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Terrestrial runoff influences the transport and contamination levels of *Toxoplasma gondii* in marine organisms

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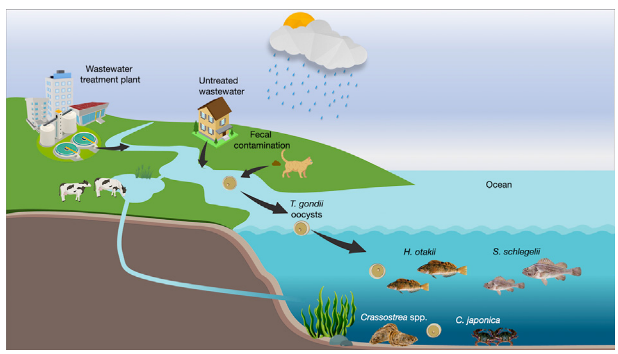
HIGHLIGHTS

- We investigated the prevalence of *Toxoplasma gondii* in marine animals from coastal waters in eastern China.
- Higher prevalence was detected in terrestrial runoff samples compared with those from non-terrestrial runoff.
- Prevalence of *T. gondii* was correlated with temperature.
- Oysters can serve as bioindicators of *T. gondii* contamination of marine environments.

GRAPHICAL ABSTRACT



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ABSTRACT

There is a growing concern regarding the potential adverse impact of *Toxoplasma gondii* contamination of the marine environment on marine wildlife and public health. Terrestrial runoff is a significant route for dissemination of *T. gondii* oocysts from land to sea. Yet, the influence of terrestrial runoff on *T. gondii* prevalence in marine animals in China is largely unknown. To address this concern, we examined the presence of *T. gondii* in marine oysters *Crassostrea* spp., rockfish *Sebastes schlegelii* (*S. schlegelii*), fat greenling fish *Hexagrammos otakii* (*H. otakii*), and Asian paddle crab *Charybdis japonica* (*C. japonica*) using a PCR assay targeting *T. gondii B1* gene. A total of 1920 samples were randomly collected, in Jan-Dec 2020, from terrestrial runoff areas (TRA, TRB, and TRC) and non-terrestrial runoff area (Grape bay) in Weihai, China. *T. gondii* prevalence in TRB and TRC was 6.04 % and 5.83 %, respectively, which was higher than 2.29 % detected in the non-terrestrial runoff area. The highest prevalence was detected in *Crassostrea* spp., and a correlation was observed between *T. gondii* prevalence and weight of *Crassostrea* spp. The temperature, but not precipitation, significantly correlated with *T. gondii* prevalence. Understanding the fate of *T. gondii* delivered to oceans by terrestrial runoff is critical for predicting future disease risks for marine wildlife and humans.

1. Introduction

The anthropogenic impact on marine environment has been progressively increasing and the levels of pollutants entering the seas have caused adverse consequences on the marine ecosystems and the public health (Li et al., 2014; O'Hara et al., 2021; Trathan et al., 2015). Zoonotic

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protozoan parasites carried by surface runoff are emerging pollutants for estuarine and coastal marine environments (Liu et al., 2019; McCallum et al., 2004). The protist *Toxoplasma gondii*, which infects humans and a broad range of animals (Dubey, 2010), has been detected in marine habitats and marine organisms worldwide (Dubey et al., 2020; Li et al., 2022; van de Velde et al., 2016). Felines, the only known definitive host, can produce and excrete environmentally resistant *T. gondii* oocysts, which are highly infectious to humans and many animals (Elsheikha et al., 2020; Frenkel et al., 1975). These oocysts can mature and remain infective in seawater for months (Lindsay and Dubey, 2009; Lindsay et al., 2003).

Fecal materials from humans, pets and livestock are directly discharged into surface water after simple or no treatment, contaminating the terrestrial runoff and inevitably the coastal waters (Fayer et al., 2004; Liu et al., 2019; Shuval, 2003). The flux of organic materials, including pathogens, from land through coastal watersheds facilitates the transport of terrestrial pathogens into the marine ecosystems and therefore increasing the risk of contamination of beaches, seawater, and seafloors with zoonotic pathogens. Waterborne infections by *T. gondii* have been reported in many countries with significant socioeconomic impacts (Blaizot et al., 2020; Ma et al., 2022; Minuzzi et al., 2021; Shuval, 2003).

Several studies have examined the transport process of *T. gondii* oocysts from land to sea, however the results are inconsistent and sometimes contradictory due to differences in the geographical conditions and environmental factors. For example, one study showed that runoff caused by rainfall increases the number of oocysts transported to the ocean (VanWormer et al., 2016). Another study found no significant correlation between the time of the death of sea otters caused by *T. gondii* and land runoff indicators (Shapiro et al., 2012).

Monitoring the level of *T. gondii* oocysts in a huge volume of seawater can be challenging (Karanis et al., 2013). Some marine animals including shellfish, fish or marine mammals can act as bioindicators of *T. gondii* contamination of marine environment (Hamza-Chaffai, 2014; Zuykov et al., 2013). Because of their ability to filter and retain microorganisms or organic particles from large volumes of water, marine filter-feeders can be used as sentinels to investigate the contamination of aquatic environments with *T. gondii* (Géba et al., 2020; Moratal et al., 2020; Palos Ladeiro et al., 2014; Staggs et al., 2015).

In the present study, we have used four common marine animal species as biological indicators to investigate the impact of terrestrial runoff on the abundance of *T. gondii* in the coastal marine environment in Weihai, China. Specifically, we determined *T. gondii* prevalence in marine animals collected from three terrestrial runoff areas versus one non-terrestrial runoff area. The prevalence data was correlated with environmental parameters – temperature and precipitation – in an effort to identify those factors that may increase the risk of marine animals for *T. gondii* infection.

2. Materials and methods

2.1. The study areas

The present study was carried out in Weihai city (36°41′ ~ 37°35′ N, 121°11′ ~ 122°42′ E), located in Shandong province, eastern China. Weihai includes over 20 islands and has a coastline of 95 km. Besides being a touristic destination, Weihai is a significant base for fishery and marine aquaculture. In this study, terrestrial runoffs were selected from three areas in Weihai, including terrestrial runoff A (TRA), terrestrial runoff B (TRB) and terrestrial runoff C (TRC) (Fig. 1). These runoffs flow through urban residential and commercial areas, and eventually into the Yellow Sea of China. Additionally, a bay known as Grape bay (GB) without nearby terrestrial runoff was included as a control (Fig. 1).

2.2. Sample collection and meteorological parameters

From January to December 2020, four common marine animal species were collected from coastal areas of the Yellow Sea of China. These included oysters *Crassostrea* spp., rockfish *Sebastes schlegelii* (*S. schlegelii*), fat greenling fish *Hexagrammos otakii* (*H. otakii*), and Asian paddle crab *Charybdis japonica* (*C. japonica*). The samples of TRA, TRB and TRC were collected at the interface between terrestrial runoff and ocean to ensure that their habitats are directly affected by the runoffs. Local fishermen were contracted to catch the marine animals used in this study. Every month, 10 specimens were randomly selected from the harvest of each of the 4 marine animal species and from each of the 4 sampling areas (i.e. $10 \times 4 \times 4 = 160$ samples/month). This sampling strategy resulted in a total of 1920 samples collected over 12 months. These included 480 samples from each sampling area, including 120 samples from each of the four marine animal species. All 1920 samples were quickly placed in separate bags, rinsed with sterile water and transported to the laboratory in ice box for processing. Meteorological information about the studied areas, such as monthly temperature (°C) and precipitation (mm) was gathered from Weihai Statistical Yearbook 2021.

2.3. Sample processing

The body weight of each sample was determined to the nearest 0.01 g using a digital balance. The length of *S. schlegelii* and *H. otakii* was determined to the nearest mm using a caliper. Then, the hemolymph, gills, hepatopancreas, stomach and gonads of each specimen were blended to obtain a mixed tissue homogenate from each sample. The samples were kept in cryogenic vials at −80 °C until analysis.

2.4. DNA extraction and detection of *T. gondii*

Each pooled sample (~5 g) was subjected to freezing in liquid nitrogen (5 min) followed by thawing at 80 °C (5 min), for five cycles and centrifuged at 9750*g* for 2 min. Total genomic DNA isolation was performed using E.Z.N.A.® Stool DNA Kit (Omega Biotek Inc., Norcross, GA, USA) as per the manufacturer's recommendations. *T. gondii* DNA was detected by using a nested PCR assay that targets the parasite *B1* gene as previously described (Monteiro et al., 2019; Yai et al., 2003).

2.5. Data analysis

All statistical analyses were performed using the SPSS 26.0 software (IBM, Armonk, NY, USA) and R-based software v3.6.3. The differences in *T. gondii* prevalence between marine animal species collected from terrestrial runoff and non-terrestrial runoff areas were detected using the chi-square test. Pearson's correlation test was used to examine the association between *T. gondii* prevalence in marine animal and epidemiological (temperature and precipitation) and biological variables (body length of *S. schlegelii* and *H. otakii*, and body weight of all four marine species). *P*-values <0.05 were considered statistically significant.

3. Result and discussion

Terrestrial runoff in coastal cities represents a key mechanism for contamination of the aquatic habitats and infection of marine organisms with *T. gondii* (Conrad et al., 2005; Miller et al., 2002; Miller et al., 2008; Shapiro et al., 2015;). In the present study, we examined the differences in *T. gondii* prevalence between four species of marine organisms collected from three terrestrial runoff areas versus one non-terrestrial runoff area in Weihai city in eastern China.

3.1. *T. gondii* prevalence in terrestrial versus non-terrestrial runoff areas

As shown in Table 1, the highest prevalence was found in samples collected from terrestrial runoff areas TRB (6.04 %), followed by TRC (5.83 %), and TRA (3.96 %). *T. gondii* prevalence at TRB and TRC was significantly higher compared with that of the non-terrestrial runoff area (Grape bay; 2.29 %) ($p < 0.05$). The land to sea discharge of *T. gondii* oocysts excreted by felines can be facilitated by runoff contaminated with untreated sewage or by the scouring effect of rainfall on soil, which lead

Fig. 1. Map of the four sampling sites in Weihai, China, showing the estuaries of three terrestrial runoffs A, B and C, and one non-terrestrial runoff D (Grape bay). The insert figure shows the overall location of the sampling areas in Weihai, China.

to contamination of coastal and estuarine areas (Miller et al., 2002; Simon et al., 2013b; VanWormer et al., 2016). ToxoDB#1 and ToxoDB#9, the commonest genotypes found in animals and humans in China (Pan et al., 2017; Rong et al., 2014; Tian et al., 2014), are detected in marine bivalve shellfish (Cong et al., 2021a; Cong et al., 2021b). The presence of the same *T. gondii* genotypes in terrestrial animals and in shellfish further reiterates the ability of shellfish to concentrate *T. gondii* oocysts from marine environments and is consistent with the terrestrial sources of the fecally transmitted *T. gondii* oocysts flowing from land to contaminate the marine ecosystem.

It is intriguing that a higher prevalence of *T. gondii* was detected in TRB and TRC compared with that of TRA. TRB and TRC are located in the city center, in densely populated residential and commercial areas, which are considerably affected by anthropogenic activities, potentially increasing the level of oocysts' contamination in the environment. This result is consistent with previous studies showing that more *T. gondii* oocysts can be found in the environment of areas with a high level of economic growth (VanWormer et al., 2016) and that coastal development and loss of vegetation increase land-to-sea transport of oocysts via increasing stormwater runoff caused by rainfall events (Shapiro et al., 2010).

On the other hand, the low prevalence in TRA is perhaps attributed to the fact that estuary of TRA is adjacent to Yangting River Wetland Park, which is sparsely populated and rich in vegetation. The coastal wetlands can remove protozoa from surface water and the presence of vegetation can reduce pathogens in wastewater effluent (Daniels et al., 2014; Graham et al., 2021; Hogan et al., 2013; Shapiro et al., 2010). In addition, TRA is connected to a bay before it meets the ocean. Bays are at the interface of three different environments: land, freshwater, and seawater. The interactions occurring at the freshwater-land-seawater interface are complex (Malham et al., 2014), and may result in dilution of *T. gondii* oocysts. The relationship between the abundance of oocysts in terrestrial runoff and the contamination level of *T. gondii* in the marine environment and its spatial distribution in watershed-estuary-offshore biosphere merit further investigation.

3.2. Climatic factors influence T. gondii prevalence in marine animals

We examined the correlation between *T. gondii* prevalence in marine animals and climatic factors, such as temperature and precipitation. As shown in Fig. 2, prevalence of *T. gondii* in samples collected from TRB and the total number of samples was correlated with temperature ($p < 0.05$),

Table 1
Toxoplasma gondii prevalence in marine animals collected from terrestrial runoff and non-terrestrial runoff areas in Weihai, China.

Sampling site[a]	*Crassostrea* spp.		*Sebastes schlegelii*		*Hexagrammos otakii*		*Charybdis japonica*		Total	
	No. positive (Prevalence %)	*p*-Value	No. positive (Prevalence %)	*p*-Value	No. positive (Prevalence %)	*p*-Value	No. positive (Prevalence %)	*p*-Value	No. positive (Prevalence %)	*p*-Value
TRA	10 (8.33)	0.301	4 (3.33)	0.175	4 (3.33)	0.175	1 (0.83)	0.313	19 (3.96)	0.138
TRB	14 (11.67)	0.062	4 (3.33)	0.175	6 (5.00)	0.055	5 (4.17)	0.472	29 (6.04)	0.004
TRC	13 (10.83)	0.094	4 (3.33)	0.175	8 (6.67)	0.017	3 (2.50)	1	28 (5.83)	0.005
Grape bay	6 (5.00)	Reference	1 (0.83)	Reference	1 (0.83)	Reference	3 (2.50)	Reference	11 (2.29)	Reference

[a] Sampling sites TRA, TRB and TRC are located at the estuary of terrestrial runoffs. Sampling site Grape bay represents the non-terrestrial runoff area. A total of 480 samples were examined from rom each sampling site, including 120 samples from each of the four marine animal species.

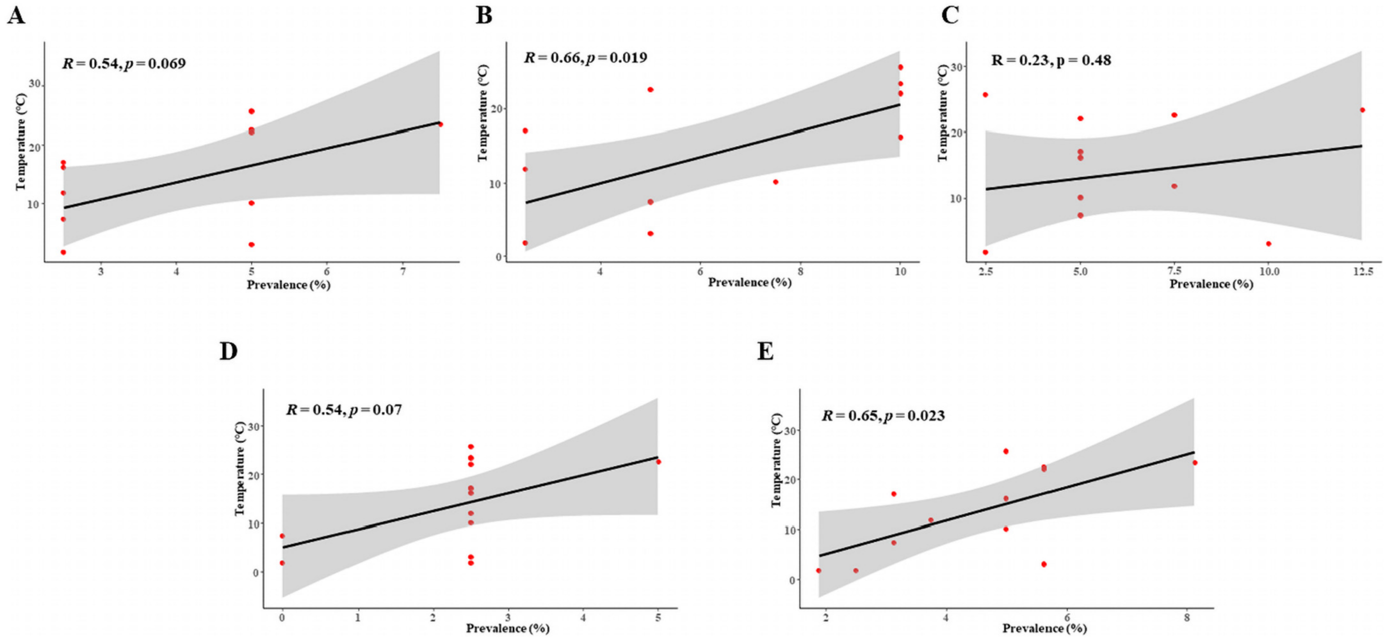

Fig. 2. Correlation between *T. gondii* prevalence in marine animals collected from different study areas and the temperature in Weihai, China. A-E represent terrestrial runoff A, terrestrial runoff B, terrestrial runoff C, Grape bay, and total, respectively.

however no correlation was detected between prevalence and precipitation at any sampling area (Fig. 3). Temperature increases the survival and maturation of oocysts (Kakakhel et al., 2021; Patz et al., 2000; Yan et al., 2016). The prevalence of *T. gondii* DNA in soil in China is significantly higher in autumn and summer than in the other two seasons, further highlighting the effect of temperature on seasonal occurrence of *T. gondii* (Cong et al., 2020).

Higher levels of precipitation and weather extremes can increase water contamination with *T. gondii* oocysts. Simulation of the transport of oocysts during snow melting indicates that snowmelt runoff can be a source of *T. gondii* infection of marine animals (Simon et al., 2013a; Simon et al., 2013b). Toxoplasmosis outbreaks reported in British Columbia in Canada are linked to heavy rainfall and contamination of the incriminated water reservoir (Bowie et al., 1997). The scouring effect of rainwater on soil may increase the level of pathogens in terrestrial runoff (Liu et al., 2019; VanWormer et al., 2016; Zhu et al., 2021). Freshwater runoff may increase the risk of infection in marine animals, such as the southern sea otters along the coast of California (Miller et al., 2002). Precipitation can have a significant impact on the occurrence of *T. gondii* oocysts in marine bivalve shellfish (Cong et al., 2021a). In disagreement with previous studies, our results did not reveal any significant correlation between precipitation and

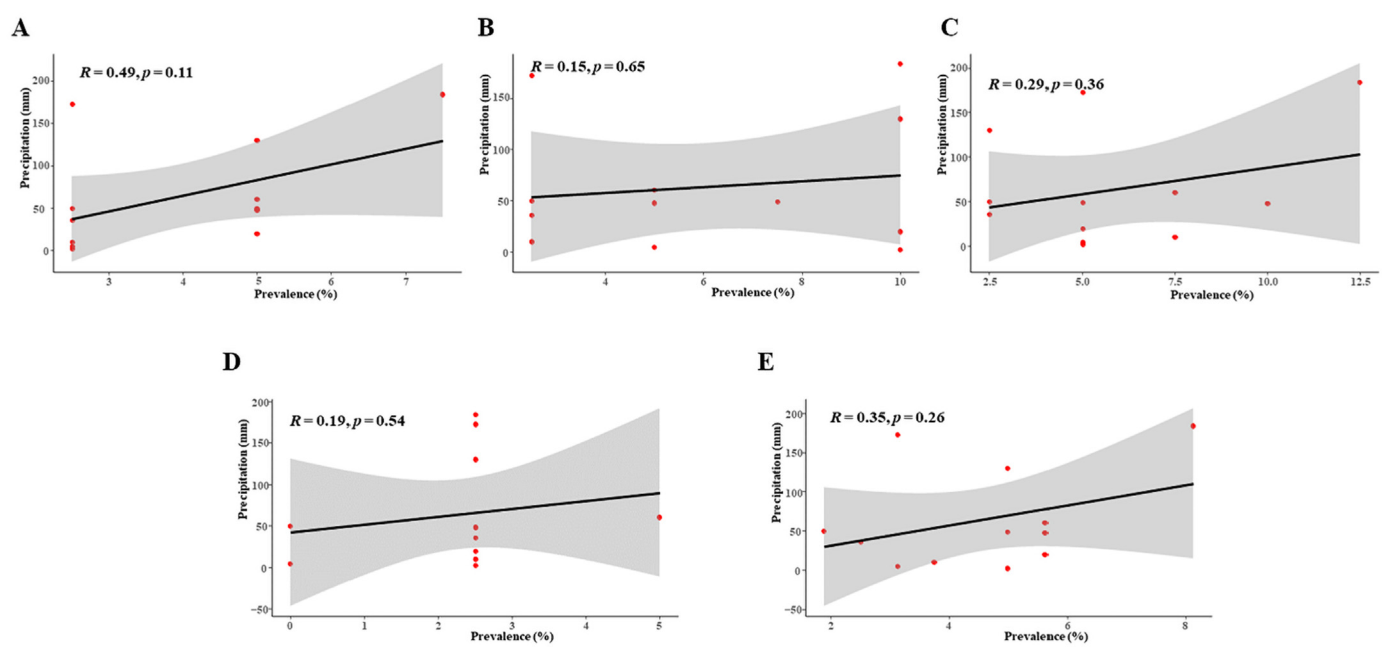

Fig. 3. Correlation between *T. gondii* prevalence in marine animals from different study areas and the precipitation in Weihai, China. A-E represent terrestrial runoff A, terrestrial runoff B, terrestrial runoff C, Grape bay, and total, respectively.

T. gondii prevalence. Further studies are required to better understand the effect of heavy rains, as a trigger for freshwater runoff, on the levels of *T. gondii* in marine animals.

3.3. *T. gondii prevalence is different between animal species*

We detected differences in *T. gondii* prevalence between sampling areas and between four marine species (*Crassostrea* spp., *S. schlegelii*, *H. otakii*, and *C. japonica*). A significantly higher prevalence of *T. gondii* was detected in TRC (6.67 %) than that detected in Grape bay (0.83 %) in *H. otakii* ($p < 0.05$). The highest prevalence of *T. gondii* in all sampling areas was detected in *Crassostrea* spp. (Table 1). This result is consistent with a previous study in Weihai showing the highest prevalence of *T. gondii* in *Crassostrea* spp. compared to three other bivalve shellfish species (Cong et al., 2021a). The high prevalence observed in *Crassostrea* spp. may be attributed to their water filtering ability and concentration of oocysts. Interestingly, we observed a correlation between *T. gondii* prevalence and *Crassostrea* spp. weight (Fig. 4). The increase in the filtration rate of filter-feeding shellfish correlates with the increase in their body weight (Guo et al., 2013), suggesting that *Crassostrea* spp. are more efficient in accumulating oocysts than the other studied marine species. Given the challenge associated with detection of *T. gondii* in marine ecosystems (Karanis et al., 2013), the competence of these filter-feeders to retain *T. gondii* oocysts and their limited activity makes benthic shellfish appropriate sentinels for monitoring *T. gondii* contamination in coastal waters (Fung et al., 2021; Robertson, 2007; Staggs et al., 2015).

3.4. Limitations

Differences in the prevalence of *T. gondii* between sampling areas and marine animal species may be attributed to differences in geographical and environmental factors. However, it is worth noting that nektons, such as *S. schlegelii* and *H. otakii*, are good swimmers and can travel freely between the sampling areas. Additionally, ocean currents may influence the dispersion of *T. gondii* oocysts in seawater and in turn the contamination levels of *T. gondii* in marine environment (Li et al., 2022; Poulle et al., 2021). Therefore, it is possible that differences in the swimming and travel abilities of the examined marine species and in the water currents between the sampling areas may have confounded the results.

3.5. Implications for public health policies

T. gondii has a worldwide distribution and can reach coastal waters in contaminated runoffs. The flux of this terrestrial parasite to coastal waters imposes a health risk to marine animals and the public who utilize the near-shore for seafood harvest and recreational activities (Poulle et al., 2021). Cold-blooded marine organisms can accumulate oocysts through filter-feeding and hence become a potential source of infection to larger marine organisms and humans (Moratal et al., 2020). *T. gondii* oocysts can maintain its viability in the digestive tract of migratory filter-feeding fish (Massie et al., 2010). The consumption of infected marine organisms can have adverse health effects (Vail et al., 2020) and lead to foodborne outbreaks (Cruz et al., 2015; Potasman et al., 2002). Given the ubiquitous nature of *T. gondii*, the present study corroborates previous studies (Li et al., 2022; Moratal et al., 2020; Poulle et al., 2021; Tedde et al., 2019) emphasizing the risk associated with terrestrial runoff into the ocean on marine ecosystems and public health.

Marine organisms, particularly nektons, are not limited by a space or confined to a certain aquatic niche, and thus conventional approaches such as vaccines and drugs are not effective in protecting them from *T. gondii* infection (Glidden et al., 2022; Liu et al., 2018; Secrieru et al., 2020). Therefore, efficient measures should be implemented to prevent the discharge of *T. gondii* oocysts from the terrestrial environment into the sea. Coastal areas are strongly affected by fecal contamination from terrestrial sources. Therefore, feline fecal pollution should be mitigated with proper education regarding cat waste disposal. Identifying the sources of pollution by fecally-transmitted, zoonotic pathogens such as *T. gondii* in coastal environments is relevant to public health and marine ecosystem.

4. Conclusions

This study provided novel data on *T. gondii* prevalence in *Crassostrea* spp., *S. schlegelii*, *H. otakii*, and *C. japonica* from eastern China. Out of the four examined marine species, *Crassostrea* spp. had the highest prevalence

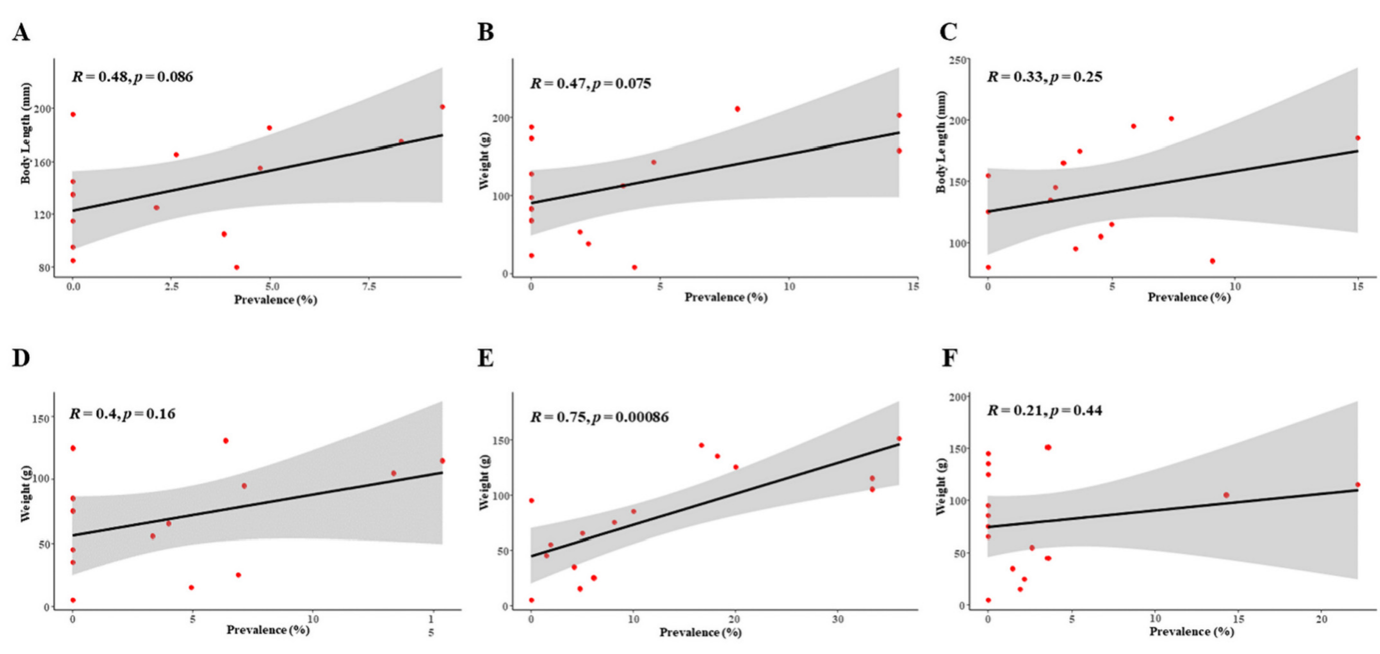

Fig. 4. Correlation between *T. gondii* prevalence, and the body length and weight of the examined marine organisms. (A-B) Correlation with body length and body weight of *Sebastes schlegelii*, respectively; (C—D) Correlation with body length and body weight of *Hexagrammos otakii*, respectively; (E) Correlation with body weight of *Crassostrea* spp.; (F) Correlation with body weight of *Charybdis japonica*.

and more potential to serve as biosentinels for monitoring *T. gondii* contamination in coastal waters. The results substantiate the hypothesis that terrestrial runoff is a significant factor for contamination of the ocean with *T. gondii* oocysts. It is hoped that our data inform management measures for mitigating *T. gondii* risk in marine habitats. Future investigations should explore how spatial–temporal dynamic changes in terrestrial runoff affect the carriage of oocysts into the ocean, and their prevalence and distribution in marine environment.

CRediT authorship contribution statement

Man-Yao Li: Investigation, Methodology, Software, Formal analysis, Writing- Original draft preparation.

Yuan-Huan Kang: Investigation, Methodology, Writing- Original draft preparation.

Wen-Chao Suna: Formal analysis.

Zhi-Peng Hao: Investigation, Methodology.

Hany M. Elsheikha: Formal analysis, Visualization, Writing - Review & Editing.

Wei Cong: Conceptualization, Visualization, Funding acquisition, Writing - Review & Editing.

Ethical approval

No ethical approval was required.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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