

Supplementary Material

Table S1: Discharge characteristics of the wastewater treatment plant (WWTP) Bempflingen-Riederich (3-stage-WWTP) in the year 2020. WWTP-out = WWTP effluent, COD = chemical oxygen demand

Monthly average	WWTP-out COD in mg/l	COD efficiency in %	WWTP-out NH ₄ -N in mg/l	WWTP-out N _{anorg} in mg/l	WWTP-out P _{total} in mg/l
January	15,8	97,8	0,94	8,11	0,45
February	16,1	88,8	0,07	8,57	0,31
March	15,3	97,2	0,26	4,87	0,36
April	20,3	95,4	1,16	7,16	0,24
May	16,3	94,3	0,25	7,59	0,18
June	18,6	94,7	0,1	6,58	0,15
July	13,2	92,4	0,06	5,73	0,26
August	12,8	95,0	0,06	6,14	0,63
September	15,1	96,1	0,03	10,45	0,53
October	13,9	97,1	0,17	8,05	0,54
November	15,9	98,2	0,12	9,26	0,33
December	16,1	98,2	0,44	6,95	0,26
Annual average	15,8	95,4	0,30	7,50	0,40

Table S2: Discharge characteristics of the wastewater treatment plant (WWTP) Albstadt-Lautlingen (4-stage-WWTP) in the year 2020. WWTP-out = WWTP effluent, COD = chemical oxygen demand

Monthly average	WWTP-out COD in mg/l	COD efficiency in %	WWTP-out NH ₄ -N in mg/l	WWTP-out N _{anorg} in mg/l	WWTP-out PO ₄ -P in mg/l
January	6	96,5	0,08	7,36	0,07
February	5	94,2	0,03	4,62	0,03
March	5	95,8	0,02	5,62	0,03
April	5	98,4	0,01	8,60	0,05
May	7	97,6	0,03	6,49	0,04
June	6	95,1	0,02	5,12	0,05
July	6	97,8	0,01	8,59	0,05
August	6	97,2	0,01	5,04	0,04
September	4	98,7	0,01	7,38	0,09
October	7	97,0	0,01	3,89	0,07
November	6	97,9	0,02	7,46	0,03
December	6	96,5	0,08	5,75	0,03
Annual average	6	97,3	0,03	6,32	0,05

Table S3: Limnological parameters measured during gammarid sampling in July (sampling of precopula pairs) and August (sampling of gammarids in the non-reproductive phase) 2020, up- and downstream of the 3-stage-WWTP as well as up- and downstream of the 4-stage-WWTP. July 2020 = 13.07.2020; August 2020 = 04.08.2020. Values that represent a very good ecological status according to the “Oberflächengewässerverordnung” (OGewV. Verordnung zum Schutz der Oberflächengewässer (Oberflächengewässerverordnung - OGewV) 2016) are marked with a green colour. Values indicating good ecological status are shown in yellow. If no good ecological status can be achieved, the value is marked in orange. Values that are not marked are not used to classify ecological status. Nitrate values are assessed using the AA_EQS. Values exceeding the AA-EQS are marked in red. Values below the AA-EQS are marked green.

	July 2020				August 2020			
	3-stage-WWTP upstream	3-stage-WWTP downstream	4-stage-WWTP upstream	4-stage-WWTP downstream	3-stage-WWTP upstream	3-stage-WWTP downstream	4-stage-WWTP upstream	4-stage-WWTP downstream
Oxygen in mg/l	9,87	9,56	10,02	9,12	9,27	9,00	9,49	9,06
Oxygen in %	99,0	97,3	108,6	100,2	97,9	94,7	103,4	100,2
Water temperature	14,3	15,0	15,7	16,4	16,1	16,2	15,4	15,4
Conductivity in µS/cm	657	654	486	671	350	345	503	457
pH		8,26	83,42	8,03	8,02	8,02	8,22	7,9
NH4-N in mg/l	0,04	0,04	<0,04	<0,04	0,1	0,1	<0,04	<0,04
PO4-P in mg/l	<0,05	<0,05	<0,05	<0,05	0,09	0,1	<0,05	<0,05
Chloride in mg/l	41	45	36	71	19	20	36	38
NO2-N in mg/l	0,017	0,013	<0,003	<0,003	0,024	0,027	0,003	<0,003
NO3-N in mg/l	3,4	3,7	0,3	4,8	1,8	1,8	0,5	1,6
Carbonate hardness in °dH	17	14	14	14	9	9	12	6
Total hardness in °dH	21	21	18	19	12	10	15	10

Table S4: Limnological parameters at the end of exposure of gammarids, separated from precopula pairs up- and downstream of the 3-stage-WWTP as well as up- and downstream of the 4-stage-WWTP, at 15 °C and 25 °C. Exposure duration: 13.07.-20.07.2020. F = female aquaria, M = male aquaria. Values that represent a very good ecological status according to the “Oberflächengewässerverordnung” (OGewV. Verordnung zum Schutz der Oberflächengewässer (Oberflächengewässerverordnung - OGewV) 2016) are marked with a green colour. Values indicating good ecological status are shown in yellow. If no good ecological status can be achieved, the value is marked in orange. Values that are not marked are not used to classify ecological status. Nitrate values are assessed using the AA_EQS. Values exceeding the AA-EQS are marked in red. Values below the AA-EQS are marked green.

	15 °C								25 °C							
	3-stage-WWTP upstream		3-stage-WWTP downstream		4-stage-WWTP upstream		4-stage-WWTP downstream		3-stage-WWTP upstream		3-stage-WWTP downstream		4-stage-WWTP upstream		4-stage-WWTP downstream	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Oxygen in mg/l	9,62	9,13	9,64	9,03	9,3	9,46	9,48	9,57	7,99	7,85	8,04	8,01	7,93	7,97	7,96	7,94
Oxygen in %	101,9	89,8	102,2	98,1	100,3	101,1	101,4	101	101	99,7	101,2	101,7	100,7	100,5	100,7	101,1
Water temperature	15,6	16,6	15,7	16,8	16,4	16,0	16,0	15,5	24,4	24,7	24,2	24,7	24,7	24,3	24,5	24,8
Conductivity in µS/cm	619	501	650	639	427	399	527	538	447	466	459	441	395	408	557	562
pH	8,11	6,73	8,17	8,06	8,31	8,14	7,85	8,06	7,21	7,5	7,25	7,22	7,77	7,93	7,67	7,8
NH4-N in mg/l	0,08	0,06	0,07	0,07	0,29	0,18	0,35	0,07	<0,04	0,91	0,04	0,89	0,36	2,12	1	0,36
PO4-P in mg/l	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	0,06	<0,05	<0,05	<0,05
Chloride in mg/l	43	44	45	47	38	38	75	75	43	44	36	46	38	38	72	74
NO2-N in mg/l	0,029	0,011	0,01	0,008	0,011	0,009	0,033	0,008	0,083	0,144	0,088	0,099	0,055	0,054	>0,460	>0,460
NO3-N in mg/l	3,8	3,4	3,5	3	0,7	0,6	5,1	5	3,1	2,9	3,2	3,4	0,5	0,3	2	2
Carbonate hardness in °dH	14	9	17	14	10	9	7	8	7	7	7	6	8	9	9	8
Total hardness in °dH	20	12	20	21	13	12	14	12	10	11	11	10	11	11	12	11

Table S5: Limnological parameters at the end of exposure of gammarids in the non-reproductive phase, sampled up- and downstream of the 3-stage-WWTP as well as up- and downstream of the 4-stage-WWTP, at 18 °C and 25 °C. Exposure duration: 04.08.-11.08.2020.

	18 °C				25 °C			
	3-stage-WWTP upstream	3-stage-WWTP downstream	4-stage-WWTP upstream	4-stage-WWTP downstream	3-stage-WWTP upstream	3-stage-WWTP downstream	4-stage-WWTP upstream	4-stage-WWTP downstream
Oxygen in mg/l	8,68	8,7	8,84	8,88	7,82	7,91	7,92	7,93
Oxygen in %	99,4	98,5	99,9	100,5	101	101,2	101,3	101,5
Water temperature	18,8	18,4	18,7	18,7	25,6	25	25,0	25,1
Conductivity in µS/cm	361	347	370	389	360	359	390	399
pH	8,29	8,18	8,26	8,19	8,3	8,05	8,16	8,13
NH4-N in mg/l	0,09	<0,04	0,15	0,25	0,1	0,27	0,37	0,13
PO4-P in mg/l	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05
Chloride in mg/l	21	21	38	41	23	25	40	41
NO2-N in mg/l	0,029	0,006	0,008	0,01	0,11	0,018	0,017	0,01
NO3-N in mg/l	1,5	1,4	0,5	1,5	1,5	1,4	0,5	1,5
Carbonate hardness in °dH	9	8	7	7	9	9	9	8
Total hardness in °dH	10	9	9	9	10	11	11	11

Table S6: Exposure of male and female gammarids separated from precopula pairs, sampled up- and downstream of the 3-stage-WWTP as well as up- and downstream of the 4-stage-WWTP. Numbers of individuals at the beginning of exposure (13.07.2020 “# alive start exposure”) and at the end of exposure (20.07.2023 “# alive end”, “# dead end”).

Temperature	Sampling site	Sex	# alive start exposure	# alive end	# dead end
15 °C	3-stage-WWTP _{upstream}	Female	50	41	9
15 °C	3-stage-WWTP _{upstream}	Male	50	46	4
15 °C	3-stage-WWTP _{downstream}	Female	50	44	6
15 °C	3-stage-WWTP _{downstream}	Male	50	47	3
15 °C	4-stage-WWTP _{upstream}	Female	50	36	14
15 °C	4-stage-WWTP _{upstream}	Male	50	42	8
15 °C	4-stage-WWTP _{downstream}	Female	50	31	19
15 °C	4-stage-WWTP _{downstream}	Male	50	45	5
25 °C	3-stage-WWTP _{upstream}	Female	50	4	46
25 °C	3-stage-WWTP _{upstream}	Male	50	11	39
25 °C	3-stage-WWTP _{downstream}	Female	50	6	44
25 °C	3-stage-WWTP _{downstream}	Male	50	9	41
25 °C	4-stage-WWTP _{upstream}	Female	50	0	50
25 °C	4-stage-WWTP _{upstream}	Male	50	0	50
25 °C	4-stage-WWTP _{downstream}	Female	50	0	50
25 °C	4-stage-WWTP _{downstream}	Male	50	0	50

Table S7: Exposure of gammarids in the non-reproductive phase, sampled up- and downstream of the 3-stage-WWTP as well as up- and downstream of the 4-stage-WWTP. Numbers of individuals at the beginning of exposure (04.08.2020 “# alive start exposure”) and at the end of exposure (11.08.2023 “# alive end”, “# dead end”).

Temperature	Sampling site	# alive start exposure	# alive end	# dead end
18 °C	4-stage-WWTP _{upstream}	50	47	3
18 °C	4-stage-WWTP _{downstream}	52	51	1
18 °C	3-stage-WWTP _{upstream}	50	48	2
18 °C	3-stage-WWTP _{downstream}	50	50	0
25 °C	4-stage-WWTP _{upstream}	50	23	27
25 °C	4-stage-WWTP _{downstream}	50	35	15
25 °C	3-stage-WWTP _{upstream}	50	19	31
25 °C	3-stage-WWTP _{downstream}	50	23	27