Supplement

Effect of prey selectivity and trophic cascades induced by mesozooplankton on the dynamics of phytoplankton

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Text S1. MATERIALS and METHODS

S1.1. Analysis of ciliates by FlowCAM

We used a benchtop continuous imaging flow cytometer (FlowCAM VS-IV, Fluid Imaging Technologies, Inc., Maine, USA) to capture the images of particles in our samples. FlowCAM has been used for the quantification and size estimation of phytoplankton and zooplankton, which provide indications about the abundance, size structure, and community composition of plankton groups (Álvarez et al., 2014; Buskey and Hyatt, 2006; Sieracki et al., 1998; Wang et al., 2017). Previous studies on copepod clearance on ciliates and phytoplankton suggested good agreements in rates derived from FlowCAM and microscopic methods (Ide et al., 2008; Liu et al., 2005). Samples were analyzed under the AutoImage mode with a 10X objective (100X overall magnification) and a 100 μm flow cell. Prior to sample analysis, focus and size calibration were performed with 1 mL of 20 μm standard beads under auto focus mode. Samples were manually primed to eliminate any bubbles and debris inside the flow cell. 1–5 mL samples were then processed with a flow rate of 0.15 mL/min, a camera frame rate of 20 frames s^{-1}, and an estimated efficiency of particle capture at ~25%. The fluid flow rate and camera frame rate were selected to maximize the capture efficiency while avoiding image duplication.

Image analysis for filtering ciliates was performed with the Visual Spreadsheet software (version 3.7.5). Two filtering function are available with the software: the value filter results in highly identical particles to the library but often overlook other target particles that have lower similarity to the library; while the Statistical filter tends to include non-target particles but seldom leave out the targeted particles. Prior to ciliates filtering, we removed invalid images (e.g., detritus, bubbles, and duplicate images) with the combination of the Value and Statistical filters. Ciliates were identified from the images according to published literature that described free-living ciliates in the Pearl River Estuary and other coastal areas (Li et al., 2019; Song et al., 2009; Zhang et al., 2012). Initially, we built the library for filtering the ciliates by selecting 15-20 ciliates which different in size and shape from our samples, then we used this library to filter all the samples to build a more representative filter. The final library has 30 organisms in it to achieve the balance of selecting all the ciliates while omitting other particles (*see* Fig. S1). Ciliates were filtered with the library under the Statistical mode, with the results then visually checked to remove any non-target particles. The cell biovolume was calculated from the equivalent spherical diameter of each ciliate. The carbon content of ciliates was then estimated based on different volume-to-carbon conversion factors for aloricate and loricate ciliates (Menden-Deuer and Lessard, 2000).
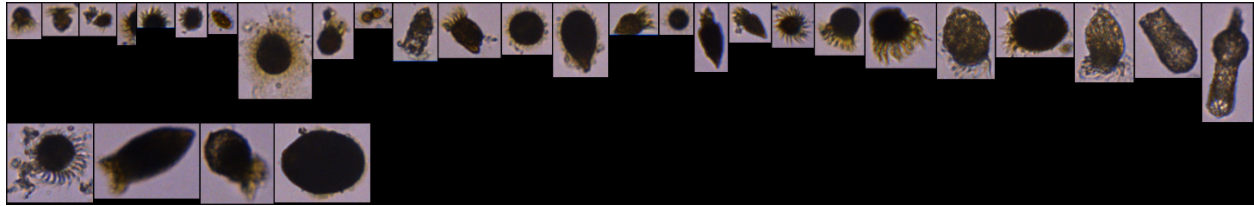
Fig S1. A FlowCAM library comprising of both aloricate and loricate ciliates in various shapes and sizes built from our samples. All images were taken under 100X magnification.

S1.2. Calculations of grazing rates

The mesozooplankton grazing rate g_{meso} (d^{-1}) on phytoplankton was calculated by the following equations (Frost, 1972):

$$k_t = k_{intrinsic} - g_{micro} - g_{meso}, \qquad (1)$$

$$k_c = k_{intrinsic} - g_{micro}, \qquad (2)$$

where k_t and k_c represent the net growth rate of phytoplankton in the treatments and controls, respectively. $k_{intrinsic}$ is the intrinsic growth rate of phytoplankton, g_{micro} is the grazing rate of microzooplankton, and g_{meso} is the grazing rate of mesozooplankton. Assuming the microzooplankton grazing rate on phytoplankton is not affected by the existence of mesozooplankton, then the grazing rate of mesozooplankton can be calculated based on Equations (1) and (2). In such an aspect, the difference in the net growth rate of phytoplankton between treatments and controls is only affected by mesozooplankton grazing.

$$g_{meso} = k_c - k_t = ln(P_c/P_t)/t - ln(P_t/P_0)/t = ln(P_c/P_t)/t \qquad (3)$$

Here, P_0 is the initial concentration of phytoplankton, P_t and P_c stand for the concentrations of phytoplankton in treatments and controls at the end of the incubation, respectively, and t is the incubation time ($t = 1$ d for our experiment).

Mesozooplankton clearance rate (F, L mg^{-1} d^{-1}) and ingestion rate (I, µg mg^{-1} d^{-1}) can be calculated such as:

$$F = g_{meso}/(d_w / V) = V \times g_{meso} / d_w, \qquad (4)$$

$$I = F \times P_{mean}, \qquad (5)$$

where V (l) is the volume of the incubation bottle, d_w (mg) is the dry weight of mesozooplankton, and P_{mean} (µg L^{-1}) is the mean concentration of phytoplankton throughout the incubation period

that is calculated such as:

$$P_{mean} = (P_t - P_0) \,/\, (lnP_t - lnP_0). \tag{6}$$

The calculation of the grazing rate of mesozooplankton from Equations (1) and (2) is based on the hypothesis that g_{micro} is not affected by the existence of mesozooplankton. However, under field conditions, mesozooplankton can generally affect the microzooplankton population through ingestion; thus, g_{micro} is changed to g'_{micro} in the treatments. Thus, Equation (1) should be corrected as follows.

$$k_t = k_{intrinsic} - g'_{micro} - g_{meso} \tag{7}$$

g_{meso} should be corrected using the following formula:

$$g_{meso} = k_c - k_t + g_{micro} - g'_{micro} = \ln(P_c/P_t)/t + TC, \tag{8}$$

where trophic cascade effect (TC) is calculated as the difference between the microzooplankton grazing rates of the treatment and control samples in mesozooplankton incubation experiments ($TC = g_{micro} - g'_{micro}$), which represents the indirectly promoted effect of mesozooplankton on phytoplankton through ingesting microzooplankton.

Because g_{micro} and g'_{micro} are unresolved by the mesozooplankton grazing experiment, a parallel study of microzooplankton grazing experiment was conducted. g_{micro} and g'_{micro} can then be estimated from the regression relations between g_{micro} and phytoplankton concentrations.

Assuming the intrinsic growth rate of phytoplankton (k) is not changed by dilution, and the grazing rate of microzooplankton is linearly related to the proportion of unfiltered water. Then, the microzooplankton grazing rate can be calculated based on the changes in phytoplankton growth rate over incubation time t, as follows:

$$ln(P_t/P_0)/t = k - d \times g, \tag{9}$$

where P_0 and P_t are Chl *a* concentrations before and after the incubation, respectively. d indicates the dilution factor of each bottle. g represents the microzooplankton grazing rate, which is estimated by a linear regression (Landry and Hassett, 1982). The regression relationship between prey concentration and microzooplankton ingestion rate was fitted to the Michaelis-Menten equation:

$$I = I_{max} \times \frac{P}{k_d + P}, \tag{10}$$

where I (cells predator^{-1} d^{-1}) is the ingestion rate of microzooplankton, I_{max} is the maximum

ingestion rate, k_d is the half-saturated concentration, and P is the concentration of phytoplankton (an independent variable in the function). As the ingestion rate is calculated by the clearance rate and grazing rate ($I_{micro} = F_{micro} \times P_{mean} = g_{micro}/M_{mean} \times P_{mean}$), that is

$$g_{micro} = M_{mean} \times I_{micro}/P_{mean}, \tag{11}$$

where M_{mean} and P_{mean} are the average concentrations of microzooplankton and phytoplankton during the incubation, respectively. By combining Equations (10) and (11), the regression relationship among microzooplankton grazing rate $g(x, y)$ and average concentrations of predator (y) and prey (x) is calculated as follows.

$$g(x,y) = \frac{y}{x} \times \left[I_{max} \times \frac{x}{k_d+x} \right] = y \times \frac{I_{max}}{k_d+x} \tag{12}$$

Finally, trophic cascade rate and corrected mesozooplankton grazing rate can be calculated by combining Equation (8) and Equation (12).

Text S2. RESULTS

In microzooplankton grazing experiments, the mean growth rate of phytoplankton was 4.25 ± 2.69 d^{-1} and the mean grazing rate of microzooplankton on phytoplankton was 2.86 ± 1.34 d^{-1} (Fig. S1). On average, $86 \pm 48\%$ of phytoplankton growth was grazed by microzooplankton, with a highly variable range of 23–209%. Overall, microzooplankton grazing rates on phytoplankton were higher in the spring and summer than those in the autumn and winter. Based on regression relations between microzooplankton ingestion rates on individual size fractions of phytoplankton and their mean Chl a concentration during the incubation, and subsequent fitting to a Michaelis-Menten equation, the maximum ingestion rate of microzooplankton (I_{max}, represented by ciliates in this study) on total phytoplankton was estimated to be 3.963 µg C ciliate^{-1} d^{-1} and the half-saturated prey concentration (k_d) was 5987 µg C L^{-1} (Table 2). However, the highest phytoplankton concentration was recorded at 1500 µg C L^{-1}, far below the half-saturated concentration, during the experiments. Indeed, ciliates had a much higher maximum ingestion rate (reaching 3 µg C ciliate^{-1} d^{-1}) on the nano-size category (2–20 µm) than those (less than 0.8 and 1.2 µg C ciliate^{-1} d^{-1}) on micro- (> 20 µm) and pico-phytoplankton (< 2 µm), showing a feeding selectivity on particular prey sizes.
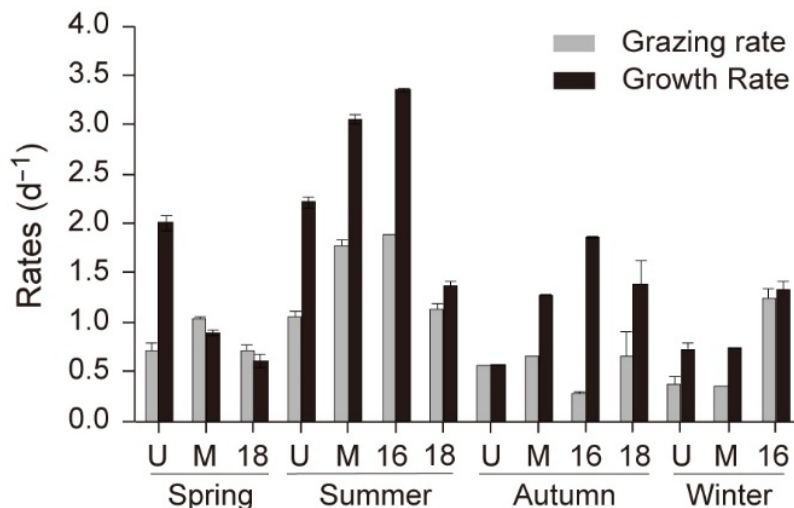
Fig. S2. Growth rate of phytoplankton and gazing rate of microzooplankton in the Pearl River estuary.

Table S1. Nutrient concentrations (µM) of the surface and bottom water in the Pearl River estuary

Season	Station	Surface			Bottom		
		DIN	Silicate	Phosphate	DIN	Silicate	Phosphate
Spring	1#	282.49	120.71	2.14	258.49	117.86	2.31
	2#	253.09	59.29	1.93	237.52	120.00	1.71
	3#	284.00	137.14	2.59	263.39	125.71	2.18
	4#	227.55	92.86	1.55	250.13	98.57	1.54
	5#	160.08	105.71	1.30	159.54	97.86	1.22
	6#	245.95	82.14	1.72	239.89	75.71	1.70
	7#	221.79	75.71	1.15	192.05	71.43	1.26
	8#	159.87	76.43	1.10	132.16	60.71	0.98
	9#	124.35	63.57	1.19	119.85	60.71	1.18
	10#	100.11	39.29	0.80	85.35	36.07	0.72
	11#	144.57	75.00	1.30	141.50	82.14	1.35
	12#	83.16	41.43	0.89	84.74	38.57	0.87
	13#	65.30	5.36	0.06	62.08	6.43	0.10
	14#	60.08	20.36	0.44	41.62	14.29	0.31
	15#	74.99	47.14	0.23	52.46	29.29	0.21
	16#	38.84	6.43	0.15	38.33	6.79	0.10
	18#	12.85	6.07	0.13	8.54	11.79	0.16
Summer	1#	174.48	105.00	1.85	185.59	117.14	1.93
	2#	183.30	117.14	1.57	182.84	94.64	1.61
	3#	143.85	112.50	1.20	119.74	96.79	1.50
	4#	161.31	67.86	1.20	155.03	99.29	1.00
	5#	129.45	105.00	1.09	135.91	99.64	0.98
	6#	151.21	111.07	1.41	139.02	105.36	1.85
	7#	98.31	59.64	0.99	100.44	101.79	1.24
	8#	111.41	98.21	0.84	72.10	79.64	0.88

Season	Station						
	9#	126.53	91.79	1.12	112.98	53.93	1.18
	10#	129.21	64.29	2.23	91.61	88.57	1.46
	11#	112.51	111.43	1.18	110.81	114.29	1.19
	12#	89.85	101.43	0.79	81.87	95.71	0.79
	13#	104.27	110.00	0.91	92.96	95.71	0.91
	14#	111.42	112.86	1.11	26.45	39.29	0.66
	15#	91.65	101.43	0.61	68.48	80.00	0.54
	16#	61.36	78.57	0.50	40.42	60.00	0.75
	17#	71.99	84.29	0.32	34.83	47.50	0.33
	18#	75.03	97.14	0.34	19.51	32.50	0.26
Autumn	4#	144.21	70.00	2.12	158.46	63.41	2.27
	5#	124.37	64.96	1.42	124.50	57.21	1.53
	6#	155.07	51.78	1.29	154.97	54.88	2.18
	7#	111.56	58.76	2.09	109.27	52.95	2.86
	8#	102.97	48.68	2.82	82.02	38.60	3.05
	9#	117.15	48.68	2.41	114.40	51.78	2.18
	10#	87.31	44.81	1.73	78.28	32.02	1.63
	11#	122.74	44.03	1.59	121.95	45.97	1.58
	12#	98.34	46.36	1.59	117.63	46.36	1.58
	13#	80.58	45.58	1.17	62.85	38.22	1.25
	14#	88.30	28.53	2.80	40.31	15.74	2.45
	15#	71.85	25.04	2.56	61.50	20.78	2.40
	16#	64.13	30.47	2.43	67.98	0.62	2.51
	17#	64.69	21.94	2.46	17.11	9.15	2.03
	18#	35.80	13.41	2.21	12.83	7.98	1.95
Winter	1#	193.21	101.40	2.27	201.36	102.17	2.73
	2#	196.48	102.56	1.97	203.10	102.56	2.00
	3#	179.12	83.57	1.92	181.76	83.95	1.90
	4#	170.43	80.85	1.99	156.02	90.16	2.02
	5#	132.68	64.96	1.67	118.08	75.04	1.41
	6#	148.69	68.06	2.25	134.28	64.96	2.67
	7#	105.03	62.64	1.61	100.13	61.09	1.55
	8#	75.31	48.68	1.22	69.51	52.22	1.26
	9#	138.48	80.08	1.67	135.36	82.02	1.61
	10#	42.49	21.94	1.32	41.19	20.78	1.23
	11#	114.09	80.25	1.56	110.38	79.63	1.58
	12#	87.90	68.47	1.50	97.59	73.12	1.54
	13#	66.59	43.26	1.08	57.87	43.26	0.54
	14#	26.64	20.67	0.83	24.36	18.19	0.82
	15#	54.18	45.40	1.14	43.38	36.40	1.00
	16#	39.17	32.45	0.97	31.37	27.80	0.92
	17#	8.48	12.06	0.58	8.30	10.59	0.64
	18#	8.54	12.64	0.28	7.98	12.25	0.26

Table S2. Environmental parameters for mesozooplankton feeding experiments

Season	Station	S	T (°C)	> 20 µm Chl *a*	2–20 µm Chl *a*	< 2 µm Chl *a*	Dry weight in field	Dry weight in bottles
Spring	#7	2.75	27.72	5.91	4.76	0.71	2.2	0.43
	#13	17.37	28.29	7.82	4.21	3.70	27.0	0.69
	#18	30.28	27.55	0.49	0.29	0.70	10.1	3.10
Summer	#8	10.42	29.17	2.20	3.20	1.80	4.6	1.33
	#13	6.60	30.37	1.24	3.79	1.82	12.9	0.78
	#16	21.13	28.01	1.69	3.39	0.95	9.1	1.35
	#18	15.99	29.87	5.59	2.82	5.11	13.4	2.25
Autumn	#8	0.07	27.69	0.50	0.31	1.09	5.1	0.42
	#13	8.04	24.84	0.64	1.42	0.18	2.6	0.91
	#16	10.8	25.36	0.20	0.70	0.15	1.5	0.25
	#18	26.93	26.28	0.43	2.59	1.32	8.9	0.60
Winter	#8	23	19.53	0.38	0.92	0.18	9	1.29
	#9	4.79	17.1	1.61	2.56	0.28	54.5	1.49
	#13	23.5	18.9	0.42	1.67	0.88	13.6	1.06
	#16	28.16	19.31	0.16	0.67	0.62	40.3	2.25

S, salinity; T, temperature; Chl *a*, chlorophyll *a* concentration in µg l^{-1}; Dry weight of mesozooplankton in mg m^{-3}

Table S3. Initial prey concentrations of grazing experiments in the Pearl River estuary

Season	Station	Ciliates abundance (Cells l^{-1})	Ciliates biomass (µg C l^{-1})	Phytoplankton concentration (µg Chl *a* l^{-1})	Phytoplankton biomass (µg C l^{-1})
Spring	#7	1783	5.25	11.39	706.2
	#13	2337	16.79	15.74	975.9
	#18	2060	1.31	1.47	91.1
Summer	#8	1702	48.56	7.20	446.4
	#13	1581	12.58	6.85	424.7
	#16	2694	24.12	6.04	374.5
	#18	1734	9.66	13.51	837.6
Autumn	#8	3801	0.99	1.91	118.4
	#13	3235	0.92	2.24	138.9
	#16	4721	1.12	1.05	65.1
	#18	3448	6.67	4.34	269.1
Winter	#8	3558	11.62	1.48	91.8
	#13	7274	4.63	2.49	154.4
	#16	2409	9.70	2.97	184.1

Table S4. Mesozooplankton compositions in experimental stations in the Pearl River estuary

Groups	Species	Abundance of mesozooplankton in dfferent sasons and stations (ind. m⁻³)											
		Spring			Summer				Autumn				Winter	
		#7	#13	#18	#8	#13	#16	#18	#8	#13	#16	#18	#8	#13
Cnidarians	*Solmundella bitentaculata*											0.7		
	Liriope tetraphylla			4.22		2.14	0.7						1.1	
	Aglaura hemistoma											0.7	3.3	
	Bougainvillia muscus								0.7			0.7		
	Turritopsis nutricula	0.73									0.3			
	Podocorynoides minima						0.7							
	Euphysora sp													
	Ectopleura xiamenensis	2.93					5.2						1.1	
	Eirene conica											0.7		
	Eucheilota menoni	1.46					2.2							
	Clytia folleata	10.98	1.54						0.7				5.5	1.5
	Obelia spp	0.73			0.99		6.7	0.8					1.1	
	Nanomia bijuga			0.84										1.5
	Lensia subtiloides							2.5						
	Diphyes chamissonis						1.5	0.8						
Ctenophores	*Pleurobrachia globosa*		0.77											
Pteropods	*Creseis clava*							2.5						
Cladocerans	*Diaphanosoma leuchtenbergianum*	0.73												
	Penilia avirostris	10.24	131.18	504.83	13.80	57.66	42.3	113.7						
	Evadne tergestina	16.10	3.86	21.12	11.83	17.08	7.4	120.3						
Copepods	*Calanus sinicus*													
	Canthocalanus pauper						9.1					1.4		1.5

	Species													
	Undinula vulgaris											31.8		
	Subeucalanus crassus													
	Subeucalanus subcrassus	6.59		0.42	1.97		3.7	4.1			1.4	17.7	1.1	1.5
	Acrocalanus gracilis										3.1	113.2		
	Paracalanus aculeatus	2.20									0.3			
	Paracalanus gracilis											106.1		
	Paracalanus parvus	73.91	46.30		22.18	34.17	8.9	22.3	1.4	0.4		7.1	4.4	6.2
	Parvocalanus crassirostris	248.79	3.86		71.45	113.18	69.7	34.6	3.4					
	Clausocalanus furcatus													
	Euchaeta copepodite larvae												1.1	
	Temora discaudata											3.5		
	Temora turbinata	8.05	23.15	16.90	2.96	17.08	5.9	38.7			3.1	42.4		1.5
	Centropages furcatus			0.42				3.3			0.3	7.1		0.0
	Centropages tenuiremis													6.2
	Pseudodiaptomus marinus							0.8	2.1	0.4				
	Pseudodiaptomus penicillus										0.7			
	Pseudodiaptomus poplesia										0.3			
	Candacia bradyi					2.14		3.3				0.7		
	Labidocera bipinnata		34.72											1.5
	Pontella chierchiae		0.00	0.42										
	Pontellina plumata											3.5		
	Acartia erythraea						5.9	14.0						
	Acartia pacifica			16.90		21.35						53.1		
	Acartia spinicauda	31.47	540.16		34.99				258.4	68.7	114.4		225.8	284.3
	Acartiella sinensis	90.74	3.86		31.54				161.9	15.5	3.1	7.1	21.0	3.1
	Tortanus gracilis					4.27		8.2	1.4		0.3	7.1	2.2	
	Oithona brevicornis	31.47			1.48			3.3					1.1	
	Oithona plumifera							4.1					1.1	
	Oithona similis		3.86			29.90	33.4	64.3	3.4		0.7	31.8		

	Oithona simplex	10.24			2.46	36.30	26.0							
	Microsetella norvegica	0.73					0.7						3.5		
	Euterpina acutifrons					1.5	4.9						0.0		
	Oncaea venusta					2.14	2.2	2.5					3.5		
	Sapphirina nigromaculata												0.7		
	Corycaeus dahli	0.73			0.49		0.8					7.1	6.6	1.5
	Corycaeus pacificus			0.42							0.3			2.2	
	Caligus sp								0.7						
Ostracods	*Euconchoecia aculeata*			0.42				2.5					0.7		
Mysidaceans	*Acanthomysis laticauda*	1.46													
Cephalopods	Gammaridea spp												3.5		
Euphausiids	*Pseudeuphausia sinica*													1.1	1.5
Decapods	*Lucifer hanseni*	3.66		0.42	4.44			9.9	10.3	1.8	0.7	1.4	1.1	
	Acetes japonicus			0.84	0.00										
Polychaetes	*Krohnitta pacifica*														
	Aidanosagitta regularis										0.3	10.6			
	Flaccisagitta enflata			3.4		4.3	0.7	3.3	2.8		1.7	63.7	6.6	12.4
	Zonosagitta bedoti		0.8			2.1			3.4	2.1	1.0	10.6		
	Oikopleura interrmedia	33.7		4.2	1.0		24.5	42.9				3.5	3.3	4.6
Tunicates	*Dolioletta gegenbauri*														0.0
	Doliolum denticulatum	1.5		111.9	0.5		3.7	41.2						1.5
Planktonic larvae	Hydroidomedusae larvae	30.0			3.4		0.7								
	Pilidum larvae (Nemertea)														
	Polychaeta larvae	5.9	1.5	0.4	2.5	4.3	11.9	6.6				7.1		3.1
	Bivalvia larvae	2.2			0.5			1.6						
	Gastropoda larvae		1.5					0.8						
	Nauplius larvae (Copepoda)	18.3			4.4	14.9	10.4	12.4				3.5		1.5
	Nauplius larvae (Cirripedia)	18.3	0.8		16.3	44.8	17.8	59.3						1.5
	Cypris larvae	0.7						1.6						

	Macrura larvae	16.1	6.2	14.8	18.2	21.4	14.8	33.8				10.6		
	Zoea larvae (Porcellana)	0.0	3.1									3.5		
	Zoea larvae (Brachyura)	19.0	3.9	2.1	21.7	23.5	19.3	23.9				0.7		1.5
	Alima larvae		3.1					5.8				2.8		
	Cyphonautes larvae													
	Actinotrocha larvae (Phoronida)	0.7												
	Auricularia larvae	8.0					1.5							
	Ophiopluteus larvae	6.6		2.1	1.5		39.3	23.9						3.1
	Ophiuroidea larvae													
	Tornaria larvae						2.2							
	Tadpole larvae							0.8						
	Fish eggs	13.9	2.3	1.3	9.9	6.4	3.0	4.1				1.4		
	Fish larvae	5.1	2.3		9.9	6.4	0.7	3.3						1.5

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