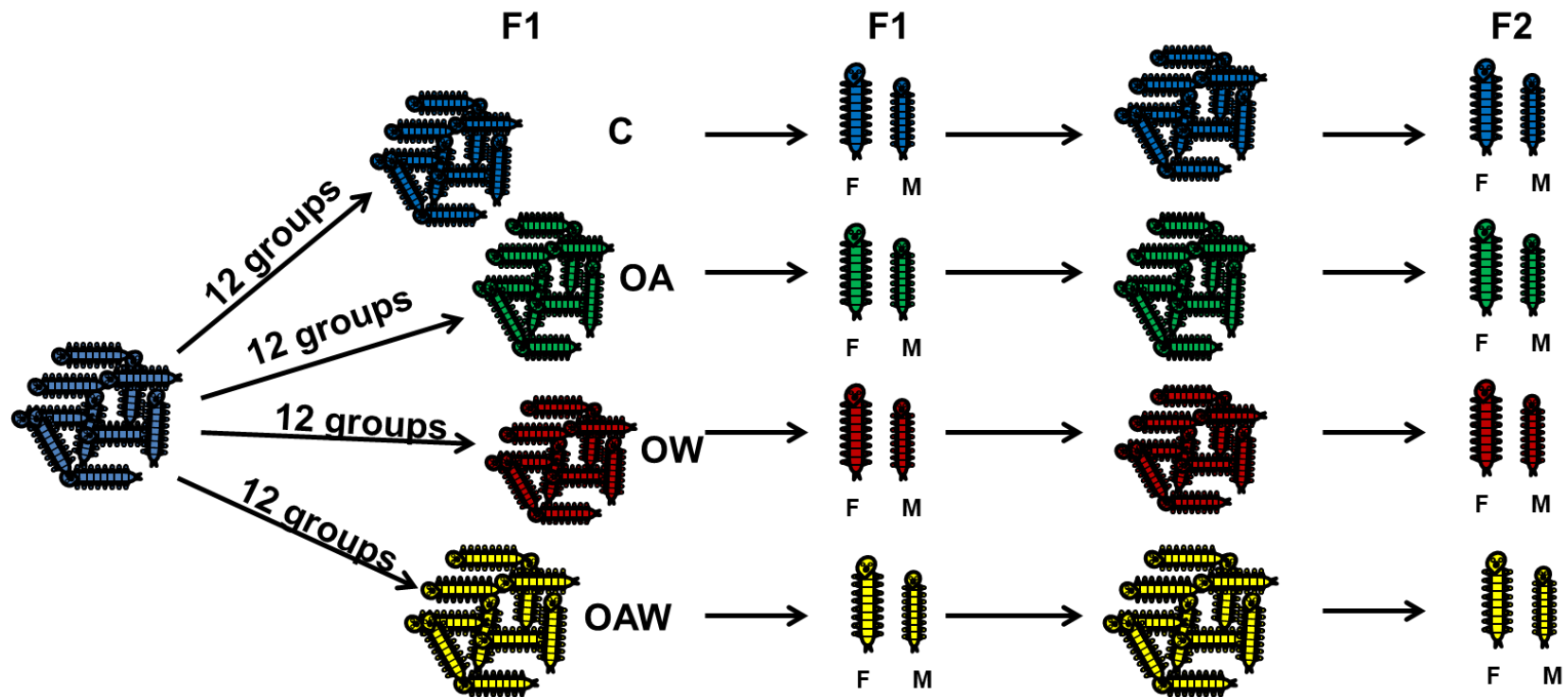


1 **Within- and trans-generational responses to combined global changes are highly divergent in two congeneric species of marine annelids**

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3 plate

4 **Appendix S1: Experimental design. Appendix S1: Experimental design.** Individuals of *Ophryotrocha robusta* and *Ophryotrocha japonica* were exposed
5 for two generations (F1-F2) to control (C; 24 °C and pH 8.2, blue), ocean acidification (OA; 24 °C and pH 7.7, green), ocean warming (OW; 28 °C and pH
6 8.2, red) and ocean acidification and warming combined (OAW; 28 °C and pH 7.7, yellow) scenarios. For each species, pairs ($n = 12$) of females (F) and
7 males (M) were assigned to each scenario for life-history and physiological measurements.



9 **Appendix S2:** Mean \pm SE of the life-history traits measured for *Ophryotrocha japonica* and *O. robusta* under control, ocean warming and acidification
 10 scenarios, in isolation and combined. Total number of breeding pairs, % of females that produced viable offspring, % of female survival after 49 days of
 11 exposure, and mean growth rate, fecundity and egg volume are provided by scenario, species and generation.

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Scenario	Species	Generation	# Breeding pairs	% female produced viable offspring	% female survival till 49 days of exposure	Growth rate (#chaetiers/day)	Fecundity (# eggs)	Eggs volume (mm ³)
Control	<i>O. japonica</i>	F1	12	100,00	100,00	-0.0003 \pm .03	164.08 \pm 2.87	0.15 \pm .001
		F2	12	100,00	100,00	0.13 \pm .004	70.73 \pm 5.79	0.10 \pm .001
	<i>O. robusta</i>	F1	12	100,00	100,00	0.11 \pm .01	206.00 \pm 6.86	0.15 \pm .0002
		F2	11	90,91	90,91	0.17 \pm .01	98.70 \pm 14.91	0.06 \pm .003
Ocean acidification	<i>O. japonica</i>	F1	12	100,00	100,00	0.04 \pm .003	109.85 \pm 5.50	0.17 \pm .01
		F2	12	100,00	100,00	0.10 \pm .004	95.61 \pm 3.56	0.07 \pm .001
	<i>O. robusta</i>	F1	12	75,00	58,33	0.11 \pm .01	50.56 \pm 11.61	0.15 \pm .0004
		F2	7	57,14	71,43	0.16 \pm .01	284.25 \pm 13.32	0.08 \pm .004
Ocean warming	<i>O. japonica</i>	F1	12	91,67	100,00	0.08 \pm .01	111.82 \pm 1.55	0.23 \pm .001
		F2	11	100,00	90,91	0.15 \pm .01	63.27 \pm 3.79	0.05 \pm .001
	<i>O. robusta</i>	F1	12	0,00	0,00	0.07 \pm .01	X	X
		F2	0	X	X	X	X	X
Ocean acidification and warming	<i>O. japonica</i>	F1	12	83,33	100,00	0.08 \pm .01	107.10 \pm 2.11	0.22 \pm .001
		F2	12	100,00	100,00	0.07 \pm .01	50.64 \pm 0.19	0.12 \pm .001
	<i>O. robusta</i>	F1	12	0,00	0,00	-0.09 \pm .02	X	X
		F2	0	X	X	X	X	X

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19 **Appendix S3:** Results for the Chi-square test (χ^2) carried out on within- (F1) and trans-generational (F1-F2)
 20 responses (degeneration events, percentage of females producing viable offspring) of *O. robusta* and *O. labronica*
 21 under ocean acidification (OA), ocean warming (OW), in isolation and combined (OAW). Degrees of freedom (df),
 22 χ^2 value and probability level (*P*) are provided.

23

1) Within-generational comparison of *O. robusta* and *O. japonica* exposed to C, OA, OW and OAW

a) Degeneration events		b) % of female producing viable offspring	
χ^2	11.28	χ^2	121.97
df	3	df	3
<i>P</i>	0.01	<i>P</i>	> 0.001
c) % of females surviving 49 days exposure			
χ^2	10.56		
df	2		
<i>P</i>	0.005		

2) Trans-generational comparison of *O. robusta* and *O. japonica* exposed to C and OA

a) Degeneration events		b) % of female producing viable offspring	
χ^2	15.46	χ^2	7.59
df	3	df	3
<i>P</i>	0.003	<i>P</i>	0.06
c) % of females surviving 49 days exposure			
χ^2	9.58		
df	2		
<i>P</i>	0.01		

3) Trans-generational responses of *O. japonica* to C, OA, OW and OAW

a) Degeneration events		b) % females produced viable offspring	
χ^2	95.23	χ^2	1.07
df	3	df	3
<i>P</i>	> 0.001	<i>P</i>	0.78

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25 **Appendix S4:** Results of the pairwise comparisons (paired *t*-test) performed on the effect of ‘species’, ‘scenario’ and
 26 ‘generation’ and their interaction on life-history traits. Degrees of freedom (df), *t*-values (*t*), standard error (SE) and
 27 probability level (*P*) are provided. Bold numbers indicate the presence of significant differences.
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	Df	SE	<i>t</i>	<i>P</i>		df	SE	<i>t</i>	<i>P</i>
1) Within-generational comparison of <i>O. robusta</i> and <i>O. japonica</i> exposed to C, OA, OW and OAW									
a) Fecundity									
<i>O. robusta</i>					<i>O. japonica</i>				
C – OA	85	26.67	5.03	<0.0001	C – OA	85	23.47	1.87	0.25
C – OAW	85	23.74	8.05	<0.0001	C – OAW	85	25.004	1.92	0.23
C – OW	85	24.59	7.56	<0.0001	C – OW	85	24.19	1.90	0.24
OA – OAW	85	26.02	2.19	0.13	OA – OAW	85	25.30	0.16	1.00
OA – OW	85	25.85	2.002	0.20	OA – OW	85	24.58	0.08	1.00
OAW – OW	85	24.06	-0.22	01.00	OAW – OW	85	25.64	-0.08	1.00
<i>O. japonica</i> – <i>O. robusta</i>									
C	85	22.21	-1.94	0.06					
OA	85	27.72	1.70	0.09					
OW	85	25.46	3.81	0.0003					
OAW	85	25.31	3.96	0.0002					
b) Egg volume									
<i>O. robusta</i>					<i>O. japonica</i>				
C – OA	380	0.02	-0.12	0.99	C – OA	380	0.01	-2.25	0.11
C – OAW	380	0.05	2.18	0.13	C – OAW	380	0.01	-7.53	< 0.0001
C – OW	380	0.05	2.18	0.13	C – OW	380	0.01	-8.51	< 0.0001
OA – OAW	380	0.05	2.14	0.14	OA – OAW	380	0.01	-5.93	< 0.0001
OA – OW	380	0.05	2.14	0.14	OA – OW	380	0.01	-6.98	< 0.0001
OAW – OW	380	0.07	0.00	1.00	OAW – OW	380	0.01	-1.001	0.75
<i>O. japonica</i> – <i>O. robusta</i>									
C	380	0.01	0.92	0.36					
OA	380	0.02	1.23	0.22					
OW	380	0.05	3.94	0.0001					
OAW	380	0.05	3.72	0.0002					
	df	SE	<i>t</i>	<i>P</i>		df	SE	<i>t</i>	<i>P</i>

2) Trans-generational comparison of *O. robusta* and *O. japonica* exposed to C and OA

a) Fecundity

<i>F1 – F2</i>									
C	69	27.42	2.53	0.01					
OA	69	33.55	-3.68	0.001					
<i>F1</i>					<i>F2</i>				
C – OA	69	27.05	3.32	0.002	C – OA	69	32.02	-3.17	0.002

O. japonica – O. robusta

F1	69	27.19	0.27	0.79
F2	69	35.09	-2.69	0.01

O. robusta

F1 – F2	69	33.90	-2.30	0.03
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O. japonica

F1 – F2	69	27.19	0.27	0.79
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b) Egg volume

O. japonica – O. robusta

C	478	0.01	2.47	0.01
OA	478	0.01	0.18	0.86

O. robusta

C – OA	478	0.01	0.18	0.42
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O. japonica

C – OA	478	0.01	1.15	0.25
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F1 – F2

C	478	0.01	9.02	<0.0001
OA	478	0.01	7.39	<0.0001

F1

C – OA	478	0.01	-0.67	0.50
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F2

C – OA	478	0.01	0.59	0.56
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O. japonica – O. robusta

F1	478	0.01	1.26	0.21
F2	478	0.01	0.94	0.35

O. robusta

F1 – F2	478	0.01	6.28	<0.0001
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O. japonica

F1 – F2	478	0.01	11.72	<0.0001
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	df	SE	t	P		df	SE	t	P
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3) Trans-generational responses of *O. japonica* to C, OA, OW and OAW

a) Egg volume

F1 – F2

C	519	0.01	5.95	<0.001
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OA	519	0.01	12.43	<0.001
OW	519	0.01	16.83	<0.001
OAW	519	0.01	9.97	<0.001

F1

C – OA	519	0.01	-2.15	0.14
C – OAW	519	0.01	-7.51	<0.001
C – OW	519	0.01	-8.63	<0.001
OA – OAW	519	0.01	-5.73	<0.001
OA – OW	519	0.01	-6.82	<0.001
OAW – OW	519	0.01	-0.93	0.79

F2

C – OA	519	0.01	2.99	0.02
C – OAW	519	0.01	-1.61	0.37
C – OW	519	0.01	4.31	0.0001
OA – OAW	519	0.01	-4.98	<0.001
OA – OW	519	0.01	1.67	0.34
OAW – OW	519	0.01	6.23	<0.001

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33 **Appendix S5.** MANOVA results for the effect of ‘species’, ‘scenario’ and ‘generation’, and their interaction on
 34 metabolomic profiles. Degrees of freedom (df), Pillai, *F*-ratio (*F*) and probability level (*P*) are provided. Bold
 35 numbers indicate the presence of significant differences.

36

1) Within-generational comparison of *O. robusta* and *O. japonica* exposed to OW and OAW scenarios for the following metabolites: ATP, NAD, Aspartate, C18:2, C18:1, C20:1, C18:3

	df	Pillai	<i>F</i>	<i>P</i>
Species	1	0.92	20.30	< 0.001
Scenario	1	0.65	3.06	0.06
Species * Scenario	1	0.19	0.38	0.88
Residuals	15			
Total	18			

2) Trans-generational responses of *O. japonica* to C, OA, OW and OAW scenarios for the following metabolites: ATP, NAD, Aspartate, C16:3, ADP, Glutamate, AMP, Fumarate, Malate, Glucose, C18:2, C18:1, C20:1, C18:3

	df	Pillai	<i>F</i>	<i>P</i>
Scenario	1	1.52	1.76	0.02
Generation	3	0.72	3.94	0.002
Scenario*Generation	2	0.74	0.959	0.54
Residuals	35			
Total	38			

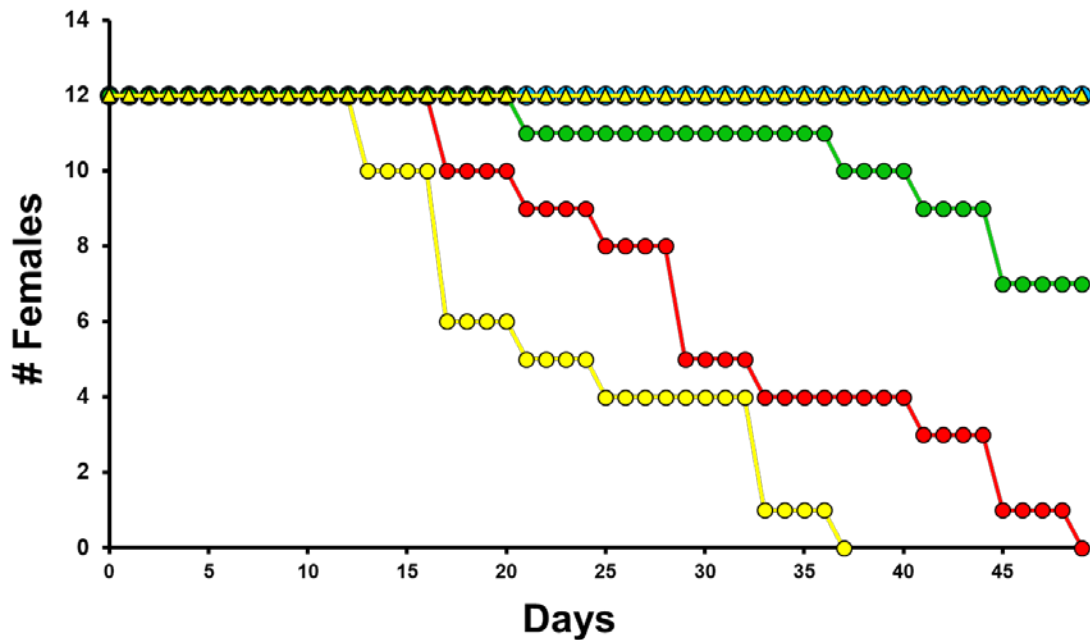
3) Within-generational responses of *O. robusta* to C, OA, OW and OAW scenarios for the following metabolites: ATP, C16:3, ADP, AMP, Glucose, C18:2, C18:1, C20:1, C18:3

	df	Pillai	<i>F</i>	<i>P</i>
Scenario	3	2.30	2.90	0.01
Residuals	11			
Total	12			

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39 **Appendix S6.** Cumulative mortality curves for F1 individuals of *Ophryotrocha robusta* (rare species; circles) and *O.*
40 *japonica* (common species; triangles) along a 49 d exposure to control (blue), ocean acidification (green) and ocean
41 warming (red) scenarios, in isolation and combined (yellow).



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