



Fate of antibiotic resistance in estuaries and marine environment

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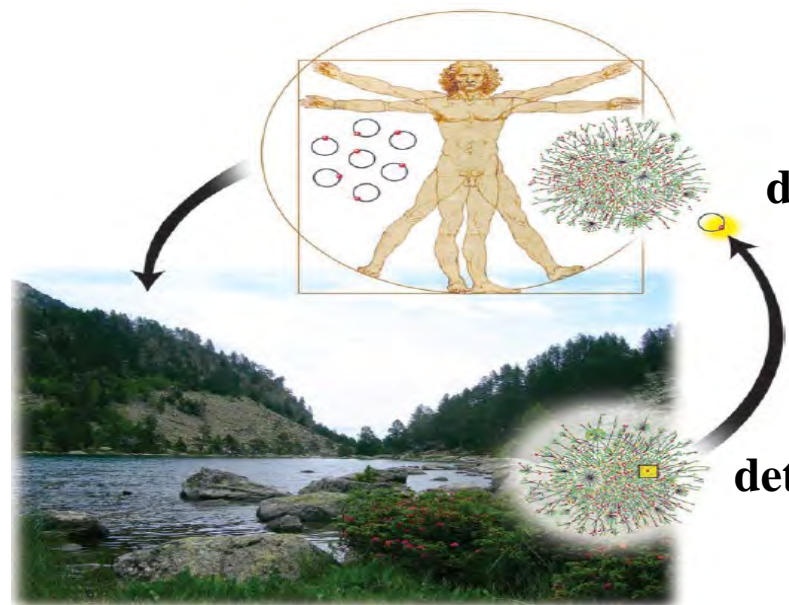
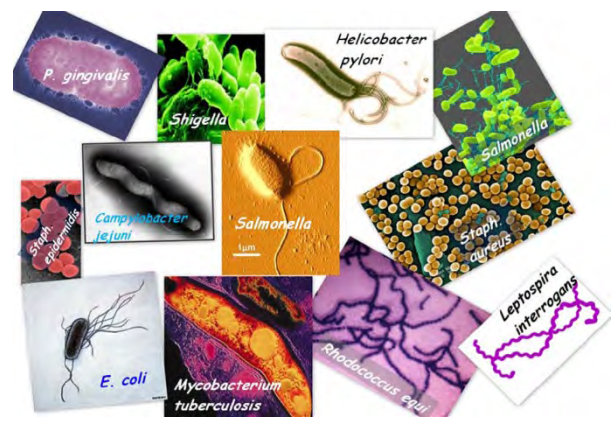




Research background

With the increase in resistance levels, at least **700,000** people die due to antibiotic-resistant infections each year, and this figure will exceed **10,000,000** in **2050**.

----- WHO, 2014

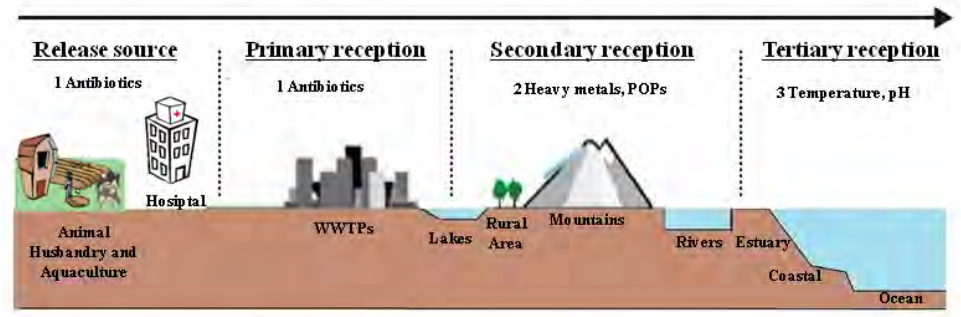


From Natural to Clinical Environments: A Functional Shift

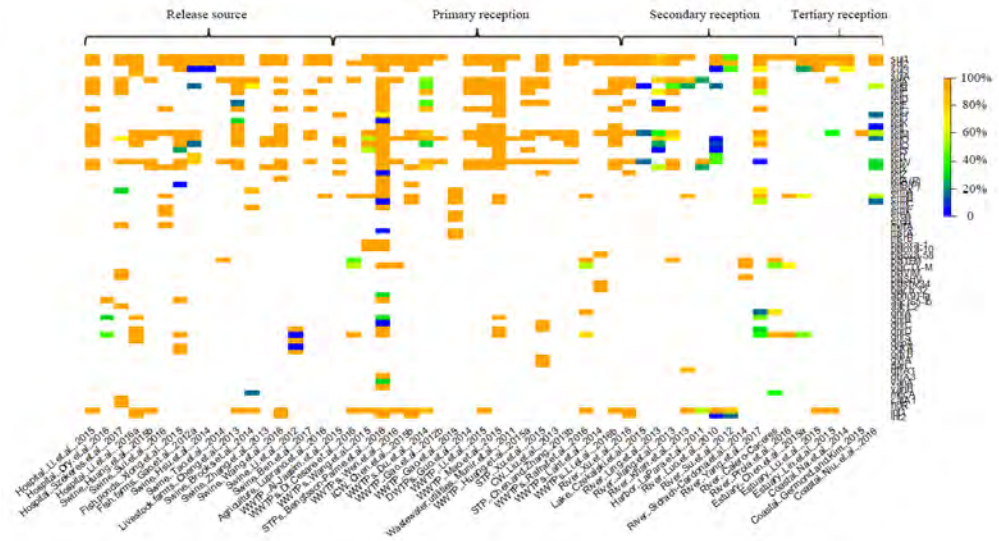
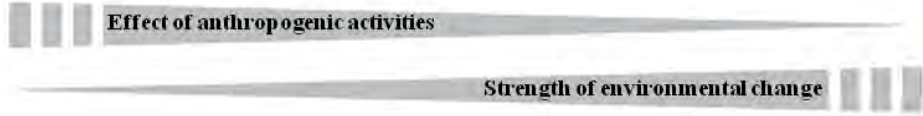
Transfer of antibiotic resistance genes from natural clinical environments.



Research background



ARGs are widely distributed in various environmental compartments.



The detection rates of ARGs in the release sources and receiving systems both reached 80%.

The detection frequency of ARGs



Research background

- ◆ Little information exist on the migration and attenuation of ARGs in estuary ecosystem, which is central to predicting their fate after release into marine environment. **Disappearance kinetics / Fate mechanisms ?**



Sulfonamide antibiotics in the Northern Yellow to resistant bacteria: Implications for antibiotic

Guangshui Na^{a,b,*}, Wanru Zhang^{a,c}, Shiyao Zhou^d, Hui Gao Yaqi Cai^b, Ziwei Yao^a



Fate of sulfonamide resistance genes in estuary environment and effect of anthropogenic activities

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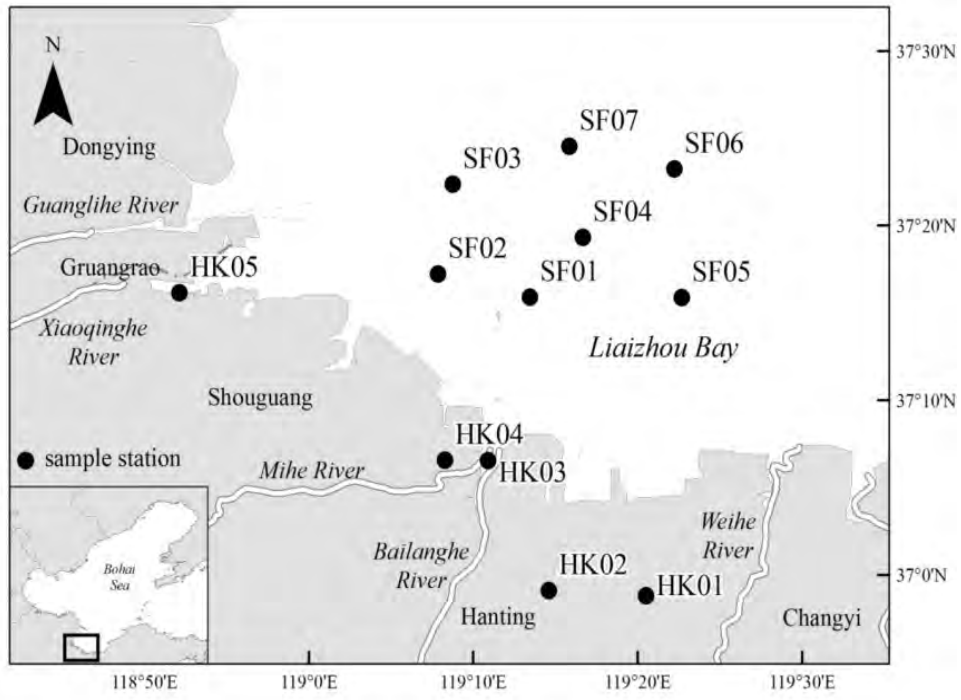
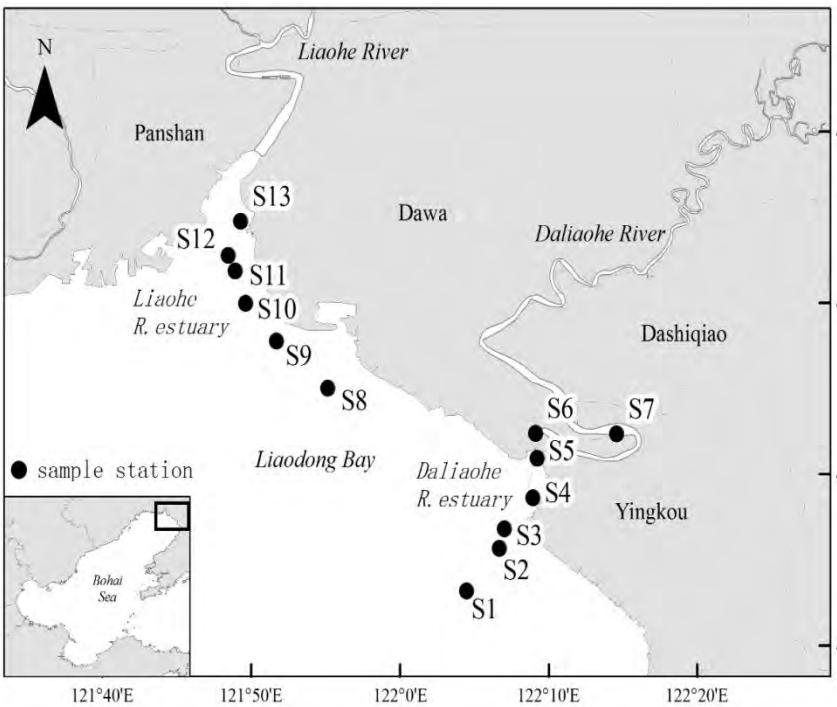
The present study aimed to :

- (1) investigate the fate of antibiotic resistance in estuaries and marine environment;
- (2) clarify the effect of estuarine and marine physicochemical conditions on the maintenance and propagation of antibiotic resistance.



Research regions and methodology

● Research regions



Location of the sampling sites



Research regions and methodology

● Methodology

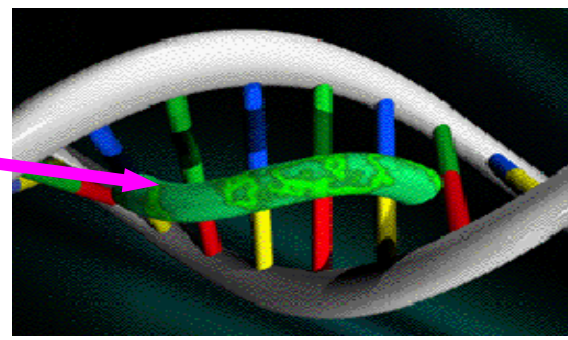


High-performance liquid chromatography-mass spectrometry analysis of antibiotics.



The target gene was obtained by real-time PCR (RT-PCR) with SYBR Premix Ex Taq II (Tli RNaseH Plus) and a light cycler 480 real-time PCR instrument.

SYBR Green I

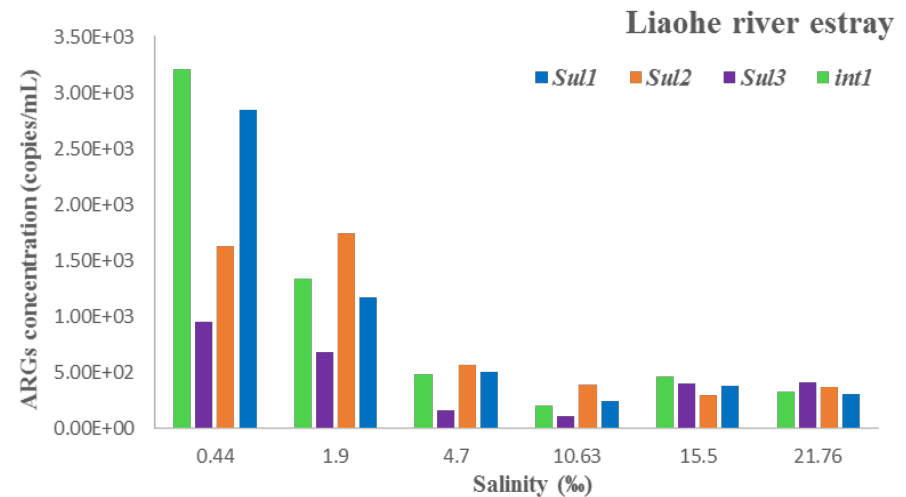
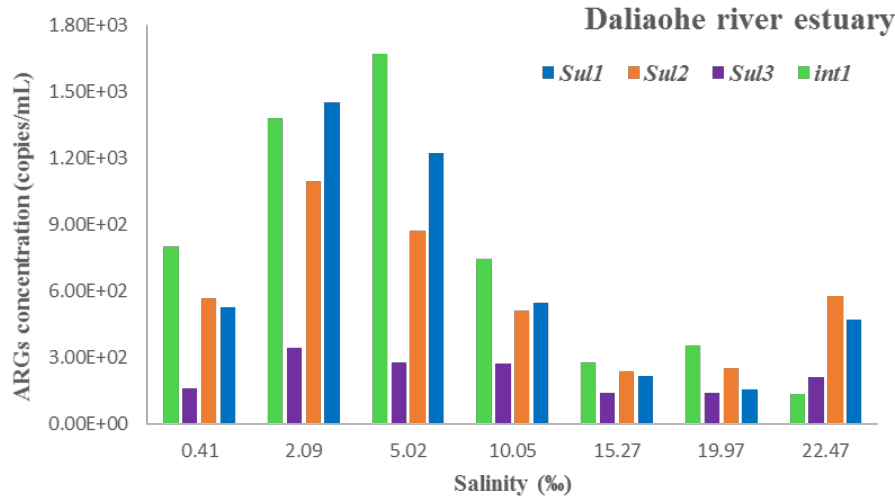


Antibiotic resistance in *E. coli* was measured using a modified chromogenic agar (MI agar).



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

● *sul*-ARGs

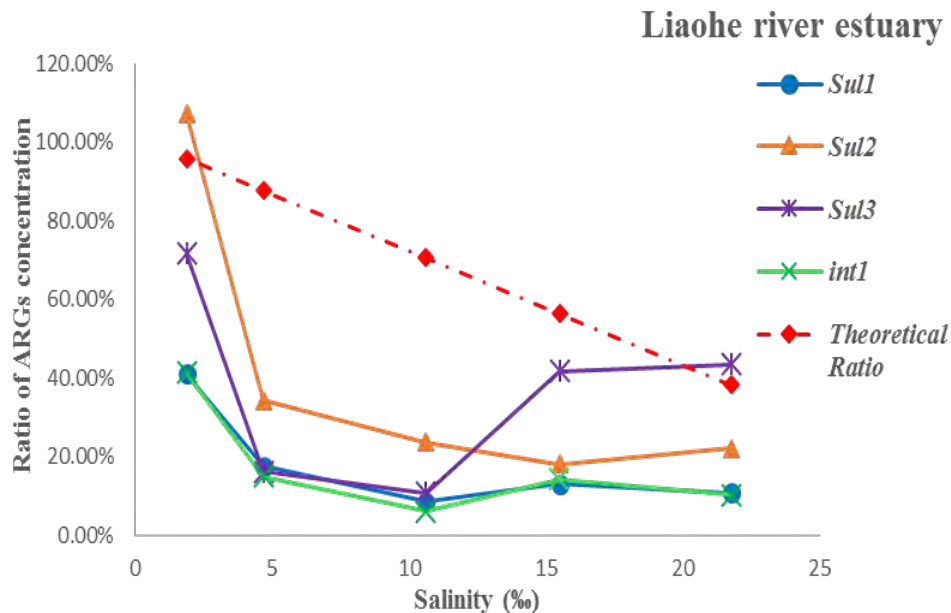
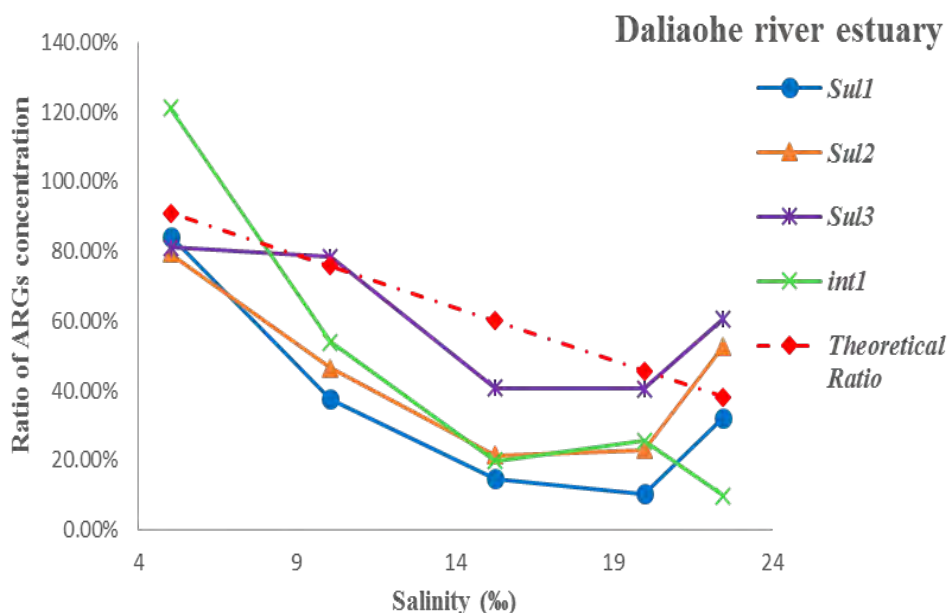


Distribution of *sul1*, *sul2*, *sul3*, and the integrase gene of class 1 integrons (*int1*) in the Daliaohe River and Liaohe River estuaries.

- *Sul1* and *sul2* were the major resistance genes detected, whereas *sul3* was relatively less in water samples from both estuaries.
- The pattern of *int1* genes was very similar to that observed in *sul1*.
- The spatial distribution of *sul*-ARGs and *int1* was largely affected by dilution of seawater.



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries



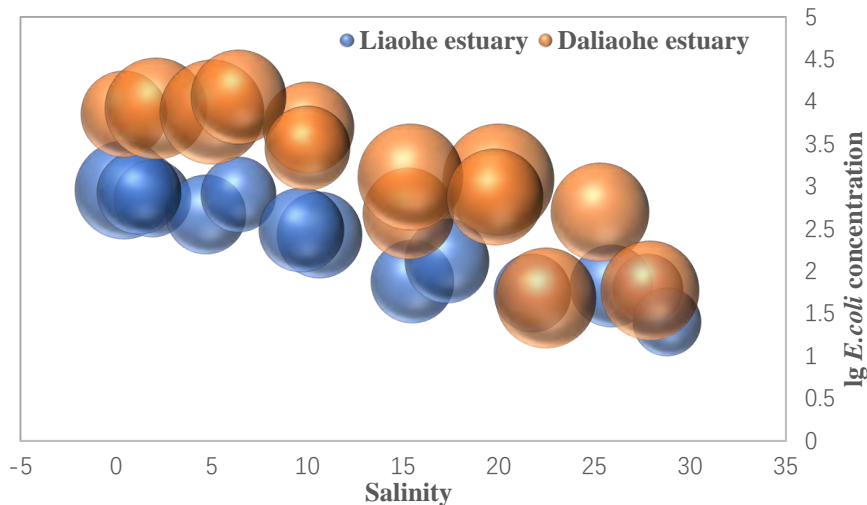
Trends in change rate of *sul1*, *sul2*, *sul3*, and the class 1 integrons (*int1*) concentration in Daliaohe River and Liaohe River estuaries.

➤ Along with increased salinity, the adaptability of most of the original carriers of sulfonamide resistance genes which thrived in the inland water environment was reduced, causing an eventual weakening in the vertical transmission of ARGs in the estuary.

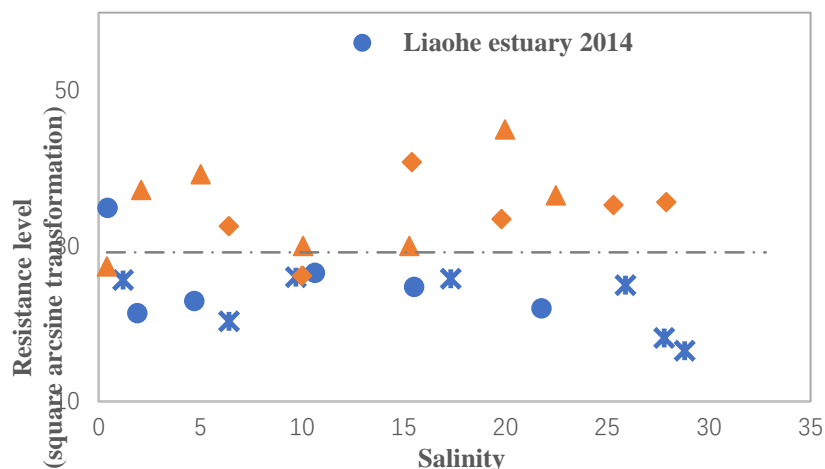


Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

● antibiotic-resistant *Escherichia coli*



Distribution and sulfonamide-resistance levels of *E. coli* in the Daliaohe and Liaohe estuaries. Bubble size indicates relative level of antibiotic resistance.



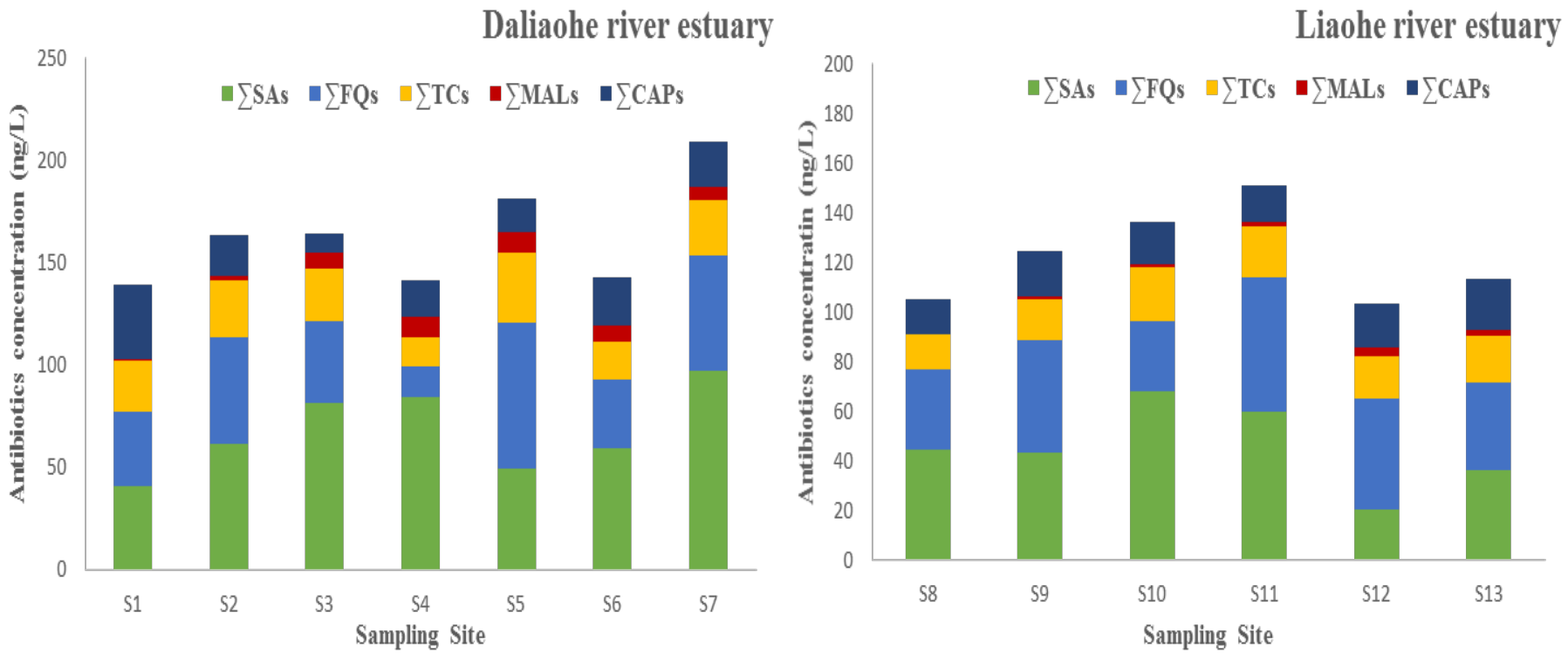
- There was significant attenuation of *E. coli* abundances in the process of migration from the inland rivers to the ocean.
- The average attenuation rates of *E. coli* were **99.45%** and **95.45%** in the Daliaohe and Liaohe estuaries, respectively.
- The theoretical attenuation rates of *E. coli* abundances in the two estuaries were **69.48%** and **71.67%**, and the average contribution to the total attenuation rate was 72.48%.

- The sulfonamide-resistance levels of *E. coli* were not significantly different among transects along the estuarine gradients.
- The resistance rate of Daliaohe estuary was higher than Liaohe estuary. The average resistance level within the Liaohe estuary was only **51.49%** of that in the Daliaohe estuary.



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

● Antibiotics

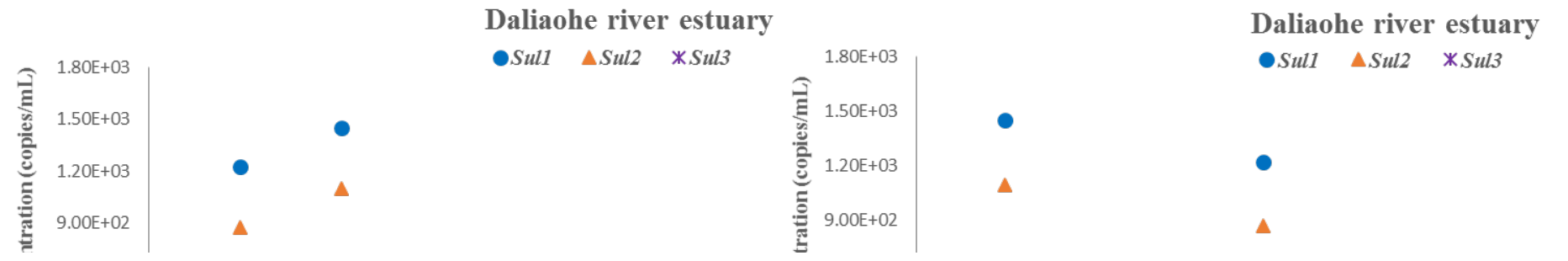


Spatial distribution of five types of antibiotics in Daliaohe River and Liaohe River estuaries.

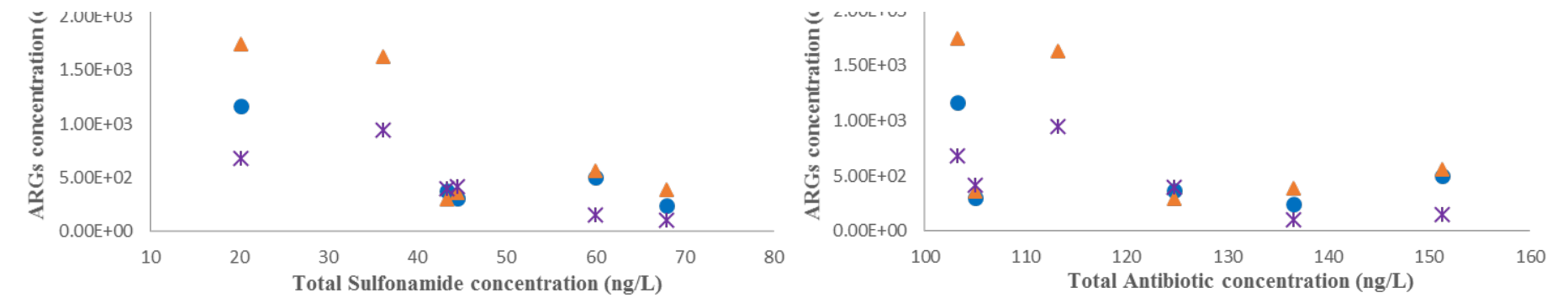
◆ Although both estuaries suffered from pollution of antibiotics to a certain extent, their concentrations were very low (ng/L).



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries



- No statistically significant correlations of *sul*-ARGs were observed with sulfonamide antibiotic concentration and total antibiotic concentration.
- A demarcation point probably exists such that the spread of ARGs are mainly determined by the corresponding antibiotic when antibiotic concentration is high. By contrast, other external factors may share or substitute the role of antibiotic when antibiotic concentration is low.



Correlation between the concentrations of ARGs and the concentrations of antibiotics in two estuaries.



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

Correlation analysis of ARGs, *16S-rRNA* genes and heavy metal concentrations in the Daliaohe River estuary

control variable		Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Ti	Pb
S	Sul1	0.314	0.759	0.538	0.333	0.726	0.957	0.973	0.946	0.414	0.088	0.957
		0.544	0.080	0.271	0.519	0.102	0.003	0.001	0.004	0.414	0.868	0.003
	Sul2	0.458	0.832	0.335	0.488	0.552	0.843	0.898	0.846	0.610	-0.140	0.960

Correlation analysis of ARGs, *16S-rRNA* genes and heavy metal concentrations in the Liaohe River estuary

control variable		Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Ti	Pb
S	Sul1	-0.200	-0.331	0.555	-0.739	0.329	0.414	0.371	0.781	0.229	0.060	-0.418
		0.747	0.521	0.332	0.154	0.524	0.415	0.538	0.067	0.711	0.923	0.484
	Sul2	-0.294	-0.480	-0.047	-0.697	0.465	0.443	-0.058	0.847	-0.049	0.087	0.140

➤ The higher concentration of heavy metals provided a stronger pressure to select for bacteria, thereby possibly causing more significant correlation.

	Temperature	pH	DO	COD	Ammonia	Nitrate	Nitrite	Phosphate	EI		
Sul1	0.956	0.465	0.041	0.881	0.066	0.014	0.058	0.031	0.947	0.222	0.138
16S-rRNA	0.125	0.631	0.174	0.201	0.545	0.561	0.607	0.499	0.580	-0.235	0.675
rRNA	0.813	0.179	0.742	0.703	0.264	0.247	0.201	0.314	0.228	0.655	0.141

	Temperature	pH	DO	COD	Ammonia	Nitrate	Nitrite	Phosphate	EI		
Sul1	0.710	0.499	0.317	0.128	0.799	0.456	0.512	0.059	0.672	0.878	0.466
16S-rRNA	-0.198	-0.573	0.703	-0.659	0.333	0.005	0.514	0.465	0.339	0.109	-0.590
rRNA	0.750	0.234	0.185	0.226	0.584	0.994	0.375	0.430	0.577	0.861	0.295

Correlation analysis of ARGs, *16S-rRNA* genes and environmental factor levels in the Daliaohe River estuary

	Temperature	pH	DO	COD	Ammonia	Nitrate	Nitrite	Phosphate	EI
Sul1	0.179	-0.037	0.379	-0.064	0.258	-0.045	0.171	0.480	0.387
16S-rRNA	0.700	0.938	0.402	0.892	0.576	0.923	0.714	0.276	0.391
rRNA	0.220	0.011	0.122	0.105	0.205	0.110	0.120	0.505	0.121
int1	0.320	0.132	0.303	-0.203	0.008	-0.023	-0.091	0.120	0.017
16S-rRNA	0.476	0.778	0.248	0.565	0.885	0.960	0.846	0.788	0.971
rRNA	-0.477	-0.540	-0.253	0.477	0.715	0.002	0.761	0.844	0.851
int1	0.279	0.210	0.584	0.279	0.071	0.996	0.047	0.017	0.015

Correlation analysis of ARGs, *16S-rRNA* genes and environmental factor levels in the Liaohe River estuary

	Temperature	pH	DO	COD	Ammonia	Nitrate	Nitrite	Phosphate	EI
Sul1	0.732	0.240	0.728	0.298	-0.219	0.379	-0.322	-0.154	0.533
16S-rRNA	0.098	0.647	0.101	0.566	0.677	0.459	0.533	0.771	0.276
rRNA	0.220	0.011	0.122	0.105	0.205	0.110	0.120	0.505	0.121
int1	0.712	0.233	0.706	0.293	-0.209	0.383	-0.323	-0.164	0.541
16S-rRNA	0.113	0.657	0.117	0.573	0.691	0.451	0.529	0.756	0.267
rRNA	0.552	-0.113	0.696	0.358	-0.333	0.513	-0.430	-0.197	0.670
int1	0.256	0.832	0.124	0.486	0.519	0.298	0.394	0.708	0.146

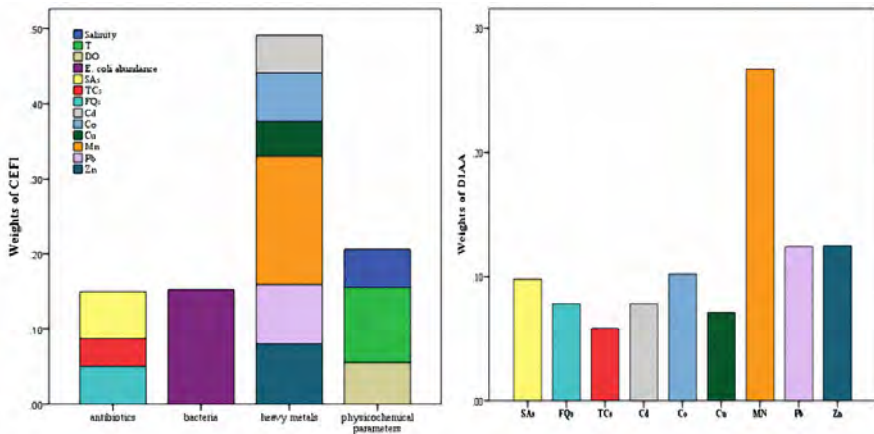
➤ No significant correlation was found between the abundance of sulfonamide resistance genes and the water quality constituents.

In each cell, the top value indicates the Pearson correlation coefficient (*r*), and the bottom value in italics indicates the *p*-value. Bold values indicate statistical significance (*p* < 0.05).



Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

In order to explore the influencing factors in the distribution and migration of sulfonamide-resistant *E. coli* in estuaries, the environmental factors index and the disturbance index of anthropogenic activities were calculated.



$$CEFI_i = \sum_{j=1}^m w_j \times y_{ij}$$

(w: weights of each indicator, y: standardization of environmental indicators)

$$DIAA_i = \sum_{j=1}^m w'_j \times p_{ij}$$

(w': weights of each pollutant, p: standardization of pollutants)

The weights of each indicator in CEFI and the weights of each pollutant in DIAA

Correlation analysis of CEFI, DIAA and the resistance levels of *E. coli* in estuary environment

	SAs	TCs	FQs	Mn	Co	Cu	Zn	Cd	Pb	CEFI	DIAA
Resistance levels of <i>E. coli</i>	0.25	0.47	0.23	0.37	0.26	0.10	0.41	-0.03	0.26	0.47	0.43
Sulfonamide-resistant <i>E. coli</i> abundance	0.21	0.02	0.26	0.06	0.20	0.63	0.04	0.88	0.19	0.02	0.03
	0.39	0.20	-0.09	0.60	0.56	0.24	0.63	0.03	0.47	0.85	0.64
	0.05	0.32	0.68	0.00	0.00	0.23	0.00	0.90	0.02	0.00	0.00

A significant positive correlation between CEFI, DIAA and the resistance levels of *E. coli*.

** The overall effect of environmental factors on *E. coli* resistance and contamination is independent of spatial distribution.



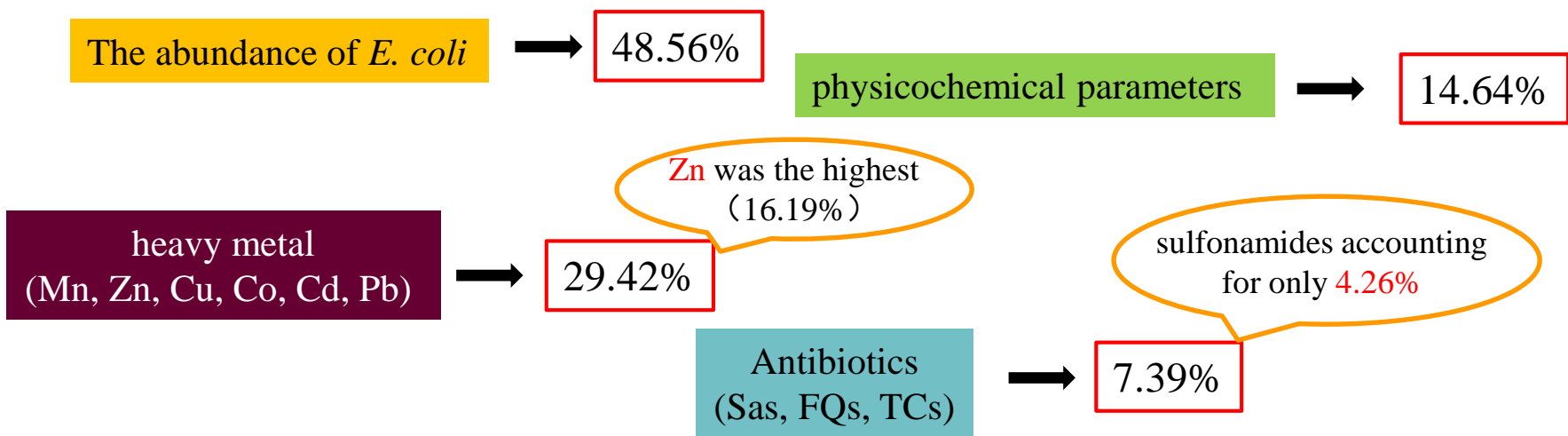
Antibiotic resistance in the typical marine environment – Daliaohe River and Liaohe River estuaries

◆ Multivariable linear regression model analysis

➤ Analysis the linear relationship between environmental indicators and antibiotic resistant *E. coli*.

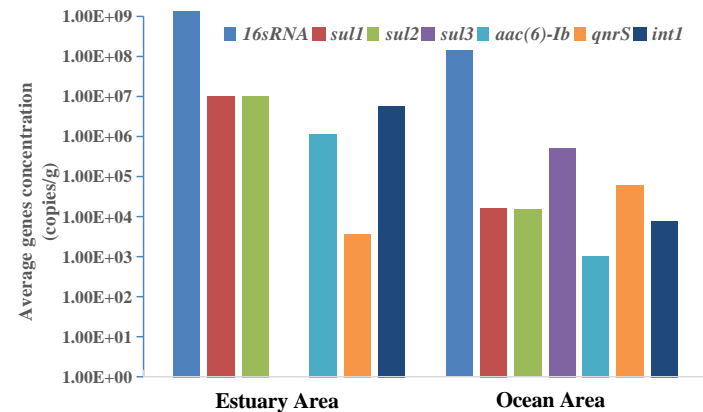
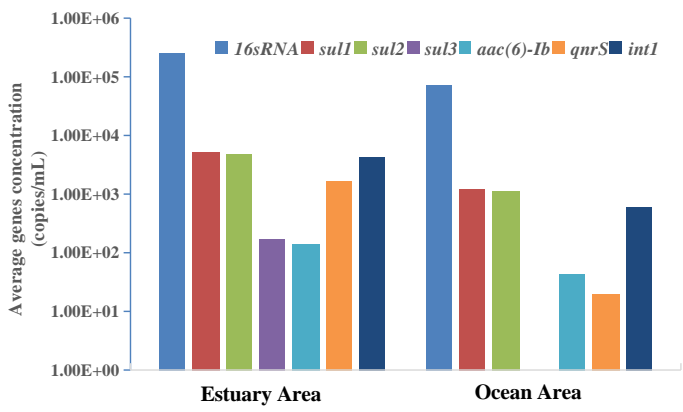
A strong multivariate linear fitting relationship was observed between antibiotic resistant *E. coli* abundances and environmental indicators ($R^2=0.986, p<0.01$).

➤ Assessing the contribution of environmental indicators on the distribution and migration of antibiotic resistant *E. coli* by partial regression coefficient.





Antibiotic resistance in the typical marine environment – Layzhou Bay



Distribution of 16S-rRNA genes, *sul1*, *sul2*, *sul3*, *aac(6)-Ib*, *qnrS* and the integrase gene of class 1 integrons (*int1*) in water and sediment samples collected from Laizhou Bay.

- The abundance of *sul*-genes was more than *qnr*-genes in different samples.
- *Sul1* and *sul2* were the major resistance genes detected, whereas *sul3* was relatively less in samples from Laizhou Bay.
- The pattern of *int1* genes was very similar to that observed in *sul1*, and the spatial distribution of ARGs and *int1* was largely affected by dilution of seawater.

The effect of estuarine physicochemical conditions on the distribution and migration of ARGs

ΣHeavy metals physicochemical parameters ΣOrganic pollutants ΣAntibiotic



Strong

Weak



Conclusions

- The concentration of antibiotics was **very low** in estuary and marine environment ;
- Antibiotic resistance was widespread in estuary and marine environment, and exhibited a **declining trend** from the inner estuary to coastal area. Of all the tested genes, *sul1* and *sul2* were the **most prevalent** in the estuary and marine environment;
- No statistically significant correlations existed between antibiotic resistance and antibiotic residues in the estuary and marine environment. The fate of antibiotic resistance was **most affected** by environmental pressures from **non-antibiotic contaminants**.



Thank You for your attention!

