# Marked seasonality and high spatial variation in estuarine ciliates are driven by exchanges between the 'abundant' and 'intermediate' biospheres 

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Figure S1 Rarefaction curve for individual samples (left) as well as for the total community (right).



Figure S2 Variations of the taxonomic composition of the total community along salinity zones.


Figure S3 Venn diagram of OTUs from three delineated salinity groups: Freshwater, Oligohaline and Mesohaline, and Polyhaline and Euhaline.


Figure S4 Location of sampling sites in Jiulong River Estuary. Inset depicts Fujian coast near southwestern Taiwan Strait. The sampling map was generated with Surfer version 7 (Golden Software, http://www.goldensoftware.com/products/surfer). The inset was generated with Ocean Data View version 4 (Schlitzer, R., Ocean Data View, odv.awi.de, 2017).


Table S1 Information of the sampling sites in Jiulong River Estuary.

| Sampling sites | Latitude | Longitude | Sampling <br> year | Sampling frequency |
| :---: | :---: | :---: | :---: | :--- |
| A5 | 24.447 | 117.824 | 2014 | Every two months |
| A6 | 24.431 | 117.849 | 2014 | Every two months |
| A7 | 24.407 | 117.888 | 2014 | Every two months |
| A9 | 24.401 | 117.961 | 2014 | Every two months |
| JY0 | 24.423 | 118.004 | 2014 | Every two months |
| JY1 | 24.421 | 118.035 | 2014 | Every two months |
| JY2 | 24.418 | 118.067 | 2014 | Every two months |
| KM2 | 24.366 | 118.133 | 2014 | Every two months |

Table S2 Variations of the environmental factors across the sampling period in Jiulong River Estuary.

| Sampling <br> date | Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Salinity <br> $(\mathrm{PSU})$ | pH | $\mathrm{NO} 3-\mathrm{N}$ <br> $(\mu \mathrm{mol} / \mathrm{L})$ | $\mathrm{NO} 2-\mathrm{N}$ <br> $(\mu \mathrm{mol} / \mathrm{L})$ | $\mathrm{NH} 4-\mathrm{N}$ <br> $(\mu \mathrm{mol} / \mathrm{L})$ | DTN <br> $(\mu \mathrm{mol} / \mathrm{L})$ | DRP <br> $(\mu \mathrm{mol} / \mathrm{L})$ | $\mathrm{Chl}-a$ <br> $(\mu \mathrm{~g} / \mathrm{L})$ | Bacteria <br> $\left(10^{5} \mathrm{cells} / \mathrm{ml}\right)$ |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 2014.02 .12 | $12.5-15.1$ | $0.4-28.4$ | - | $51.1-224.9$ | $3.6-14.9$ | $18.1-241.8$ | $153.5-563.4$ | $1.74-9.70$ | $1.65-19.85$ | $7.87-47.87$ |
| 2014.04 .17 | $19.5-23.0$ | $0.5-30.0$ | $6.92-8.00$ | $27.8-167.7$ | $3.4-40.4$ | $27.3-166.5$ | - | $1.20-5.36$ | $0.89-8.82$ | $16.12-42.05$ |
| 2014.06 .04 | $25.3-27.7$ | $0.0-27.0$ | $6.91-8.09$ | - | - | $36.7-84.3$ | $186.0-368.9$ | $2.35-7.00$ | $0.47-4.02$ | $10.00-43.41$ |
| 2014.08 .07 | $30.1-31.9$ | $0.0-29.8$ | $6.73-7.99$ | $27.6-163.8$ | $3.5-19.0$ | $63.5-123.8$ | $168.7-419.3$ | $1.20-4.89$ | $1.14-15.6$ | $36.08-86.34$ |
| 2014.10 .10 | $26.0-26.4$ | $2.7-31.1$ | $7.02-8.08$ | $14.8-178.7$ | $6.9-50.1$ | $12.7-53.3$ | $104.9-370.6$ | $0.05-1.10$ | $0.36-10.95$ | $9.75-45.53$ |
| 2014.12 .12 | $17.7-18.6$ | $4.0-29.5$ | $7.54-8.05$ | $0.70-2.64$ | $0.07-0.48$ | $0.4-1.4$ | - | $0.05-0.13$ | $0.75-5.12$ | $7.43-16.87$ |

Table S3 Similarity within and between the three salinity groups $\pm$ standard deviation (ANOSIM, $\mathrm{P}<0.001$ ) as determined by Bray-Curtis similarity coefficient. F: freshwater; OM: oligohaline and mesohaline; PE: polyhaline and euhaline.

|  | Freshwater | OM | PE |
| :--- | :---: | :---: | :---: |
| Freshwater | $45.6 \pm 16.4$ |  |  |
| OM | $27.7 \pm 12.6$ | $38.8 \pm 12.9$ |  |
| PE | $11.1 \pm 10.4$ | $26.0 \pm 11.3$ | $40.1 \pm 13.0$ |

Table S4 ANOSIM statistics tests of the groupings of communities according to salinity, temperature, and depth. Abbreviations: F, freshwater; OM, oligohaline and mesohaline; PE, polyhaline and euhaline. Community turnover was based on the Bray-Curtis distance.

|  | Total |  |
| :--- | :---: | :---: |
| Grouping by | R | P |
| Salinity (global test) | 0.660 | $<0.001$ |
| F versus OM | 0.527 | $<0.001$ |
| OM versus PE | 0.587 | $<0.001$ |
| F versus PE | 0.917 | $<0.001$ |
| Temperature (global test) | 0.252 | $<0.001$ |
| Depth (global test) | -0.066 | $>0.05$ |

Table S5 Relative abundances of reads and OTUs in the abundant, intermediate and rare groups.

|  | Minimum | Maximum | Average | SD |
| :--- | :---: | :---: | :---: | :---: |
| Relative abundance of abundant OTU | 2.597 | 18.382 | 11.617 | 3.114 |
| Relative abundance of intermediate OTU | 50.000 | 76.147 | 63.925 | 5.686 |
| Relative abundance of rare OTU | 11.010 | 44.286 | 24.459 | 6.240 |
| Relative abundance of abundant reads | 79.764 | 98.293 | 90.165 | 3.562 |
| Relative abundance of intermediate reads | 1.646 | 19.980 | 9.674 | 3.544 |
| Relative abundance of rare reads | 0.061 | 0.304 | 0.162 | 0.052 |

Table S6 BVSTEP and BIOENV analyses showing the correlations between environmental variables and the total/bimonthly collected community (which was randomly resampled from 14,825 to 100 sequences per sample).

| Environment | BV-STEP factors | $\rho$ | BIO-ENV factors | $\rho$ |
| :---: | :---: | :---: | :---: | :---: |
| All samples | Salinity, Temperature, Chl $a$, Violaxanthin | 0.589 | Salinity | 0.519 |
|  |  |  | Violaxanthin | 0.368 |
| February samples only | Salinity, Temperature, DRP | 0.851 | Salinity | 0.797 |
|  |  |  | DRP | 0.669 |
| April samples only | Bacteria, $\quad \mathrm{NH}_{4}, \quad$ DRP, | 0.740 | Salinity | 0.629 |
|  |  |  | Temperature | 0.219 |
| June samples only | Salinity, Temperature, $\mathrm{NH}_{4}$, Neoxanthin, Violaxanthin | 0.863 | Salinity | 0.780 |
|  |  |  | DRP | 0.755 |
| August samples only | Salinity, Bacteria, Fucoxanthin, Alloxanthin, Diadinoxanthin | 0.929 | Salinity | 0.915 |
|  |  |  | Diadinoxanthin | 0.848 |
| October samples only | Temperature, Neoxanthin | 0.505 | Temperature | 0.366 |
|  |  |  | $\text { Chl } b$ | 0.339 |
| December samples only | Salinity, Temperature | 0.854 | Salinity | 0.932 |
|  |  |  | $\mathrm{NH}_{4}$ | 0.770 |

