

Bioenergetic responses in green lipped mussels (*Perna viridis*) as indicators of pollution stress in Xiamen coastal waters, China

Wang Shuhong^{a,b}, Hong Huasheng^{a,*}, Wang Xinhong^a

^a Key Lab of Marine Environmental Science (Xiamen University), Ministry of Education, Environmental Science Research Center, Xiamen University, Xiamen 361005, Fujian, China

^b Fisheries college, Jimei University, Xiamen, China

Abstract

Samples of green lipped mussels (*Perna viridis*) were collected from three sites (Huoshao, I'Maluan and Tong'an) around Xiamen coastal waters, where levels of various trace organic pollutants have been studied. Samples were also collected at a "cleaner" reference site near Dongshan Island for comparison. Clearance rate, absorption efficiency and oxygen consumption of the mussels were measured under controlled laboratory condition; organic pollutants in their tissues were also analyzed. Scope for growth (SFG) was employed as a general biomarker to evaluate the stress of pollutants. Specimens from Tong'an site had the lowest SFG values (1.14 J/h/g); while specimens from the I'Maluan site and Huoshao site had SFG values of 5.01 and 6.72 J/h/g, respectively. Specimens from Dongshan (reference site) had a relatively high SFG value of 10.96 J/h/g. There was a significant negative correlation between the SFG of the different populations of green lipped mussels and the concentration of DDT in their tissues.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Scope for growth; Biomarker; Green lipped mussels; Xiamen coastal waters

1. Introduction

Xiamen, one of the earliest of the five "special economic zones" in China has developed rapidly in recent years. The region has experienced an increased population (metropolitan pop: 2.14 million in 2002) and enormous economic growth (18.4% increase in GNP per year) in the past 20 years. The extraordinarily rapid industrial development and population growth has led to significant increases in the ambient levels of pollution and environmental damage.

The concentrations of trace organic contaminants such as PAHs, PCBs and pesticides present in water and accumulated in sediments and biota have been studied in Xiamen coastal waters (Hong et al., 1995; Chen

et al., 1998; Wu et al., 1999; Zhang et al., 2000; Yuan et al., 2001). Total DDT values of 5–60 ng/g dry weight in sediments have been reported. The DDT content (500–1500 ng/g wet weight) in fish liver was biomagnified above sediment levels by up to two orders of magnitude (Klumpp et al., 2002).

To date, most studies in Xiamen coastal waters have focused on the levels of pollutants in seawater, sediment and biota. Only a few studies have considered the biological effects of these pollutants. Scope for growth (SFG) relates the energetic balance between processes of energy acquisition (feeding and related absorption percentage) and energy expenditure (respiration and excretion) of the organisms and it is one of the most sensitive measures of stress and has been successfully used as a biomarker in many laboratory and field situations, detecting and quantifying pollution effects (Magnusson et al., 1988; Nelson, 1990; Widdows et al., 1995a, 2002; Cranford et al., 1999; Elfving and Tedengren,

* Corresponding author. Tel.: +86 592 2182216; fax: +86 592 2095242.

E-mail address: hshong@xmu.edu.cn (H. Hong).

2002). SFG value can range from maximum positive under optimal conditions, declining to negative when animal is severely stressed and utilizing body reserves (Widdows et al., 1995b). The stress here includes natural stress, such as lack of feeding, and pollutant stress. The reproductive cycle also can affect the measurement of SFG. The preferred season for measuring SFG is during the period of maximum growth potential.

Green lipped mussels are widely distributed in the Indo-Pacific region, extending from Japan to New Guinea and from the Persian Gulf to the South Pacific Islands (Siddall, 1980). It has been selected as an indicator species in the sub-tropical zone (Nicholson, 1999). Li et al. (2002) used green lipped mussels' SFG to indicate the influence of harmful algal blooms and found that SFG is a sensitive biomarker to monitor the influence of harmful algal blooms on bivalve growth. The objectives of this study were to use the SFG of green lipped mussels as physiological stress indicators to evaluate the degree of pollution and to investigate the possibility of using SFG to indicate pollution in the area.

2. Materials and methods

2.1. Study area

The sampling sites are shown in Fig. 1. The site of Huoshao is located in the Xiamen western sea area and very near to Dongdu harbor, the main port area in Xiamen, which is considered the most polluted site. I'Maluán site has limited tidal exchange with the adjoining Xiamen western sea via a small channel. It was mostly occupied by a high density of cages for shellfish and fish culture during sampling time. The Tongan site is zoned for aquaculture use, but in recent years industrialization and urbanization have developed rapidly, resulting in increased waste and sewage discharges to the sea. Dongshan Island, located 200 km to the south



Fig. 1. Sample sites.

of Xiamen, was selected as a reference site since it is a clean area for tourism, and less developed compared to Xiamen coastal areas.

Mussels were collected in May 2001 from the above four sites, five replicate mussel visceral samples were collected at each site for DDT or PAHs analysis, respectively. For SFG measurement, 20 mussels were collected and transported back to laboratory within 3 h. Surface water and sediment were sampled at the same time using methods described in Zhang et al. (2003).

2.2. Chemical analyses

Water samples were extracted using a solid-phase extraction (SPE) system from Supelco, following established procedures (Zhou et al., 1996, 2000). Sediment samples were extracted by ultrasonication, a method developed by the authors (Hong et al., 2000). Mussel visceral were homogenized and extracted with acetonitrile, then elutes were clean up on a silica column. Conditions of measurement are described by Hong et al. (1995) and Zhou et al. (2000). Briefly, detection of organochlorinated pesticides was performed with a HP 5890GC-ECD. PAHs were identified and quantified using a HP 6980 (Hewlett Packard) gas chromatograph equipped with HP 5973A mass selective detector. Quantification of the PAH was carried out using a five-point calibration curve of a mixture of 16 PAHs (TLC PAHs Mix, SUPELCO Corp.). All data were subject to strict quality control procedures, including the analysis of procedural blanks and spiked samples with each set of samples analyzed (for details, see Zhang et al., 2003). Detection limits were 0.01–0.05 ng/g dry weight for organochlorinated pesticides and 0.2–2.2 ng/g dry weight for PAHs.

2.3. Physiological measurements

Mussels (shell length of 6–7 cm) were collected from the four sites and transported to the laboratory within 4 h. Mussels were maintained in water at a salinity of 30‰ with the temperature at 23–25 °C for more than 24 h as recommended by Widdows (1993). Mussels were fed with about 1×10^5 cell/ml of the microalga *Chlorella* sp. as food.

2.3.1. Clearance rate (CR) and consumed energy (C)

Mussels were individually placed in 700 ml chambers with flowing sand filtered aerated seawater with inflow concentrations of *Chlorella* sp. at approximately 1×10^5 cell/ml. One chamber without mussels was used as a control. Seawater samples (200 ml) were collected from inflow and outflow, cell concentrations were measured by microscope. The CR, expressed as l/h (liter per hour), was calculated using the following equation:

$$CR = \text{Flow rate (in l/h)} \times (C_1 - C_0) / C_1$$

where C_1 is the inflow concentration represented by cell concentration in the control, and C_0 is the outflow concentration from each experimental chamber. The clearance rate of each mussel was determined on four occasions at 1 h intervals.

The amount of ingested or consumed energy (C) was calculated for each individual by multiplying CR by the amount of organic material (POM) per liter in input water samples and assuming an energy content of algal material of 23 J/mg (Widdows and Johnson, 1988; Widdows et al., 1990).

2.3.2. Absorption efficiency

Absorption efficiency (AE) represents the efficiency with which organic material is absorbed from food. Filters (GFC, 1- μ m pore size) were preweighed and heated to 500 °C for 2 h before use. One liter of seawater was filtered and then salts collected on the filter were removed by gentle washing with distilled water. The filters were then dried at 100 °C for 48 h, weighed to calculate dried weights, ashed at 500 °C for 2 h, and reweighed to calculate particulate organic matter (POM) weight (ash-free material). Feces were collected by pipette from each exposure tank, filtered on GFC filters, dried, weighed, and ashed as described above. The AE was calculated by the following equation (Conover, 1966), it represents the efficiency with which organic material is absorbed from the ingested food material.

$$AE(F - E) / [(1 - E) \times F]$$

where F is the ratio of ash-free dry weight to the dry weight (AFDW/DW) of the food; E is the ratio of ash-free dry weight to the dry weight of the feces.

2.3.3. Respiration rate measurement

The respiration rate (mmol O₂/g/h) of mussels was measured in enclosed chambers (700 ml). Each individual was placed into a chamber before the chamber was sealed without air. Seawater in the chamber was mixed by a magnetic stirrer placed on the bottom. The oxygen concentration in the seawater was detected by an oxygen electrode and meter 85/10 FT (YSI). Measurements started only when the animals had opened their shell valves and the measurement period lasted for 0.5 h. The respiration rate was calculated from the slope of the decrease of oxygen concentration over time. The amount of energy used in respiration (R) was calculated assuming an energy content of 0.456 J/ μ mol O₂ (Gnaiger, 1983).

2.3.4. Calculation of scope for growth (SFG)

The SFG, which defined the energy available for growth and reproduction and was calculated by the following equation (Widdows et al., 1995b):

$$SFG = C \times AE - R$$

where C is the consumed energy, AE is absorption efficiency, and R is respired energy.

2.4. Statistical analysis

Dunnett T test was used to compare physiological responses of the mussels from the sites around Xiamen coastal waters with those from the reference Dongshan site. $P < 0.05$ was considered to be significantly different. The Pearson correlation factor was used to analyze the relationship between SFG and the pollutant content in mussel viscera.

3. Results and discussion

From our analysis, Tong'an site showed the highest levels of organochlorinated pesticides in surface water, sediment and mussels viscera (Table 1). In the coastal waters of Xiamen, DDT was found to be the dominant organochlorinated pesticides concentrated in sediment, water and biota. These results are in close agreement with previous studies (Hong et al., 1995; Chen et al., 1998; Wu et al., 1999; Yuan et al., 2001; Klumpp et al., 2002). Mussel bioaccumulate DDT from seawater, and the bio-concentration factor was more than 100 at Huoshao site. Unexpectedly, the PAHs concentrations in surface water from Dongshan were relatively high, but the levels of PAHs in the visceral mass of mussels from Dongshan were not higher than that in tissues from the three sites in Xiamen coastal waters. Small craft used by local fishermen may be a possible source of petroleum contamination, however, the level of PAHs in Dongshan was not serious when compared with levels in Xiamen coastal waters, as shown by the mussel visceral mass analyses (Table 1).

The clearance rate measured in green lipped mussels collected from Tong'an site (2.62 l/g/h) was significantly different from the reference site, Dongshan ($P < 0.05$). Estimations have been recorded that relate the presence of pesticides with a reduction in filtration rates (Donkin et al., 1989; Widdows et al., 1990; Widdows and Salkeld, 1992). The absorption rate showed the same tendency as the clearance rate: mussels from Dongshan had the highest value of 0.50 ± 0.03 , while those in Tong'an site were significantly lower, the value being from 0.41 ± 0.03 . The significant decrease in the absorption efficiency observed in mussels from Tong'an site when compared with those from Dongshan, indicated deficiencies in the functioning of the digestive system in mussels from the polluted area. These results are comparable to results obtained in other studies, where the absorption efficiency declined in the presence of organic chemical compounds, mainly aromatic hydrocarbons (Widdows et al., 1987; Toro et al., 2003). There were

Table 1
Concentrations of PAHs, HCHs and DDT in surface water, sediment and viscera of green lipped mussels from four sites

Site	Surface water (ng/l)			Sediment (ng/g)			Mussels viscera (ng/g dry weight)			
	PAHs	HCHs	DDT	PAHs	HCHs	DDT	DDT	<i>n</i>	PAHs	<i>n</i>
Tong'an	32.8	6.8	43.9	56.8	0.1	17.5	183.0 ± 56.7	5	64 ± 25.6	5
Maluan	71.6	1.0	6.4	96.9	ND	0.3	137.8 ± 35.4	5	45 ± 16.7	5
Huoshao	36.0	ND	1.0	42.5	ND	0.6	123.2 ± 38.5	5	85 ± 27.9	5
Dongshan	413	2.9	6.8	49.6	ND	3.8	67.24 ± 21.7	5	18 ± 6.5	5

ND: under the detection limited of method.

no significant differences in respiration rate between mussels from sites around Xiamen coastal waters and mussels from the Dongshan site.

Fig. 2 shows the components of the energy budget of mussels from the four sites. Mussels from the Dongshan site had the most total consumed energy (38.6 J/g/h) and the fecal energy, SFG, respired energy were 49%, 29%, 22% percentage respectively. Mussels from Tong'an site showed the lowest consumed energy, 23.9 J/g/h, and the SFG was only 5%. It was surprising that mussels from Tong'an site showed the lowest SFG values (1.14 J/h/g) and some individuals' SFG was negative. The reduction of the SFG to negative values is a result of the low clearance rate observed for individuals from Tong'an site which were unable to obtain enough energy to fulfill their metabolic requirements. Some natural factors such as lack of food and phase of reproductive cycle also can affect the SFG value. For green lipped mussels, from late June to September is the reproductive season in south of China. We sampled mussels on early May, which belongs to the fast growth period. So, the decline of SFG should not be caused by the "quiescence" prior to the onset of gametogenesis. In the present study, we measured SFG of mussels from four different sites under "standard laboratory conditions": same temperature, same salinity and same algae concentrations. Thus it appears that the difference of SFG was mainly caused by the different pollutant load in their body. Widdows et al. (1995b) performed the detailed study on the SFG and contaminant levels in North Sea mussels *Mytilus edulis* and concluded that after 24 h of recovery mussels

still retained the pollution induced stress effects, when measured under "standard laboratory conditions".

From the view of Widdows et al. (1995b), SFG values of >15 J/g/h represent pristine environments with low pollution stress. Nevertheless, SFG values from 5 to 15 J/g/h are typical of areas influenced by moderate levels of pollution; and values of <5 are more typical of areas with significant levels of pollution (Table 2). Negative SFG values are indicative of severe stress conditions where organisms do not gain enough energy (negative energy balance) and have to utilize body reserves in order to survive (Bayne et al., 1985). SFG of mussels from three sites around Xiamen were significantly lower than those from Dongshan. Even mussels collected from the reference site, Dongshan, were in moderate stress as indicated by that SFG. This may be due to the relatively high levels of PAHs in Dongshan (Fig. 2).

A highly significant negative correlation was found between SFG and DDT in viscera of the green lipped mussel (Table 3, $r = -0.998$, $P < 0.05$, $n = 4$). Several studies have shown a negative relationship between PAHs concentrations in mussel and SFG (Widdows et al., 1997, 2002; Toro et al., 2003), however, we did not observe any significant relationship between SFG and PAHs concentrations. The PAHs concentrations recorded in this study were much lower than that recorded in other studies (ng/g vs. $\mu\text{g/g}$). In our study, DDT was the dominant pollutant in water, while in above studies, the DDT levels were relatively low, for example, in the study of Widdows et al. (2002), almost no DDT were detected. It is possible that the response to DDT

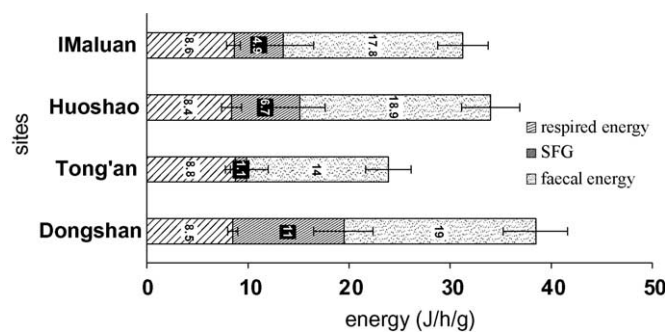


Fig. 2. Components of the energy budget of mussels from four sites, 1999. Total bar height = energy ingested, which is partitioned into respiratory energy loss, the net energy gain termed scope for growth and faecal energy loss.

Table 2

Physiological response parameters and SFG values of *Perna viridis* from four sites (mean values \pm SD)

Site	<i>n</i>	Length (cm)	Tissue dry weight (g)	Clearance rate (l/g/h)	Absorption efficiency	Respiration rate (O ₂ μ mol/g/h)	SFG (J/g/h)	95% Confidence interval for SFG
Tong'an	18	6.91 \pm 0.23	0.39 \pm 0.09*	2.62 \pm 0.45*	0.41 \pm 0.04*	19.20 \pm 2.27	1.14 \pm 2.12*	0.07–2.2
ΓMaluan	10	6.78 \pm 0.13	0.55 \pm 0.18*	3.41 \pm 0.68	0.43 \pm 0.03	18.65 \pm 1.14	5.01 \pm 3.05	2.8–7.4
Huoshao	20	6.83 \pm 0.24	0.81 \pm 0.12	3.68 \pm 0.34	0.45 \pm 0.06	18.41 \pm 1.27	6.72 \pm 2.6	5.5–7.9
Dongshan	13	7.00 \pm 0.29	0.83 \pm 0.12	4.21 \pm 0.60	0.50 \pm 0.03	18.66 \pm 1.57	10.96 \pm 2.95	9.2–12.7

* Indicate significant difference compared with reference site: Dongshan ($P < 0.05$).

Table 3

Pearson correlation coefficients between SFG (J/g/h) and concentrations (ng/g dry weight) of DDT or PAHs in viscera of *Perna viridis*

	DDT (viscera)	PAHs (viscera)	<i>n</i>
SFG	−0.998*	−0.564	4

* $P < 0.05$.

obscured any effects of low levels of PAHs on SFG in our study.

In summary, mussels from Tong'an site were highly stressed, because the environmental quality in this sea area was deteriorating and if no remedial action is taken, the area will no longer be suitable for aquaculture.

Acknowledgement

This work was supported by the Chinese National Science Foundation (project no. 40106012). We thank David Klumpp from Australia for teaching us how to measure SFG and Zhang Zhiguo (an undergraduate student in Fisheries college, Jimei University) for assisting with sampling of mussels.

References

- Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A.R.D., Widdows, J., 1985. Effects of Stress and Pollution on Marine Animals. Greenwood Press, Westport, CT, 384 pp.
- Chen, W., Zhang, L., Xu, L., Wang, X., Hong, H., 1998. Persistent organochlorine residues in sediments of Hong Kong Victoria Harbour and Xiamen Western Bay. Chinese Journal of Oceanology & Limnology 16, 22–30.
- Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnology and Oceanography 11, 338–345.
- Cranford, P.J., Gordon, D.C., Lee, K., Armsworthy, S.L., Tremblay, G.H., 1999. Chronic toxicity and physical disturbance effects of water- and oil-based drilling fluids and some major constituents on adult sea scallops (*Placoepecten magellanicus*). Marine Environmental Research 48, 225–256.
- Donkin, P., Widdows, J., Evans, S.V., Worrall, C.M., Carr, M., 1989. Quantitative structure-activity relationships for the effect of hydrophobic organic chemicals on rate of feeding by mussels (*Mytilus edulis*). Aquatic Toxicology 14, 277–294.
- Elfwing, T., Tedengren, M., 2002. Effects of copper on the metabolism of three species of tropical oysters, *Saccostrea cucullata*, *Crassostrea lugubris* and *C. belcheri*. Aquaculture 204, 157–166.

- Gnaiger, E., 1983. Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. Journal of Experiment and Zoology 228, 471–490.
- Hong, H., Xu, L., Zhang, L., Chen, J.C., Wong, Y.S., Wan, T.S.M., 1995. Environmental fate and chemistry of organic pollutants in the sediment of Xiamen and Victoria Harbours. Marine Pollution Bulletin 31, 229–236.
- Hong, H., Wang, X., Xu, L., Chen, W., Zhang, L., Zhang, Z., 2000. Trace organic pollutants in the southeast estuarine environments of China. Journal of Environmental Science & Health Part A—Toxic/Hazardous Substances & Environmental Engineering 35, 1833–1847.
- Klumpp, D.W., Hong, H., Humphrey, C., Wang, X., Codi, S., 2002. Toxic contaminants and their biological effects in coastal waters of Xiamen, China. I. Organic pollutants in mussel and fish tissues. Marine Pollution Bulletin 44, 752–760.
- Li, S., Wang, W., Hsieh, D.P.H., 2002. Effects of toxic dinoflagellate *Alexandrium tamarens* on the energy budgets and growth of two marine bivalves. Marine Environmental Research 53, 145–160.
- Magnusson, K., Berggren, M., Granmo, A., 1988. Energy budget of caged common mussels (*Mytilus edulis*) in the vicinity of an industrial centre on the Swedish west coast. Vatten Water 44, 59–64.
- Nelson, W.G., 1990. Use of the blue mussel “*Mytilus edulis*” in water quality toxicity testing and in situ marine biological monitoring. Ecological Research Series. USEPA, pp. 1–12.
- Nicholson, S., 1999. Cardiac and lysosomal responses to periodic copper in the mussel *Perna viridis* (Bivalvia: Mytilidae). Marine Pollution Bulletin 38, 1157–1162.
- Siddall, S.E., 1980. A clarification of the genus *Perna* (Mytilidae). Bulletin of Marine Science 30, 858–870.
- Toro, B., Navarro, J.M., Palma-Fleming, H., 2003. Relationship between bioenergetics responses and organic pollutants in the giant mussel, *Choromytilus chorus* (Mollusca: Mytilidae). Aquatic Toxicology 63, 257–269.
- Widdows, J., 1993. Marine and estuarine invertebrate toxicity tests. In: Calow, P. (Ed.), Handbook of Ecotoxicology, vol. 1. Blackwell Scientific, Oxford, pp. 145–146.
- Widdows, J., Johnson, D., 1988. Physiological energetics of *Mytilus edulis*: scope for growth. Marine Ecology Progress Series 46, 113–121.
- Widdows, J., Salkeld, P.N., 1992. Role of scope for growth in environmental toxicology and pollution monitoring. FAO/UNEP training workshop on “the techniques for monitoring biological effects of pollutants on marine organism”. Plymouth Marine Laboratory, Plymouth, United Kingdom.
- Widdows, J., Donkin, P., Evans, S.V., 1987. Physiological responses of *Mytilus edulis* during chronic oil exposure and recovery. Marine Environmental Research 23, 15–32.
- Widdows, J., Burns, K.A., Menon, N.R., Page, D.S., Soria, S., 1990. Measurement of physiological energetics (scope for growth) and chemical contaminants in mussels (*Arca zebra*) transplanted along a contamination gradient in Bermuda. Journal of Experimental Marine Biology and Ecology 138, 99–117.

- Widdows, J., Donkin, P., Brinsley, M.D., Evans, S.V., Page, D.S., Salkeld, P.N., 1995a. Sublethal biological effects and contaminant monitoring of Sullom Voe (Shetland) using mussels (*Mytilus edulis*). Proceedings of the Royal Society of Edinburgh 103B, 99–112.
- Widdows, J., Donkin, P., Brinsley, M.D., Evans, S.V., Salkeld, P.N., Franklin, A., Law, R.J., Waldock, M.J., 1995b. Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Marine Ecology Progress Series 127, 131–148.
- Widdows, J., Nasci, C., Fossato, V.U., 1997. Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy. Marine Environmental Research 43, 69–79.
- Widdows, J., Donkin, P., Staff, F.J., Matthiessen, P., Law, R.J., Allen, Y.T., Thain, J.E., Allchin, C.R., Jones, B.R., 2002. Measurement of stress effects (scope for growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. Marine Environmental Research 53, 327–356.
- Wu, Y., Zhang, J., Zhou, Q., 1999. Persistent organic residues in sediments from Chinese river/estuary systems. Environmental Pollution 105, 143–150.
- Yuan, D., Yang, D., Wade, T., Qian, Y., 2001. Status of persistent organic pollutants in the sediment from several estuaries in China. Environmental Pollution 114, 101–111 (in Chinese with English abstract).
- Zhang, Z., Hong, H., Khalid, M., Zhou, J., 2000. Study on organochlorine pesticide and PCBs at surface water in Xiamen Harbour. Marine Environmental Science 19, 48–52 (in Chinese with English abstract).
- Zhang, Z., Hong, H., Zhou, J., Huang, J., Yu, G., 2003. Fate and assessment of persistent organic pollutants in water and sediment from Minjiang River Estuary, Southeast China. Chemosphere 52, 1423–1430.
- Zhou, J.L., Fileman, T.W., Evans, S.V., Donkin, P., Mantoura, R.F.C., Rowland, S.J., 1996. Seasonal distribution of dissolved pesticides and polynuclear aromatic hydrocarbons in the Humber Estuary and Humber Coastal Zone. Marine Pollution Bulletin 32, 599–608.
- Zhou, J.L., Hong, H., Zhang, Z., Maskaoui, K., Chen, W., 2000. Multi-phase distribution of organic micropollutants in Xiamen, China. Water Research 34, 2132–2150.