moderate correlation with Coniferyl aldehyde, glycine (0.77). An aspartate showed a very strong positive correlation with sorbitol, glucose, hypotaurine (0.83) but a moderate correlation with Coniferyl aldehyde, glycine (0.77). Creatine showed a very strong positive correlation with sorbitol, glucose, hypotaurine (0.83) but a moderate correlation with Coniferyl aldehyde, glycine (0.77). Sucrose showed a very strong positive correlation with ethanolamine, aspartate, creatine, acetylcholine, hypotaurine (0.94), lactic acid (0.83) moderate correlation with glucose (0.77), Coniferyl aldehyde, glycine, choline (0.71). An acetylcholine showed a very strong positive correlation with sorbitol, glucose, hypotaurine (0.83) but a moderate correlation with Coniferyl aldehyde, Glycine (0.77).Sorbitol showed a very strong positive correlation with glucose, galactose (0.89) but a moderate correlation with Coniferyl aldehyde, glycine (0.77). Glucose showed a very strong positive correlation with Coniferyl aldehyde, glycine (0.94), choline, hypotaurine (0.83) but a moderate correlation with betaine (0.77). Choline showed a very strong positive correlation with hypotaurine (0.83), but a moderate correlation with L-Cysteinesulfinic acid (0.77), Coniferyl aldehyde, glycine (0.71). Hypotaurine showed a moderate correlation with lactic acid, glycine (0.77). Triethanolamine showed a moderate correlation with glutamine (0.71). Betaine showed a very strong positive correlation with Coniferyl aldehyde, glycine (0.89) whereas a moderate correlation with glutamic acid (0.71). γ-Amino-butyrate (GABA) showed a very strong positive correlation with isethionic acid, creatinine (0.83). Cysteine showed a very strong positive correlation with isethionic acid, creatinine, γ-Amino-butyrate (GABA) (0.89). Malic acid showed a very strong positive correlation with tryptophan (0.89), proline (0.83). Succinic acid showed a moderate correlation with threonine (0.71). glutamic acid showed a very strong positive correlation with proline, α-ketoglutaric acid, lactate (0.83). lactate showed a very strong positive correlation with threonine (0.89).

Table S7. Spearman correlation among metabolites in determination phase with degree of correlation

Metabolites	Correlated metabolites	Perfect correlation (1)	Very strong correlation (+0.9)	Very strong correlation (+0.8)	Strong correlation (+0.7)
Tryptophan	Proline, alanine	-	-	0.83	-
Lactic acid	Galactose, ethanolamine, aspartate, creatine, acetylcholine, sorbitol, glucose	-	0.94	-	-
Lactic acid	Coniferyl aldehyde, glycine, L-Cysteinesulfinic acid	-	-	0.89	-
L-Cysteinesulfinic acid	Galactose, sorbitol, glucose	-	0.94	-	-
L-Cysteinesulfinic acid	Coniferyl aldehyde, glycine	-	-	0.83	-
L-Cysteinesulfinic acid	Betaine, acetylcholine, ethanolamine, aspartate, creatine	-	-	-	0.71
Galactose	Ethanolamine, aspartate, creatine, acetylcholine, choline, hypotaurine	-	-	0.83	-
Galactose	Coniferyl aldehyde, glycine	-	0.94	-	-
Galactose	betaine, sucrose	-	-	-	0.77
Ethanolamine	Sorbitol, glucose, hypotaurine	-	-	0.83	-
Ethanolamine	Aspartate, creatine, acetylcholine	1	-	-	-
Ethanolamine	Coniferyl aldehyde, glycine	-	-	-	0.77
Aspartate	Acetylcholine, creatine	1	-	-	-
Aspartate	Sorbitol, glucose, hypotaurine	-	-	0.83	-
Aspartate	Coniferyl aldehyde, glycine	-	-	-	0.77
Creatine	Sorbitol, glucose, hypotaurine	-	-	0.83	-
Creatine	Coniferyl aldehyde, glycine	-	-	-	0.77
Sucrose	Lactic acid	-	-	0.83	-

Sucrose	Ethanolamine, aspartate, creatine, acetylcholine, hypotaurine	-	0.94	-	-
Sucrose	Glucose	-	-	-	0.77
Sucrose	Coniferyl aldehyde, glycine, choline	-	-	-	0.71
Acetylcholine	creatine	1	-	-	-
Acetylcholine	Sorbitol, glucose, hypotaurine	-	-	0.83	-
Acetylcholine	Coniferyl aldehyde, glycine	-	-	-	0.77
Sorbitol	Glucose, galactose	-	-	0.89	-
Sorbitol	Coniferyl aldehyde, glycine	-	-	-	0.77
Glucose	Coniferyl aldehyde, glycine	-	0.94	-	-
Glucose	Galactose	1	-	-	-
Glucose	Choline, hypotaurine	-	-	0.83	-
Glucose	Betaine	-	-	-	0.77
Glycine	Coniferyl aldehyde	1	-	-	-
Choline	L-Cysteinesulfinic acid	-	-	-	0.77
Choline	Coniferyl aldehyde, glycine	-	-	-	0.71
Choline	Hypotaurine	-	-	0.89	-
Hypotaurine	Lactic acid, glycine	-	-	-	0.77
Triethanolamine	Glutamine	-	-	-	0.71
Betaine	Coniferyl aldehyde, glycine	-	-	0.89	-
Betaine	Glutamic acid	-	-	-	0.71
Isethionic acid	Creatinine	1	-	-	-
γ-Amino-butyrate (GABA)	Isethionic acid, creatinine	-	-	0.83	-
Cysteine	Isethionic acid, creatinine, γ-Amino-butyrate (GABA)	-	-	0.89	-
Malic acid	Tryptophan	-	-	0.89	-
Malic acid	Proline	-	-	0.83	-
Succinic acid	Threonine	-	-		0.71
Glutamic acid	Proline, a-ketoglutaric acid, lactate	-	-	0.83	-
Lactate	threonine	-	-	0.89	-

Box S10: Spearman correlation among metabolites in determination phase with degree of correlation

In the differentiation phase (Fig. 4c, Table S8 in the supportive information) tryptophan showed a very strong positive correlation with betaine, glutamic acid (0.83). L-Cysteinesulfinic showed moderate correlation with acetylcholine (0.71). Lactic acid showed a moderate correlation with creatine (0.83). Galactose showed a very strong positive correlation with succinic acid (0.83). Creatine showed a moderate correlation with sucrose, sorbitol (0.77). Sucrose showed a very strong positive correlation with sorbitol (0.89) and a moderate correlation with isethionic acid (0.77). Acetylcholine showed a moderate correlation with glycine, cysteine (0.77), threonine (0.71). Sorbitol showed a moderate correlation with triethanolamine (0.71). Glucose showed a very strong positive correlation with coniferyl aldehyde (0.89). Coniferyl aldehyde showed a moderate correlation with triethanolamine (0.77). Choline showed a moderate correlation with threonine (0.77). Hypotaurine showed a moderate correlation with α-ketoglutaric acid, lactate, galactose (0.77). Betaine showed a very strong positive correlation with hypotaurine (0.83) while a moderate correlation with succinic acid acetate (0.77). Cysteine showed a very strong positive correlation with glycine (0.89) while a moderate correlation with L-Cysteinesulfinic acid (0.77). Malic acid showed a very strong positive correlation with betaine (0.88), α-ketoglutaric acid (0.83) while a moderate correlation with lactate, galactose (0.71). Succinic acid showed a moderate correlation with glutamic acid (0.77), tryptophan (0.71). α-ketoglutaric acid showed a very strong positive correlation with galactose (0.89), betaine (0.83) but a moderate correlation with glutamic acid (0.71). Glutamic acid showed a very strong positive correlation with betaine (0.89) but a moderate correlation with alanine (0.71). Glutamine showed a moderate correlation with proline (0.71).

Table S8. Spearman correlation among metabolites in differentiation phase with degree of correlation

Metabolite	Correlated metabolite	Perfect correlation (1)	Very strong correlation (+ 0.9)	Very strong correlation (+ 0.8)	Strong correlation (+0.7)
Tryptophan	Acetate	-	0.94	-	-
Tryptophan	Betaine, glutamic acid	-	-	0.83	-
Lactic acid	Creatine	-	-	-	0.71
L-Cysteinesulfinic acid	Glycine	-	0.94	-	-
L-Cysteinesulfinic acid	Acetylcholine	-	-	-	0.71
Galactose	Succinic acid,	-	-	0.83	-
Creatine	Sucrose, sorbitol	-	-	-	0.77
Sucrose	Isethionic acid	-	-	-	0.77
Sucrose	Sorbitol	-	-	0.89	-
Acetylcholine	Threonine	-	-	-	0.71
Acetylcholine	Glycine, cysteine	-	-	-	0.77
Sorbitol	Triethanolamine	-	-	-	0.71
Glucose	Triethanolamine	-	0.94	-	-
Glucose	Coniferyl aldehyde	-	-	0.89	-
Coniferyl aldehyde	Triethanolamine	-	-	-	0.77
Choline	Threonine	-	-	-	0.77

Hypotaurine	a-ketoglutaric acid, lactate, Galactose	-	-	-	0.77
Betaine	Succinic acid, acetate	-	-	-	0.77
Betaine	Hypotaurine	-	-	0.83	-
Cysteine	Glycine	-	-	0.89	-
Cysteine	L-Cysteinesulfinic acid	-	-	-	0.77
Malic acid	Hypotaurine	-	0.94	-	-
Malic acid	a-ketoglutaric acid	-	-	0.83	
Malic acid	Betaine	-	-	0.88	-
Malic acid	Lactate, galactose	-	-	-	0.71
Succinic acid	Tryptophan	-	-	-	0.71
Succinic acid	Glutamic acid	-	-	-	0.77
a-ketoglutaric acid	Succinic acid	-	0.94	-	-
a-ketoglutaric acid	Galactose	-	-	0.89	-
a-ketoglutaric acid	Betaine	-	-	0.83	-
a-ketoglutaric acid	Glutamic acid	-	-	-	0.71
Glutamic acid	Betaine	-	-	0.89	-
Glutamic acid	Alanine	-	-	-	0.77
Glutamine	Proline	-	-	-	0.71