

Molecular response of larval Pacific cod to warming, acidification

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Seattle - Ingrid Spies



UW - Steven Roberts

Pacific cod workshop

Seattle, WA

November 28-29, 2023

Larval Pacific cod are sensitive to warming - Why?

Larval Pacific cod are sensitive to warming - Why?

What are mechanisms for mortality in warm temperatures?

Larval Pacific cod are sensitive to warming - WHY?

Gene expression analysis

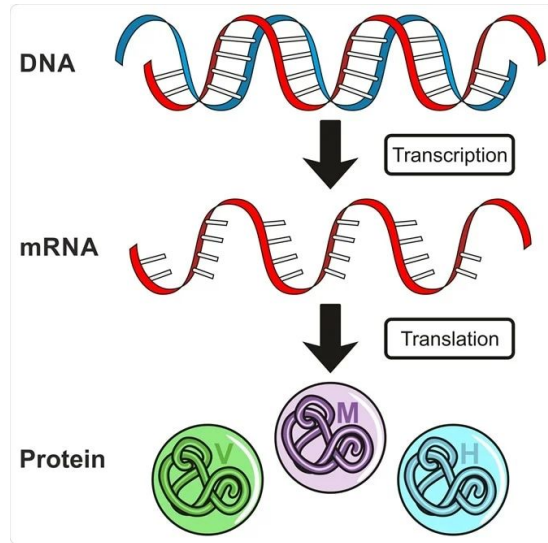


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Larval Pacific cod are sensitive to warming - WHY?

Gene expression analysis

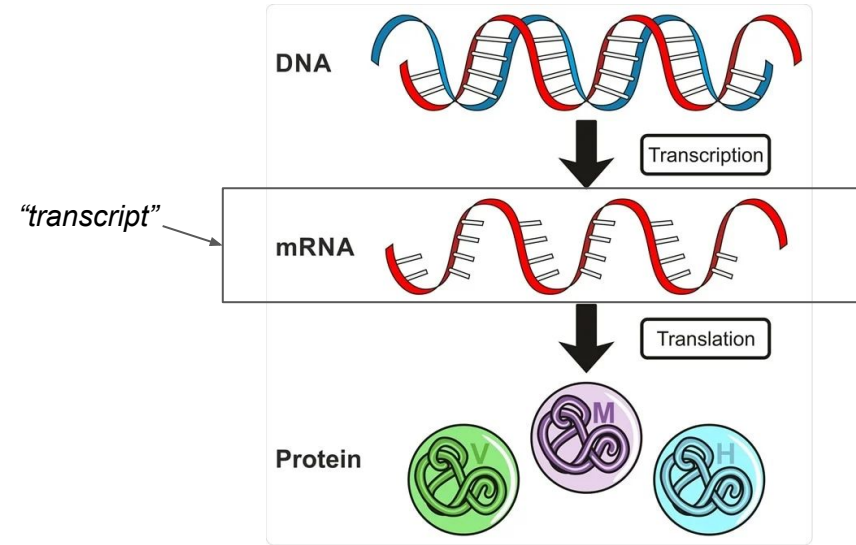


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Larval Pacific cod are sensitive to warming - WHY?

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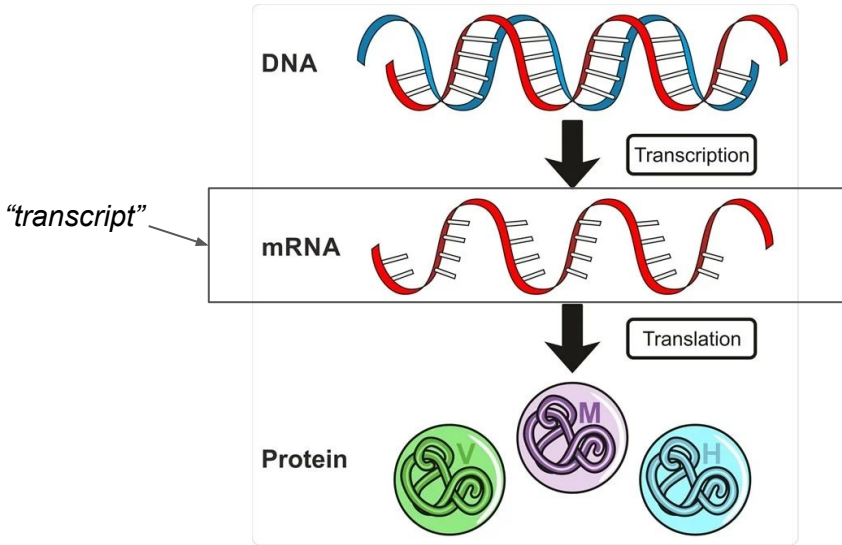
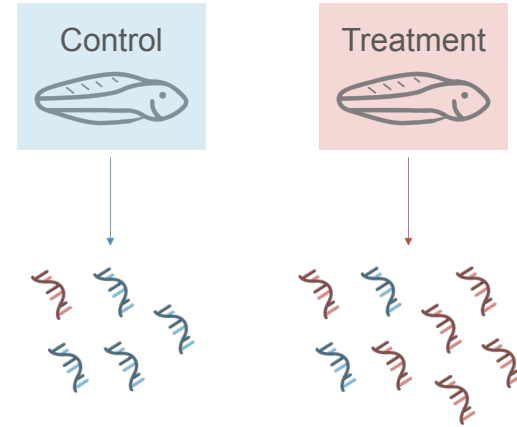


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Gene expression analysis

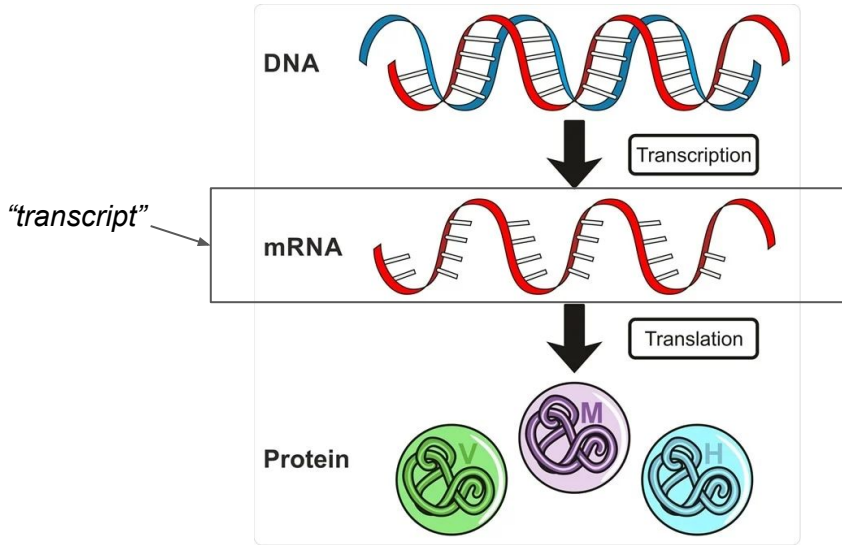
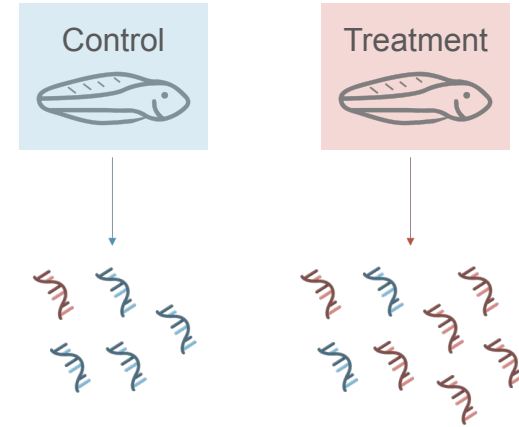


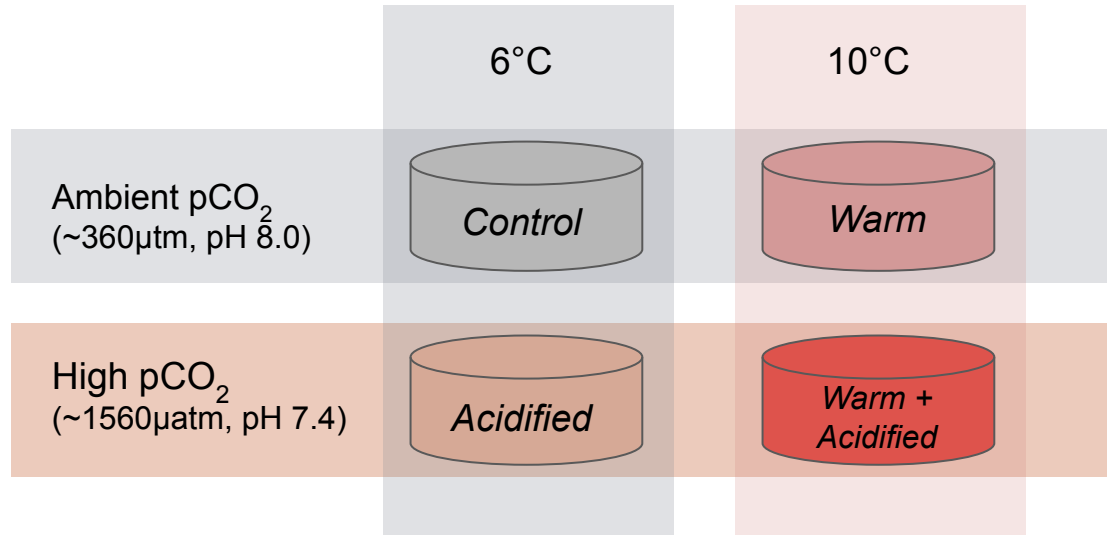
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- Measure gene activity, "expression"
- Compare expression among treatments
- Snapshot of energy allocation
- Simultaneously measure many physiological processes

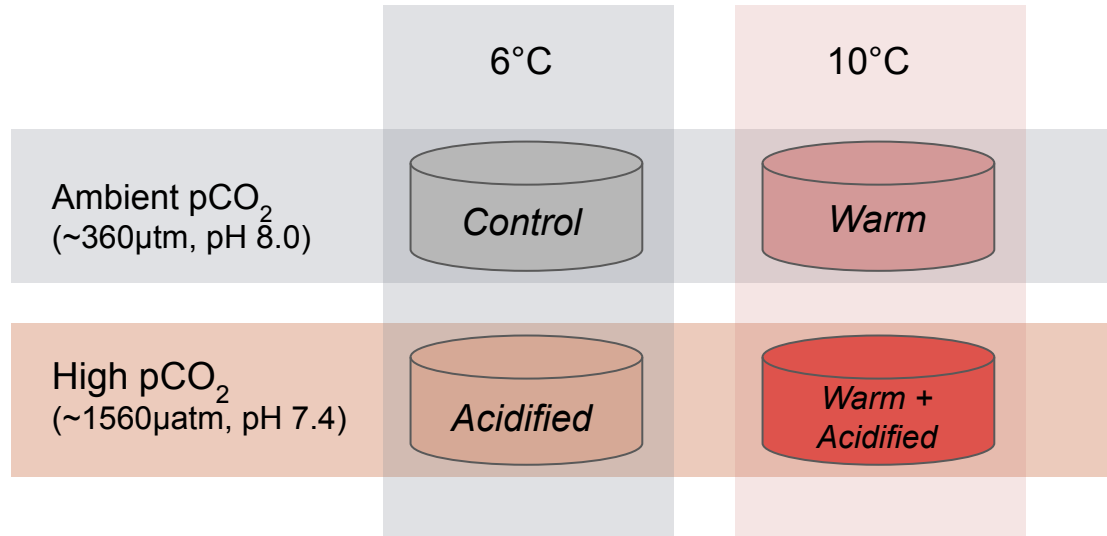
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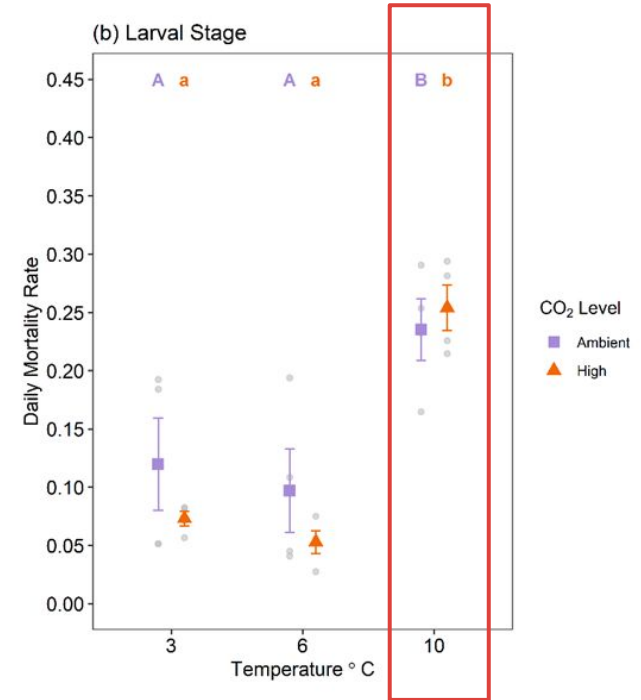
~9 week exposure, fertilized eggs → post-flexion stage (~6-8mm)

Larval Pacific cod are sensitive to warming - WHY?



~9 week exposure, fertilized eggs → post-flexion stage (~6-8mm)

High larval mortality in
Warm, Warm+Acidified



Slesinger et al. *in revision*

WHY does warming affect larval cod?

- What are mechanisms for mortality in warm temperatures?

Different if also exposed to acidification?

- What are mechanisms for thinner fish in acidification?

WHY does warming/acidification affect larval cod?

Gene expression analysis

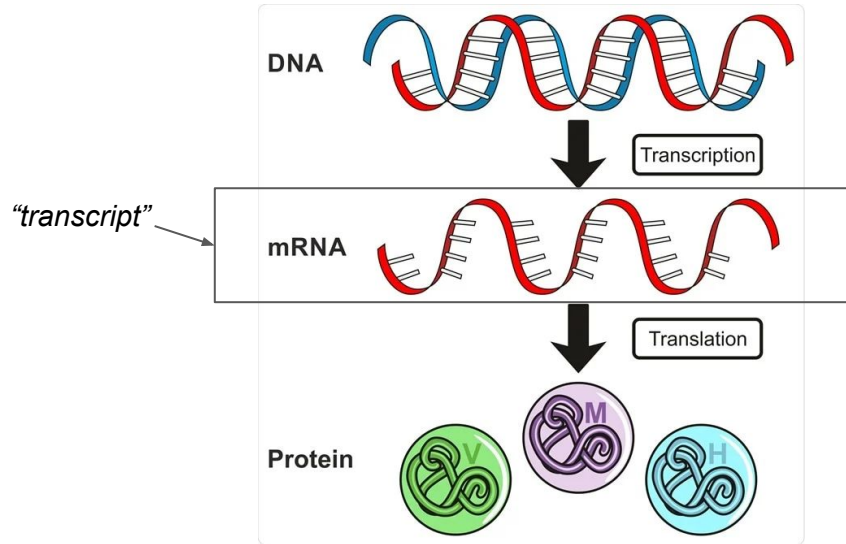


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- RNA-Seq, measures *all* transcripts
- 73 larval cod individuals, whole-body tissue (n=11-14 / treatment)
- Aligned to Atlantic cod genome
- Analyzed the same 21,076 genes in all individuals
- Identified differentially expressed genes and their functions

What does my gene expression data look like?

Gene ID in genome

Samples

gene_gadmor	PCG001	PCG004	PCG011	PCG015	PCG017	PCG020	PCG029	PCG035	
ND1	8276	8202	8327	7750	5508	4351	5673	6385	
ND2	39142	43297	57032	31681	33489	30275	38921	36460	
COX1	555463	631876	917827	551062	378628	403956	438595	455514	
COX2	315625	309958	493176	294979	189346	229132	227614	219894	
ATP6	82892	105415	89100	73253	77770	58061	86703	78859	
COX3	165275	189321	235193	135559	112870	81377	135462	126615	
ND3	10710	13595	10404	7013	10244	2567	9792	8231	
ND4L	19364	31196	42855	16261	20566	17877	21021	16269	
ND4	37081	42648	67118	30275	29274	37939	35681	34155	
ND5	16299	21803	28231	Gene counts			19960	19262	18187
ND6	4102	3787	3187				1774	3772	3391
CYT8	176843	211635	277391				114784	137070	144369
LOC115539476	260	228	333	219	270	266	312	266	
LOC115539709	890	849	919	777	561	461	945	1000	
LOC115538781	586	596	576	630	417	450	704	730	
abhd14a	1197	1381	1629	1031	774	757	1158	1108	
acy1	1626	1244	1670	1245	1224	1386	1566	1642	
LOC115537228	2106	2402	2555	2008	1845	2826	2127	2235	
LOC115537019	659	544	911	564	505	611	696	444	
LOC115538651	727	674	630	564	479	599	554	523	
LOC115538267	57	192	81	153	104	137	110	104	
kbtbd12	1003	743	646	766	715	875	301	216	

What does my gene expression data look like?

Gene ID in genome

Samples

functional info for each gene

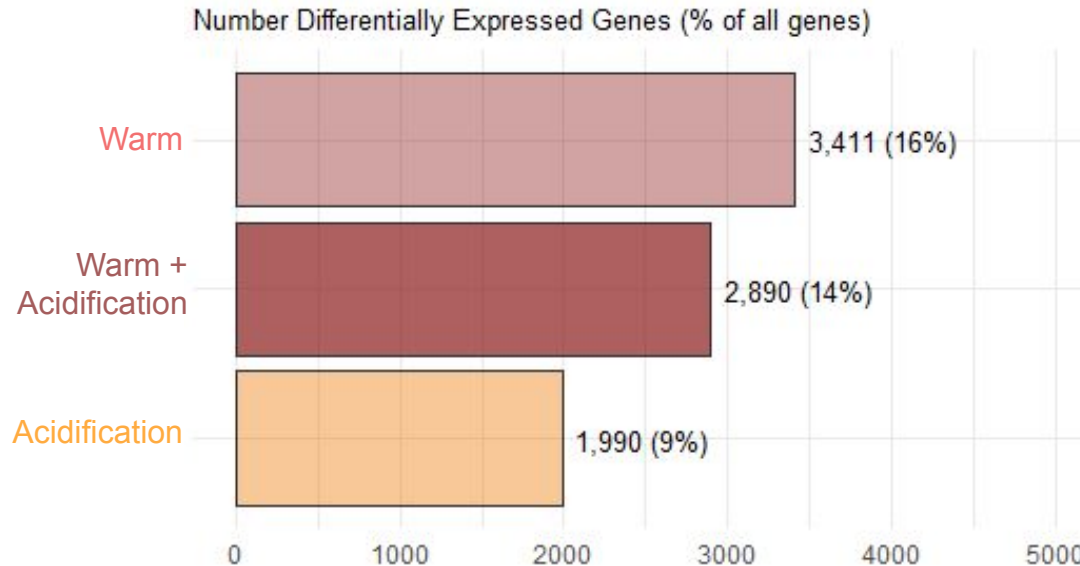
gene_gadmor	PCG001	PCG004	PCG011	PCG015	PCG017	PCG020	PCG029	PCG035	spid	species	evalue	protein_names	
ND1	8276	8202	8327	7750	5508	4351	5673	6385	P55779	GADMO	0.00e+00	NADH-ubiquinone oxidoreductase chain 1 (EC 7.1.1.2) (NA	
ND2	39142	43297	57032	31681	33489	30275	38921	36460	P55780	GADMO	2.40e-158	NADH-ubiquinone oxidoreductase chain 2 (EC 7.1.1.2) (NA	
COX1	555463	631876	917827	551062	378628	403956	438595	455514	Q36775	GADMO	0.00e+00	Cytochrome c oxidase subunit 1 (EC 7.1.1.9) (Cytochrome c	
COX2	315625	309958	493176	294979	189346	229132	227614	219894	Q37741	GADMO	1.12e-132	Cytochrome c oxidase subunit 2 (EC 7.1.1.9) (Cytochrome c	
ATP6	82892	105415	89100	73253	77770	58061	86703	78859	P55778	GADMO	2.60e-104	ATP synthase subunit a (F-ATPase protein 6)	
COX3	165275	189321	235193	135559	112870	81377	135462	126615	P55777	GADMO	5.84e-152	Cytochrome c oxidase subunit 3 (EC 7.1.1.9) (Cytochrome c	
ND3	10710	13595	10404	7013	10244	2567	9792	8231	P15957	GADMO	1.48e-32	NADH-ubiquinone oxidoreductase chain 3 (EC 7.1.1.2) (NA	
ND4L	19364	31196	42855	16261	20566	17877	21021	16269	P23633	GADMO	1.08e-41	NADH-ubiquinone oxidoreductase chain 4L (EC 7.1.1.2) (N	
ND4	37081	42648	67118	30275	29274	37939	35681	34155	P55781	GADMO	0.00e+00	NADH-ubiquinone oxidoreductase chain 4 (EC 7.1.1.2) (NA	
ND5	16299	21803	28231	Gene counts			19960	19262	18187	P55782	GADMO	0.00e+00	NADH-ubiquinone oxidoreductase chain 5 (EC 7.1.1.2) (NA
ND6	4102	3787	318				1774	3772	3391	P55783	GADMO	1.86e-42	NADH-ubiquinone oxidoreductase chain 6 (EC 7.1.1.2) (NA
CYT8	176843	211635	277391				114784	137070	144369	Q37080	GADMO	0.00e+00	Cytochrome b (Complex III subunit 3) (Complex III subunit
LOC115539476	260	228	333	219	270	266	312	266	Q99MK9	MOUSE	1.66e-27	Ras association domain-containing protein 1 (Protein 123F	
LOC115539709	890	849	919	777	561	461	945	1000	Q9WVF8	MOUSE	5.41e-27	Tumor suppressor candidate 2 (Fusion 1 protein) (Fus-1 pr	
LOC115538781	586	596	576	630	417	450	704	730	Q12891	HUMAN	3.51e-104	Hyaluronidase-2 (Hyal-2) (EC 3.2.1.35) (Hyaluronoglucosar	
abhd14a	1197	1381	1629	1031	774	757	1158	1108	Q1LV46	DANRE	4.34e-34	Protein ABHD14A (EC 3.-.-.-) (Alpha/beta hydrolase domai	
acy1	1626	1244	1670	1245	1224	1386	1566	1642	Q6PTT0	RAT	2.57e-16	Aminoacylase-1B (ACY-1B) (EC 3.5.1.14) (ACY 1B) (N-acyl-L-	
LOC115537228	2106	2402	2555	2008	1845	2826	2127	2235	Q6PHS9	MOUSE	1.07e-34	Voltage-dependent calcium channel subunit alpha-2/delta	
LOC115537019	659	544	911	564	505	611	696	444	Q90339	CYPCA	1.94e-172	Myosin heavy chain, fast skeletal muscle	
LOC115538651	727	674	630	564	479	599	554	523	Q9NXG6	HUMAN	5.61e-20	Transmembrane prolyl 4-hydroxylase (P4H-TM) (EC 1.14.11	
LOC115538267	57	192	81	153	104	137	110	104	Q8CIW6	MOUSE	3.91e-23	Solute carrier family 26 member 6 (Anion exchange transp	
kbtbd12	1003	743	646	766	715	875	301	216	Q3ZB90	DANRE	0.00e+00	Kelch repeat and BTB domain-containing protein 12 (Kelch	

Which genes are affected by treatments compared to control?

Differential gene expression analysis

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Differential gene expression analysis



LARGE effect of temperature on expression

Biggest effect: Warming

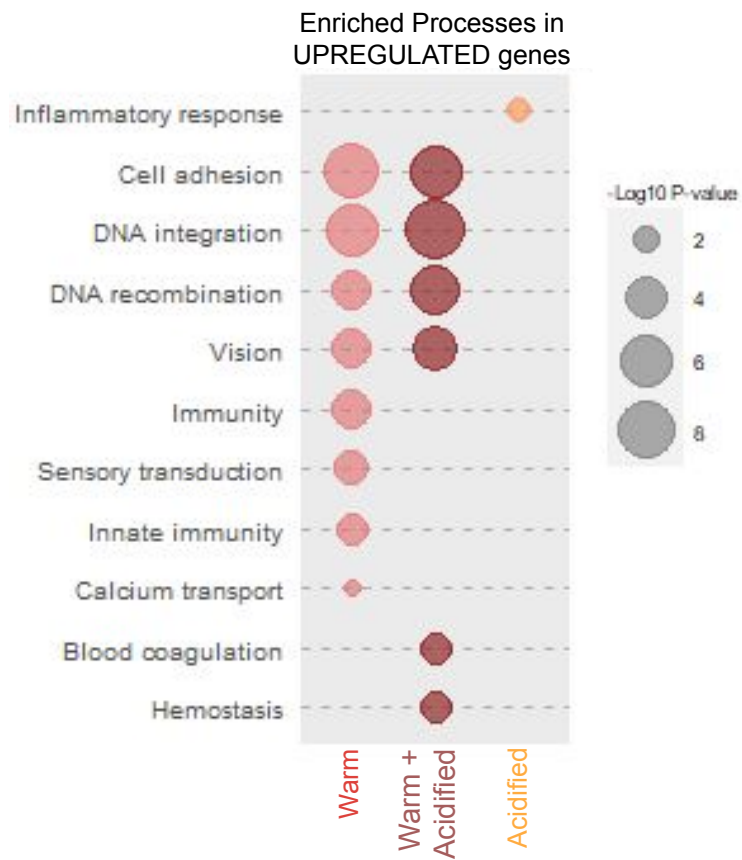
Acidification did not amplify effect of warming

Which biological processes are affected by treatments?

Enrichment Analysis

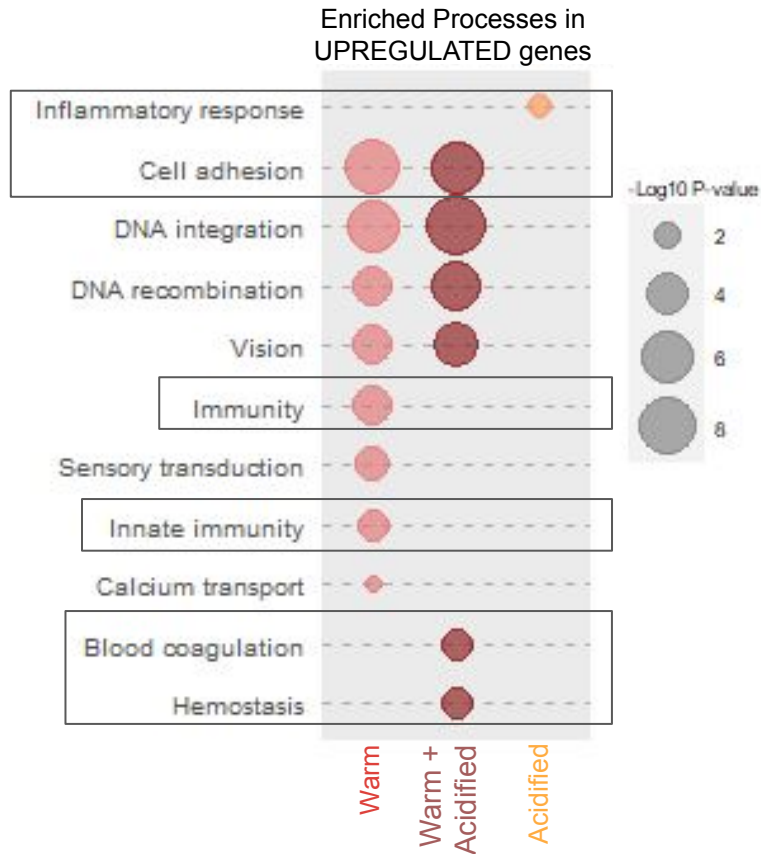
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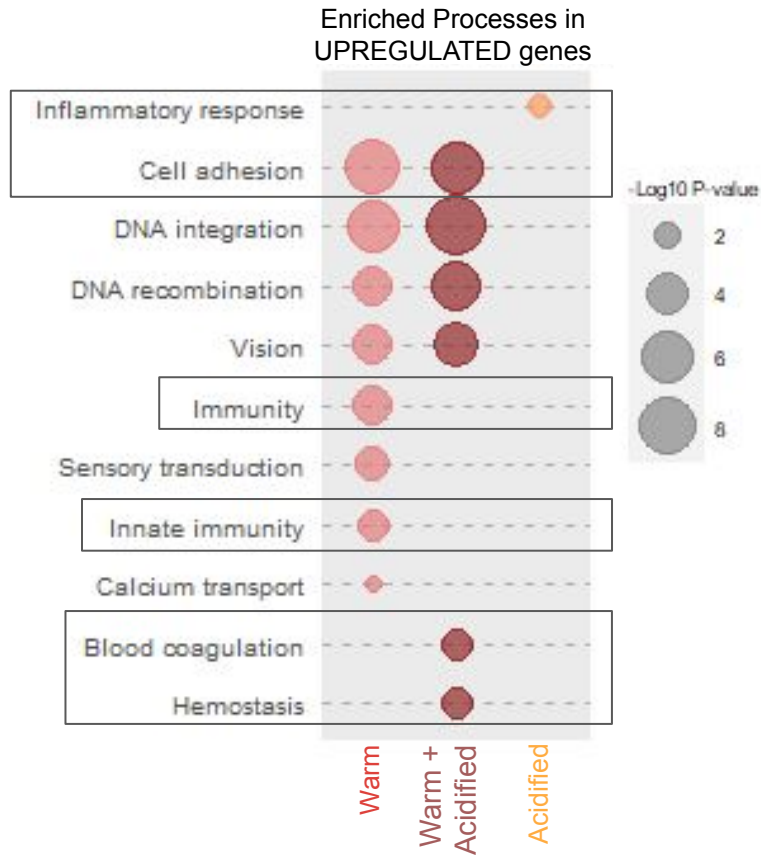
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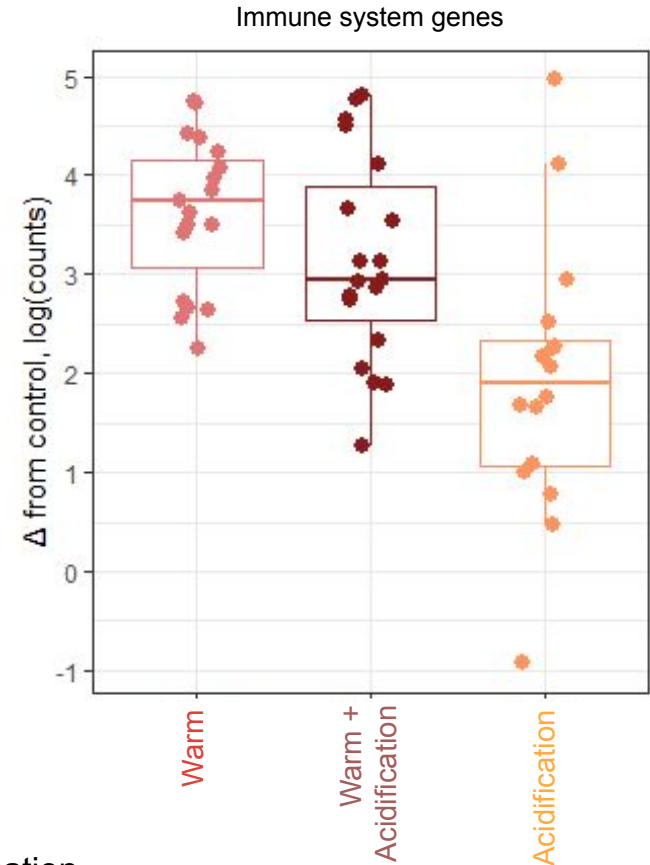


Warming increased immune system activity, weaker effect in acidification

Which biological processes are affected by treatments?

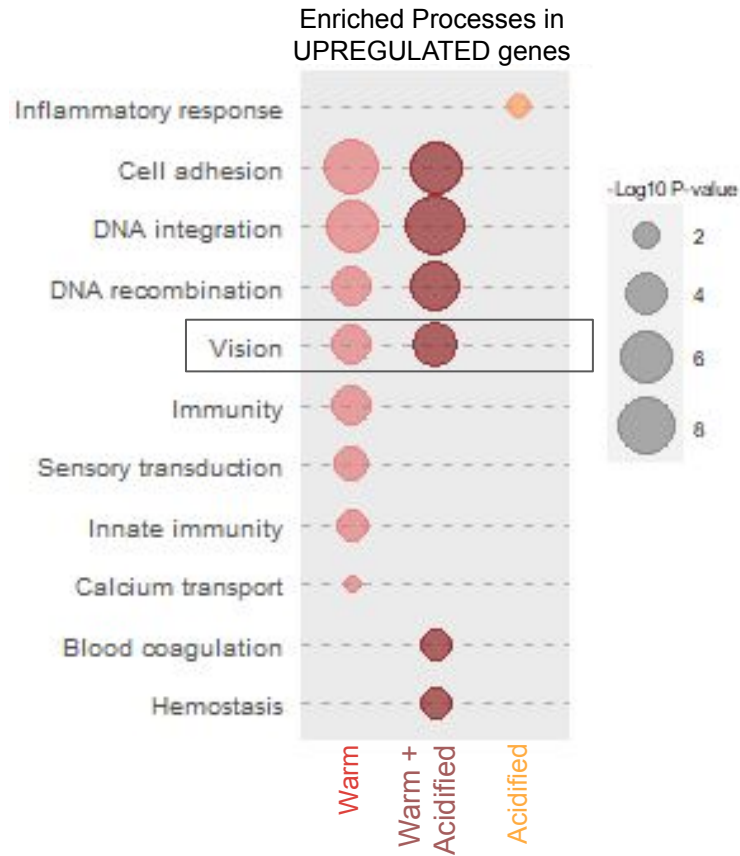


Enrichment Analysis

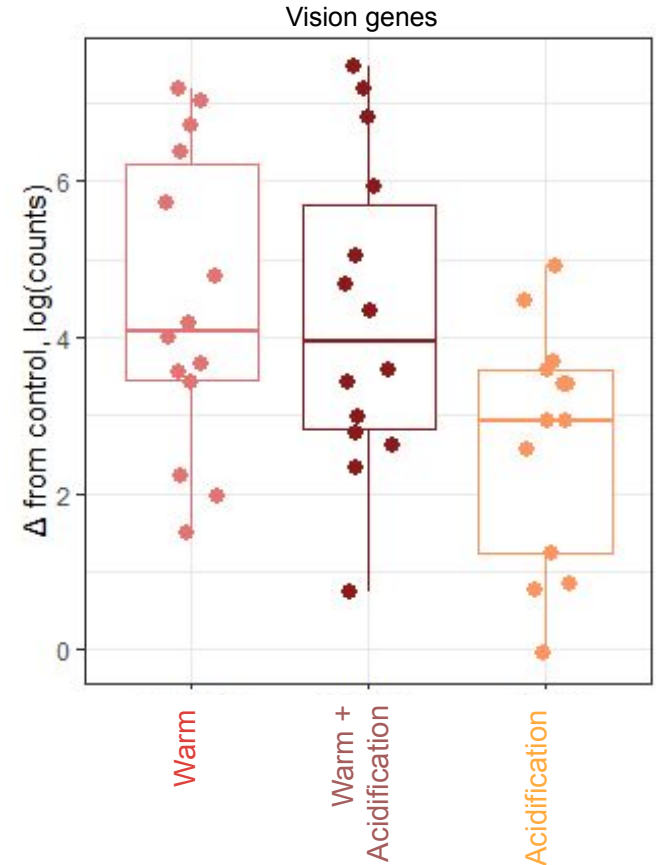


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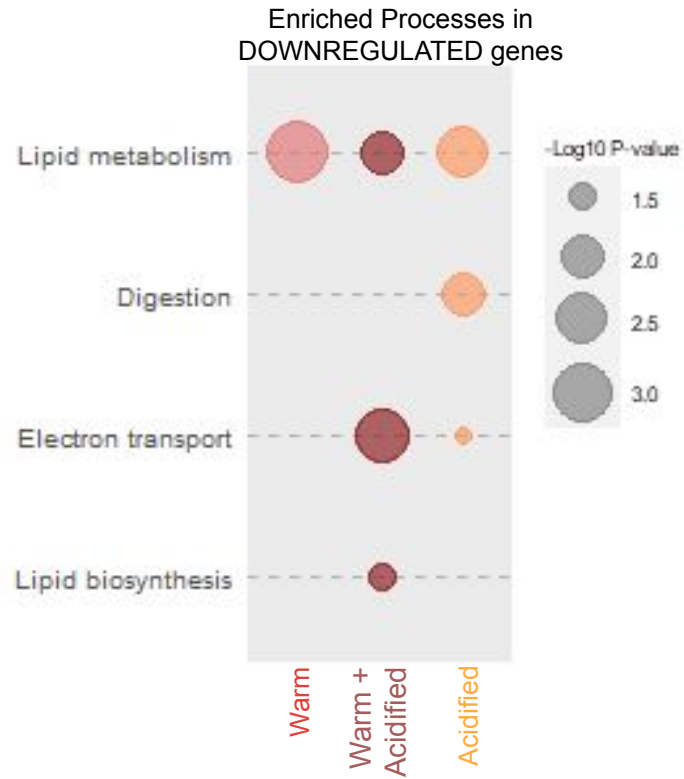
Enrichment Analysis



Warming increased activity of vision-related genes

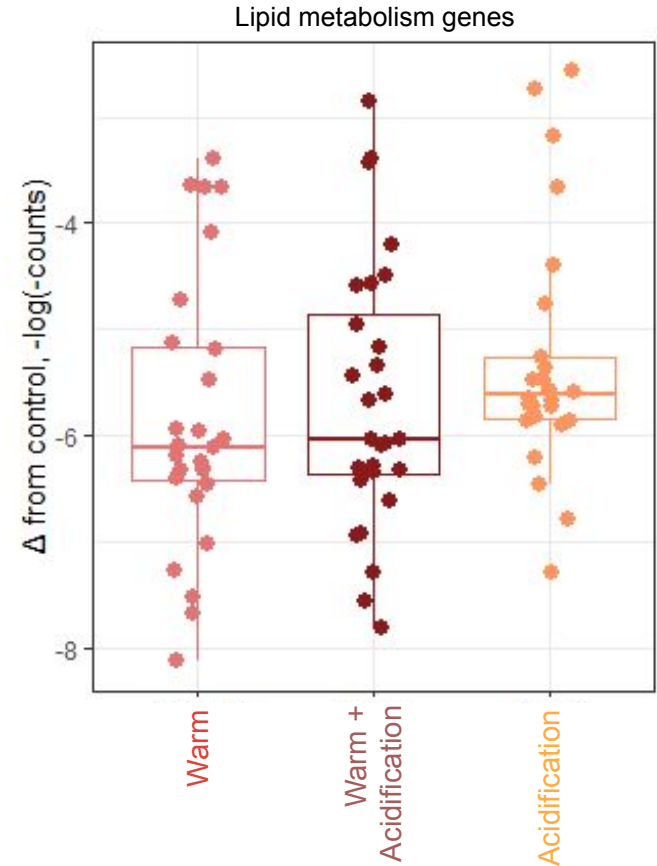
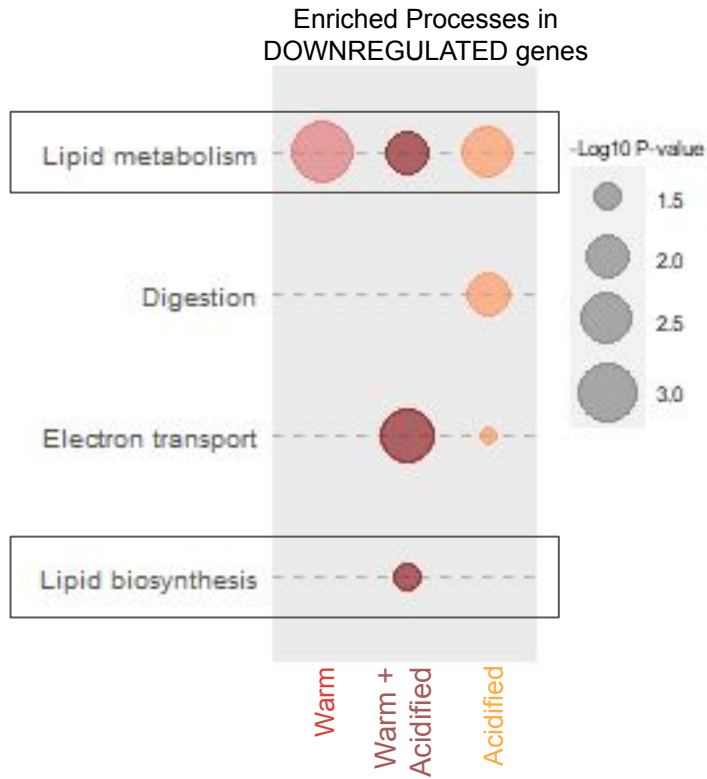
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Enrichment Analysis



Which biological processes are affected by treatments?

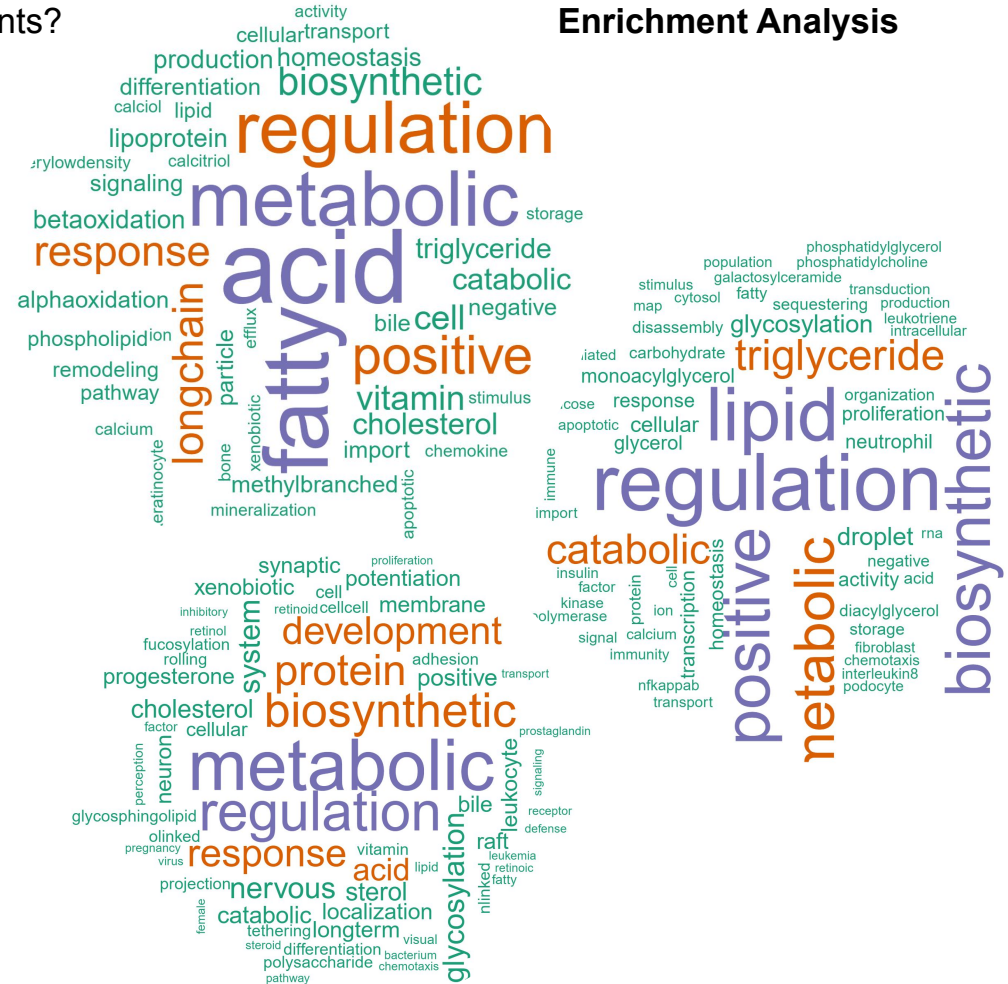
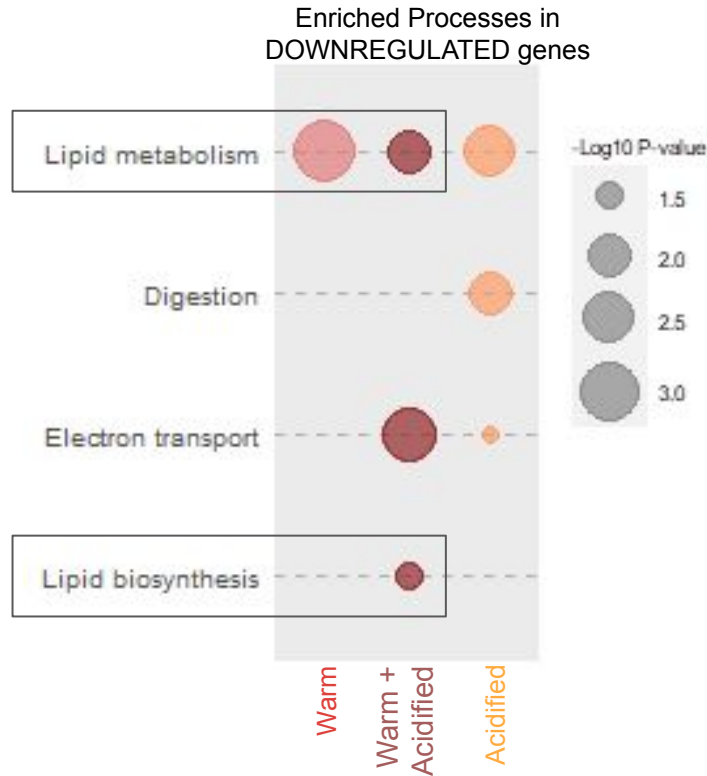
Enrichment Analysis



Lipid metabolism is sensitive to both warming & OA

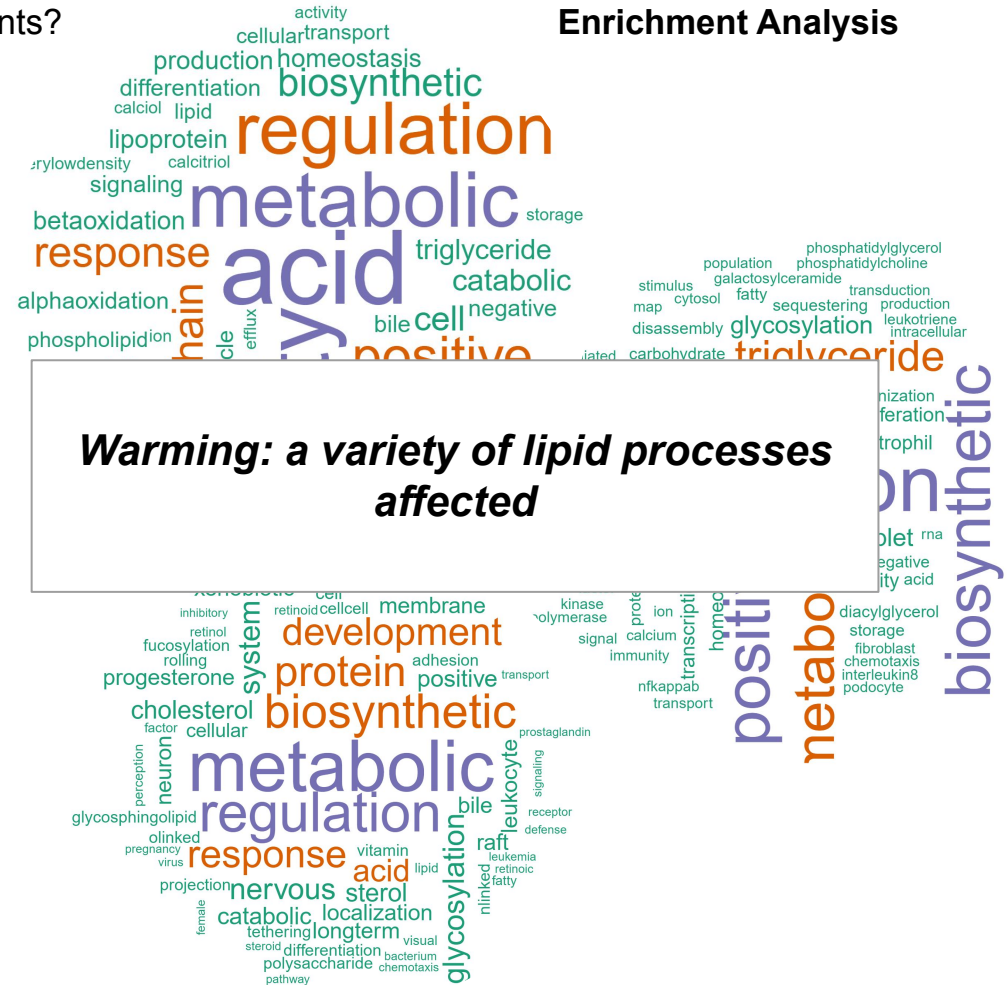
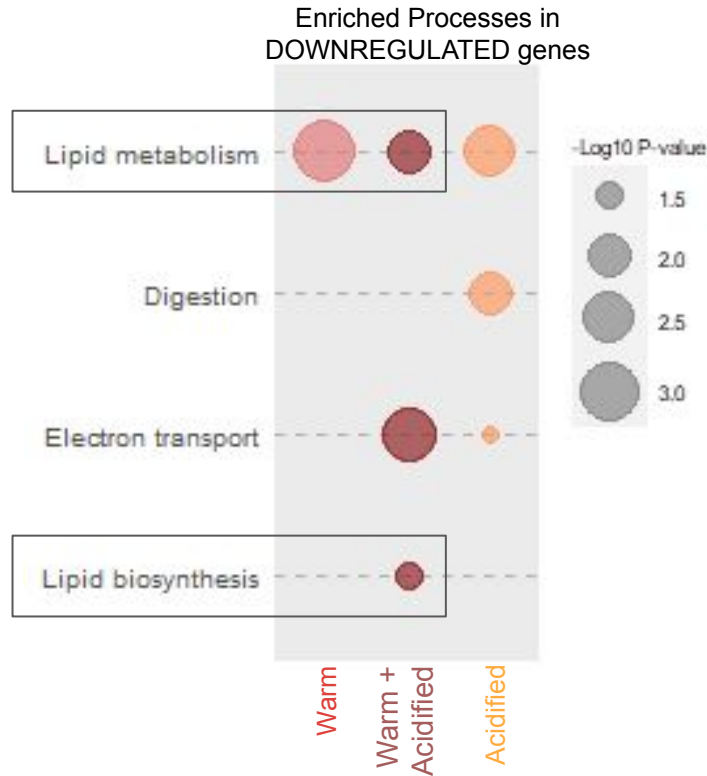
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Enrichment Analysis



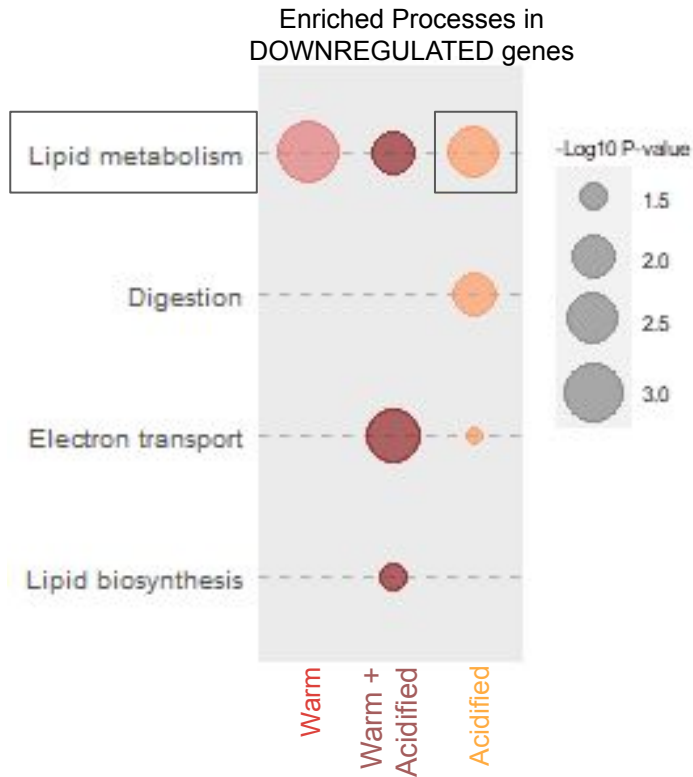
Which biological processes are affected by treatments?

Enrichment Analysis



Which biological processes are affected by treatments?

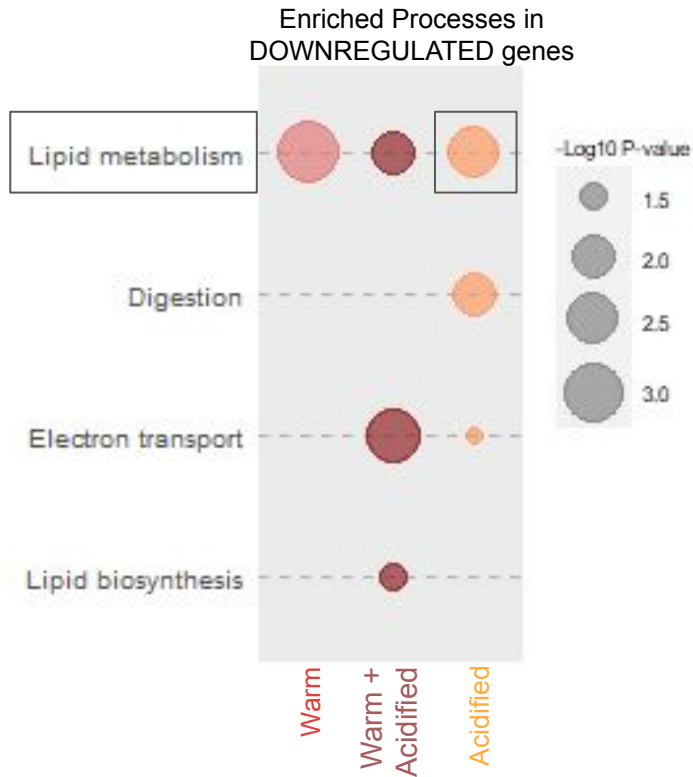
Enrichment Analysis



positive
biosynthetic
sphingomyelin
catabolic
lipid
negative
intestinal
absorption
metabolic
regulation

Which biological processes are affected by treatments?

Enrichment Analysis

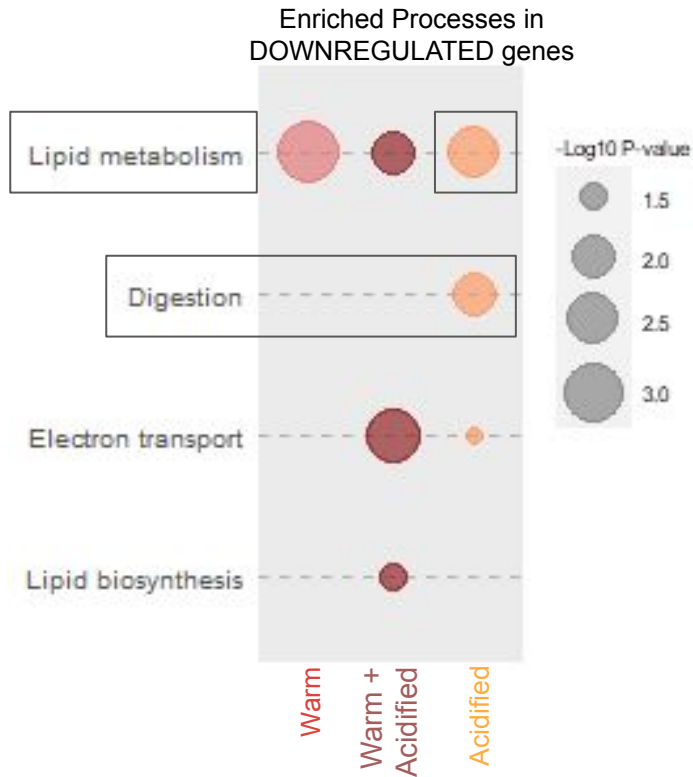


Acidification: decreased lipid digestion/absorption in intestine

positive
biosynthetic
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Which biological processes are affected by treatments?

Enrichment Analysis



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Acidification: decreased lipid digestion/absorption in intestine

Summary of results

Warming may cause high mortality by combination of heightened immune activity and lipid dysregulation.

- Immune activity a temperature-dependent response, or reflective of infection?
- Lipid metabolism sensitive to both warming and acidification:
 - Warming = broad changes to variety of lipid metabolism processes
 - Acidification = decreased lipid digestion in intestine
- Visual activity sensitive to warming, possibly to enable foraging?
- Combined warming + acidification = many similar impacts as warming alone, but more muted.

Next steps - Juvenile study in more extreme temperatures (including cold exposure)

Questions?