



Article

The pathogen *Aeromonas salmonicida achromogenes* induces fast immune and microbiota modifications in rainbow trout

Baptiste Redivo ¹, Nicolas Derôme ², Patrick Kestemont ¹ and Valérie Cornet ^{1,*}¹ Research Unit in Environmental and Evolutionary Biology (URBE), Institute of Life, Earth & Environment (ILEE), University of Namur, 5000 Namur, Belgium² Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, Québec, QC G1V 0A6, Canada

* Correspondence: valerie.cornet@unamur.be

Supplementary materials

Supplementary Materials S1. Mean Ct values of 3 technical replicates obtained by qPCR of several genes expressed in trout head kidney (K) at 0, 6, 24 and 72 hpi.

| | | Mean Ct values of 3 technical replicates | | | | | | | | | |
|------|----------------|--|---------|-------|-------|-------|-------|-------|-------|-------|-------|
| Time | Identification | 18S | B-actin | mpo | lyso | C3-4 | tgfb | il10 | vapa | il1b | tnfa |
| 0h | K_0h_1 | 12,50 | 21,34 | 18,56 | 21,98 | 31,00 | 22,30 | 29,50 | 34,39 | 29,68 | 30,52 |
| 0h | K_0h_2 | 12,55 | 21,58 | 19,48 | 22,08 | 29,94 | 22,39 | 31,00 | 33,97 | 29,69 | 31,11 |
| 0h | K_0h_3 | 12,25 | 21,73 | 19,42 | 22,93 | 30,92 | 22,63 | 28,23 | 34,92 | 30,67 | 30,31 |
| 0h | K_0h_4 | 11,85 | 20,90 | 19,27 | 22,39 | 30,80 | 22,57 | 30,57 | 34,70 | 30,40 | 30,59 |
| 6h | K_6h_1 | 12,81 | 21,77 | 19,74 | 22,65 | 30,34 | 22,35 | 29,28 | 28,92 | 29,35 | 30,36 |
| 6h | K_6h_2 | 12,32 | 21,49 | 20,12 | 22,23 | 30,68 | 21,95 | 29,51 | 28,68 | 29,69 | 31,06 |
| 6h | K_6h_3 | 12,69 | 21,88 | 20,08 | 22,96 | 29,69 | 22,42 | 29,01 | 28,84 | 29,24 | 29,94 |
| 6h | K_6h_4 | 12,85 | 22,10 | 20,22 | 23,04 | 28,78 | 22,51 | 31,58 | 29,47 | 29,44 | 32,41 |
| 6h | K_6h_5 | 12,47 | 21,67 | 20,24 | 22,27 | 31,91 | 22,27 | 29,80 | 29,45 | 28,97 | 31,12 |
| 6h | K_6h_6 | 12,89 | 22,29 | 20,43 | 23,05 | 31,45 | 22,50 | 29,21 | 30,07 | 30,17 | 32,05 |
| 24h | K_24h_1 | 13,00 | 23,27 | 21,66 | 23,64 | 33,49 | 23,79 | 31,43 | 29,55 | 30,41 | 33,08 |
| 24h | K_24h_2 | 12,54 | 21,58 | 19,05 | 21,08 | 29,87 | 21,68 | 30,57 | 23,05 | 28,14 | 30,15 |
| 24h | K_24h_3 | 12,57 | 21,91 | 19,74 | 22,06 | 30,04 | 22,70 | 28,96 | 29,93 | 29,12 | 31,84 |
| 24h | K_24h_4 | 12,73 | 21,87 | 19,47 | 21,45 | 30,24 | 22,65 | 31,20 | 30,35 | 29,29 | 30,50 |
| 24h | K_24h_5 | 12,63 | 21,79 | 19,76 | 21,24 | 29,28 | 22,40 | 32,35 | 27,30 | 30,31 | 32,35 |
| 24h | K_24h_6 | 12,53 | 21,91 | 19,85 | 22,90 | 30,26 | 22,65 | 30,57 | 29,56 | 29,86 | 30,50 |
| 72h | K_72h_1 | 12,55 | 22,19 | 19,93 | 22,21 | 30,07 | 23,16 | 29,38 | 29,66 | 28,32 | 30,42 |
| 72h | K_72h_2 | 12,78 | 22,60 | 19,85 | 22,87 | 31,81 | 22,88 | 28,32 | 28,78 | 29,85 | 31,35 |
| 72h | K_72h_3 | 12,73 | 22,33 | 19,68 | 23,66 | 30,96 | 22,67 | 31,20 | 30,32 | 30,44 | 31,33 |
| 72h | K_72h_4 | 12,64 | 22,22 | 19,67 | 22,52 | 31,12 | 22,82 | 30,31 | 30,05 | 28,99 | 30,93 |
| 72h | K_72h_5 | 12,29 | 21,14 | 18,65 | 20,59 | 30,07 | 22,46 | 28,71 | 30,27 | 25,81 | 30,48 |
| 72h | K_72h_6 | 12,30 | 22,22 | 19,92 | 21,77 | 30,63 | 22,50 | 31,56 | 31,22 | 29,59 | 29,51 |

Supplementary Materials S2. Pairwise comparisons of the different microbiotas (beta-diversity) using Permanova with Benjamini-Hochberg correction. These Permanova were obtained using Bray-Curtis distance (dissimilarity matrix).

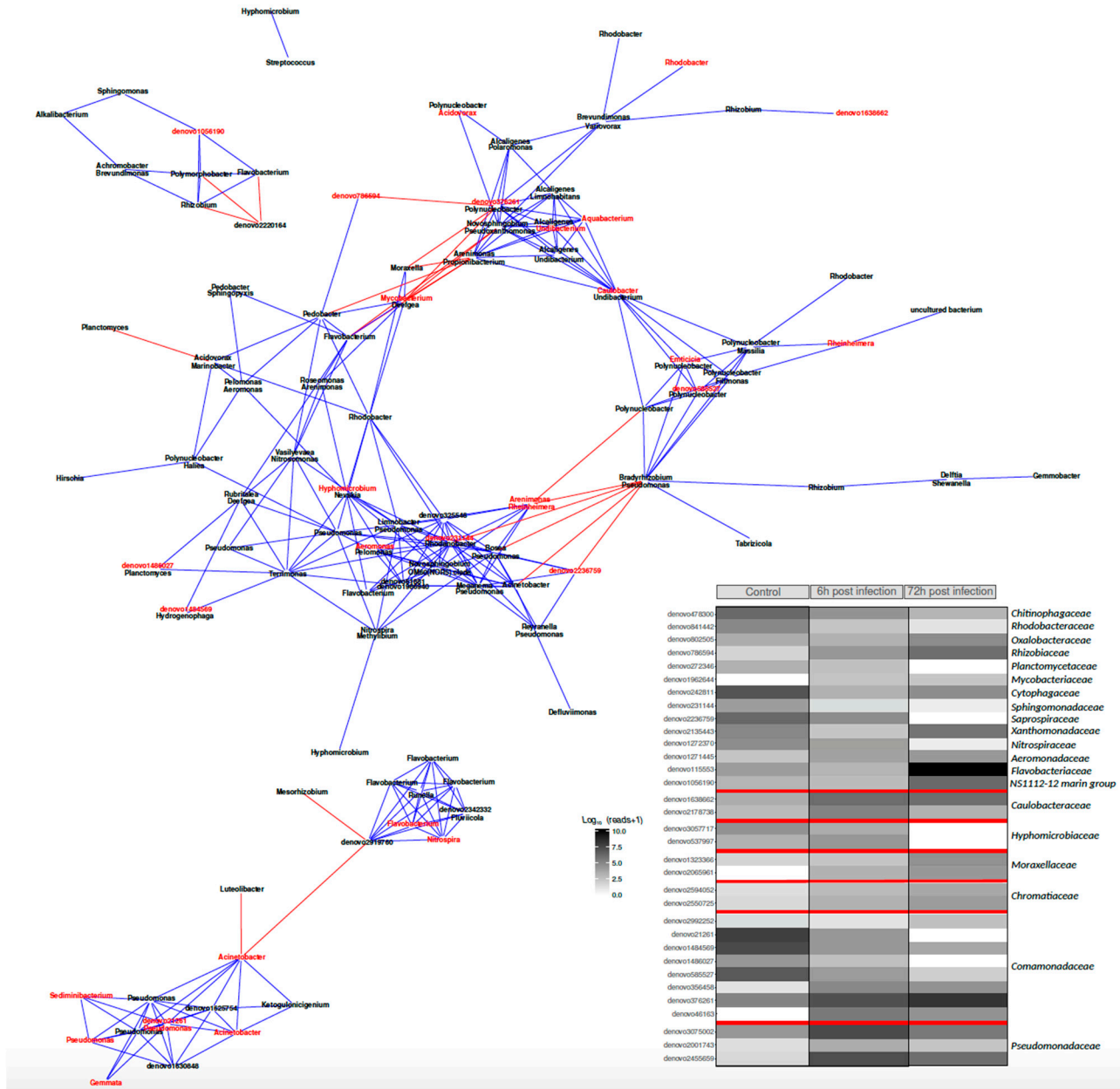
| pairs | F.Model | R2 | p.value | p.adjusted | sig |
|------------------------|-----------|-----------|---------|------------|-----|
| Fin.0h vs Skin.0h | 10.358585 | 0.3652716 | 0.00004 | 0.0005000 | *** |
| Fin.0h vs Fin.6h | 1.566467 | 0.0843708 | 0.14677 | 0.1467700 | |
| Fin.0h vs Fin.72h | 4.100124 | 0.2265246 | 0.00250 | 0.0057692 | ** |
| Fin.0h vs Gills.0h | 7.449027 | 0.3046758 | 0.00008 | 0.0005400 | *** |
| Fin.0h vs Water.0h | 8.130346 | 0.4249973 | 0.00354 | 0.0072000 | ** |
| Skin.0h vs Skin.6h | 1.974775 | 0.1098637 | 0.14220 | 0.1467700 | |
| Skin.0h vs Gills.0h | 20.917466 | 0.5516578 | 0.00002 | 0.0005000 | *** |
| Skin.0h vs Water.0h | 39.690005 | 0.7829947 | 0.00360 | 0.0072000 | ** |
| Skin.0h vs Skin.72h | 9.069959 | 0.3931502 | 0.00057 | 0.0019000 | ** |
| Water.6h vs Fin.6h | 13.189114 | 0.5687632 | 0.00498 | 0.0078632 | ** |
| Water.6h vs Skin.6h | 46.588421 | 0.8380958 | 0.00603 | 0.0086286 | ** |
| Water.6h vs Water.0h | 45.152813 | 0.9186211 | 0.10000 | 0.1071429 | |
| Water.6h vs Gills.6h | 10.944665 | 0.5487515 | 0.00604 | 0.0086286 | ** |
| Water.6h vs Water.72h | 6.172906 | 0.6067987 | 0.10000 | 0.1071429 | |
| Fin.6h vs Fin.72h | 4.317148 | 0.2492990 | 0.00641 | 0.0087409 | ** |
| Fin.6h vs Skin.6h | 19.874060 | 0.5698809 | 0.00005 | 0.0005000 | *** |
| Fin.6h vs Gills.6h | 11.178522 | 0.4270112 | 0.00009 | 0.0005400 | *** |
| Fin.72h vs Gills.72h | 8.159242 | 0.4493162 | 0.00224 | 0.0057250 | ** |
| Fin.72h vs Water.72h | 15.809192 | 0.6931062 | 0.01162 | 0.0141240 | * |
| Fin.72h vs Skin.72h | 10.850875 | 0.5204038 | 0.00226 | 0.0057250 | ** |
| Gills.72h vs Gills.0h | 10.869866 | 0.4553803 | 0.00023 | 0.0009857 | *** |
| Gills.72h vs Gills.6h | 14.281280 | 0.5434012 | 0.00031 | 0.0011625 | ** |
| Gills.72h vs Water.72h | 31.632594 | 0.8188058 | 0.01177 | 0.0141240 | * |
| Gills.72h vs Skin.72h | 24.979485 | 0.7141181 | 0.00229 | 0.0057250 | ** |
| Skin.6h vs Gills.6h | 16.101487 | 0.5349067 | 0.00015 | 0.0007500 | *** |
| Skin.6h vs Skin.72h | 8.346270 | 0.4102113 | 0.00446 | 0.0074333 | ** |
| Gills.0h vs Water.0h | 6.921160 | 0.4090240 | 0.00439 | 0.0074333 | ** |
| Gills.0h vs Gills.6h | 5.347262 | 0.2628001 | 0.00423 | 0.0074333 | ** |
| Water.0h vs Water.72h | 49.779669 | 0.9256225 | 0.10000 | 0.1071429 | |
| Water.72h vs Skin.72h | 65.716471 | 0.9037357 | 0.01170 | 0.0141240 | * |

Supplementary Materials S3: Pairwise comparisons of the different microbiotas (beta-diversity) using Permanova with Benjamini-Hochberg correction. These Permanova were obtained using Weighted Unifrac distance.

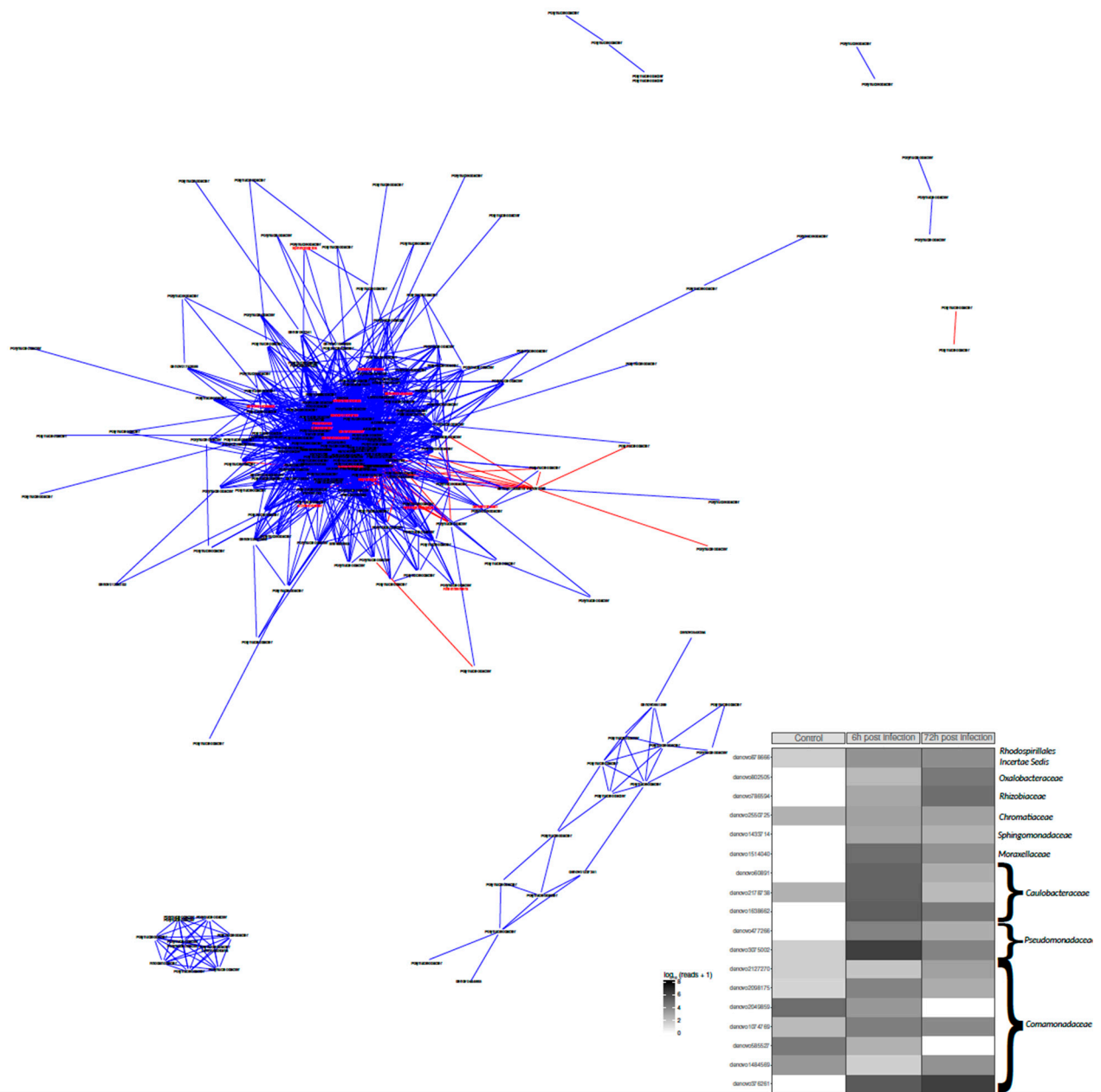
| pairs | F.Model | R2 | p.value | p.adjusted | sig |
|------------------------|------------|-----------|---------|------------|-----|
| Fin_0h vs Skin_0h | 9.9305163 | 0.3555436 | 0.0001 | 0.0007500 | *** |
| Fin_0h vs Fin_6h | 0.6263704 | 0.0355360 | 0.6491 | 0.6491000 | |
| Fin_0h vs Fin_72h | 6.2363156 | 0.3081745 | 0.0024 | 0.0051429 | ** |
| Fin_0h vs Gills_0h | 13.4897227 | 0.4424351 | 0.0003 | 0.0012857 | ** |
| Fin_0h vs Water_0h | 11.1362262 | 0.5030770 | 0.0037 | 0.0065294 | ** |
| Skin_0h vs Skin_6h | 5.2289337 | 0.2463117 | 0.0333 | 0.0384231 | * |
| Skin_0h vs Gills_0h | 27.2732772 | 0.6160212 | 0.0002 | 0.0012000 | ** |
| Skin_0h vs Water_0h | 56.8449344 | 0.8378656 | 0.0034 | 0.0063750 | ** |
| Skin_0h vs Skin_72h | 12.8292470 | 0.4781814 | 0.0001 | 0.0007500 | *** |
| Water_6h vs Fin_6h | 15.2702898 | 0.6042784 | 0.0045 | 0.0075000 | ** |
| Water_6h vs Skin_6h | 70.2698943 | 0.8864638 | 0.0057 | 0.0090000 | ** |
| Water_6h vs Water_0h | 89.0882374 | 0.9570300 | 0.1000 | 0.1034483 | |
| Water_6h vs Gills_6h | 11.7017937 | 0.5652551 | 0.0078 | 0.0117000 | * |
| Water_6h vs Water_72h | 24.5996082 | 0.8601379 | 0.1000 | 0.1034483 | |
| Fin_6h vs Fin_72h | 7.9795159 | 0.3803480 | 0.0023 | 0.0051429 | ** |
| Fin_6h vs Skin_6h | 26.5388160 | 0.6388920 | 0.0001 | 0.0007500 | *** |
| Fin_6h vs Gills_6h | 15.5322927 | 0.5087169 | 0.0004 | 0.0015000 | ** |
| Fin_72h vs Gills_72h | 11.9047400 | 0.5434778 | 0.0022 | 0.0051429 | ** |
| Fin_72h vs Water_72h | 11.6622707 | 0.6249117 | 0.0135 | 0.0168750 | * |
| Fin_72h vs Skin_72h | 9.8588025 | 0.4964450 | 0.0026 | 0.0052000 | ** |
| Gills_72h vs Gills_0h | 9.4213331 | 0.4201950 | 0.0003 | 0.0012857 | ** |
| Gills_72h vs Gills_6h | 21.5927105 | 0.6427796 | 0.0008 | 0.0026667 | ** |
| Gills_72h vs Water_72h | 33.9004332 | 0.8288527 | 0.0099 | 0.0135000 | * |
| Gills_72h vs Skin_72h | 42.9811153 | 0.8112535 | 0.0016 | 0.0043636 | ** |
| Skin_6h vs Gills_6h | 15.0460699 | 0.5180071 | 0.0001 | 0.0007500 | *** |
| Skin_6h vs Skin_72h | 14.1412549 | 0.5409555 | 0.0009 | 0.0027000 | ** |
| Gills_0h vs Water_0h | 6.7629673 | 0.4034469 | 0.0086 | 0.0122857 | * |
| Gills_0h vs Gills_6h | 4.9807517 | 0.2492775 | 0.0165 | 0.0198000 | * |
| Water_0h vs Water_72h | 77.1953903 | 0.9507361 | 0.1000 | 0.1034483 | |
| Water_72h vs Skin_72h | 65.5781663 | 0.9035523 | 0.0129 | 0.0168261 | * |

Supplementary materials S4: Pairwise comparisons of the different microbiotas (beta-diversity) using Permanova with Benjamini-Hochberg correction. These Permanova were obtained using Unweighted Unifrac distance.

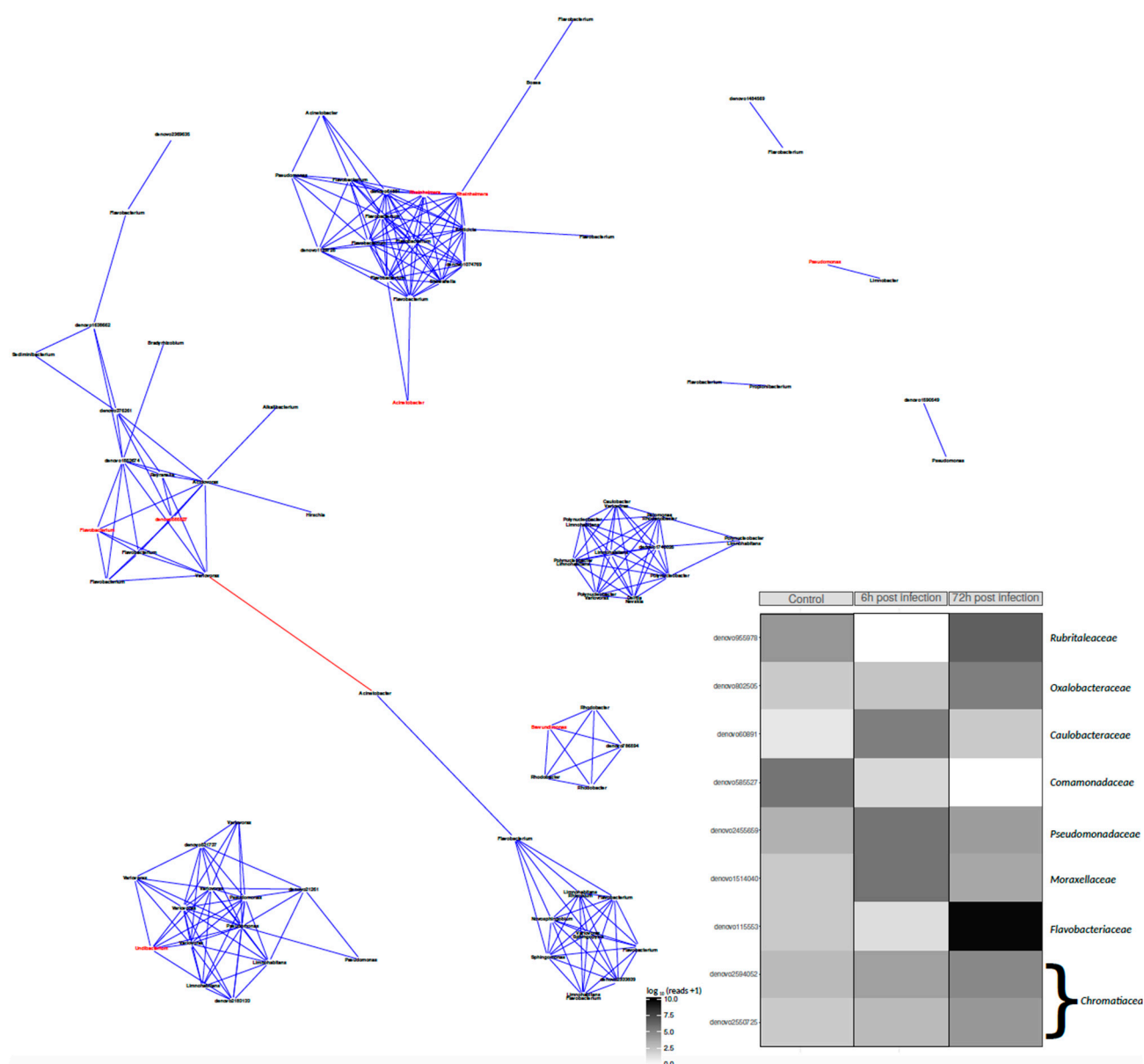
| pairs | F.Model | R2 | p.value | p.adjusted | sig |
|------------------------|----------|-----------|---------|------------|-----|
| Fin_0h vs Skin_0h | 2.038372 | 0.1017234 | 0.0409 | 0.0454444 | * |
| Fin_0h vs Fin_6h | 3.806033 | 0.1829293 | 0.0012 | 0.0032727 | ** |
| Fin_0h vs Fin_72h | 3.887796 | 0.2173435 | 0.0003 | 0.0012857 | ** |
| Fin_0h vs Gills_0h | 3.314471 | 0.1631581 | 0.0050 | 0.0093750 | ** |
| Fin_0h vs Water_0h | 2.729833 | 0.1988249 | 0.0277 | 0.0319615 | * |
| Skin_0h vs Skin_6h | 3.945899 | 0.1978301 | 0.0003 | 0.0012857 | ** |
| Skin_0h vs Gills_0h | 6.202457 | 0.2673190 | 0.0001 | 0.0010000 | *** |
| Skin_0h vs Water_0h | 5.176748 | 0.3200117 | 0.0039 | 0.0082000 | ** |
| Skin_0h vs Skin_72h | 5.468346 | 0.2808840 | 0.0003 | 0.0012857 | ** |
| Water_6h vs Fin_6h | 4.434158 | 0.3071989 | 0.0107 | 0.0152857 | * |
| Water_6h vs Skin_6h | 6.044596 | 0.4017786 | 0.0061 | 0.0096316 | ** |
| Water_6h vs Water_0h | 16.16550 | 0.8016415 | 0.1000 | 0.1034483 | |
| Water_6h vs Gills_6h | 4.479516 | 0.3323203 | 0.0057 | 0.0095000 | ** |
| Water_6h vs Water_72h | 1.164209 | 0.2254380 | 0.5000 | 0.5000000 | |
| Fin_6h vs Fin_72h | 3.531183 | 0.2136074 | 0.0026 | 0.0060000 | ** |
| Fin_6h vs Skin_6h | 3.271963 | 0.1790701 | 0.0056 | 0.0095000 | ** |
| Fin_6h vs Gills_6h | 4.647942 | 0.2365613 | 0.0001 | 0.0010000 | *** |
| Fin_72h vs Gills_72h | 2.975787 | 0.2293338 | 0.0041 | 0.0082000 | ** |
| Fin_72h vs Water_72h | 9.155362 | 0.5667073 | 0.0134 | 0.0160800 | * |
| Fin_72h vs Skin_72h | 2.727101 | 0.2142751 | 0.0128 | 0.0160000 | * |
| Gills_72h vs Gills_0h | 9.570329 | 0.4240226 | 0.0001 | 0.0010000 | *** |
| Gills_72h vs Gills_6h | 8.477811 | 0.4139999 | 0.0005 | 0.0018750 | ** |
| Gills_72h vs Water_72h | 14.90379 | 0.6804206 | 0.0122 | 0.0159130 | * |
| Gills_72h vs Skin_72h | 5.480372 | 0.3540207 | 0.0021 | 0.0052500 | ** |
| Skin_6h vs Gills_6h | 6.725415 | 0.3245009 | 0.0003 | 0.0012857 | ** |
| Skin_6h vs Skin_72h | 4.919325 | 0.2907518 | 0.0007 | 0.0021000 | ** |
| Gills_0h vs Water_0h | 2.935376 | 0.2269262 | 0.0077 | 0.0115500 | * |
| Gills_0h vs Gills_6h | 5.307294 | 0.2613491 | 0.0006 | 0.0020000 | ** |
| Water_0h vs Water_72h | 16.21523 | 0.8021294 | 0.1000 | 0.1034483 | |
| Water_72h vs Skin_72h | 13.28458 | 0.6549104 | 0.0117 | 0.0159130 | * |



Supplementary Materials S5. Gills correlated network and negative binomial GLM associated on the bottom right. For the network graph, red OTU represent the OTU that are significantly impacted by the bath infection. Blue links represent the positive correlations between OTU's. ($r > 0.8$) while red ones represent the negative correlations ($r < -0.8$).



Supplementary Materials S6. Skin correlated network and negative binomial GLM associated on the bottom right. For the network graph, red OTU represent the OTU that are significantly impacted by the bath infection. Blue links represent the positive correlations between OTU's ($r > 0.8$) while red ones represent the negative correlations ($r < -0.8$).



Supplementary Materials S7. Fin correlated network and negative binomial GLM associated on the bottom right. For the network graph, red OTU represent the OTU that are significantly impacted by the bath infection. Blue links represent the positive correlations between OTU's ($r > 0.8$) while red ones represent the negative correlations ($r < -0.8$).

Supplementary Materials S8. Pairwise comparisons of the different functional profiles of microbiotas through Tax4Fun using Permanova with Benjamini-Hochberg corrections. These Permanova were obtained using Bray-Curtis distance.

| pairs | F.Model | R2 | p.value | p.adjusted | sig |
|--------------------------------|------------|------------|---------|------------|-----|
| Fin.Control vs Fin.6h | 0.6387335 | 0.03621198 | 0.51143 | 0.51143 | |
| Fin.Control vs Fin.72h | 0.74680378 | 0.05064174 | 0.46902 | 0.4851931 | |
| Fin.6h vs Fin.72h | 0.87285857 | 0.06291844 | 0.3676 | 0.39385714 | |
| Skin.Control vs Skin.6h | 4.82989152 | 0.2318731 | 0.04332 | 0.06188571 | |
| Skin.Control vs Skin.72h | 1.60867633 | 0.10306296 | 0.219 | 0.25269231 | |
| Skin.6h vs Skin.72h | 13.0080842 | 0.52015517 | 0.00586 | 0.1276 | |
| Gills.Control vs Gills.6h | 6.86167476 | 0.31386775 | 0.01351 | 0.020265 | * |
| Gills.Control vs Gills.72h | 11.3843176 | 0.46687046 | 0.00045 | 0.00192857 | ** |
| Gills.6h vs Gills.72h | 22.4477997 | 0.65164684 | 0.00042 | 0.00192857 | ** |
| Water.Control vs Water.6h | 64.9053121 | 0.94194932 | 0.1 | 0.12 | |
| Water.Control vs Water.72h | 52.1536002 | 0.92876681 | 0.1 | 0.12 | |
| Water.6h vs Water.72h | 34.8999517 | 0.89717211 | 0.1 | 0.12 | |
| Fin.Control vs Gills.Control | 10.5575615 | 0.38310942 | 0.00019 | 0.001425 | ** |
| Fin.Control vs Skin.Control | 21.4651701 | 0.54390162 | 0.00008 | 0.0012 | ** |
| Fin.Control vs Water.Control | 12.5389474 | 0.53268938 | 0.00334 | 0.00949091 | ** |
| Skin.Control vs Water.Control | 192.387503 | 0.94591605 | 0.00348 | 0.00949091 | ** |
| Gills.Control vs Skin.Control | 14.3194159 | 0.45720571 | 0.0004 | 0.00192857 | ** |
| Gills.Control vs Water.Control | 7.12997162 | 0.41622787 | 0.00468 | 0.0117 | * |
| Fin.6h vs Gills.6h | 20.3739965 | 0.5759597 | 0.00014 | 0.0014 | ** |
| Fin.6h vs Skin.6h | 40.4192883 | 0.72933611 | 0.00003 | 0.0009 | *** |
| Fin.6h vs Water.6h | 3.83279519 | 0.27708031 | 0.04986 | 0.06799091 | |
| Gills.6h vs Skin.6h | 4.96227501 | 0.26169197 | 0.00747 | 0.01400625 | * |
| Gills.6h vs Water.6h | 12.8436689 | 0.5879813 | 0.00598 | 0.01276 | * |
| Skin.6h vs Water.6h | 157.893818 | 0.9460735 | 0.00638 | 0.01276 | * |
| Fin.72h vs Gills.72h | 1.2447364 | 0.11069503 | 0.28324 | 0.31471111 | |
| Fin.72h vs Skin.72h | 52.8780717 | 0.84096204 | 0.00211 | 0.0072 | ** |
| Fin.72h vs Water.72h | 13.9288544 | 0.66553353 | 0.01265 | 0.01997368 | * |
| Gills.72h vs Skin.72h | 28.6355463 | 0.741171 | 0.00216 | 0.0072 | ** |
| Gills.72h vs Water.72h | 7.86194649 | 0.52899844 | 0.01191 | 0.01985 | * |
| Skin.72h vs Water.72h | 158.637587 | 0.95773906 | 0.01166 | 0.01985 | * |