The following supplement accompanies the article

Physiological tolerance as a tool to support invasion risk assessment of tropical ascidians

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Supplement 1

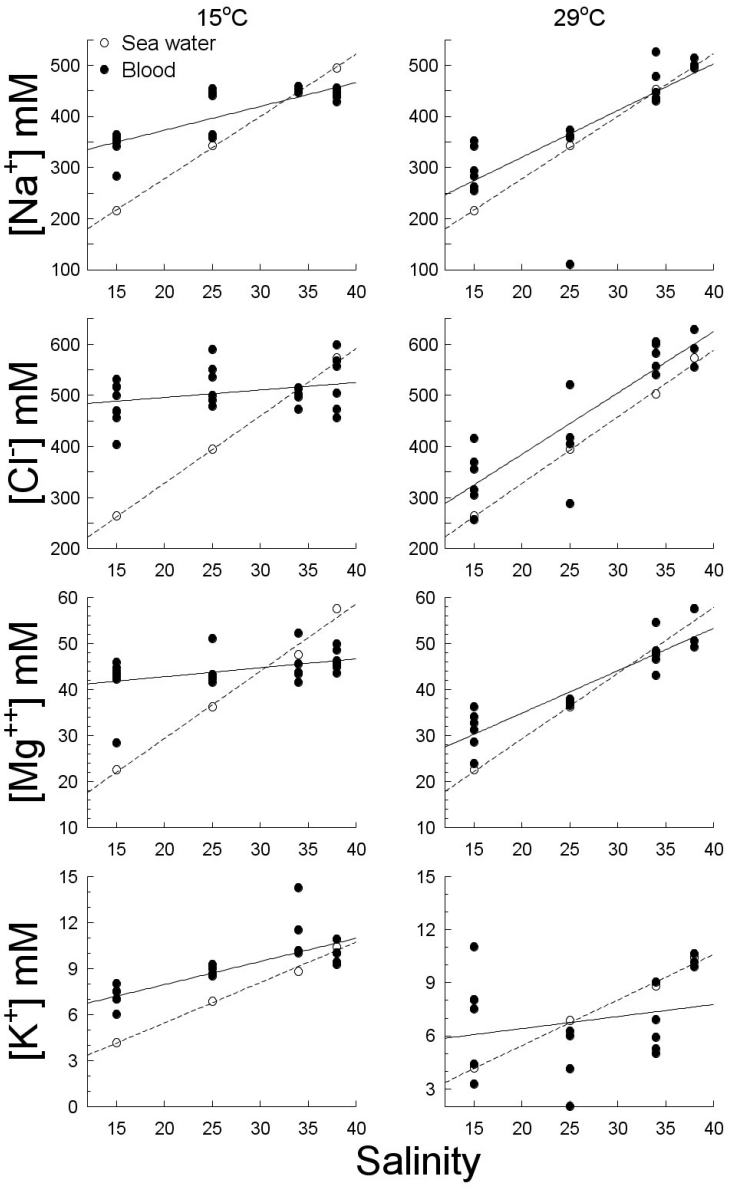

Fig. S1 – Regression of ion concentration in *Ascidia curvata* blood (solid line) and sea water (hatched line) against salinity challenges (15, 25, and 38. Control – 34 ppt) in 15°C (n = 25) and29°C (n = 18), after two hours of immersion. Each dot represents a different individual. Test results for significance in Table S1.

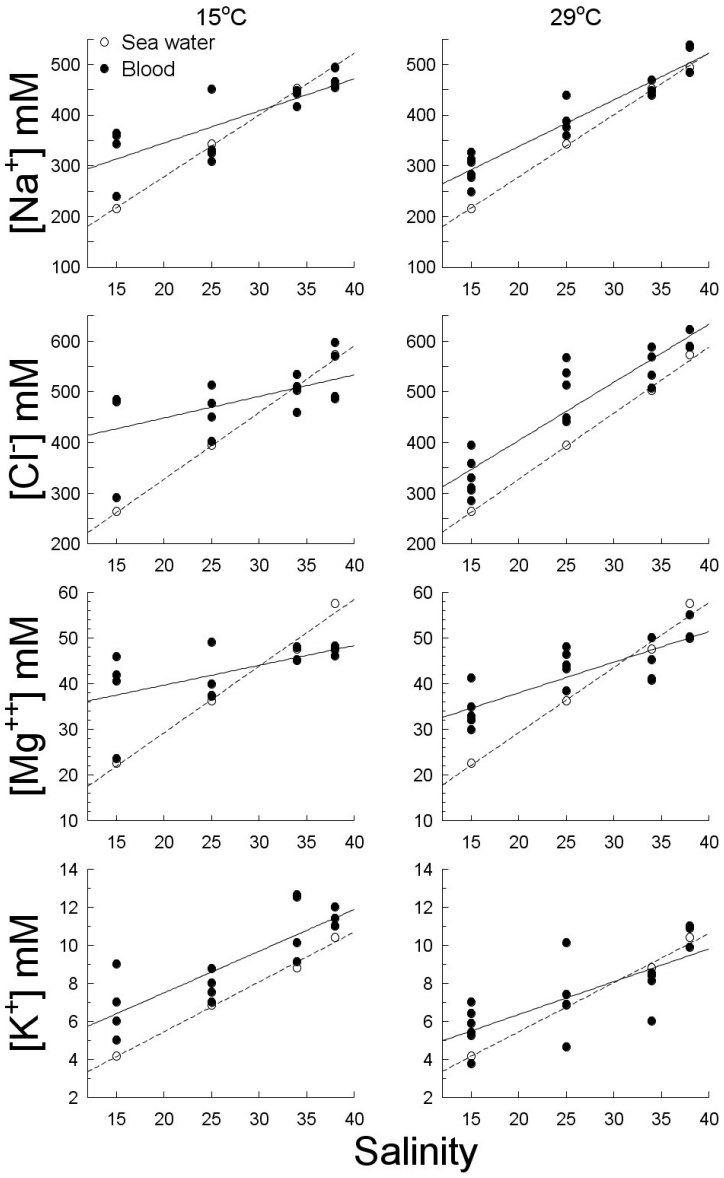

Fig. S2 Regression of ion concentration in *Phallusia nigra* blood (solid line) and sea water (hatched line) against salinity challenges (15, 25, and 38. Control – 34 ppt) in 15°C (n = 16) and 29°C (n = 18), after two hours of immersion. Each dot represents a different individual. Test results for significance in Table S1.

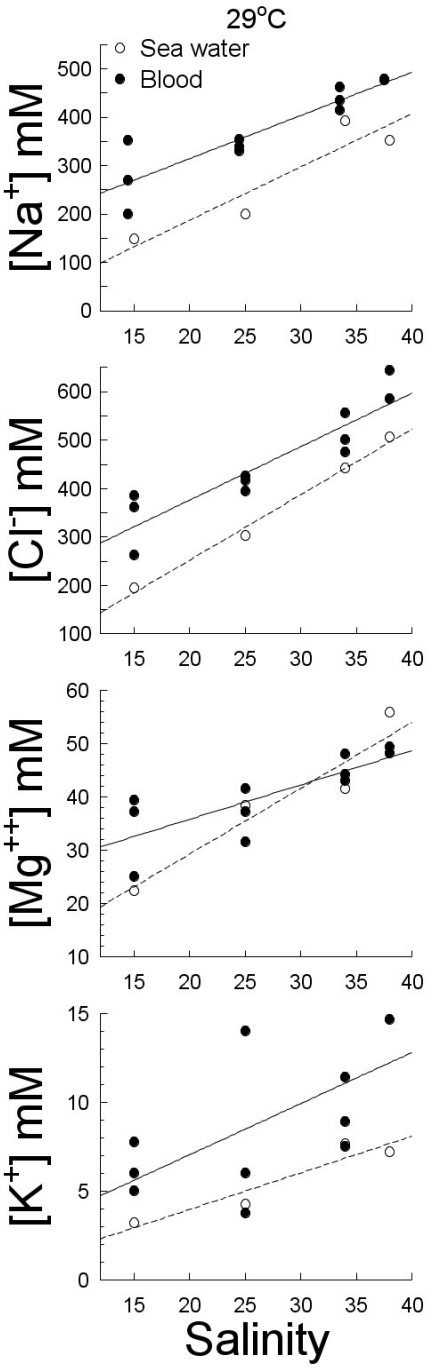

Fig. S3 Regression of ion concentration in *Ascidia panamensis* blood (solid line) and sea water (hatched line) against salinity challenges (15, 25, and 38. Control – 34 ppt) in 29°C (n = 11), after two hours of immersion. Each dot represents a different individual. Test results for significance in Table S1.

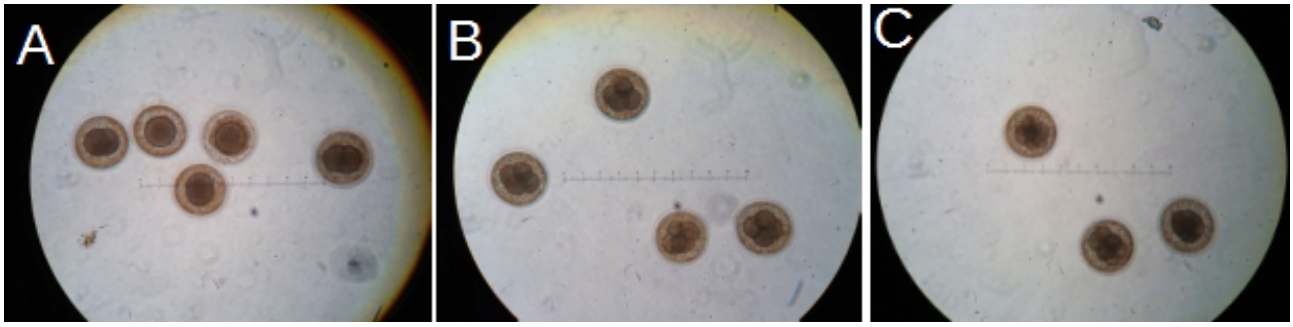

Fig. S4. Eggs of *Ascidia sydneiensis* 1 h after fertilization. A. 15 ppt, B. 25 ppt, C. 34 ppt.

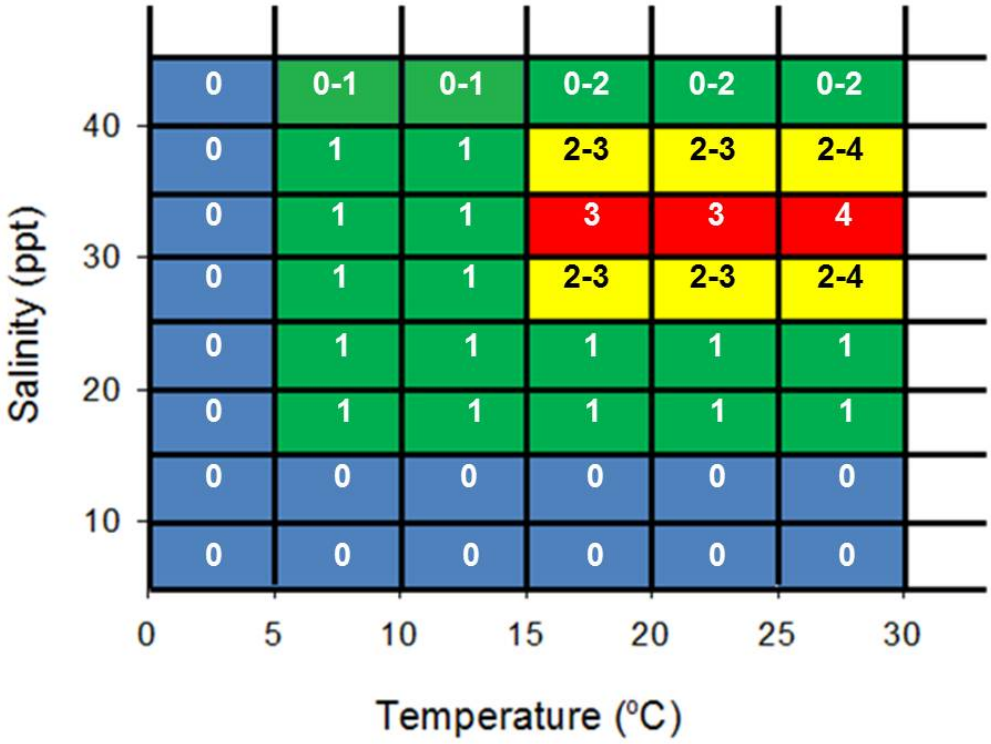

Fig. S5. Colonization pressure (number of species with potential risk of introduction, sensu Lockwood, 2009) in each combination of salinity and temperature determined by the physiological tolerances (Fig. 3, main text) of adults and gametes of *A. sydneiensis, A. curvata, Phallusia nigra* and *A. panamensis* (decreasing order of tolerance). High colonization pressure (high probability of being invaded by 3-4 species) ports are indicated by red cells. At the other end of the spectrum, zero colonization pressure is indicated by blue cells. Yellow (follows red) and green (before blue) cells indicate some colonization pressure. These results were used as an intermediate step in the assessment of colonization pressure of ports connected with Atlantic Panamanian ports (see Table 5 and Figure 4, main text, and Supplement 2).

Table S1. Regression analysis for the difference (dif [ ]) in ion concentration between the blood and seawater against salinity (S) for the ascidians *Ascidia curvata*, *A. panamensis*, *A sydneiensis* and *Phallusia nigra* in each of two temperatures.

		15°C					**29°C**				
Species	**Ion**	**equation**	**r^2**	**n**	**F**	**P**	**equation**	**r^2**	**n**	**F**	**P**
Ascidia sydneiensis	Na^+	dif $[Na^+]$ = 156.18 – 6.04 S	0.60	19	27.79	**<0.01**	dif $[Na^+]$ = 90.95 – 3.96 S	0.56	18	20.98	**<0.01**
	Cl^-	dif $[Cl^-]$ =274.98 – 9.60 S	0.62	19	30.33	**<0.01**	dif $[Cl^-]$ = 111.71 – 4.32 S	0.37	18	11.11	**<0.01**
	Mg^{++}	dif $[Mg^{++}]$ = 23.95 – 0.98 S	0.76	19	57.44	**<0.01**	dif $[Mg^{++}]$ = 20.29 – 0.82 S	0.68	18	37.84	**<0.01**
	K^+	dif $[K^+]$ = 1.05 – 0.01 S	-0.05	19	0.15	0.71	dif $[K^+]$ = 2.07 – 0.10 S	0.44	18	13.67	**<0.01**
Ascidia curvata	Na^+	dif $[Na^+]$ = 245.52 – 7.53 S	0.87	25	155.71	**<0.01**	dif $[Na^+]$ = 125.74 – 3.34 S	0.47	18	14.98	**<0.01**
	Cl^-	dif $[Cl^-]$ = 402.45 – 11.72 S	0.87	25	163.18	**<0.01**	dif $[Cl^-]$ = 90.83 – 1.17 S	-0.01	18	0.87	0.37
	Mg^{++}	dif $[Mg^{++}]$ = 38.80 – 1.27 S	0.89	25	191.60	**<0.01**	dif $[Mg^{++}]$ = 15.84 – 0.51 S	0.58	18	23.06	**<0.01**
	K^+	dif $[K^+]$ = 4.74 – 0.11 S	0.39	25	16.67	**<0.01**	dif $[K^+]$ = 5.18 – 0.19 S	0.37	18	10.49	**<0.01**
Phallusia nigra	Na^+	dif $[Na^+]$ = 183.48 – 5.84 S	0.57	16	20.71	**<0.01**	dif $[Na^+]$ = 121.17 – 3.05 S	0.48	18	16.02	**<0.01**
	Cl^-	dif $[Cl^-]$ = 298.94 – 8.91 S	0.66	16	29.80	**<0.01**	dif $[Cl^-]$ = 108.53 – 1.61 S	0.04	18	1.67	0.21
	Mg^{++}	dif $[Mg^{++}]$ = 31.01 – 1.03 S	0.73	16	41.85	**<0.01**	dif $[Mg^{++}]$ = 23.97 – 0.76 S	0.74	18	49.27	**<0.01**
	K^+	dif $[K^+]$ = 2.91 – 0.04 S	0.01	16	1.20	0.29	dif $[K^+]$ = 2.66 – 0.09 S	0.21	18	5.27	**<0.05**
Ascidia panamensis	Na^+	-	-	-	-	-	dif $[Na^+]$ = 97.42 – 3.30 S	0.32	11	5.67	**<0.05**
	Cl^-	-	-	-	-	-	dif $[Cl^-]$ = 88.67 – 2.01 S	0.06	11	1.66	0.23
	Mg^{++}	-	-	-	-	-	dif $[Mg^{++}]$ = 22.38 – 0.79 S	0.68	11	22.10	**<0.01**
	K^+	-	-	-	-	-	dif $[K^+]$ = 1.01 + 0.03 S	-0.10	11	0.07	0.80

Table S2. Literature review of temperature and salinity variation physiological tolerance of ascidian species. IR: Introduction records; G/A = Gradual or abrupt exposition; T = temperature (°C); S = salinity (ppt).

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Phlebobranchia									
Ascidia callosa		Adult	S: Natural occurrence			survival		S low = 30	Dybern 1969
Ascidia ceratodes		Adult	T: 15, 24.5	3 days	A	mortality	100% mortality at 24.5	T high < 24.5	Sorte et al. 2010
Ascidia ceratodes		Adult	T: Natural occurrence S: Natural occurrence			survival		T low = 11 T high = 21.5 S low = 30 S high = 35	Tracy & Reyns 2014
Ascidia conchilega		Adult	S: Natural occurrence			survival		S low = 30	Dybern 1969
Ascidia mentula		Adult	S: Natural occurrence			survival		S low = 20	Dybern 1969
Ascidia virginea		Adult	S: Natural occurrence			survival		S low = 20	Dybern 1969
Ascidia zara	X	Adult	T: Natural occurrence S: Natural occurrence			survival		T low = 11 T high = 23 S low = 30 S high = 35	Tracy & Reyns 2014
Ascidiella aspersa	X	Adult	S: Natural occurrence			survival		S low = 18	Dybern 1969
Ascidiella aspersa	X	Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	No development at 25°C. Settlement success at 25°C. Metamorphosis and post-metamorphosis was most successful between 20-25°C.	T low = 15 T high= 25	Rius et al. 2014
Ascidiella scabra		Adult	S: Natural occurrence			survival		S low = 18	Dybern 1969
Phallusia mamillata		Adult	T: 7, 10, 15, 20, 25	12 h	G	Pumping, filtration and digestion rates	Pumping higher at 15ºC and ceased at 7°C; filtering efficiency decreased at 20°C; digestion rate max at 15°C	T min = 7	Fiala-Médioni 1978
Ciona intestinalis	X	Adult	S: Natural occurrence			survival		S low = 12	Dybern 1969

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Ciona intestinalis	X	Adult	S: 9.6, 20.8 / 32	24 h	G, A	Oxygen consumption, pumping activity, heart rate	Oxygen consumption decreased with water dilution, and ceased in S = 10. Pumping decreased and ceased in S = 19. Heart rate became irregular with water dilution.	S low = 10	Shumway 1978
Ciona intestinalis	X	Embryo, Larvae	S: 21, 23, 25, 27, 29, 31, 33, 35, 37, 39 / 25 or 31	2, 96 h	A	# cleaved eggs (2h), # metamorphosed individuals (96h)	Acclimation S influences the developmental parameters	S low = 21 S high= 37	Renborg et al. 2014
Ciona intestinalis	X	Juveniles	T: low 8, medium 16 and high 24°C S: low 20, medium 25 and high 35	12 weeks	G	Growth, survival	100% motality in combination of T 24ºC, S = 20. Growth rate decrease with salinity decrease	T low = 8 T high= 16 S low = 25 S high= 35	Vercaemer et al. 2011
Ciona intestinalis	X	Adult	S = 0	1 min	A	survival	90 % survival	S low = 0	Carver et al. 2003
Ciona intestinalis	X	Embryos Larva	T: -0.6 – 20.5 (field)				Settlement occurred from June to November,	Recruits: T low = 5 T high = 20 Adults: T low = -0.6 T high = 20.5	Harris et al. 2017
Ciona robusta	X	Gametes Juvenile Adults	T: 7,10,15,20,25,30 S: 13,17,21,29,37,45,50	15 – 30 days	G	survival	Survival of at least 10% at combined values of temperature and salinty	juveniles T = 7 - 25 S = 21 - 50 adults T = 12 - 23 S = 25 - 50	Marin et al. 1987
Ciona robusta	X	Embryo, Larvae	T: 9 - 25 / 20 S: 26 - 42 ppt / 34 ppt pH: 6.5 - 9.5 / 8	20 h	A	Larval development, fertilization rates	Optimum development at 18-23°C, 34-42 ppt, and pH 7.4-8.8	T low = 16 T high= 24 S low = 32	Bellas et al. 2003
Ciona robusta	X	Adult	T: 13, 16, 18, 20, 22, 23, 25, 28 / 13 or 16	1:6h in T = 22, 25, 28, and 16h in control (13). 2: 6h in T = 18, 20, 23, and 16h in control (16).	G	Protein expression	Sensitivity to thermal stress was lower to cytoskeletal proteins and ATP-synthase than to the other proteins		Serafini et al. 2011

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Ciona robusta	X	Adult	T: 10.3, 17.2, 25.2 / 17.2 S: 15, 25, 35, 45 /35	10 days	T: G S: A	Survival, growth	Reduction in growth at S 25 and 45, and at 25°C. Growth increased at 10°C.	T low = 10 T high = 25 ~30% survival S low = 25 ~30% survival S high = 45	Madariaga et al. 2014
Ciona robusta	X	Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	Development faster in T > 20°C. No larval development at 30°C. Best settlement success at 25°C Metamorphosis and post-metamorphosis most successful = 20-25°C.	T low = 15 T high = 25	Rius et al. 2014
Ciona robusta	X	Adult	T: Natural occurrence S: Natural occurrence			survival		T low = 11 T high = 23 S low = 5 S high = 35.5	Tracy & Reyns 2014
Ciona savignyi	X	Embryo, larvae	T: 22-26 (summer), 12-17 (winter)	Variable	G	Early cleavage, metamorphosis, time of hatching	Cleavage T_{opt} = 14-27 (summer) and 10-20°C (winter) Metamorphosis T_{opt} = 15-25 (summer) and 12-20°C (winter).	T low = 12 T high = 25	Nomaguchi et al. 1997
Ciona savignyi	X	Adult	T: 13, 16, 18, 20, 22, 23, 25, 28 / 13 or 16	1:6h in T = 22, 25, 28, and 16h in control (13). 2: 6h in T = 18, 20, 23, and 16h in control (16).		Protein expression	Sensitivity to thermal stress was lower to cytoskeletal proteins and ATP-synthase than to the other proteins		Serafini et al. 2011
Corella parallelogramma		Adult	S: Natural occurrence			survival		S low = 18	Dybern 1969
Ecteinascidia turbinata	X	Larvae	S: 10, 16, 22, 26, 33 / 33	Variable (until larvae stop swimming for several min)	A	Swimming behavior to haloclines of different S	Haloclines of S 10, 16, 22 were barriers to larvae. Halocline of S 26 were crossed without difficult.	S low = 26	Vázquez & Young 1996

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Ecteinascidia turbinata	X	Larvae	Attachment, S: 10, 16, 22, 26 / 33 Metamorphosis and mortality, S: 10, 13, 16, 19, 22, 24, 26, 30	Attachment: 24h Metamorphosis and mortality: 10 days	A	Larval attachment and metamorphosis, and juvenile mortality	80% attached at S 22 and 100% at S>22. Metamorphosis: complete at S 22 or higher	S low = 22	Vázquez & Young 2000
Styelidae									
Botrylloides leachi	X	Adult	S: Natural occurrence			Survival		S low = 16	Dybern 1969
Botrylloides leachi	X	Young and adult colonies	Young, T: 11, 16, 18, 22, 26 / 18 S: 20, 24, 33, 38, 44 / 30-35 Adults, T: 3, 10, 18, 24, 28 / 18 S: 16, 24, 38, 44/30-35	Variable (1-31 days)	G	Survival, colony growth index, life quality index, zooids average length	Colonial growth was equal in young and adult colonies. T and S equally influence life quality in young colonies, but in the adults S exerts more influence than T	Young: T low = 11 T high = 26 S low = 20 S high44 Adult : T low = 3 T high = 28 S low = 24 S high = 38	Brunetti et al. 1980
Botrylloides nigrum	X	Adult	Chronic, S: 20, 24, 28, 32 and 35 Episodic, S: 20, 24	Chronic exposure: 9days Episodic exposure: 3 or 12h	A	Heart beat, assexual division, mortality	Colonies exposed to 24 and 28 ppt lost part of the body wall exposing their pharynx; lower salinity resulted in reduced heart rates and fewer asexual reproductive phases. No recover to pre-test heart beat rates at S = 20	S low = 24 (20% survival).	Dijkstra & Simkanin 2016
Botryllus planus		Adult	Chronic, S: 20, 24, 28, 32 and 35 Episodic, S: 20, 24	Chronic exposure: 9days Episodic exposure: 3 or 12h	A	Heart beat, assexual division, mortality	Colonies exposed to 24 ppt lost part of the body wall exposing their pharynx; lower salinity resulted in reduced heart rates and fewer asexual reproductive phases. Recover to pre-test heart beat rates.	S low = 20 (60% survival).	Dijkstra & Simkanin 2016

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Botryllus schlosseri	X	Young and adult colonies	Young, T: 11, 16, 18, 22, 26 / 18 S: 20, 24, 33, 38, 44 / 30-35 Adults, T: 3, 10, 18, 24, 28 / 18 S: 16, 24, 38, 44 / 30-35	Variable (11-77 days)	G	Survival, colony growth index, life quality index, zooids average length	Growth was greater in young than in adult colonies. T influences the life quality more than S. Length of zooids is heavily influenced by T.	Young colonies: T low = 11 T high = 26 S low = 20, S high = 44 Adult colonies: T low = 3 T high = 28 S low = 16, S high = 44	Brunetti et al. 1980
Botryllus schlosseri	X	Adult	T: 13, 19, 25 S: 25, 33, 40		A	Gonadal formation and maturation, colonial growth	No influence in the initial growth and the gonad formation. High T and the intermediate S stimulate gonadal maturation, and low T with extreme S stimulates colonial growth. T influences reproductive process more than S	T low = 13, T high = 25 S low = 25, S high = 40	Brunetti et al. 1984
Botryllus schlosseri	X	Adult	T: Ambient (19-21), cold (3-4<ambient), warm 1 (2 > ambient), warm 2 (4-5> ambient)	1 week	A	Growth rate	Growth was equal in all treatments		McCarthy et al. 2007
Botryllus schlosseri	X	Adult	S: 5, 10, 15, 20, 25 / 30	10 days	A	Heart rate, mortality	Heart rate decreased with S reduction to 10 100% mortality at S 5 in the 1st day, and at S 10 in the 7th day.	S low = 15	Dijkstra et al. 2008
Botryllus schlosseri	X	Young colonies	T: 5, 10, 15, 20, 25 / 13 S: 14, 20, 26, 32, 38/29	8 weeks	G	Growth, largest colonies (reproduction), survival	Grew in 10-25°C, and S 20-38, reached largest colonies in 15-20 and S 20-38	T low = 10, T high = 25 S low = 14, S high = 38	Epelbaum et al. 2009
Botryllus schlosseri	X	Adult	T: 21, 25, 29, 34	24 h	G	LT50 values	East/west coast USA LT50 = 29.4/28.3	T high = 29	Sorte et al. 2011
Botrylloides violaceus	X	Adult	T: Ambient (19-21), cold (3-4<ambient), warm 1 (2 > ambient), warm 2 (4-5> ambient)	1 week	A	Growth rate	Growth was equal in all treatments	T low = 15, T high = 26	McCarthy et al. 2007

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Botrylloides violaceus	X	Adult	S: 5, 10, 15, 20, 25 / 30	10 days	A	Heart rate, mortality	Heart rate decreased with the S reduction to 15. 100% mortality at S 5 in the 1st day, and at S 10 in the 3rd day. The mortality at S 15 was of about 35% since the 4th day	S low = 15	Dijkstra et al. 2008
Botrylloides violaceus	X	Young colonies	T: 5, 10, 15, 20, 25 / 13 S: 14, 20, 26, 32, 38 / 29	8 weeks	G	Growth, largest colonies (reproduction), survival	Grew in 15-25°C, and S 26-38, reached largest colonies in 20-25°C, and S 26-38	T low = 5, T high = 25 S low = 20, S high = 38	Epelbaum et al. 2009
Botrylloides violaceus	X	Adult	T: 15, 24.5 °C	3 days	A	mortality	100% mortality at 24.5	T high < 24.5	Sorte et al. 2010
Botrylloides violaceus	X	Adult	T: 21, 25, 29, 34	24 h	G	LT50 values	East/west coast USA LT50 = 27.4/25.3 °C	T high = 34	Sorte et al. 2011
Dendrodoa grossularia		Adult	S: Natural occurrence			Survival		S low = 7-8	Dybern 1969
Dendrodoa grossularia		Larvae	T: 9.6, 10.3, 14, 18.1 / 14	> 30 min	A	Tail beat frequency, swimming speed, stride length	All parameters increased linearly with T		Batty et al. 1991
Polyandrocarpa zorritensis	X	Larvae	S: 10, 16, 22, 26, 33 / 33	Variable (until larvae stop swimming for several min)	A	Swimming behavior to haloclines of different S	Haloclines of S 10, 16, 22 were barriers to larvae. The halocline of S 26 was crossed with some difficulty	S low= 26	Vázquez & Young 1996
Styela clava	X	Adult	S: 17.5, 26.3, 38.5 / 35	17.5: 70 h 26.3: 65 h 35: 148 h 38.5: 70 h	A	Change in body weight, pericardial fluid osmotic and ionic (Na^+ and K^+) concentra-tions	Corporal volume regu-lation in hyposmotic solutions, but low capacity in the hyper-osmotic. Osmocon-formist pattern, with osmotic gradient maintenance under low S. Moderate capacity to maintain Na^+ gradients, and moderate/ great capacity to maintain K^+ gradients	S low= 17.5, S high= 38.5	Sims 1984

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Styela clava	X	Adult	T: 12, 16, 20, 24, 28	10-12 h	A	Energy of respiration, excretion, feces, ingestion, and energy absorption	Respiration energy increased with T. Energy of feces and excretion reach critical values at 24°C, and energy of ingestion and absorption at 20°C		Jiang et al. 2008
Styela clava	X	Adult	T: 12, 16, 20, 24, 28 S: 20, 24, 28, 36 / 32	2 h	A	Oxygen consumption rate	Oxygen consumption rate increased with T and S increasing		Ai-li et al. 2008
Styela clava	X	Adult	T: 10, 27-29 – air exposure 15-16 control immersed	24-96 h		Survival and water loss under air exposure			Hillock & Costello 2013
Styela montereyensis		Adult	S: 17.5, 26.3, 38.5 / 35	17.5: 70 h 26.3: 65 h 35: 148 h 38.5: 70 h	A	Change in body weight, pericardial fluid osmotic and ionic (Na^+ and K^+) concentrations	Low corporal volume regulation. Osmoconformer. Low capacity to maintain Na^+ gradients, and moderate/ great capacity to maintain K^+ gradients	S low = 17.5, S high = 38.5	Sims 1984
Styela plicata	X	Adult	S: 17.5, 26.3, 38.5 / 35	17.5: 70 h 26.3: 65 h 35: 148 h 38.5: 70 h	A	Change in body weight, pericardial fluid osmotic and ionic (Na^+ and K^+) concentrations	Moderate capacity to regulate corporal volume. Regulates osmotic concentration under low S, and conforms under the high S. Moderate/ high capacity to maintain Na^+ gradients, and moderate/ great capacity to maintain K^+ gradients	S low = 17.5, S high = 38.5	Sims 1984

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Styela plicata	X	Embryo Larvae Juvenile	T: 18, 22, 26, 30 S: 22, 26, 30, 34	Embryonic development: until hatching or for 24 h. Larval experiments: 24 h plus the time to events evaluated occur	A	Duration of embryonic development, duration of larval metamorphosis, time to develop functional siphons	No development at S = 22, 26. In S 30, 34 the duration of embryonic development increased when T decreased (>50% of the larvae attached and metamorphosed. Siphon developed in 72 h in S 30, 34, and 22, 26, 30°C; 90 h at 18°C (30% of individual)	T low = 18 S low = 30	Thiyagarajan & Qian 2003
Styela plicata	X	Adult	T: <20, 20-25, >25 S: <28, 28-32, >32	Seasonal, during 2 years	G	Hsp 70 gene expression	Higher expression under the combination of the highest T with the lowest S		Pineda et al. 2012
Styela plicata	X	Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	Development was faster in T higher than 20°C. Successful development at 25°C. No larval development at 30°C. Best settlement success at 25°C. Metamorphosis and post-metamorphosis was most successful between 20-25°C.	T high = 25	Rius et al. 2014
Styela rustica	X	Larvae	S: 4, 8, 10, 12, 16, 18, 36, 40, 45, 50 / 23-24	2 weeks	A	Survival, metamorphose	At S = 16 there was 36-40% survival and delayed metamorphose	S low = 16	Saranchova et al. 2006
Halocynthia roretzi		Adult	T = 5, 10, 15, 20, 25		G	Filtration rate	Survival at all temperatures, with increased filtration rate in higher temperatures		Jeong & Cho 2013
Halocynthia roretzi		Adult	S = 6.6, 13.2, 19.8, 26.4, 36.3/ 33	6 days	G	Osmotic control, mortality	LS_{50} = 25.4, S = 6.6 (100% mortality day 4); S = 13.2 (100% mortality day 6)	S min = 25	Shin et al. 2007

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Halocynthia aurantium		Embryo, Larvae	T: 8, 12, 16, 20 S: 20, 25, 30, 34			Larval survival and growth	Survival at 8 – 20°C and S = 25-34; positive growth at 8-16°C, S = 30-34	T low = 8 T high = 20 S low = 25 S high = 34	Lee et al. 2009
Microcosmus exasperatus	X	Adult	T: 12–13 (low) 23–24 (control) 32–33 (high). S: 33–35 (low); 37–40 (control); 43–45 (high)	T: 5 weeks S: 2 weeks	T: G S: A	Survival	Poor survival T > 32ºC and S = 33-35	T low = 12 T high = 32 S low = 33 S high = 45	Nagar & Shenkar 2016
Microcosmus squamiger	X	Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	Development faster in T > 20°C. No larval development at 30°C. Best settlement success at 25°C. Metamorphosis and post-metamorphosis was most successful between 20-25°C.	T low = 20 T high = 25	Rius et al. 2014
P dalbyi		Adult	T: 16 S: 30, 23.5 / 33.5	40 min, 80-100 min for recovery	A	Ciliary pumping, squirt rate	Total cessation of pumping at 23.5 ppt. No recovery of pumping and some animals later died. Reduction of squirting rate and temporary in siphon diameter	S min = 23.5	Evans & Huntington 1992
Pyura herdmani		Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	Development faster in T>20°C. No develo-pment at 25 and 30°C. Best settlement success 20°C. Metamorphosis and post-metamorphosis most successful 20-25°C.	T low = 20 T high = 20	Rius et al. 2014

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Pyura stolonifera	X	Embryo, Larvae	T: 10, 15, 20, 25, 30 / 15-20	Embryonic and larval development: 10-48h after fertilization Larval settlement and metamorphosis: 72 h	A	Time of embryonic development, larval development success, larval settlement and metamorphosis	Development faster T > 20°C. No develop-ment at 25°C and 30°C. Best settlement success at 20°C. Metamorphosis and post-metamorphosis most successful 20-25°C.	T low = 20 T high = 20	Rius et al. 2014
Molgula manhattensis	X	Adult	S: Natural occurrence			Survival		S min ≤ 10	Dybern 1969
Molgula manhattensis	X	Adult	S: 9, 11, 16, 20, 32, 40	8h, 24h, 1 week.	G	Urine and blood osmotic, sodium and chloride concentrations.	Body fluids isosmotic in S = 16-40 ppt, and hyperosmotic in S < 16. Blood isoionic in S > 16, hyperosmotic in S < 16. Urine hyposmotic S = 11-40	S low = 9 S high = 40	Gaill & Lasserre 1977
Molgula socialis	X	Young (3 days old), adult (>1 month old)	T and S combinations. Young: T: 5, 10, 15, 20, 25, 30, 35; S: 15, 20, 25, 30, 35, 40, 45 Adult: T: 7, 10, 18, 25, 30; S: 15, 20, 25, 30, 35, 40, 45	15 days	G	Mortality, growth.	More tolerant to changes in T than to S. Young are more tolerant to high T, and adults to low T	T low = 5 T high = 30 S low = 15 S high = 45	Brunetti et al. 1985
Aplousobran-chia									
Clavelina huntsmani		Adult	T: 4, 7.5, 17.5, 22.5 / 12.5 S: 28, 35, 37.6 / 35			Cardiac function: beat rate, period of beating, reversal frequency	Beat rate increased with T, and period of beat decreased. Reversal frequency increased in low S. In high S the beat rate and the reversal frequency decreased.	T low = 4 T high = 22.5 S low = 28 S high = 37.6	Ponec 1982
Clavelina lepadiformis	X	Adult	S: Natural occurrence			Survival		S low = 14	Dybern 1969

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Eudistoma hepaticum		Larvae	S: 10, 16, 22, 26, 33 / 33	Variable (until larvae stop swimming for several min)	A	Swimming behavior to haloclines of different S	Haloclines of S 10, 16 were barriers to larvae. Halocline of S 26 was crossed without difficulty, but larvae soon sank or swam back to control halocline.	S low = 22	Vázquez & Young 1996
Eudistoma hepaticum		Larvae Juvenile	Attachment, S: 10, 16, 22, 26 / 33 Metamorphosis and mortality, S: 10, 16, 22, 26/ 33	Attachment: 24h Metamorphosis and mortality: 10 days	A	Larval attachment and metamorphosis, and juvenile mortality	Attachment: 100% at S $\geq$ 22, <20% in S 16, and 0% in S 10. Metamorphosis: complete at S $\geq$ 24	S low = 16	Vázquez & Young 2000
Eudistoma olivaceum		Larvae	S: 10, 16, 22, 26, 33 / 33	Variable (until larvae stop swimming for several min)	A	Swimming behavior to haloclines of different S	Haloclines of S 10, 16, 22 were barriers to larvae. Halocline of S 26 was crossed without difficulty.	S low = 26	Vázquez & Young 1996
Eudistoma olivaceum		Larvae Juvenile	Attachment, S: 10, 16, 22, 26 / 33 Metamorphosis and mortality, S: 10, 16, 22, 26 /33	Attachment: 24h Metamorphosis and mortality: 10 days	A	Larval attachment and metamorphosis, and juvenile mortality	Attachment: 80% at S $\geq$ 26, 40% in S = 22, and <20% in S $\leq$ 16. Metamorphosis: complete at S $\geq$ 26	S low = 26	Vázquez & Young 2000
Distaplia occidentalis		Adult	T: 15, 24.5	3 days	A	mortality	100% mortality at 24.5	T high < 24.5	Sorte et al. 2010
Didemnum perlucidum		Adult	T: Natural occurrence S: Natural occurrence			survival		T low = 13 T high = 30 S low = 27 S high = 39.5	Simpson et al. 2016
Didemnum vexillum		Adult	T: Natural occurrence S: Natural occurrence			survival		T low = -2 T high = ~24	Bullard et al. 2007
Didemnum vexillum	X	Adult	T: Ambient (19 - 21), cold (3 - 4<ambient), warm 1 (2 > ambient), warm 2 (4 -5>ambient)	1 week	A	Growth rate	Growth was greater in ambient than in the warmers treatments		McCarthy et al. 2007
Didemnum vexillum	X	Adult	T: <1-24 (daily range of ~ 11) (field)	2 years	G	Tolerance		T low = 1 T high = 24	Valentine et al. 2007

Species	IR	Life cycle stage	Variable: range / control	Exposure time	G/A	Parameter	Physiological results	Tolerance results [1]	Reference
Didemnum vexillum	X	Larvae	T: 12 – 22 (field) S: 28.5 – 31.3 (field)	1 year		Recruitment	First recruitment	T low = 18.4 T high = 22 S low = 28.5 S high = 31.3	Auker & Oviatt 2008
Didemnum vexillum	X	Adult	S: 26-30, 15-28, 10-26 (field)	2 weeks	G	Survival, growth	Growth was greater in high S, than in medium and low S	S low = 10 S high = 30	Bullard & Whitlatch 2009
Didemnum vexillum	X	Larvae	T: 3.4-22.6 (field)	2 years	G	Larval recruitment	Recruitment occurs in 14-20°C, and ceased in 9-11°C.	T low = 14 T high = 20	Valentine et al. 2009
Didemnum vexillum	X	Adult	T: 15, 24.5	3 days	A	mortality	100% mortality at 24.5	T high < 24.5	Sorte et al. 2010
Didemnum vexillum	X	Adult	2 hours, S: 10 / 34 2 weeks, S: 20, 27 / 34	2 h/every second day during 2 weeks 2 weeks	A	Growth, survival	Reduced growth in S < 34; 72% survived at S = 27, and 55% at S = 20; 100% survival in short exposure to S = 10	S low= 10 S high= 34	Gröner et al. 2011
Didemnum vexillum	X	Adult	S: 20, 27 / 34	2 weeks	A	Survival	82% survival at S = 27; 62% survival at S = 20	S low = 20 S high = 34	Lenz et al. 2011
Didemnum vexillum	X	Adult	Diluted water, S: 0, 5, 20	0,5, 1, 5, 10 min	A	Fouling on oysters	increase in D. vexillum fouling over time	S low = 0 S high = 20	Rolheiser et al. 2012
Didemnum vexillum	X	Adult	Freshwater	Freshwater : 4, 24h.		Mortality	100% mortality after h in freshwater		McCann et al. 2013
Diplosoma listerianum	X	Adult	2 hours, S: 10 / 34 2 weeks, S: 20, 27 / 34	2 h/every second day during 2 weeks 2 weeks	A	Growth, survival	Growth only in S = 34; 100% mortality at S <34, in long or short exposure	S low = 34 S high = 34	Gröner et al. 2011
Diplosoma listerianum	X	Adult	S: 20, 27 / 34	2 weeks	A	Survival	0% survival after 10 days at S = 20 and 27	S low = 34 S high = 34	Lenz et al. 2011
Diplosoma listerianum	X	Adult	T: 15, 24.5	3 days	A	mortality	100% mortality at 24.5	T high < 24.5	Sorte et al. 2010
Diplosoma listerianum	X	Adult	T: 21, 25, 29, 34	24 h	G	LT50 values	East/west coast USA LT50 = 29.1/27.9 °C	T high = 29.5	Sorte et al. 2011

[1]Tolerance results show figures of maximum and minimum temperature and salinity conditions in which the species survived, even for a short time in the conditions of the study. In many cases the authors did not test the species beyond their limits, thus the numbers given are probably not true limits, but rather are experimental results.

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