Supplementary information

Viability of coastal fish larvae under ocean alkalinity enhancement: from organisms to communities
by Goldenberg et al.

Detailed description of metabolic rate estimates in the laboratory experiment:

Routine metabolic rate (RMR) was measured on individual larvae following Berg et al. (2020). Larvae were collected from the tanks before the lights went on in the morning (i.e. with empty guts) and placed in a container with filtered seawater from the tanks (0.2 µm). Then they were introduced individually in screw-top glass vials with integrated oxygen sensor (4 or 20 mL). Oxygen consumption was tracked for 1.5 h in dark conditions using a 4-channel fiber-optic oxygen meter (FireSting O_2, PyroScience GmbH). Temperature was kept constant throughout the measurements (8.4 - 8.5 °C). Oxygen sensors were calibrated before each trial day. Oxygen partial pressure was continuously measured and never decreased below 80%. Background respiration was assessed through vials filled with filtered water (0.2 µm) from each tank. Oxygen respiration in these procedural controls was <20% (typically <5%) of those in vials with larvae. The mean respiration across all procedural controls from each day was used to correct the routine metabolic rate of the larvae (nmol O_2 ind^{-1} h^{-1}). After each trial, larvae were anesthetized with MS222 (0.1 g L^{-1}), photographed, euthanized by an anesthetic overdose and frozen at -20 °C. Dry mass was estimated for each larva after freeze drying. Mass-specific routine metabolic rate (RMRm, nmol O_2 mg $fish^{-1}$ h^{-1}) was estimated following Howald et al. (2022). Routine metabolic rate was divided by larval dry weight (resulting in RMRraw) and then corrected for allometric scaling with the following formula:

$$RMR_m = RMR_{raw} \cdot \left(\frac{DM}{DM_{mean}}\right)^{1-coeff_{Larvae}}$$

DM is larval dry mass (mg), DM_{mean} the mean DM of all tested larvae and $coeff_{Larvae}$ (0.8859) a general allometric scaling coefficient for herring larvae estimated in Moyano et al. (2018).

Table S1: Linear models to establish that *mineral* was not a dominant driver of fish in the mesocosms. *ΔTA* (continuous), *mineral* (categorical) and their interaction were employed as explanatory variables (type III test). In all further analyses, only ΔTA is used as explanatory variable and simply referred to as OAE.

Response variable	Source of variation	MS	df	F-ratio	p-value
a) Mortality longer-term (all taxa days 7-53)	ΔTA	12	1	0.06	0.818
	Mineral	192	1	0.93	0.373
	ΔTA × Mineral	0	1	0.00	0.976
	Residuals	207	6		
b) Abundance (all taxa day 54)	ΔTA	2	1	0.00	0.949
	Mineral	333	1	0.82	0.401
	ΔTA × Mineral	180	1	0.44	0.532
	Residuals	409	6		
d) Per capita size (all taxa day 54) *log 10 transformed*	ΔTA	0.270	1	5.00	0.067
	Mineral	0.038	1	0.69	0.437
	ΔTA × Mineral	0.063	1	1.17	0.321
	Residuals	0.054	6		
e) Biomass (all taxa day 54)	ΔTA	11.9	1	10.92	0.016
	Mineral	0.0	1	0.00	0.994
	ΔTA × Mineral	0.8	1	0.78	0.412
	Residuals	0.8	6		

MS = mean squares; df = degrees of freedom

Table S2: Statistical tests for response of herring larvae to OAE in the laboratory, to accompany figure 3**. a-c)** Linear mixed models with individuals fish as lowest level of replication employing *OAE* as categorical fixed effect and *tank* as random effect (random intercept, restricted maximum likelihood fit, Satterthwaite approximation, (Bates et al. 2015; Kuznetsova et al. 2017). **d)** Simple pairwise comparison with tanks as lowest level of replication.

Response variable	Day	Source of variation	MS	df_{Num}	df_{Den}	F-ratio	p-value	Tank effect p-value
a) Routine metabolic rate	22	OAE	143	1	19	1.90	0.184	1
	27	OAE	8.39	1	22	0.01	0.911	1
	37	OAE	323	1	8	2.23	0.174	1
b) Time moving	27	OAE	24.8	1	27	1.84	0.187	1
	31	OAE	3.17	1	4	0.16	0.708	0.040
	35	OAE	367	1	4	2.08	0.223	0.354
Pause frequency	27	OAE	10.2	1	4	0.59	0.485	0.230
	31	OAE	2.76	1	4	0.18	0.692	0.048
	35	OAE	0.757	1	4	0.01	0.911	0.525
Pause duration	27	OAE	0.0777	1	3.7	7.09	0.058	0.451
	31	OAE	0.00107	1	4.2	0.05	0.836	0.069
	35	OAE	0.0484	1	4	0.24	0.648	0.046
c) Growth	22	OAE	1.86	1	4	1.96	0.235	0.806
	28	OAE	0.0146	1	4.1	0.01	0.928	0.582
	37	OAE	0.0528	1	4	0.05	0.832	0.272
d) Survival	37	OAE	24.0	1	4	0.65	0.465	na

MS = mean squares; df_{Num} and df_{Den} = numerator and denominator degrees of freedom

Table S3: Linear regressions for the response of fish to OAE in the mesocosms, to accompany figure 4.

Response variable	Source of variation	MS	df	F-ratio	p-value
a) Mortality longer-term (all taxa, days 7-53)	OAE	192	1	1.19	0.306
	Residuals	161	8		
b) Mortality shorter-term (herring only, days 7-15)	OAE	5.0	1	0.11	0.747
	Residuals	44.7	8		
c) Per capita size (all taxa day 54)	OAE	0.270	1	5.56	0.046
	Residuals	0.049	8		
d) Abundance (all taxa day 54)	OAE	2	1	0.01	0.945
	Residuals	349	8		
e) Biomass (all taxa day 54)	OAE	11.9	1	10.53	0.012
	Residuals	1.1	8		

MS = mean squares; df = degrees of freedom

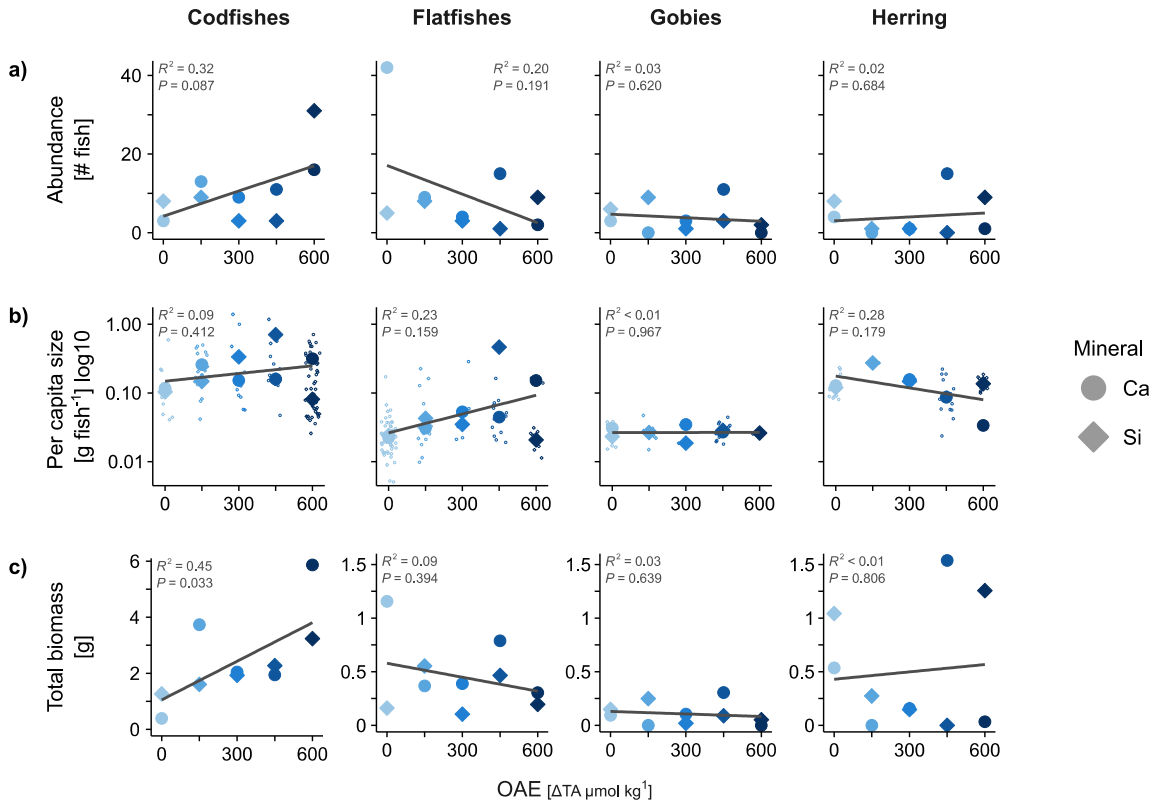

Figure S1: Responses of individual fish taxa to OAE, assessed at the end of the mesocosm experiment. Count **(a)**, individual size **(b)** and total biomass **(c)** of live fish. Larger points represent mesocosms and smaller points in b single individuals. Linear regressions test for an OAE effect.

Table S4: Linear regressions for the response of other functional groups (a-c) and predation on herring (d) under OAE in the mesocosms, to accompany figure 5.

Response variable	Source of variation	MS	df	F-ratio	p-value
a) Chlorophyll a (days 7-53)	OAE	0.0034	1	0.147	0.711
	Residuals	0.0233	8		
b) Copepods (days 7-53)	OAE	0.5	1	0.05	0.832
	Residuals	10.4	8		
c) Hydrozoa (days 7-53)	OAE	16.0	1	0.47	0.511
	Residuals	33.8	8		
d) Fish missing (days 7-54)	OAE	51	1	0.44	0.524
	Residuals	115	8		

MS = mean squares; df = degrees of freedom

References

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