The following supplement accompanies the article

Individual and population level effects of ocean acidification on a predator-prey system with inducible defenses: bryozoan-nudibranch interactions in the Salish Sea

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Text S1 *Membranipora* population dynamics model equations and assumptions

Zooids increase their available energy content via feeding such that

$$q_{f_j} = A \cdot c_j \cdot F \cdot f \cdot S_j \cdot D_j \,.$$

Here, A is the algal concentration of the environment (cells/ml), c_J is the clearance rate of zooid j (ml/day), F is the food quality of algal cells (J/cell), f is the assimilation efficiency associated with trophic energy transfer, and S_j is a spination coefficient varying between 0 – 1.0. Zooids that are undefended have high S_j values whereas defended zooids have lower S_j values to reflect interference with feeding currents by induced spines (Grünbaum, 1997). Feeding is also constrained proportionally to developmental state, D_j, because newly formed zooids slowly gain the capacity to feed as they develop lophophores (McKinney and Jackson, 1991). In zooids with energy available for growth D_j increases up to the fully developed state D_{max} as

$$\frac{dD_j}{dt} = \begin{Bmatrix} d_j\,;\ D < D_{max}\,;\quad E_{tot_j} > 0 \\ otherwise: 0 \end{Bmatrix}.$$

Available energy decreases as it is used to form tissue and to metabolize. The rate of energy allocated for growth is described by the equation:

$$q_{g_j} = g_j \cdot Q_m \cdot (M_{max} - M_j)$$

where g_j is the rate of energy put towards tissue and mass production (day^{-1}), Q_m is the mass to energy conversion rate (J/g), M_{max} is the maximum possible zooid mass (g) and M_j is the current zooid mass. As zooids mature, maximum size is attained and q_{g_j} approaches zero. Zooid mass, M_j, increases as described by the following relationship:

$$\frac{dM_j}{dt} = \frac{q_{g_j}}{Q_m}$$

such that mass no longer increases when $q_{g_j} = 0$. E_{tot_j} is also decreased via metabolism whereby

$$q_{m_j} = q_0$$

where q_0 is the basal metabolic rate of a zooid (J/day).

Translocation of energy between zooids

Zooids can import energy from up to four neighbors: two upstream axial neighbors, and both lateral neighbors. They can also export energy to up to four neighbors, two downstream axial neighbors and both lateral neighbors. Zooids can translocate energy if their available energy content is above a threshold energy requirement, E_r. The rate of energy input to zooid j due to translocation is described by

$$q_{in_j} = r_{t_a} \cdot a_{j-1,j}(E_{tot_{j-1}} - E_r) + r_{t_a} \cdot a_{j-2,j}(E_{tot_{j-2}} - E_r) + r_{t_l} \cdot l_{lat1,j}(E_{tot_{lat1}} - E_r) + r_{t_l} \cdot l_{lat2,j}(E_{tot_{lat2}} - E_r)$$

Here r_{t_a} is the energy translocation rate (day^{-1}) between axially-connected zooids and r_{t_l} is the energy translocation rate between laterally-connected zooids. $a_{j-i,j}$ and $l_{lat1,j}$ are the fractions of available energy transferred axially, from zooid z_{j-1} to zooid z_j, and laterally, from zooid z_{lat1} to zooid z_j, respectively (see Figure 2) and sum to 0.5. A similar relationship describes the energy export rate.

$$q_{out_j} = r_{t_a} \cdot a_{j+1,j}(E_{tot_j} - E_r) + r_{t_a} \cdot a_{j+2,j}(E_{tot_j} - E_r) + r_{t_l} \cdot l_{j,lat1}(E_{tot_j} - E_r) + r_{t_l} \cdot l_{j,lat2}(E_{tot_j} - E_r)$$

where a zooid's own excess available energy is distributed among four downstream neighbors based on the respective translocation rates of the neighbor's orientation to the focal zooid. Zooids can translocate energy if they are located in the colony interior, or form daughters in addition to transferring energy if they are on the colony edge, if they have sufficient energy available above E_r. Daughter zooids are born with an initial energy content, E_{min}, which is initially subtracted from the energy pool of the zooid that produced it, and an initial mass, M_{min}. Energy translocation cannot result in negative energy transfer for any zooid in a colony. In the model, zooids can detect existing zooids, including those belonging to other colonies, and do not create daughters if unoccupied space is unavailable.

Table S1 *Membranipora* population dynamics model parameters. Parameter values controlling zooid-level energy processes were informed by experiments (parts 1-3) or approximated and calculated from literature ranges and values.

Parameter	Definition	Value	Units	Source
p	Zooid side length	0.54041	mm	Experimental estimate
θ	Acute zooid angle	π /6	radians	Experimental estimate
F	Food quality	1.15 x 10^{-6} (1e-10 g dry wt/cell T.iso[1]) x (5.5 g cal/g dry wt[2])	J/cell	[1]Yoshioka et al., 2012 [2]Parsons et al., 1977
S_j	Spination coefficient of zooid j	1.0 unspined zooid 0.7 spined zooids	none	Grünbaum, 1997
A	Algal density	50,000	cells/ml	Experimental value
E_r	Threshold energy needed to send energy downstream	7.0	J	
M_{min}	Starting mass of zooid j	1 x 10^{-8}		Experimental estimate

Parameter	**Definition**	**Value**	**Units**	**Source**
M_{max}	Maximum size (tissue mass) of any zooid	1.96×10^{-7} From g skeleton / colony area estimates assuming skeleton is 70% of the total weight	g	Experimental estimate Hyman, 1959
Q_m	Mass – energy conversion factor	4.184×10^4 1 gC ~ 10-12 Kcal general conversion	g/J	McLusky, 1981
D_{min}	Starting developmental state of zooid *j*	0.1	none	
D_{max}	Maximum size developmental state of any zooid	1.0	none	
E_{min}	Starting energy content of every zooid	0.001	J	
f	Assimilation efficiency	0.7	none	
c_j	Clearance rate of zooid *j* (feeding)	22.08 (0.92 ml/hr/zooid)	ml/day	Riisgard and Manriquez 1997
$a_{j,k}$	Fraction of available energy transferred between axially connected zooid *j* – zooid *k*	0.25	none	Miles et al., 1995
$l_{j,k}$	Fraction of available energy transferred between laterally connected zooid *j* – zooid *k*	0.25	none	Miles et al., 1995
r_{t_a}	Energy translocation rate between axial zooids	2.0	day^{-1}	Miles et al., 1995
r_{t_l}	Energy translocation rate between lateral zooids	6.0	day^{-1}	Miles et al., 1995
g_j	Rate of energy used for growing tissue and mass	0.5	day^{-1}	
d_j	Developmental rate of zooid *j*	0.3	day^{-1}	Experimental estimate
q_0	Basal metabolic rate of zooid *j*	0.05 (0.025 – 0.144 J/day for *Bugula neritina*)	day^{-1}	Petterson et al., 2016

Table S1 References

Grünbaum D (1997) Hydromechanical mechanisms of colony organization and cost of defense in an encrusting bryozoan, *Membranipora membranacea*. Limnology and Oceanography. 42:741-752.

Hyman LH (1959) The Invertebrates: Smaller Coelomate Groups, Chaetognatha, Hemichordata, Pogonophora, Phoronida, Ectoprocta, Brachiopoda, Sipunculida, the Coelomate Bilateria, Vol. 5. McGraw- Hill, New York, NY.

McKinney FK, Jackson JB (1991) Bryozoan evolution. University of Chicago Press. Chicago, IL.

McLusky DS (1981) The estuarine ecosystem. Blackie Press, Glasgow, Scotland.

Miles JS, Harvell CD, Griggs CM, Eisner S (1995) Resource translocation in a marine bryozoan: quantification and visualization of 14C and 35S. Marine Biology. 122:439-445.

Parsons TR, Takahashi M, Hargrave B (1977) Biological oceanographic processes. Pergamon Press, Oxford, UK.

Pettersen AK, White CR, Marshall DJ (2016) Metabolic rate covaries with fitness and the pace of the life history in the field. Proc. R. Soc. B. 283:20160323.

Riisgard HU, Manriquez P (1997) Filter-feeding in fifteen marine ectoprocts (Bryozoa): particle capture and water pumping. Marine Ecology Progress Series. 154:223–239.

Yoshioka M, Yago T, Yoshie-Stark Y, Arakawa H, Morinaga T (2012) Effect of high frequency of intermittent light on the growth and fatty acid profile of *Isochrysis galbana.* Aquaculture. 338:111-117.

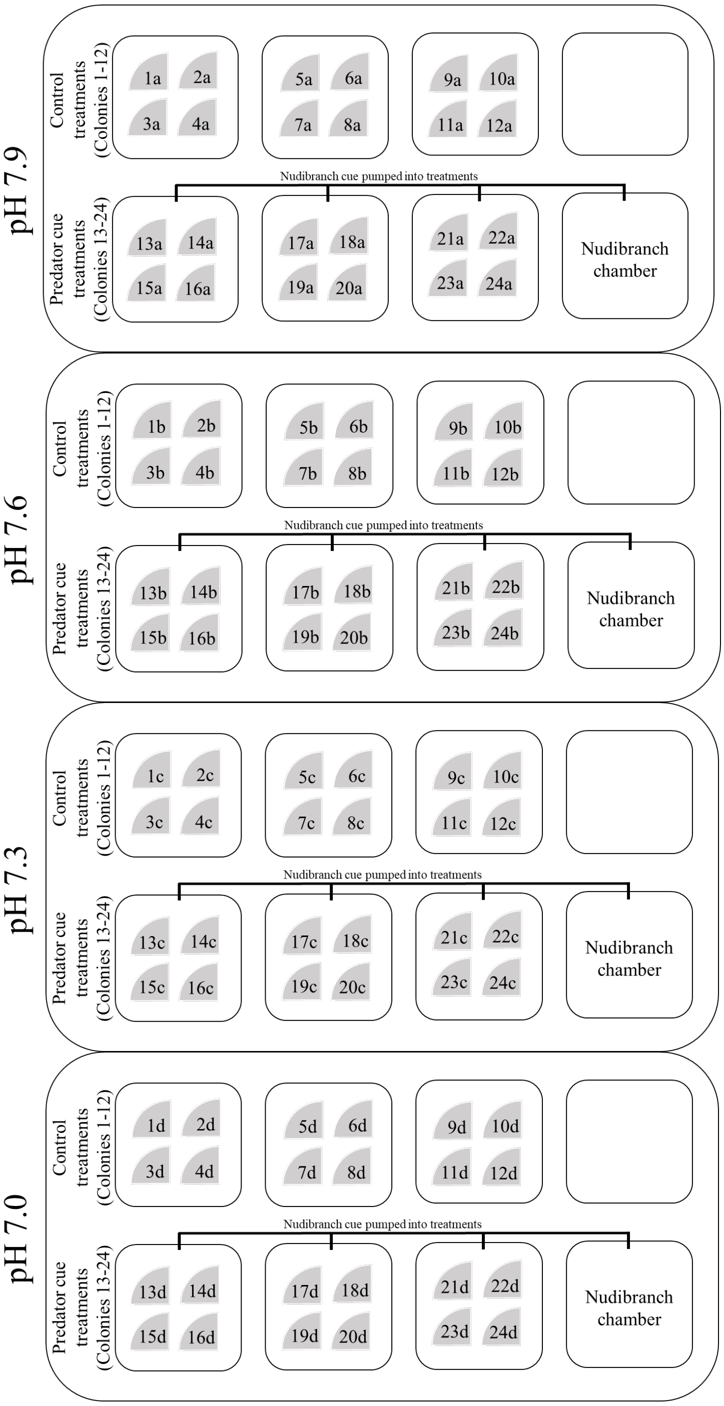

Figure S1: Experimental setup diagram depicting four pH treatments in larger tanks each with 8 chambers. Twelve colonies were divided into quarters and distributed across four pH treatments either exposed or not exposed to predator cues. Colony section positions in diagram do not reflect actual locations in chambers as colony section locations were randomized and rotated across chambers of the same predation treatment within pH treatment every two days to control for any chamber effects. Numbers refer to individual colonies (genotypes) and letters refer to the subsection of that genotype placed in a pH treatment (a = pH 7.9, b = pH 7.6, c = pH 7.3, d = pH 7.0). Nudibranch chambers contained predators to create waterborne cue pumped to predator treatments.

Table S2.1 Bryozoan growth rate models and AIC values used for model selection. Best fit model indicated in bold.

Model	**AIC**
Growth rate ~ 1 + (1|colony)	-465.2
Growth rate ~ pH + (1|colony)	-485.5
Growth rate ~ predation cue + (1|colony)	-467.8
Growth rate ~ pH + predation cue + (1|colony)	-488.1
Growth rate ~ pH * predation cue + (1|colony)	-498.3
Growth rate ~ pH + pH^2 + (1|colony)	-508.4
Growth rate ~ pH * predation cue + pH^2 + (1|colony)	-527.2
Growth rate ~ pH * predation cue + pH^2 * predation cue + (1|colony)	**-537.5**

Table S2.2 Best Fit Model Summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	**Estimate**	**SE**	**T**	**P**
Intercept	-10.32290	1.34700	-7.664	< 0.0001 *
pH	2.73056	0.35941	7.597	< 0.0001 *
Predation cue	7.42088	1.94776	3.810	0.0003 *
pH^2	-0.17888	0.02395	-7.470	< 0.0001 *
pH : predation cue	-1.94605	0.51971	-3.745	0.0004 *
pH^2 : predation cue	0.12721	0.03463	3.674	0.0005 *

Random effects

Effects	**Variance**	**SD**
Colony	7.684e-05	0.008766
Residual	1.006e-04	0.010032

Deviance = -553.5, df = 84

Table S3.1 Colony senescence models and AIC values used for mortality model selection. Best fit model indicated in bold. *Note: pH was centered to allow for model convergence*

Model	**AIC**
Proportion dead ~ 1 + (1|colony)	29344.84
Proportion dead ~ pH + (1|colony)	12290.83
Proportion dead ~ predation cue + (1|colony)	29345.49
Proportion dead ~ pH + predation cue + (1|colony)	12289.96
Proportion dead ~ pH * predation cue + (1|colony)	10794.83
Proportion dead ~ pH + pH^2 + (1|colony)	7862.155
Proportion dead ~ pH * predation cue + pH^2 + (1|colony)	6614.426
Proportion dead ~ pH * predation cue + pH^2 * predation cue + (1|colony)	**5485.83**

Table S3.2 Best Fit Model Summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	**Estimate**	**SE**	**Z**	**P**
Intercept	-3.50941	0.20079	-17.48	< 0.0001 *
pH (centered)	-3.85900	0.04126	-93.53	< 0.0001 *
Predation cue	0.68312	0.29032	2.35	0.0186 *
pH^2 (centered)	14.21632	0.21023	67.62	< 0.0001 *
pH: predation cue	1.59555	0.06386	24.99	< 0.0001 *
pH^2: predation cue	-10.26004	0.30616	-33.51	< 0.0001 *

* indicates significant P values at alpha = 0.05

Random effects

Effects	**Variance**	**SD**
Colony	0.4759	0.6898

Deviance = 5471.8, df = 85

Table S4.1 Spine length models and AIC values used for model selection. Best fit model indicated in bold.

Model	AIC
Spine length ~ 1 + (1|colony) + (1|colony : colony section)	13232.26
Spine length ~ pH + (1|colony) + (1|colony : colony section)	13227.55
Spine length ~ predation cue + (1|colony) + (1|colony : colony section)	13187.92
Spine length ~ pH + predation cue + (1|colony) + (1|colony : colony section)	**13183.21**
Spine length ~ pH * predation cue + (1|colony) + (1|colony : colony section)	13184.73

Table S4.2 Best Fit Model Summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	Estimate	SE	T	P
Intercept	-141.896	61.274	-2.316	0.02346 *
pH	21.527	8.108	2.655	0.00983 *
Predation cue (defended)	117.104	9.580	12.223	< 0.0001 *

Random effects

Effects	Variance	SD
Colony section	425.8	20.63
Colony	405.6	20.14
Residual	853.5	29.22

Deviance = 13171.2, df = 1344

Figure S2 To further explore differences in the abundance of spines with respect to pH in predation cue (black) and control treatments (grey), the following plot shows the proportion of spines present at random sampling locations along photo transects, with plotted prediction from the best fit model as determined by AIC. Models used a binomial response distribution and logit link function and considered colony a random effect.

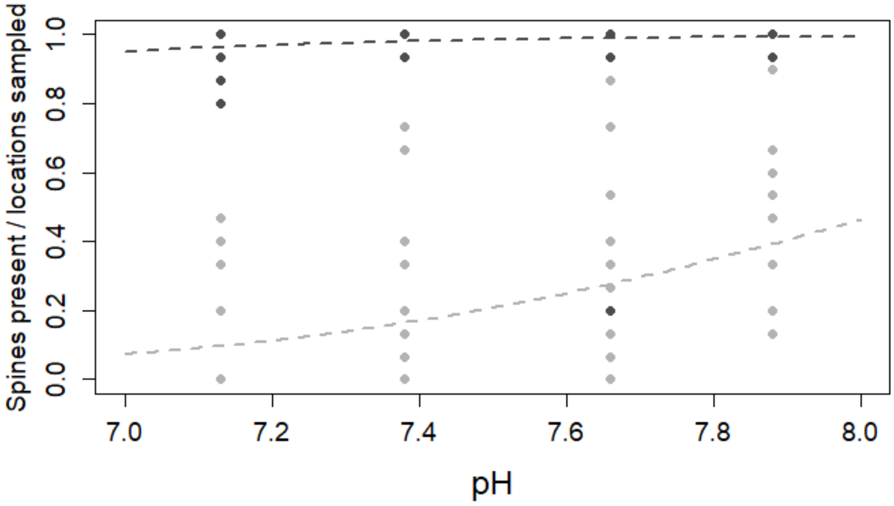

Table S5.1 Spine metric models and AIC values used for model selection. Best fit model indicated in bold.

Model	**AIC**
Spination metric ~ 1 + (1|colony)	101.46
Spination metric ~ pH + (1|colony)	100.05
Spination metric ~ predation cue + (1|colony)	69.13
Spination metric~ pH + predation cue + (1|colony)	**67.42**
Spination metric ~ pH * predation cue + (1|colony)	67.79

Table S5.2 Best Fit Model Summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	**Estimate**	**SE**	**T**	**P**
Intercept	-19.215	10.122	-1.898	0.0576
pH	2.383	1.328	1.795	0.0726
Predation cue	5.493	1.282	4.285	< 0.0001 *

* indicates significant P values at alpha = 0.05

Random effects

Effects	**Variance**	**SD**
Colony	0.4826	0.6947

Deviance = 59.4, df = 88

Table S6.1 Nudibranch zooid consumption models and AIC values for model selection. Best fit model indicated in bold.

Model	AIC
Zooids consumed ~ nudibranch length + (1|colony) + (1|nudibranch) + (1|colony size)	978.03
Zooids consumed ~ nudibranch length + pH + (1|colony) + (1|nudibranch) + (1|colony size)	979.76
Zooids consumed ~ nudibranch length + predation cue + (1|colony) + (1|nudibranch) + (1|colony size)	976.85
Zooids consumed ~ nudibranch length + pH + predation cue + (1|colony) + (1|nudibranch) + (1|colony size)	978.56
Zooids consumed ~ nudibranch length + pH * predation cue + (1|colony) + (1|nudibranch) + (1|colony size)	**975.09**

Table S6.2 Best fit model summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	Estimate	SE	Z	P
Intercept	0.65689	3.02767	0.217	0.82824
Nudibranch length	0.30969	0.09478	3.267	0.00109 *
pH	0.24648	0.40458	0.609	0.54238
Predation cue (defended)	6.82416	2.98450	2.287	0.02222 *
pH: predation cue	-0.95386	0.39693	-2.403	0.01626 *

Random effects

Effects	Variance	SD
Colony	0.2534	0.5034
Nudibranch	0.3468	0.5889
Colony size	0.1211	0.3480

Deviance = 959.1, df = 84

Table S7.1 Skeletal density (Ca per unit mm of skeleton) models and AIC values for model selection. Best fit model indicated in bold.

Model	**AIC**
Ca per area ~ 1 + (1|colony)	-1051.2
Ca per area ~ pH + (1|colony)	-1069.3
Ca per area ~ predation cue + (1|colony)	-1049.2
Ca per area ~ pH + predation cue + (1|colony)	-1067.3
Ca per area ~ pH * predation cue + (1|colony)	-1065.4
Ca per area ~ pH * predation cue + pH^2 + (1|colony)	-1079.0
Ca per area ~ pH * predation cue + pH^2 * predation cue + (1|colony)	-1078.1
Ca per area ~ pH + pH^2 + (1|colony)	**-1082.9**

Table S7.2 Best fit model summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	**Estimate**	**SE**	**T**	**P**
Intercept	-0.1339793	0.0310058	-4.321	< 0.0001 *
pH	0.0358173	0.0083360	4.297	< 0.0001 *
pH^2	-0.0023485	0.0005594	-4.198	< 0.0001 *

Random effects

Effects	**Variance**	**SD**
Colony	1.432e-07	0.0003784
Residual	2.270e-07	0.0004764

Deviance = -1092.9, df = 85

Figure S3 To further explore differences in the skeletal quality and composition with respect to pH treatment and predation cue, the following plot shows that proportion of the skeleton weight that is calcium, as a proxy for calcium carbonate, with plotted predictions from the best fit model as determined by AIC. Models used a Gaussian response distribution and identity link function and considered colony a random effect.

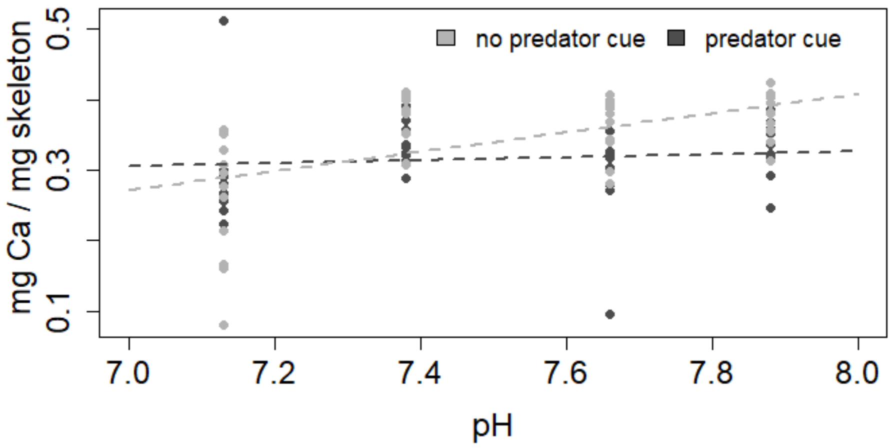

Table S8.1 Skeletal composition (mg Ca per mg of skeleton) models and AIC values for model selection. Best fit model indicated in bold.

Model	AIC
Ca per weight ~ 1 + (1|colony)	-227.6
Ca per weight ~ pH + (1|colony)	-236.3
Ca per weight ~ predation cue + (1|colony)	-228.9
Ca per weight ~ pH + predation cue + (1|colony)	-238.0
Ca per weight ~ pH * predation cue + (1|colony)	**-242.7**

Table S8.2 Best fit model summary. Asterisk indicates significant P values at alpha = 0.05

Fixed effects

Parameter	Estimate	SE	T	P
Intercept	-0.68307	0.23403	-2.919	0.0044 *
pH	0.13637	0.03113	4.381	< 0.0001 *
Predation cue	0.84465	0.33189	2.545	0.0126 *
pH: predation cue	-0.11579	0.04415	-2.623	0.0102 *

Random effects

Effects	Variance	SD
Colony	0.00	0.00
Residual	0.003456	0.05879

Deviance = -254.7, df = 84