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Biodynamic understanding of mercury accumulation in marine and freshwater fish

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Abstract. Mercury (Hg) is a global environmental pollutant that has been the cause of many public concerns. One particular concern about Hg in aquatic systems is its trophic transfer and biomagnification in food chains. For example, the Hg concentration increases with the increase of food chain level. Fish at the top of food chain can accumulate high concentrations of Hg (especially the toxic form, methylmercury, MeHg), which is then transferred to humans through seafood consumption. Various biological and physiochemical conditions can significantly affect the bioaccumulation of Hg–including both its inorganic (Hg(II)) and organic (MeHg) forms–in fish. There have been numerous measurements of Hg concentrations in marine and freshwater fish worldwide. Many of these studies have attempted to identify the processes leading to variations of Hg concentrations in fish species from different habitats. The development of a biokinetic model over the past decade has helped improve our understanding of the mechanisms underlying the bioaccumulation processes of Hg in aquatic animals. In this review, I will discuss how the biokinetic modeling approach can be used to reveal the interesting biodynamics of Hg in fish, such as the trophic transfer and exposure route of Hg(II) and MeHg, as well as growth enrichment (the increases in Hg concentration with fish size) and biomass dilution (the decreases in Hg concentration with increasing phytoplankton biomass). I will also discuss the relevance of studying the subcellular fates of Hg to predict the Hg bioaccessibility and detoxification in fish. Future challenges will be to understand the inter- and intra-species differences in Hg accumulation and the management/mitigation of Hg pollution in both marine and freshwater fish based on our knowledge of Hg biodynamics.

Keywords: mercury; fish; biodynamics; bioaccumulation; biodilution; subcellular distribution; risk assessment

1. Introduction

Mercury is a global metal pollutant and there are very few metals can match mercury in terms of attracting the public's attention and causing global concern. Mercury is third (after arsenic and lead) on the list of hazardous substances prepared by the US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry of the US Department of Health and Human Services. One of the greatest issues in Hg pollution is the trophic transfer and biomagnification in aquatic food chains.

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Among the many different toxic metals, mercury is unique in the following aspects. First, atmospheric deposition is often the dominant anthropogenic source of Hg pollution in many aquatic environments. In China, Hg emissions from non-ferrous metal smelting, coal combustion, and miscellaneous activities (e.g., battery, fluorescent lamp and cement production) contribute about 45%, 38% and 17% to total Hg emissions, respectively (Zhang *et al*. 2002). As a consequence, mercury pollution is often documented in pristine environments where there is no obvious source of industrial activity. Identifying the sources of Hg emissions and inputs has been a major research area. Second, mercury is complicated with its various chemical species. In natural environments, Hg mainly exists as elemental mercury (Hg^0), divalent mercury (Hg^{2+}), and monomethylmercury (CH_3-Hg^+, or methylmercury (MeHg)), in addition to other chemical species such as Hg_2^+ and CH_3HgCH_3. In natural waters, Hg is mainly present as inorganic Hg (Hg(II), or Hg^{2+}) and MeHg. These two species have contrasting patterns of bioaccumulation potential and toxicity, and provide ample opportunities in ecotoxicological and environmental chemistry studies. Third, inorganic and organic mercury can be transformed–through methylation and demethylation–into each other as mediated by biological activity (e.g., sulfur-reducing bacteria) or physico–chemical processes (e.g., photoreaction). Methylation is a key step in the introduction of Hg into the food chain. For Hg(II) and MeHg, speciation is complicated by their binding to various ligands (e.g., chloride and dissolved organic carbon, Fitzgerald *et al*. 2007). Differences in Hg speciation may considerably affect its bioavailability and bioaccumulation in aquatic organisms. Fourth, MeHg is one of the few metals that are known to be biomagnified in marine food chains. Thus, fish at the top of the food chain have high MeHg concentrations and pose health threats to people who consume them.

Because of these very unique properties of Hg and its environmental importance, research on Hg has been extensive. The bioaccumulation in and toxicity of mercury to aquatic organisms are greatly dependent on the physico–chemical factors (e.g., dissolved organic matter, salinity, temperature) as well as the biological factors. There have been numerous studies on Hg bioaccumulation in different freshwater and marine organisms, but the majority of these studies have focused on Hg concentrations in the organisms. A search in the *Web of Science* using the keywords '*mercury + fish + bioaccumulation*' yields about 700 publications between 1991 and Jan. 2012. Using simply the keywords 'fish+mercury' yields a total of 4500 publications between 1991 and Jan. 2012. This simple search clearly shows that the number of publications in bioaccumulation increased almost exponentially over the past 20 years (Fig. 1), underscoring the serious concerns about Hg in fish, now considered one of the major routes through which humans are exposed to Hg.

The vast majority of these studies have focused on Hg concentrations in the organisms, with the aim mainly being to identify the potential risk to humans due to fish consumption. Over 20 years ago, Morel *et al*. (1998) concluded that 'we still have an incomplete understanding of the factors that control the bioconcentration of mercury'. Nine years later, Fitzgerald *et al*. (2007) also concluded that 'only a limited number of studies have investigated the bioaccumulation and biomagnification of MeHg in marine food webs'. Their statements remain valid to this day. Recently, there have been a substantial number of studies on the biodynamics of Hg in both freshwater and marine fish. Biodynamic studies have benefited greatly from the development of a biokinetic model over the past decade to predict the bioaccumulation of metals in aquatic organisms (see reviews by Luoma and Rainbow 2005, Wang and Rainbow 2008). In this review, I will highlight some of these recent studies and discuss the biodynamic mechanisms controlling the trophic transfer of Hg in fish. With the application of the biokinetic model, it is now possible to delineate the exposure of Hg (waterborne uptake, including gill uptake and gastrointestinal uptake, trophic transfer, as well as the

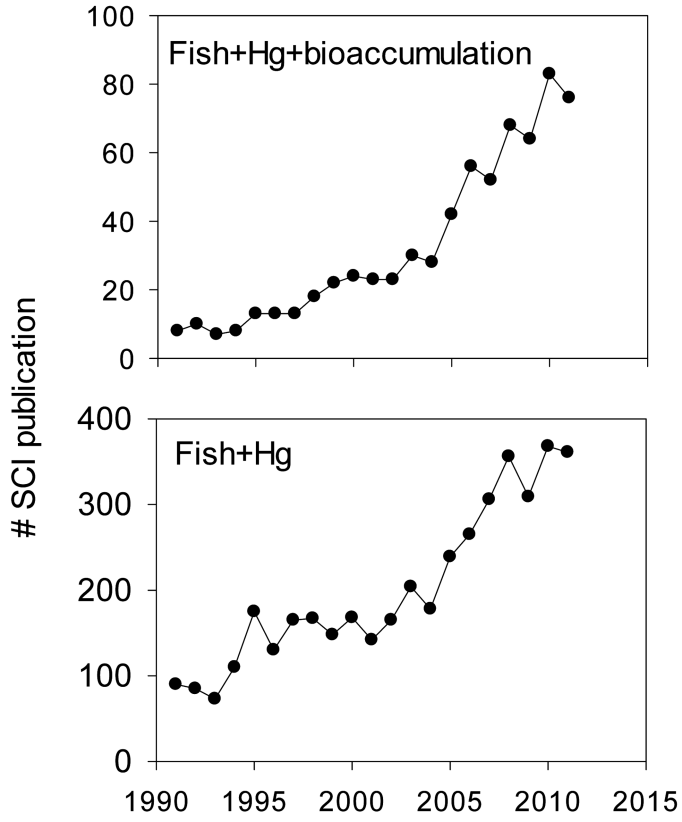

Fig. 1 Number of Science Citation Index publications over the past 20 years. Data are obtained from the Web of Science using keywords 'fish + Hg + bioaccumulation' (top) and 'fish + Hg' (bottom)

relative importance of different species of Hg) and the potential for biomagnification of both Hg(II) and MeHg in aquatic food chains. Furthermore, the application of the biokinetic model can help tremendously in understanding the bioaccumulation of Hg in fish under different environmental conditions, now considered a major topic of research in Hg ecotoxicology.

Trophic transfer and exposure in fish

Trophic transfer is now considered the dominant pathway through which many metals are accumulated in aquatic organisms (for reviews, see Wang and Fisher 1999, Wang 2002, Wang and Rainbow 2008). Numerous studies have been conducted on Hg bioaccumulation in fish, and in particular on Hg concentrations in fish tissues and their links with trophic position (e.g., Magalhaes *et al*. 2007, Levinton and Pochron 2008, Rypel *et al*. 2008). Mechanistic studies of Hg bioaccumulation such as trophic transfer, subcellular controls, and dietary exposure are relatively rare however. With the application of the radiotracer methodology (using ^{203}Hg as a radiotracer), recent studies have measured the dietary assimilation efficiency (AE) of fish feeding on different preys (Lawson and Mason 1998, Wang and Wong 2003, Pickhardt *et al*. 2006, Mathews and Fisher 2008) (Table 1). These studies have generally demonstrated that dietary AEs of MeHg are very high (up to 90%), whereas the AEs of Hg(II) are rather low or moderate.

Table 1 Biokinetic parameters for freshwater and marine fish measured using radiotracer techniques

	Hg(II)			MeHg			References
	k_u (L g^{-1} d^{-1})	AE (%)	k_e (d^{-1})	k_u (L g^{-1} d^{-1})	AE (%)	k_e (d^{-1})	
Tilapia *Oreochromis niloticus*	0.086	9-32	0.039 d^{-1}	0.333	90-99	0.0055	Wang *et al*. (2010)
Mosquitofish *Gambusia affinis*	0.052-0.078	42-51	0.021-0.042	0.185-0.338	90-94	0.016-0.019	Pickhardt *et al*. (2006)
Sunfish *Lepomis microlophus*	0.038-0.051	8-10	0.003-0.035	0.454-1.28	86-91	0.015-0.021	Pickhardt *et al*. (2006)
Sweetlips *Plectorhinchus gibbosus*	0.195	10-27	0.029-0.055	4.52	56-95	0.010-0.013	Wang and Wong (2003)
Fundulus heteroclitus					80-99	0.010-0.019	Mathews and Fisher (2008)
Menidia mendidia	0.015-0.017	8-15	0.071-0.075	1.15-4.38	82-89	0.006-0.014	Dutton and Fisher (2010)
Terapon jurbua	0.080	38	0.026	1.90	93	0.0018	Dang and Wang (2011)
*Acanthopagrus schlegeli**	$0.24W^{-0.68}$	$25.6W^{-0.68}$	$0.050W^{-0.36}$	$0.36W^{-0.54}$	80-100	$0.0062W^{-0.40}$	Dang and Wang (2012)
Atlantic cod *Gadus morhua*						0.002	Amlund *et al*. (2007)

* these biokinetic parameters were measured as a function of fish dry weight (g).

In fish, it has been well recognized that the AEs of metals are dependent on the food conditions such as the food density and food type (Ni *et al*. 2000, Wang and Wong 2003, Zhang and Wang 2006). These external conditions may significantly affect the ingestion, digestion, solubilization (Leaner and Mason 2002, Goto and Wallace 2009), membrane transport (Mason *et al*. 1996), and gut passage time (Xu and Wang 2002) and subsequently affect the dietary AEs. The AEs of Hg(II) are generally more variable than the AEs of MeHg. Hg(II) is much more particle reactive and its assimilation may be more dependent on the digestive physiology of the animals than is MeHg.

One factor that has received increasing attention is the subcellular distribution of metals in the prey in controlling the trophic transfer process. Earlier study by Lawson and Mason (1998) found that the greater assimilation of MeHg than of Hg(II) by a marine fish, sheepshead minnow (*Cyprinodon variegatus*), was due to a larger fraction of MeHg in the copepods' soft tissues. This extended previous work showing that metal assimilation by marine herbivores (copepods and bivalves) was related to its partitioning in the cytoplasm of phytoplankton cells (Reinfelder and Fisher 1991, Wang and Fisher 1996). Typically, subcellular metals are partitioned into fractions like metal-rich granules (MRG), cellular debris, organelles, heat-denatured proteins (HDP) and heat-stable proteins (HSP, presumably mainly metallothionein-like proteins) (Wallace *et al*. 2003). Wallace and Luoma (2003) proposed grouping organelles, HDP and HSP together and referred to them collectively as the trophically available metal (TAM) fraction. Goto and Wallace (2009) found that MeHg partitioned in the TAM fraction could be solubilized in the gut fluid of mummichogs (*Fundulus heteroclitus*).

Dang and Wang (2010) quantified the AEs of Hg(II) and MeHg in a marine fish, the grunt *Terapon jarbua*, based on mercury subcellular partitioning in preys (brine shrimp, clams, mussels, scallops and fish) and purified subcellular fractions of prey tissues (from mussel digestive glands and fish muscle into insoluble fraction consisting of cellular debris, MRG and organelles, HSP and HDP). Consistent with the previous measurements, the AEs of MeHg (90-94%) were much higher than those of

Hg(II). In contrast to the TAM hypothesis, Dang and Wang did not find a significant relationship between the AEs and TAM fractions for Hg(II) or MeHg, nor were they able to do so when Hg(II) and MeHg data were combined. Indeed, the AEs of MeHg were greater than its distribution in the TAM fraction, implying that MeHg bound to other fractions (MRG and/or cellular debris) was apparently bioavailable. On the other hand, Hg(II) in the TAM fraction was not completely assimilated by the fish since its AEs were smaller than its distribution in the TAM fraction. Dang and Wang's study suggested that TAM was not a reliable predictor for Hg(II) or MeHg assimilation. Recently, Rainbow *et al*. (2011) reviewed the literatures on the study of how the TAM fraction controls the trophic transfer of metals. They proposed that this term/fraction can only be considered a component of accumulated metal in food items that will vary between food items and with the feeding animals, and between different metals.

However, subcellular distribution may account for the difference in dietary bioavailability between Hg(II) and MeHg. Dang and Wang (2010) found notable differences between Hg(II) and MeHg in subcellular distribution in the cellular debris and HSP fractions. Each purified subcellular fraction had different bioavailability in fish, and appeared to explain the Hg assimilation difference. Hg(II) was less bioavailable in the insoluble fractions (e.g., cellular debris) than in the soluble fractions (e.g., HSP). It is likely that the stronger binding affinity of Hg(II) than of MeHg to the insoluble fractions explains their different AEs. However, subcellular distribution was shown to be less important for MeHg, with each fraction having comparable bioavailability.

Both the dietary and waterborne accumulation of Hg(II) and MeHg contribute to the overall Hg accumulation in fish. Earlier, Hall *et al*. (1997) found that the uptake of MeHg from diet (trophic transfer) contributed over 85% of total MeHg accumulation in freshwater finescale dace (*Phoxinus neogaeus*). In addition, due to the fact that Hg can be biomagnified, people naturally thought that food is the predominant route for Hg accumulation in fish. But this conclusion is not well substantiated. In addition, marine fish must drink seawater in saline environments for osmoregulation (e.g., gastrointestinal uptake), thus the route of exposure can be very different from that of freshwater fish. Indeed, there is a tremendous difference in the exposure of metals between marine and freshwater fish (Wang and Rainbow 2008). The relative importance of Hg(II) versus MeHg accumulation is also intriguing. Since most Hg present in fish muscle is in methylated form, people also naturally thought that MeHg is the main species accumulated by fish. This is in rather sharp contrast to findings that the majority of Hg in natural water is present as inorganic mercury, while MeHg only contributes to less than 5% of the total Hg (Watras *et al*. 1998). Devising experiments to separate these two chemical species and different exposure routes (waterborne vs. dietary) in natural settings is a daunting task, but modeling has allowed us to answer some of these key questions.

A biodynamic model can be used to identify the dominant bioaccumulation route for both Hg species under realistic environmental conditions and to characterize the relative importance of Hg(II) and MeHg to the overall Hg bioaccumulation in fish. Under steady-state conditions, the Hg(II) and MeHg concentration in fish (C_{ss}, ng g^{-1}) can be calculated by

$$C_{ss} = (k_u \times C_w + \mathrm{AE} \times \mathrm{IR} \times C_f) / k_e \quad (1)$$

where C_{ss} is the mercury concentration in fish under steady state (ng g^{-1}), k_u is the uptake rate constant following water exposure (L g^{-1} d^{-1}), C_w is the Hg concentration in dissolved phase (ng L^{-1}), AE is the dietary assimilation efficiency (%), IR is the fish daily ingestion rate (% of body weight), C_f is the Hg concentration in the prey (ng g^{-1}), and k_e is the efflux rate constant (d^{-1}). Growth is

assumed to be negligible in this calculation (but can be an important term in Hg biodynamics as will be shown later). To predict the relative importance of water vs. dietary source to mercury accumulation, C_f can be estimated from the bioconcentration factor (BCF, under steady state) of Hg in prey and the water concentration C_w ($C_f = C_w \times \mathrm{BCF}$). Then the fraction of mercury accumulation from the aqueous phase (f) can be calculated from the following equation

$$f = k_u / ((\mathrm{AE} \times \mathrm{IR} \times \mathrm{BCF}) + k_u) \quad (2)$$

To predict the fraction of total mercury (THg) accumulation due to Hg(II) or MeHg, the concentration factor (CF) of either mercury species in fish can be calculated using the following equation (Wang and Fisher 1999)

$$\mathrm{CF} = (k_u + (\mathrm{AE} \times \mathrm{IR} \times \mathrm{BCF})) / k_e \quad (3)$$

Thus, the THg accumulation due to Hg(II) ($R_{Hg(II)}$) can be calculated as

$$R_{Hg(II)} = \mathrm{CF}_{\mathrm{Hg(II)}} / (\mathrm{CF}_{\mathrm{Hg(II)}} + \mathrm{CF}_{\mathrm{MeHg}} \times C_{MeHg} / C_{Hg(II)}) \quad (4)$$

where $\mathrm{CF}_{\mathrm{Hg(II)}}$ and $\mathrm{CF}_{\mathrm{MeHg}}$ are the concentration factors of Hg(II) and MeHg in fish, respectively, and the C_{MeHg}/C_{Hg} is the ratio of MeHg concentration to Hg(II) concentration in water.

The trophic transfer factor (TTF), defined as the ratio of Hg concentration in fish to Hg concentration in prey, can also be calculated as

$$\mathrm{TTF} = (\mathrm{AE} \times \mathrm{IR}) / k_e \quad (5)$$

It is clear from these kinetic equations that the relative importance of waterborne vs. dietary, together with that of Hg(II) vs. MeHg, depends on many biokinetic parameters, thus laboratory experiments using simple exposure conditions cannot truly reflect the actual conditions in the field. These biokinetic parameters are now available for several marine and freshwater fish (Table 1). For example, Wang and Wong (2003) investigated the bioaccumulation of Hg(II) and MeHg in the marine sweetlip *Plectorhinchus gibbosus*. This study was perhaps the first to consider the variability of AEs as a function of prey (10-27% for Hg(II) and 56-95% for MeHg) and to model the exposure of Hg in fish. The dissolved uptake rate of MeHg was 23 times higher than that of Hg(II). Modeling calculation indeed found that there was no definite dominance of either dissolved exposure or dietary uptake, the latter of which depends largely on the feeding rate of the fish (IR) and the Hg concentration factor in the prey (BCF). This study demonstrated just how complicated the biodynamics of Hg in marine fish really is.

A similar modeling exercise was conducted by Dang and Wang (2011), who found that the relative importance of waterborne and dietary exposure varies with the BCF of the prey and the IR of the marine fish *T. jurbua*. At the low end of BCF, waterborne exposure dominates the Hg(II) and MeHg accumulation. In contrast, dietary exposure contributes mainly to Hg accumulation at the high end of BCF regardless of the variation in IR. Using the representative BCF values for Hg(II) and MeHg in prey found in Hong Kong (10^5 L kg^{-1} and 2.5×10^5 L kg^{-1}, respectively), it was found that more than 80% of the Hg(II) body burden and 55% of the MeHg body burden in *T. jurbua* indeed derive from dietary exposure. The relative importance of Hg(II) vs. MeHg in the overall Hg

bioaccumulation in marine fish can also be modeled. In Dang and Wang's modeling exercise, a typical IR value of 5% of fish body weight per day was chosen, but such variation should have little effect on the modeling outcome. Within the range of dissolved MeHg:Hg concentration ratios of 1-5%, the calculated likely contribution of Hg(II) accumulation in *T. jurbua* was 17-51% of total Hg in marine fish. This calculation again suggested that a significant fraction of the Hg accumulated in fish was due to Hg(II) uptake. One immediate question then is why MeHg was predominantly found in the fish muscle, with only a small fraction as Hg(II). At present, there is still no definite answer to this intriguing question.

In the freshwater tilapia (*Oreochromis niloticus*), MeHg was predominantly accumulated through dietary exposure. Hg(II) was too except when the BCF of Hg in the prey was low (Wang *et al*. 2010). The significance of trophic transfer was primarily a result of a relatively low k_u value for both Hg species (note that this fish's k_u was conspicuously lower than those of other fish species, Table 1). In contrast, the BCF of Hg in the prey is in the order of 10^4-10^6, which represents the greatest step of Hg bioaccumulation through transfer along the aquatic food chain. Modeling calculation also showed that about 60-99% of THg bioaccumulation in fish was due to MeHg uptake, which was consistent with the finding that MeHg was the major mercury species in fish muscle based on a large number of field studies (Bloom 1992). The much higher k_u and AE, and the lower k_e of MeHg than of Hg(II) all greatly contributed to the relative importance of MeHg in the overall mercury accumulation, even though MeHg constituted only a small fraction of the total dissolved Hg in the water. In this study, the consistence between the model prediction and the actual measurement may also imply that the methylation within the tilapia tissue was rather small, but this requires to be further tested.

Equation 5 shows that the TTF is dependent on the IR, AE and k_e. The calculated TTF of either mercury species generally indicated that the TTF of MeHg was greater than 1, while the TTF of Hg(II) was less than 1. Thus, MeHg has a higher potential to be biomagnified by trophic transfer, whereas Hg(II) is unlikely to be biomagnified perhaps because of its low AE and relatively high k_e. Figure 2 shows the possible biomagnification of MeHg and Hg(II) under different AE, k_e and IR scenarios. Both the high and low ends of IR for fish are used in the modeling simulation. The straight lines in the figures represent the predicted TTF of 1, thus data above the straight lines suggest biomagnification of Hg (TTF > 1), while data below the straight lines suggest biodiminishment of Hg (TTF < 1). It is clear that under most circumstances MeHg will be biomagnified, except when its k_e is at the high end (e.g., around 0.02 d^{-1}). With high k_e, the potential biomagnification of MeHg will be dependent on the IR of the fish. For Hg(II), there is still potential biomagnification when the IR of fish is at the high end.

Significance of Hg speciation and fish physiology in waterborne uptake

It is important to realize that the bioaccumulation in and toxicity of mercury to aquatic organisms are greatly dependent on physico-chemical factors (e.g., dissolved organic matter, salinity, temperature) as well as biological factors. This has been an important research area in Hg ecotoxicology. For example, metal accumulation (or toxicity) is reduced in the presence of organic matter, a phenomenon largely ascribed to the complexation of organic ligands with metals, thereby reducing the concentration of available free ionic metals. Choi *et al*. (1998) observed a decrease in MeHg uptake by the Sacramento blackfish *Orthodon microlepidotus*, as well as in MeHg levels in their gills, with increasing dissolved organic carbon concentrations. Watras *et al*. (1998) found that there was a negative relationship between the bioaccumulation factors of Hg and MeHg in microsestons,

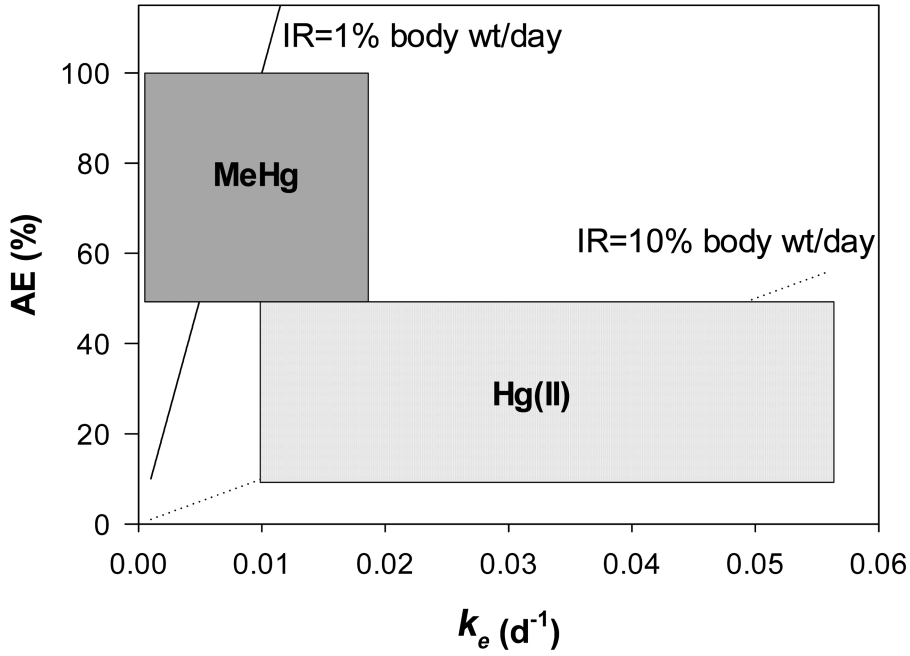

Fig. 2 Ranges of dietary assimilation efficiency (AE) and efflux rate constant (k_e) of MeHg and Hg(II) quantified for different species of freshwater and marine fish. The two lines are two different ingestion rates (IRs) of the fish (1 and 10% of body weight per day, representing the low and high ends of typical IRs) with the same predicted trophic transfer factor of 1. Above the line would indicate biomagnification of Hg (TTF > 1) at that particular IR of the fish

crustacean zooplankton, and fish and dissolved organic carbon (DOC) concentrations in Wisconsin lakes.

The underlying mechanisms of the influences of environmental factors (e.g., DOC, pH, competing ions) on Hg bioaccumulation have seldom been considered. Relatively few studies have specifically addressed the chemical species of Hg directly bioavailable to aquatic organisms, including fish. Hg speciation in the water as well as its physiological change can be greatly dependent on the water physico-chemical properties. Hg is bound to different inorganic or organic ligands in the ambient environments. Complexation of Hg with chloride (Cl) can form different species such as $HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$, depending on the Cl concentration in the water (freshwater and marine). Organic complexation with DOC is also critical for Hg due to its strong binding to the reactive thiol group (Lamborg *et al*. 2004, Haitzer *et al*. 2002). Thus, both DOC and the Cl complex are the dominant binding ligands for Hg, and can significantly affect the biological uptake of Hg by aquatic organisms. Hg-Cl can be taken up through passive diffusion as well as active transport (Laporte *et al*. 2002, Klinck *et al*. 2005, Zhong and Wang 2009), while the mercury-DOC complex has a very low bioavailability and the possible uptake mechanism likely involves ligand exchange processes. Zhong and Wang (2009) examined the effects of Cl on the bioaccumulation in a marine diatom and showed the importance of neutral Hg species. The DOC effects were found to be dependent on its origin, concentration, and size and various effects have been documented in the literature (Laporte *et al*. 2002, Klinck *et al*. 2005, Pickhardt and Fisher 2007, Zhong and Wang 2009).

In a recent study, Wang and Wang (2010) examined the roles of Hg speciation in its waterborne uptake by tilapia *O. niloticus* by controlling the salinity (the chloride concentration to be precise) and DOC in the medium. Hg speciation varies with the chloride concentration (Fitzgerald *et al*. 2007), especially within the low chloride range (0 to 0.1 M chloride). For example, neutral $HgCl_2$ is dominant at low salinity, and increases initially with salinity until it peaks at 2 practical salinity units (psu) (0.03 M chloride) and then decreases again to a constant level. Instead, the negatively charged complexes ($HgCl_3^-$, $HgCl_4^{2-}$) become more important at a higher salinity due to Cl complexation. Such a change in Hg speciation provides an excellent way to study the Hg species taken up by the fish, if the fish can be acclimated under different salinities. Wang and Wang (2010) acclimated the tilapia at different salinities and then measured the Hg uptake by the fish. They showed that only the decrease in neutral $HgCl_2$ was consistent with the uptake results, implying that neutral $HgCl_2$ was probably the most bioavailable mercury species. To further confirm this hypothesis, they measured the uptake rates at very low salinities (0-6 psu). Within this range only the abundance of neutral $HgCl_2$ changed rapidly. Hg(II) uptake initially increased and then decreased with the salinity gradient, peaking at 2 psu, consistent with the $HgCl_2$ speciation. These data were similar to those of a previous study on marine diatom suggesting that $HgCl_2^0$ was the main pathway of Hg(II) aqueous uptake (Mason *et al*. 1996, Zhong and Wang 2009).

Compared with salinity, DOC had a more dominant role in affecting the Hg accumulation. In the presence of DOC, Hg uptake decreased dramatically since mercury-DOC complexes were unlikely to transport across the biological membrane. In this case, ligand exchange and competitive binding may be required and become a limiting step in Hg uptake. Wang and Wang (2010) showed that the inhibition of DOC was dependent on Cl^-, which was less significant at middle salinity levels for Hg(II). This was consistent with the prediction by the mercury-Cl-DOC model which suggested that the organic ligand strength under higher salinity is stronger (Fitzgerald *et al*. 2007), and the dominance of mercury-DOC will then increase, leading to reduced uptake.

MeHg has a lower affinity with DOC but permeates lipophilic biolayers more easily than Hg(II), and thus the inhibitory effects of DOC on MeHg uptake were less obvious. MeHgCl and MeHgOH are the most important MeHg species, but MeHgCl dominated (> 90%) and was less abundant only at 0 psu. MeHgCl was the most bioavailable MeHg species and was taken up either by passive diffusion (Mason *et al*. 1996) due to its higher lipophilicity than MeHgOH or by active uptake (Pickhardt and Fisher 2007). Wang and Wang (2010), however, found that the increase in MeHgCl at 10 and 28 psu could not explain the sharp decrease in MeHg uptake compared to that at 0 psu. Other processes may also play a role in MeHg uptake.

In addition to Hg speciation, physiological processes are also critical for the uptake of Hg by the fish. The fish gill is not only the respiratory organ but also the major site for ion and water exchanges, thus the changes of water-pumping activity may affect the accompanying ion uptake and excretion when the Hg uptake is a rate-limiting process. The coupling relationship between Hg uptake and fish physiology has seldom been considered. Recently, Wang *et al*. (2011) examined the relationship among the uptake of MeHg and two important fish physiological processes–respiration (metabolism) and water pumping–in tilapia (*O. niloticus*) under various environmental conditions (temperature, dissolved oxygen level, and water flow). Both the MeHg uptake and respiration rate increased at a higher temperature, indicating the influence of metabolism on MeHg uptake. With a decrease in oxygen level, MeHg and water uptake rates increased simultaneously, suggesting the coupling of water flux and methylmercury uptake. In contrast, the respiration was not affected until the oxygen concentration decreased to below 1 mg L^{-1}. Finally, rapidly swimming fish had significantly

higher uptake rates of MeHg, water and oxygen, confirming the coupling relationships among respiration, water pumping, and metal uptake. All these results strongly suggested the important role of physiological processes play in mercury bioaccumulation in fluctuating aquatic environments.

Hg growth enrichment and biomass dilution in fish

One of the most interesting aspects regarding Hg in fish is 'growth enrichment'–the phenomenon that larger fish contain higher Hg (mostly MeHg) concentrations (e.g., Gilmour and Riedel 2000, Magalhaes *et al*. 2007). Different from many other trace metals, mercury is generally accumulated in fish in proportion to its size (Joiris *et al*. 2000, Wiener *et al*. 1990, Peterson and Sickle 2007, Monterio and Lopes 1990, Sonesten 2003, Storelli *et al*. 2007, Gewurtz *et al*. 2011, Staudinger 2011, Qiu *et al*. 2011). This relationship remains one of the major topics to be covered in any Hg measurement in fish. Conversely, faster fish growth could reduce MeHg concentrations by 'growth dilution', as demonstrated in wild fish (Ward *et al*. 2010a, b, Trudel and Rasmussen 2006) and aquatic invertebrates (Karimi *et al*. 2010). Such allometric (or size-dependent) mercury bioaccumulation has been relatively well documented in the literature and is well-known among the scientific community and the general public. Growth dilution has been recognized as an important factor explaining the correlations between rapid growth and reduced element concentrations (including essential and nonessential elements) in many organisms, including the reduced mercury levels in fast-growing fish (Ward *et al*. 2010a, b, Simoneau *et al*. 2005, Trudel and Rasmussen 2006).

Various hypotheses have been put forward, for example, the shift of fish prey to more contaminated prey at higher trophic levels with age (Trudel and Rasmussen 2006), and the growth of fish ('growth dilution') associated with high growth efficiency and growth rate (Ward *et al*. 2010, Karimi *et al*. 2010). Changes in diet composition with fish growth have been well recognized. For example, Eagles-Smith *et al*. (2008) found that there were clear ontogenetic shifts in foraging habitats and trophic position. Pelagic diet decreased and benthic diet increased with increasing fish length in bluegill, black crappie, inland silverside, and largemouth bass, but not in prickly sculpin or threadfin shad. As a result, it was concluded that fish THg concentrations varied with habitat-specific foraging, trophic position, and size. Lavigne *et al*. (2010) assessed the relationship between mercury (Hg) concentrations in fish muscle and fish growth rates in 54 walleye *Sander vitreus*, 52 northern pike *Esox lucius*, and 35 lake trout *Salvelinus namaycush* populations throughout the Province of Quebec, Canada. They demonstrated that the growth rates were positively related to Hg concentrations in walleyes and northern pike, whereas no correlation was observed in lake trout. Thus, slower-growing walleyes and northern pike have higher Hg concentrations for a given length. Ward *et al*. (2010a) measured concentrations of seven trace elements (As, Cd, Cs, Hg, Pb, Se and Zn) in streamdwelling Atlantic salmon from 15 sites encompassing a 10-fold range in salmon growth. Fast-growing salmon had a lower concentration of any element than slow-growing salmon, after accounting for prey concentrations, indicating that dilution of elements in larger biomass led to lower concentrations in fast-growing fish. In a second study, Ward *et al*. (2010b) examined the relationship between Hg concentration and growth rate in fish by means of a large-scale field experiment. In this study, the Atlantic salmon Salmo salar fry were released at 18 sites into natural streams, collected after one growing season, and measured for Hg concentration and growth. The large, fast-growing ones had lower Hg concentrations than the small, slow-growing ones, and the growth rate accounted for 38% of the explained variation in Hg concentration in the Atlantic salmon across sites, while the concentration of the prey accounted for 59%. Simoneau *et al*. (2005) also examined the relationship between Hg concentrations in walleye (*Sander vitreus*) muscles and

their growth rates in 12 natural lakes located in four different regions across Quebec. Similarly, the faster-growing walleyes had lower Hg concentrations than the slower-growing ones for a given length, and growth rate dominated all other environmental factors in accounting for differences in Hg concentration among walleye populations.

Dang and Wang (2012) recently addressed the same issue using size-dependent biokinetic parameters, i.e., waterborne and dietary Hg uptake kinetics and loss dynamics. The objective was to provide a biokinetic explanation for the established Hg allometric relationships in field-sampled juvenile blackhead seabream by developing size-related dissolved uptake rate constants (k_u), AEs, growth rates (g), and k_e's. As expected, the measured THg and MeHg concentrations in the field-collected juvenile blackhead seabream *Acanthopagrus schlegeli* increased with body size, with a power coefficient of 0.19 and 0.33 for THg and MeHg, respectively, over a wide size range (more than 50-fold). THg concentrations in the juvenile were 4.7-39.5 ng g^{-1} dw among individuals 34-87 mm in length. The corresponding MeHg concentrations were 1.7 to 14.4 ng g^{-1} dw. Positive (Hg(II) AE) and negative correlations (g, k_u and k_e) with fish size were found, whereas the dietary AEs of MeHg were not affected by fish size since they were generally very high (around 90%, Table 1). For example, k_u was related to fish mass with an allometric exponent of -0.68 for Hg(II) and -0.54 for MeHg. Hg(II) AE showed a significant positive relationship with body weight, which may be due to a prolonged gut passage time and a higher intestinal digestion and solubilization efficiency. The Hg k_e was related to body weight with a power of -0.36 for Hg(II) ($Y=0.05W^{-0.36}$) and a power of -0.40 for MeHg ($Y=0.006W^{-0.40}$). The growth rate was related to the -0.42 power of body weight. Therefore, for a given prey intake, since smaller individuals grow faster than larger ones, 'growth dilution' may have a greater effect in smaller individuals than in larger ones, and thus lead to higher Hg concentrations in larger fish.

Dang and Wang (2012) then used the experimentally established size-related biokinetic parameters k_u, AE, k_e and g to explain the scaling exponents of Hg concentrations observed in the field-collected fish. Accordingly, the estimated exponents were 0.21 for MeHg and 0.21-0.25 for THg, close to the independent field measurements (0.33 for MeHg and 0.19 for THg). Thus, the allometric biokinetic parameters may explain the size-dependent mercury accumulation patterns observed in juveniles in the natural system. Furthermore, Dang and Wang demonstrated that the decreases in g and k_e with increasing body size were mainly responsible for the increased Hg concentrations. The contributions of k_e and g to size-related MeHg concentrations were probably similar since their allometric exponents (-0.40 and -0.42, respectively) were also comparable. These biokinetic measurements strongly indicated that a slower growth in combination with a lower mercury efflux rate in larger fish was more responsible for the increased MeHg and THg concentrations and the positive size-dependent allometric correlations. Hence, the shift of prey composition as proposed earlier, together with the biokinetic variation, contributed to higher accumulated Hg levels in adult fish.

Another interesting finding in Hg biodynamics is the biomass dilution of Hg in the field-collected fish. Over the past few years, it has been shown that fish collected from pristine and oligotrophic lakes often contained higher Hg concentrations than fish collected from eutrophified waters (i.e., biomass dilution) (Chen and Folt 2005, Liu *et al*. 2012). However, the mechanisms underlying these observations remain unidentified or speculative at most (Pickhardt *et al*. 2002, Karimi *et al*. 2007). In addition to the difference in water chemistry (pH, salinity, DOC, Laporte *et al*. 1997, Klinck *et al*. 2005), the food condition and fish physiology can have complex effects on the dietary uptake by influencing the metal assimilation, elimination, as well as the fish growth. Among the many

biological factors, food availability stands out as one of the most important candidates since it can affect mercury bioaccumulation in fish due to its control of the fish feeding rate and growth rate. The IR of the fish may have significantly affected the Hg bioaccumulation pattern and indeed may explain the biomass dilution of Hg in the fish. In addition to its direct effect on Hg influx from the dietary phase, IR can also affect other terms in the biokinetic equation, such as the dietary assimilation, elimination, and growth rate (Xu and Wang 2002, Tsui and Wang 2004). Such complicated relationships have seldom been considered in previous studies. A second possible explanation for the biomass dilution is the growth of the fish. A high growth rate leads to a lower metal concentration in aquatic organisms such as microalgae (Miao and Wang 2004, Hill and Larsen 2005, Wang *et al*. 2005), cladocerans (Karimi *et al*. 2007), and fish (Ward *et al*. 2010a, b, Simoneau *et al*. 2005). For example, Ward *et al*. (2010b) found that a higher growth efficiency could result in lower MeHg concentrations in fish, and Karimi *et al*. (2007) reported that the consumption of high-quality algae reduced MeHg accumulation in *Daphnia* due to change in food quality and reduced consumption rate. Again, a biokinetic study can help reveal the mechanism of biomass dilution.

Whether changes in IR could result in somatic growth dilution has never been proved, because ingestion could affect not only growth, but also other biokinetic parameters. To specifically address the biomass dilution, Wang and Wang (2012) quantified the long-term accumulation process of mercury in freshwater tilapia (*O. niloticus*) under well-controlled feeding conditions and the complex relationships between IR and various biokinetic parameters (dietary assimilation, efflux and growth). In this study, the fish were accurately fed at different IRs for a period of 30 days, and the accumulation of both Hg(II) and MeHg was quantified, together with the measurement of biokinetic parameters. As expected, the IR had significant influence on the process of mercury bioaccumulation. For example, the difference in newly accumulated Hg(II) in the fish between the highest and lowest IRs decreased during the period of bioaccumulation (e.g., a difference of 5.4 times on the third day to 2.4-fold at the end of exposure). For MeHg, the difference decreased from 9.1 times on the third day to 7.2 times at the end of exposure. These results suggested that a higher IR could lead to a smaller proportion of ingested mercury being accumulated, consistent with the biomass dilution hypothesis.

The biokinetic parameters measured at different IRs showed different responses. With the increase in IR from 0.01 g g^{-1} d^{-1} to 0.12 g g^{-1} d^{-1}, the dietary AE of Hg(II) in the tilapia decreased from 47% to 27%, while the k_e increased from 0.026 to 0.048 d^{-1}, and g showed a power increase with IR. The influence of the IR on the AE and k_e of MeHg was however not significant (due to the high dietary bioavailability). The bioaccumulation of Hg(II) and MeHg at different IRs (0.08 and 0.12 g g^{-1} d^{-1}) was then simulated using the biokinetic model incorporating the quantified biokinetic parameters (Wang and Wang 2012). The model showed that the accumulated Hg(II) concentration in the fish at an IR of 0.08 g g^{-1} d^{-1} was lower than that at an IR of 0.12 g g^{-1} d^{-1} during the initial phase of accumulation, but then the two curves crossed on day 38. Under steady-state conditions, the predicted accumulated Hg(II) concentration in fish at an IR of 0.08 g g^{-1} d^{-1} was 0.585 ng g^{-1}, higher than that at a higher IR (0.12 g g^{-1} d^{-1}, C_{ss} = 0.556 ng g^{-1}), confirming the biomass dilution observed under the field conditions.

For MeHg, IR only affected the growth term instead of AE and k_e. The fish accumulated a higher concentration of MeHg at a higher IR during the initial periods of exposure, but again, after a long period of exposure (200 days), MeHg concentrations in fish at a lower IR (0.08 g g^{-1} d^{-1}, C_{ss} = 6.978 ng g^{-1}) exceeded those at a higher IR (0.12 g g^{-1} d^{-1}, C_{ss} = 6.309 ng g^{-1}). These modeling results indicated that the growth effect could not be ignored in a long-term accumulation process. Wang

and Wang (2012) then simulated the influence of IR (0.06~0.16 g g^{-1} d^{-1}) by considering the complex influence of the IR on all biokinetic parameters (AE, k_e and g). The predicted C_{ss} of MeHg decreased constantly from 9.97 to 5.94 ng g^{-1}, indicating a significant dilution at high IRs. In contrast, the predicted C_{ss} of Hg(II) increased slightly initially and then decreased with increasing IR, reaching the highest value of 0.59 ng g^{-1} at an IR of 0.09 g g^{-1} d^{-1} and the lowest value of 0.35 ng g^{-1} at the highest IR of 0.016 g g^{-1} d^{-1}. Thus, IR strongly influences the growth dilution, which is more likely to occur for MeHg than for Hg(II). Again, the bioaccumulation of MeHg is dependent on g, while the accumulation of Hg(II) depends more on AE and k_e. Such a difference could be explained by the much lower efflux rate of MeHg, making it more sensitive to growth. These results provided a mechanistic understanding of the observation that Hg concentrations in fish and invertebrates found in pristine, oligotrophic lakes are always higher than those found in eutrophic lakes. It is clear that differences in food availability, as well as the other biokinetic parameters in the model, may significantly affect the IR of fish, leading to significant, food-availability-driven, biomass dilution of Hg in fish.

Interspecies difference in Hg bioaccumulation

A wide range of mercury concentrations has been documented in different fish species. Higher levels of Hg are generally detected in predatory fish, emphasizing the importance of feeding habit on the Hg bioaccumulation from food source. Even with the same species of fish, mercury levels are dependent on the water chemistries such as Hg concentration, pH, and type and concentration of DOC (Watras *et al*. 1998, Greenfield *et al*. 2001, Gorski *et al*. 2003, Chen *et al*. 2005, Simonin *et al*. 2008), as well as the biological factors such as the body size. For example, Vieira *et al*. (2011) found that the intra- and inter-specific variability of metals (including Hg) was mainly affected by the body length of the pelagic fish from the Atlantic Ocean. Verdouw *et al*. (2011) investigated the effects of age and length on mercury contamination in four fish species (yellow-eye mullet *Aldrichetta forsteri*, black bream *Acanthopagrus butcheri*, sand flathead *Platycephalus bassensis* and sea-run brown trout *Salmo trutta*) from the Derwent Estuary, Tasmania, Australia. Age and length significantly influenced mercury levels in brown trout and sand flathead, with age being more strongly related to intraspecies differences. Movement and distribution within the estuary and trophic status also contributed importantly to interspecific variation. Regional difference in Hg concentration is also obvious from the literature. For example, Glover *et al*. (2010) presented a large dataset from a Hg analysis in fishes collected from South Carolina. Large, pelagic, piscivorous fish species had higher levels of tissue Hg than smaller omnivorous species. Estuarine species had relatively low levels of tissue Hg compared to freshwater species, while two large open ocean species, king mackerel (*Scomberomorus cavalla*) and swordfish (*Xiphias gladius*), had higher tissue Hg readings. Magalhaes *et al*. (2007) determined the concentrations of THg and MeHg in the muscle tissue of eight species of fish: *Pagellus acarne*, *Trachurus picturatus*, *Phycis phycis*, *P. blennoides*, *Polyprion americanus*, *Conger conger*, *Lepidopus caudatus* and *Mora moro*, caught in the Azores, and found that their concentrations were significantly related to trophic level, Hg concentration in the diet, and vertical distribution.

Recent measurements in Hong Kong marine fish have highlighted the interspecies and intraspecies differences in Hg concentration. In this study, we measured the mercury concentrations in wild marine fish from different locations in Hong Kong waters (Port Shelter, Castle Peak Bay, Victoria Harbour, and Tolo Harbour) (Fig. 3). MeHg concentrations were found to be rather low. The banded cardinal fish (*Apogon cookii*) had the highest MeHg concentration in muscle (about 10 times higher

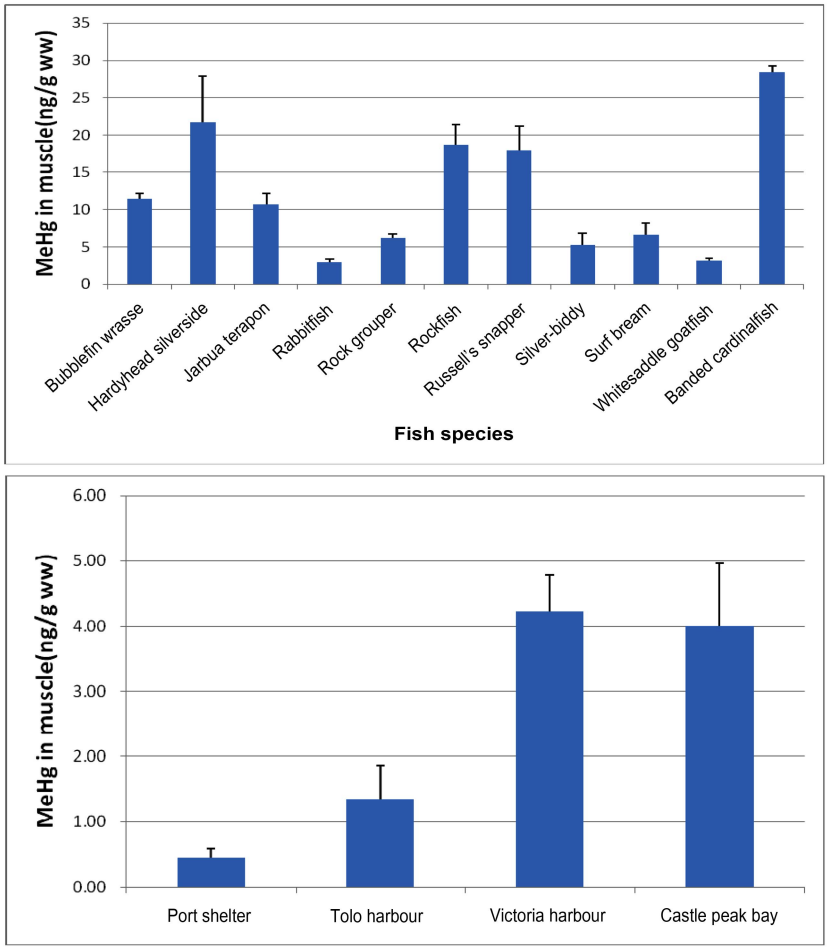

Fig. 3 The MeHg concentrations in muscle tissues of different species of marine fish collected from Hong Kong coastal waters (upper) and in muscle tissues of rabbitfish (*Siganus canaliculatus*) collected from different areas in Hong Kong

than the lowest concentration which was measured in the rabbitfish *Siganus canaliculatus*). Banded cardinal fish feed mainly on small crustaceans, which may explain why they have higher MeHg concentration than rabbitfish (which mainly feed on benthic macroalgae). However, the other species such as the rock grouper (*Epinephelus fasciatomaculosus*) and Russell's snapper (*Lutjanus russellii*) feed on large prey such as fish, prawns and gastropods, and their MeHg concentrations were not as high as expected. Thus, in addition to the trophic level (as would be expected), other factors may have an effect on the MeHg concentration in fish. We also observed a 10-fold difference in MeHg concentration in rabbitfish collected from different locations in Hong Kong. In contrast, the MeHg concentrations in muscles of silver-biddy (*Gerres macrosoma*) collected from Port Shelter were nine times higher than those collected from Tolo Harbour. Such a contrasting difference in Hg concentration within close geographical distance certainly needs to be further examined.

Subcellular distribution of Hg and its implication

The subcellular distribution of metals can provide valuable information about metal toxicity and tolerance, as well as trophic transfer and bioaccumulation. Over the past decade, a differential fractionation approach has been developed to identify the subcellular fates of metals in aquatic organisms, although it is noted that the approach is still operationally defined. Metals can be fractionated to examine their distributions in several operationally defined subcellular fractions, including MRG, cellular debris, organelles, HDP and HSP. It is believed that Hg mainly binds to the S-containing ligand since Hg^{2+} has a high affinity for thiol-complexes and always conjugates to glutathione, cysteine, homocysteine, N-acetylcysteine, metallothionein, or other S-containing molecules (Bridges and Zalups 2005). Previously, it was suggested that MRG and MTLP can be combined to form the biologically detoxified metals (BDM), while the metal soluble fractions (MTLP+HDP) and organelles can be combined to form the TAM (Wallace and Luoma 2003). Metals in the HDP and organelles are considered the metal-sensitive fraction (MSF) (Wallace *et al*. 2003). The combination of different subcellular pools has ecotoxicological relevance to the study of the subcellular distributions of metals in aquatic animals. Measurements of the subcellular distribution of Hg in marine fish may provide important information for Hg sequestration and detoxification, as well as potential bioaccessibility to human consumers.

In a large-scale study, He and Wang (2011) quantified the subcellular distribution of MeHg in different species of marine fish (rabbitfish *Siganus oramin*, grouper *Epinephelus coioides*, mullet *Mugil cephalus*, sillago *Sillago japonicus*, yellow croaker *Larimichthys crocea*, golden thread *Nemipterus virgatus*, horsehead *Branchiostegus argentatus*, mackerel *Rastrelliger faughni matsui*, and black seabream *Sparus macrocephalus*) from Hong Kong. Among these fish species, mackerel, black seabream and mullet are omnivorous and feed on detritus and copepods; rabbitfish are herbivorous and feed primarily on benthic algae; the grouper, sillago, yellow croaker, golden thread and horsehead are carnivorous and feed on other fish, octopus, crabs, and shellfish. In all the fish species, cellular debris and HSP were the major subcellular pools for MeHg, and only a small percentage of MeHg was distributed in the MRG, organelles and HDP. Earlier, MeHg has generally been found to bind to sulphydryl-rich amino acids in fish and other seafoods (Clarkson 1997). For example, Harris *et al*. (2003) found that MeHg in fish was bound to thiols, and Lemes and Wang (2009) found that it was bound to cysteine. HSP (metallothioneins) could be induced by MeHg (Nordberg 1998, Chan *et al*. 2002), which may explain the large fraction of MeHg in HSP in the fish species. Thus, binding of MeHg in HSP may be an important detoxification strategy for the marine fish. It was also difficult to conclude whether or not the subcellular distribution was dependent on the feeding habits of the fish. For example, mullet and mackerel are omnivores, and their MeHg was mainly stored in HSP in their muscles. For the herbivorous rabbitfish and carnivores such as sillago, golden thread and grouper, MeHg was predominantly stored in cellular debris. For the other carnivores (the horsehead and yellow croaker), HSP was still the dominant pool for MeHg.

In another study, Onsanit and Wang (2011) examined the concentration and distribution of total THg and MeHg in different subcellular fractions in the farmed red seabream, red drum, and black seabream from marine fish farms in Fujian, China. The concentrations of Hg in the five subcellular binding pools, including MRG, cellular debris, organelles, HDP and HSP, were then determined. Similarly, THg and MeHg were dominantly bound to the cellular debris (> 70%), followed by HSP (10-21%), MRG, HDP and organelles (< 2.7%) in that order. Significant differences in THg and MeHg distributions in all the subcellular fractions were found among the different caged sites, likely due to the different feeding habits of fish at those sites. Both THg and MeHg exhibited similar

distributions in different subcellular fractions because more than 80% of THg in all the major fractions was in MeHg form. These results and earlier studies have demonstrated that cellular debris is the major binding site for Hg in fish (Bebianno *et al*. 2007, Barghigiani *et al*. 1989). However, Amlund *et al*. (2007) found that MeHg in the Atlantic cod (*Gadus morhua*) following three months of dietary exposure to MeHg was predominantly (99%) bound to protein in the muscle. Kuwabara *et al*. (2007) performed replicate X-ray absorption near edge structure (XANES) analyses on largemouth bass and hybrid striped bass from Guadalupe Reservoir, California, and Lahontan Reservoir, Nevada, USA to determine the predominant species of mercury accumulated. They showed that mercury accumulated in both species of fish was dominated by MeHg-cysteine complexes.

Onsanit and Wang (2011) also for the first time quantified the percentage of total Hg as MeHg in each binding pool of two fishes. The highest degree of %MeHg was found in the HSP fraction (86-98% for red seabream, and 76-96% for red drums), considered the BDM pool, whereas the organelles fraction had the lowest percentage of total Hg as MeHg (30-60% for red seabream, and 32-61% for red drum). Again, mercury binding as the methylmercury-cysteine complex may thus form an important detoxification mechanism in fish muscle. It was also possible to calculate the MSF as metals in HDP and organelles. It was rather surprising that THg and MeHg in the MSF in the two fish species were both very low (4-13% for red seabream and 2-8% for red drum), which strongly suggested that Hg may present little toxicity to the fish muscle due to its low level of partitioning in the MSF.

The predominance of Hg in the cellular debris fraction may facilitate its bioavailability to humans due to fish consumption. The bioaccessibility of Hg from fish can be considered the maximum bioavailability of this pollutant to human consumers. Measurements of Hg bioaccessibility can lead to a more accurate risk assessment than measurements of THg concentrations in food. He and Wang (2011) examined the factors affecting the bioaccessibility of MeHg in nine species of marine fish with the aim of identifying ways to reduce MeHg bioaccessibility. Overall, MeHg bioaccessibility in the nine species of marine fish ranged from 16% to 68%. In general, the bioaccessibility from herbivorous and omnivorous fish (mullet, rabbitfish and mackerel) was low, while it was somewhat higher from the carnivores (yellow croaker, horsehead, sillago, grouper and golden thread). One speculation was that their different feeding habits may lead to different accumulation patterns, subcellular distributions and hence bioaccessibility. Torres-Escribano *et al*. (2010) have quantified the bioaccessibility of THg (64%) in swordfish and found that 94% of the bioaccessible mercury was MeHg, suggesting that this carnivorous fish also has a high MeHg bioaccessibility.

The bioaccessibilities of both MeHg and Hg(II) were independent on the THg concentration and the exposure route (dietary vs. dissolved exposure). In eight of the nine species studied, bioaccessibility was negatively correlated with the extent to which MeHg was partitioned into MRG and TAM fractions, but was positively correlated with its partitioning into the cellular debris fraction. Cellular debris was mainly composed of tissue fragments and cell membranes, and the much higher distribution of Hg in the cellular debris fraction indicated that the potential bioaccessibility of Hg from these marine caged fish to human consumers may also be high. He and Wang (2011) also determined the bioaccessibility of purified subcellular fractions in order to give a possible explanation for the underlying mechanisms of Hg subcellular distribution in controlling its bioaccessibility. The MeHg bioaccessibility from MRG, cellular debris and HSP fractions were all high, ranging from 84% to 91%, while those from organelles and HDP were only 17% to 29%.

2. Conclusions

To conclude, mercury contamination in fish has been a longstanding area of interest in environmental science, and there have been numerous measurements of its concentrations in different species of fish from different habitats. Extensive measurement will be continued in the future given the public concern over mercury contamination. From an academic research point of view, one fundamental question is 'why a fish can accumulate this amount of Hg in their tissue'? A biodynamic understanding of Hg bioaccumulation can certainly help to address this question. It is clear that Hg bioaccumulation is a complicated process governed by various geochemical and biological processes, but it can be studied using the powerful biokinetic modeling approach. Future challenges will be to understand the inter- and intra-species differences in Hg accumulation in both marine and freshwater fish. Given the importance of fish farming worldwide, understanding the biodynamics of Hg will certainly help improve the management of Hg pollution in farmed fish.

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