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Tracking pollutants in dietary fish oil: From ocean to table☆

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ABSTRACT

Dietary fish oil used in aquafeed transfers marine pollutants to farmed fish. However, the entire transfer route of marine pollutants in dietary fish oil from ocean to table fish has not been tracked quantitatively. To track the entire transfer route of marine pollutants from wild fish to farmed fish through dietary fish oil and evaluate the related human health risks, we obtained crude and refined fish oils originating from the same batch of wild ocean anchovy and prepared fish oil-containing purified aquafeeds to feed omnivorous lean Nile tilapia and carnivorous fatty yellow catfish for eight weeks. The potential human health risk of consumption of these fish was evaluated. Marine persistent organic pollutants (POPs) were concentrated in fish oil, but were largely removed by the refining process, particularly dioxins and polychlorinated biphenyls (PCBs). The differences in the POP concentrations between crude and refined fish oils were retained in the fillets of the farmed fish. Fillets fat content and fish growth were positively and negatively correlated to the final POPs deposition in fillets, respectively. The retention rates of marine POPs in the final fillets through fish oil-contained aquafeeds were 1.3%–5.2%, and were correlated with the POPs concentrations in feeds and fillets, feed utilization and carcass ratios. The dietary crude fish oil-contained aquafeeds are a higher hazard ratio to consumers. Prohibiting the use of crude fish oil in aquafeed and improving growth and feed efficiency in farmed fish are promising strategies to reduce health risks originating from marine POPs.

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1. Introduction

Fish are an important food, as they provide abundant nutrients, particularly n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid, which reduce the risks for cardiovascular diseases and alleviate the inflammation process (Bucher et al., 2002; Iso et al., 2006; König et al., 2005; Kris-Etherton et al., 2002). However, fish are main vectors transferring environmental pollutants to humans through food intake (Alcock et al., 1998; Harrison et al., 1998; Llobet et al., 2003). For example, fish contain the highest concentrations of polychlorinated biphenyls (PCBs) of almost all daily foods in Belgium accounting for about 50% of total daily PCB intake (Voorspoels et al., 2008). Moreover, Kiviranta et al. (2004) reported that fish are the major source of dioxins in Sweden, Norway, and Italy. Therefore, the benefits and risks of consuming fish have caught the attentions of scientists and administrators.

Notably, direct consumption of wild captured fish is decreasing and a large proportion of daily fish intake is sourced from aquaculture. Hence, the food safety of farmed fish has become particularly important health topic. Some studies indicate higher

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concentrations of pollutants, particularly persistent organic pollutants (POPs), including polycyclic aromatic hydrocarbons (PAH), organochlorine pesticides (OCPs), and PCBs are found in farmed than those in wild-caught fish (Hites et al., 2004; Rodríguez-Hernández et al., 2017). However, other studies show that farmed fish contain fewer contaminants than the corresponding wild species (Lundebye et al., 2017). The contradictory results could be explained by the fact that the pollutant concentrations in farmed fish are closely related to contaminated aquafeeds which vary depending on pollution status of different aquaculture regions (Berntssen et al., 2005; Blanco et al., 2007; Friesen et al., 2015; Hites et al., 2004; Karl et al., 2003; Lundebye et al., 2004). It has been clearly indicated that increasing dietary exposure of dioxin and dioxin-like PCB in salmon would significantly increase the concentrations of these pollutants in both salmon fillets and whole fish (Lundebye et al., 2004). Therefore, the content of pollutants in aquafeeds is an important factor affecting the safety of farmed fish.

Fish meal and fish oil, which are mainly sourced from wild-caught ocean fish, are the two main dietary ingredients in the aquafeed industry. A number of studies have demonstrated that including fish meal and fish oil in aquafeed positively correlate with the pollutants in farmed fish (Berntssen et al 2010, Berntssen et al 2005; Hamilton et al., 2005; Jacobs et al., 2002; Tacon and Metian, 2008). marine POPs tend to accumulate in the lipids of wild ocean fish because of their lipophilic characteristics and ultimately are transferred to fish oil, which is extracted from wild-caught ocean fish (Martí et al., 2010). Thus, fish oil accumulates 30-fold and 9-fold greater coplanar PCBs than bone meal, fish meat and fish meal (Guruge et al., 2005). In addition, fish oil also contains a large number of heavy metals, but the concentrations of heavy metals in fish oil are much less than those in fish meal (van de Ven, 1978). Therefore, fish oil has been identified as one of the major vectors to deliver pollutants, mainly POPs, from ocean to farmed fish through aquafeeds.

A refining process is necessary to produce fish oil for human consumption, and it can largely reduce the concentrations of environmental pollutants in the final fish oil products (Berntssen et al., 2010). However, a large number of aquafeed producers continue to use crude fish oil as a dietary oil source, especially in developing countries. One reason is that using crude fish oil decreases costs. Another more important reason is that the influence of a crude fish oil-contained diet and refined fish oil-contained diet on the potential health risks of the final farmed aquatic products have not been compared. Subsequently the use of crude fish oil in aquafeeds has been overlooked by aquafeed producers and administrators. Moreover, the transfer efficiency of the dietary fish oil-containing pollutants from ocean to farmed fish has not been precisely evaluated through the whole transmission process, including industrial and aquaculture processes. As different farmed fish species have different growth performances and biochemical compositions, whether the transfer efficiency of fish oil-containing pollutants from ocean to farmed fish varies from fish species also remains unknown. All these have caused severe difficulty to assess reliably the final health risks caused by supplementing fish oil in aquafeeds.

In the present study, we obtained crude and refined fish oils originating from the same batch of wild-caught ocean anchovy from the Chinese Yellow Sea and Bohai Sea, and performed a tracking study to investigate transfer of environmental pollutants from wild fish to farmed fish through fish oil-contained aquafeed. To distinguish the possible different transfer efficiencies among different farmed fish species, we chose Nile tilapia (*Oreochromis niloticus*) and yellow catfish (*Pelteobagrus fulvidraco*), which are omnivorous lean fish and carnivorous fatty fish, respectively, as the farmed fish. The concentrations of dioxins, PCBs, dichlorodiphenyltrichloroethane (DDTs) and heavy metals (lead, Pb and arsenic, As) in anchovy, crude and refined fish oils, dietary ingredients, fish culture water and the fillets of farmed fish were measured. We also calculated the transfer efficiencies of fish oil-containing pollutants in the two farmed fish species. The potential human health risks were assessed if the fillets of the farmed fish were consumed. The whole study design is presented in Fig. 1.

The main results of the present study indicate that: 1) differences in POPs concentrations between crude and refined fish oil were retained in the fillets of the farmed fish fed with the corresponding fish oils; 2) the deposition rates of marine POPs in fillets of farmed fish were 1.3%–5.2%, and varied slightly between fish species; 3) the use of crude fish oil in aquafeeds produces final aquatic products with a higher health risk to consumers.

2. Methods

2.1. Collection of wild anchovy and industrial fish oil preparation

In the present study, the anchovy (*Engraulis mordax*) as the raw material for fish oil were caught by using a seine net with 8 mm mesh size in Bohai Sea and Yellow Sea of China during November 2014 to January 2015. A total of 10 tons of anchovies were transported in ice cold containers to two different factories for crude fish oil (CFO) extraction in Shandong province, China, to make crude fish oil 1 (CFO1) and crude fish oil 2 (CFO2). The industrial processing protocols of both factories were the same, mainly included stewing, squeezing, alkaline neutralization and vacuum drying, and the oil extraction rate was around 2% (final fish oil/total anchovies). The obtained CFOs from two factories (around 200 kg) were reprocessed to refined fish oil (RFO) in another oil refining factory in Guangdong province, China, mainly through degumming, alkaline neutralization, vacuum drying, bleaching and winterization. Finally, 180 kg RFO was obtained. The processing protocols of crude fish oil extraction and refining used by these factories are common in the majority of crude fish oil extraction factories in China. The CFO1, CFO2 and RFO were then used as dietary lipid sources to make three purified fish diets. The study design for the whole tracking study is shown in Fig. 1. During the study, the concentrations of pollutants were detected in all components used including anchovy, crude and refined fish oils, dietary ingredients, fish culture water, and the fillets of farmed fish.

2.2. Fish, diet preparation, feeding trial and sampling

In order to evaluate the effects of fish species and body chemical composition on the pollutants accumulation, two widely cultured species, juvenile Nile tilapia (*Oreochromis niloticus*) as an omnivorous lean fish and yellow catfish (*Pelteobagrus fulvidraco*) as a carnivorous oily fish, were chosen. Fish were obtained from the Fishery Genetic Resources Experiment Station of Shanghai Ocean University (Shanghai, China). Three purified diets were formulated and prepared by using pure casein and gelatin as protein sources, pure corn starch as carbohydrate source and the processed fish oils as the unique lipid sources. Purified diets were necessary in order to avoid possible contaminations from exogenous pollutants sources. The fish oil content in the diets was set as 10%, a dietary lipid level that is commonly used in experimental or practical diets for Nile tilapia (Ng et al., 2013) and yellow catfish (Wang et al., 2014). The diets were prepared as previously described (He et al., 2015). The formulation (g/kg) and proximate analysis of the trial diets (%) are presented in the Supplementary Table S3.

Before the formal experiment, about 600 fish from each species were acclimated for one week, during which they were fed with a commercial diet (Dajiang, China). After acclimation, 180 visually

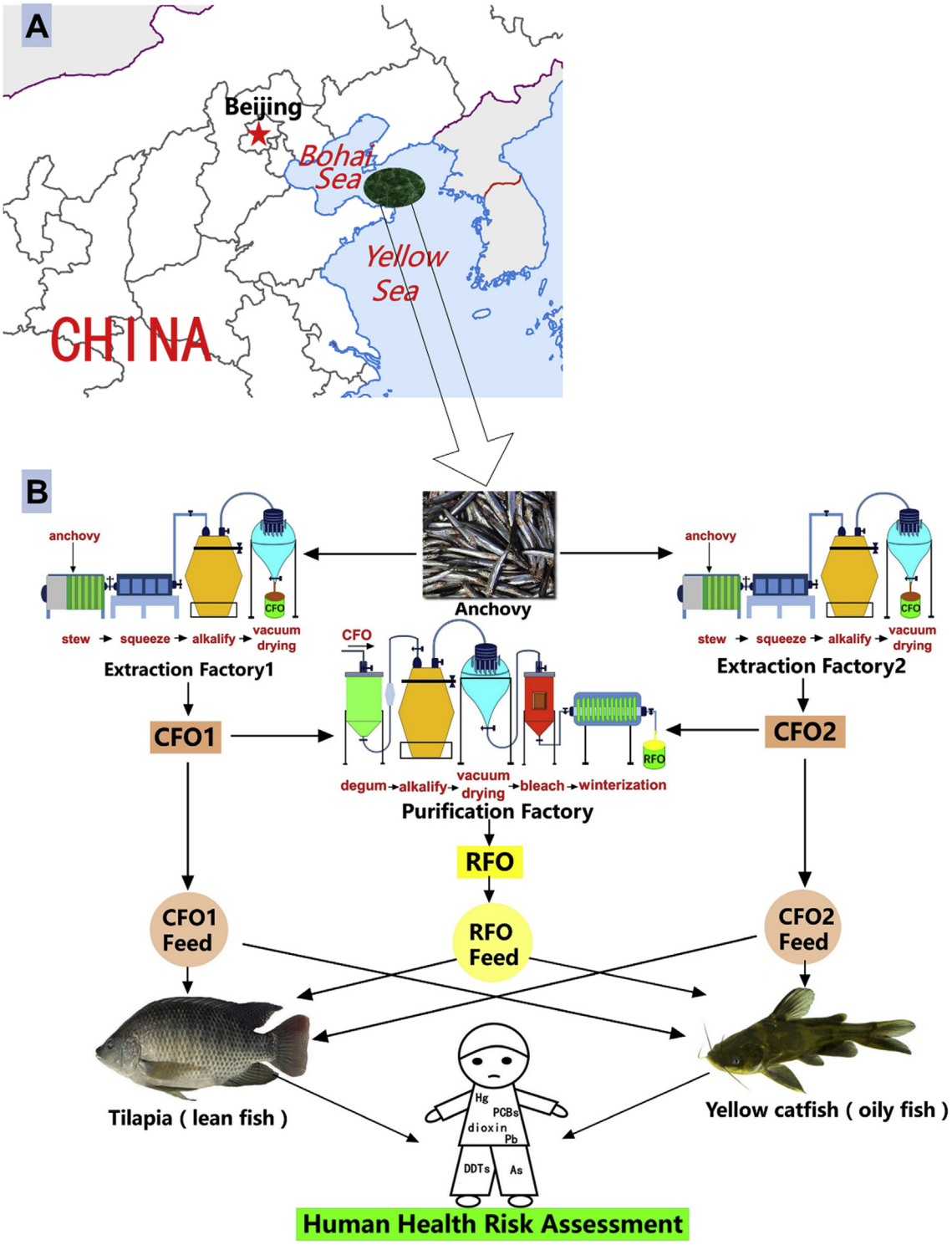


Fig. 1. The experimental strategy to track the entire transfer journey of marine pollutants from wild fish to farmed fish through dietary fish oil. CFO1, crude fish oil 1; CFO2, crude fish oil 2; RFO, refined fish oil.

healthy Nile tilapia and yellow catfish for each species, weighing approximately 10 g were independently divided randomly into three groups (60 fish per group) each containing three replicates (20 fish per replicate, 350L tank) and fed the three purified diets. Each individual fish used in the study was embedded with a tracking tag with an unique Radio-frequency identification (RFID) code, which could be recognized by a RFID machine (Boise, ID, USA) to enable precisely tracking its growth rate. During the feeding trial, Nile tilapia and yellow catfish were fed twice daily to apparent visual satiation at 9:00 and 19:00 h, the uneaten feed was used for feed intake calculations for each group on each feeding occasion. The water quality parameters such as temperature, pH, dissolved oxygen and ammonia nitrogen were maintained at $26 \pm 2\,^{\circ}C$, 7.99 ± 0.23, $5.5 \pm 1.2\ mg\ L^{-1}$ and below $0.02\ mg\ L^{-1}$, respectively.

The trial lasted for 8 weeks with a 12 h light to dark photoperiod. At the end of feeding trial, all fish were overnight fasted, and individually weighed after anesthetizing (MS-222 with 20 mg L^{-1}). A sample of 45 fish from each group (15 random fish per replicate), were collected and pooled into three samples. The edible fish fillets, including dorsal muscle, abdominal muscle, and tail muscle were collected, mixed, freeze-dried and stored at − 80 °C for biochemical assay and environmental pollutants measurements. The detailed numbers used in the feeding trial, biochemical assay and pollutants measurements were presented in Fig. S2.

All experimental protocols and animal handling in the present study were conducted strictly under the Guidance of the Care and Use of Laboratory Animals in China, and were approved by the Committee on the Ethics of Animal Experiments of East China Normal University, Shanghai.

2.3. Biochemical assays

Total protein content of fillets was determined by the Kjeldahl method using the Kjeltec™ 8200 analyzer (Foss Tecator, Kjeltec™ 8200, Sweden). Total lipid content was measured by using the classic methanol–chloroform method (Bligh and Dyer, 1959). Fatty acid composition was measured by gas chromatography (Agilent 7890B) as we previously described (Zhang et al., 2010). The detailed quality assurance of these methods is provided in the supplementary information Part 2.

2.4. Measurements of environmental pollutants

Dioxin, PCBs and DDTs (4-4′ DDT and 2–4' DDT) were chosen as the representatives for marine POPs. The species and individual concentrations of the congeners of dioxin and PCBs are listed in the Supplemental Tables S1 and S2. Samples were extracted using dichloromethane-n-hexane 1:1 (vol/vol) by an automated accelerated solvent extraction (ASE) system (ASE 200, Dionex, USA). The conditions for extraction were 1500 psi at 100 °C with a static time of 5 min for 3 cycles. The extraction cell was loaded from bottom to top: glass filters, florisil, glass filter, 1 g of sample mixed with quartz sand and sodium sulfate, and glass filter. Before the extraction, 20 ng internal standard solution was added to each fish sample. After the extraction, approximately 40 ml extraction liquid was reduced to 1 ml at 40 °C by a rotary evaporator. The extract was further shaken vigorously with concentrated sulfuric acid, and the concentrated supernatant was subjected to gas chromatography-mass spectrometer/mass spectrometer (GC-MS/MS) analysis. Analyses of dioxin, DDTs and PCBs were performed on a Waters Quattro micro™ GC-MS/MS in electron impact modes with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc.). The column oven temperature ranged from 60 °C with a hold time of 2 min–180 °C at a rate of 20 °C/min, then to 250 °C at 5 °C min followed by 310 °C/min with a final hold time of 5 min. Helium was used as carrier gas. One microliter of sample extract was injected in a splitless mode at an injection temperature of 250 °C. The metals, Pb and As were chosen as the representative marine heavy metals. To detect Pb and As contents, samples were nitrolysed by nitric acid using microwave digestion system (Multiwave 3000, Anton-paar, Austria), and then hydrogen peroxide and vitriol were added, respectively. Afterwards, the samples were filtered and determined by inductively coupled plasma-mass spectrometry (ICP-MS, X Series 2, Thermofisher, USA). The detailed quality assurance of these methods is provided in the supplementary materials Part 2.

2.5. The estimation of net contaminant deposition rates in fillets

The net deposition rates of marine pollutants in fillets of final farmed fish were calculated as the percentage of the accumulated pollutants in fillets to the total intake of the corresponding pollutants. Fish fed the RFO diet was chosen as the control (blank) in the present calculation, in order to eliminate not only the effects of the pollutants in fish culture water and feed ingredients (except for fish oil), but also initial pollutants contents in the fish fillets before the fish oil feeding trial. Therefore, the deposition rate (DR) of a given POP in Nile tilapia and yellow catfish fillets was calculated using the following equation (1):

$$DR = \frac{(W \times FC)_{CFO} - (W \times FC)_{RFO}}{(OI \times C)_{CFO} - (OI \times C)_{RFO}} \times 100\% \quad (1)$$

where W is the weight of fish fillet after feeding trial, FC is the final pollutant concentration in fillets, OI is the oil intake of fish in each group estimated as the amount of feed consumed and C is the pollutant concentration in fish oil.

In order to validate the DR calculation method, we designed an additional PCB exposure experiment. In this experiment, Nile tilapia weighing 6.1 ± 0.2 (g) were fed with a purified diet containing 12% soybean oil, supplemented with 0.4 mg Aroclor 1254 per kilogram diet, for 10 weeks under the same experimental conditions as those used for the pollutant tracking trial. The PCBs concentrations were measured in diet and Nile tilapia fillets before and after the feeding experiment. The fillet DR value from this experiment was calculated according to the equation:

$$DR = \frac{PMC_{end} - PMC_{start}}{PFC_{intake}}$$

where PMC_{end} and PMC_{start} are total muscle POP contents at the end and beginning of the feeding period, respectively and PFC_{intake} is total intake of POPs via feeding. The DR value obtained in the experiment was compared with that estimated in the fish oil pollutant tracking trial.

The net heavy metal deposition rates in fillets were not calculated, because the heavy metal concentrations of fish fillets in the RFO and CFO groups were comparable, and fish oil is not the main route of heavy metals accumulation.

2.6. Human health risk assessment

The United States Environmental Protection Agency (U.S. EPA) divided the toxicological effects of a pollutant into carcinogenic (if the pollutant is a carcinogen or possible carcinogen) and noncarcinogenic effects to human health. However, these risks are affected by the intestinal absorption of pollutants. When the chemical pollutants in fish are regarded as entirely absorbed in human body, the maximum allowable fish consumption amounts for carcinogenic and noncarcinogenic can be calculated by using equations (2) and (3), respectively. When considering the bio-availability of pollutants, the maximum allowable fish consumption amount for carcinogenic and noncarcinogenic pollutants can be calculated based on equations (4) and (5), respectively (U.S. EPA, 2000),

$$CR = \frac{BW \times ARL}{\sum_{m=1}^{X} C_m \times CSF_m} \quad (2)$$

$$CR = \frac{BW}{\sum_{m=1}^{X} \frac{C_m}{RfD_m}} \quad (3)$$

$$CR = \frac{BW \times ARL}{\sum_{m=1}^{X} C_m \times AR_m \times CSF_m} \quad (4)$$

$$CR = \frac{BW}{\sum_{m=1}^{X} \frac{C_m \times AR_m}{RfD_m}} \quad (5)$$

where CR (g/d) is the maximum allowable fish consumption amount causing carcinogenic and noncarcinogenic risk; BW is the body weight for adult and children, which are 63.1 kg and 33 kg, respectively (Tao et al., 2012; Wang et al., 2005); ARL is the maximum acceptable individual lifetime risk level approximated as 1 in 1000000 (10^{-6}). The letter C (mg/g) is the concentration of a pollutant in fish muscle (wet weight) and CSF $(mg/kg.bw/d)^{-1}$ is the cancer slope factor expressed as a cancer potency value of a carcinogen with the unit of risk exposure, for dioxin is 1.0 $(mg/kg.bw/d)^{-1}$, for PCBs is 2.0 $(mg/kg.bw/d)^{-1}$ and for DDTs is 0.34 $(mg/kg.bw/d)^{-1}$ (U.S. EPA, 2000). RfD (mg/kg.bw/d) is the noncarcinogenic reference dose of a pollutant, for dioxin is 2×10^{-5} (mg/kg.bw/d), for PCBs is 2×10^{-5} (mg/kg.bw/d), and for DDTs is 5×10^{-4} (mg/kg.bw/d) (U.S. EPA, 2000) and AR is the absorption rate of pollutants in intestine. The AR (%) for dioxin and PCBs is 3.96 $\times C_{lipid}$ (lipid content of food sample) (Li et al., 2011) while for DDTs is 1.11 $\times C_{lipid}$+37.4 (Pang, 2012).

The hazard ratio (HR) was used to evaluate the potential human health risk caused by pollutants, which is defined as the ratio of daily fish intake to the CR, and is calculated by using equation (6) (U.S. EPA, 2000; Yu et al., 2012) below:

$$HR = \frac{\mathrm{IR}}{CR} \quad (6)$$

whereby IR (g/d) is the intake ratio of daily fish. A previous research in the study area reported that the daily fish consumption in villages, normal cities and big cities were 29.6 g, 44.9 g and 62.3 g, respectively (Du et al., 2012). This study used a daily fish consumption rate of 62.3 g for adults and 37.4 g for children (Dirtu and Covaci, 2010). An HR $\geq$ 1, indicates exposure to a pollutant will cause adverse human health effects from fish consumption.

2.7. Statistical analysis

All results are expressed as mean ± SD. Statistical analyses were performed using statistical product and service solutions SPSS 19.0 software (SPSS, Chicago, IL). The significant differences in measured parameters for RFO group were compared to CFO groups within each fish species followed a comparison between similar fish oils for the two fish species by using independent sample *t*-test. The relationship between fish oils and fish fillets for dioxin was established by using a principle component analysis (PCA) (Matlab R2014a software). Differences were considered significant at the level of $p \leq 0.05$.

3. Results

3.1. Dietary fish oil concentrated POPs from marine wild fish

The concentrations of pollutants in the anchovy (dry weight, dw), fish oils, feed ingredient without fish oil, and the fish culture water are presented in Table 1 (detailed values of dioxin and PCBs concentrations are presented in Table S1 and Table S2, respectively). The pollutant concentrations in the fish culture water and feed ingredients without fish oil were marginal compared with those in the fish oils. Thus, the main source of pollutants in the present study were the fish oils. The pollutant concentrations of dioxins and PCBs in crude oil were more than 10 times higher than those in anchovy (dw). DDT concentrations in crude oil were more than four times higher than those in anchovy (dw). However, the concentrations of As and Pb were comparable between anchovy (dw) and the crude oil. These observations indicate that fish oil processing is an enrichment process for marine POPs, but not of heavy metals.

Of note, the pollutant concentrations were relatively similar between the two crude oils, which were processed in different factories with similar extraction technologies. The concentrations of dioxins, PCBs, As, and Pb in the RFO were only 20%, 70%, 6%, and ≤1% of the corresponding values in the CFOs, respectively. However, DDT concentrations were comparable between the CFO and RFO. These findings indicate that the refining process reduces pollutant concentrations by different degrees depending on the type of a pollutant.

3.2. Fatty and lean fish have different growth and diet utilizations

After the 8-week feeding trial, feed intake amounts and physiological phenotypes (including body weight, carcass ratio and fillet biochemical composition) were similar among the three fish oil groups in the same fish species (Table 2). The composition of most fatty acids was comparable among the three fish oils, and, correspondingly, no significant differences in fatty acid composition were observed in fillets from the same fish among the three fish oil groups (Fig. S1). These findings show that different fish oil processing technologies do not affect the nutritional and physiological characteristics of farmed fish.

Nile tilapia grew faster and had better feed efficiency than yellow catfish, although both fish had the same initial weights and were fed the same diets at similar feeding rates (Table 2). However, the total feed intake in Nile tilapia was higher than that in yellow catfish. The lipid and protein compositions in dry fillets were 10% and 80% in Nile tilapia, and 25% and 65% in yellow catfish, respectively. These observations indicate that fish biochemical composition, feed intake, and growth were largely species-dependent and may further affect the deposition of pollutants in contaminated feeds in different fish species. It should be noted that the growth performance of the two fish species, particularly those of yellow catfish, in the present study were much lower than those under practical farming conditions, in which commercial diets are used. This is mainly caused by the use of purified diets to avoid pollutants-contaminated dietary ingredients. However, the purified diet decreased the feed efficiency and growth rate of the experimental fish, especially yellow catfish.

3.3. Fish oil processing technology and fish fat content influence pollutant deposition in farmed fish

The deposition of dioxin, PCBs and DDTs in fish fillets of the RFO group were significantly lower than those in the CFO groups after the 8-week feeding trial (Fig. 2). The significant differences in dioxin and PCBs between the CFO and RFO groups were greater than that of the DDTs. However, the heavy metals concentrations (As and Pb) in dry fillets of fish fed the RFO were generally comparable with those fed the CFO diets. These results suggest that differences in the concentrations of POPs, but not heavy metals, between the RFO and CFO are well retained in farmed fish fillets after feeding fish oil-containing diets, meaning POPs deposition in farmed fish could be reduced by the fish oil refining process. When the POPs concentrations were calculated as per dry weight of fish fillet (pg/g), the yellow catfish fillets contained significantly higher amounts of almost all pollutants than those in Nile tilapia,

Table 1
The concentrations of environmental pollutants in anchovy, fish oils, feed materials and fish culture water.

Pollutants	Anchovy (dw)[a]	Crude fish oil 1 [b]	Crude fish oil 2 [b]	Refined fish oil [b]	Fish culture water [c]	Feed materials without fish oil [d]
Dioxin						
TEQs[e]	0.453(pg/g)	8.640(pg/g)	8.720(pg/g)	0.808(pg/g)	0.209(pg/L)	0.325(pg/g)
Content[f]	5.45(pg/g)	55.98(pg/g)	51.25(pg/g)	12.57(pg/g)	0.45(pg/L)	4.84(pg/g)
PCBs						
TEQs	0.096(pg/g)	17.300(pg/g)	15.700(pg/g)	8.570(pg/g)	0.040(pg/L)	0.009(pg/g)
Content	372.700(pg/g)	5542.000(pg/g)	5338.000(pg/g)	4039.000(pg/g)	3.500(pg/L)	7.783(pg/g)
DDTs						
content	26000(pg/g)	102000(pg/g)	99000(pg/g)	98000(pg/g)	403(pg/L)	ND
As						
content	8.140(mg/kg)	6.400(mg/kg)	4.440(mg/kg)	0.323(mg/kg)	0.005(mg/L)	0.071(mg/kg)
Pb						
content	0.159(mg/kg)	0.276(mg/kg)	0.143(mg/kg)	ND	ND	0.148(mg/kg)

AbbreviationsPCBs, polychlorinated biphenys; TEQ, toxic equivalence quantity; DDT, dichlorodiphenyltrichloroethane; As, arsenic; Pb, lead; ND, non-detected.
[a] The pollutants of anchovy were detected in dry weight. A total of 60 anchovies were collected for pollutant measurements.
[b] A total of 30 g fish oil was collected for measurements.
[c] A total of 12L fish culture water was collected for measurements.
[d] A total of 300 g feed materials without of fish oil were collected for measurements. All values in this table were from duplicate assays.
[e] The TEQs of dioxin and PCBs were calculated according to the WHO-toxic equivalence factor.
[f] The detected concentrations of pollutants.

Table 2
The biological and nutritional characteristics of the experimental fish during the 8-week feeding trial.

Treatment groups		Initial wet weight (g)	Weight Gain (g)	Average feed intake (g)	Average final wet weight(g)	Feed efficiency	Lipid content[a] (% dw)	Protein content[a] (% dw)
Tilapia	CFO1	10.5 ± 2.4	21.6 ± 7.4	33.3 ± 0.9	32.1 ± 8.5	0.65 ± 0.05	10.8 ± 1.2	79.7 ±2.2
	CFO2	10.3 ± 2.8	21.5 ± 7.7	33.4 ± 0.7	32.0 ± 9.5	0.65 ± 0.04	11.6 ± 0.4	81.0 ± 0.9
	RFO	10.6 ± 2.9	21.5 ± 9.5	32.0 ± 0.6	32.1 ± 11.7	0.67 ± 0.06	10.4 ± 0.9	83.6 ± 0.9
Yellow catfish	CFO1	10.3 ± 2.9	6.6 ± 2.9	21.2 ± 0.3	16.3 ± 3.1	0.31 ± 0.03	24.5 ± 1.7	64.9 ± 1.2
	CFO2	10.5 ± 1.7	6.0 ± 2.1	21.5 ± 0.5	14.8 ± 2.5	0.28 ± 0.03	25.4 ± 0.8	65.8 ± 0.5
	RFO	9.3 ± 1.2	5.8 ± 2.2	22.0 ± 0.3	15.4 ± 2.8	0.26 ± 0.02	25.8 ± 0.5	65.5 ± 1.7

Each treatment group contained 60 fish (20 fish per replicate). Data was expressed as mean ± SD (n = 3).
[a] Contents of lipid and protein were measured in dry fillet powder which was sourced from 45 fish of each treatment group (15 fish per replicate).

particularly dioxin, PCBs and DDTs (Fig. 2A–C). This result highlights that pollutants accumulated differently in fatty and lean fish. However, when the POPs concentrations were calculated as per weight of fillet lipid (pg/g lipid), the large differences of POPs concentrations in dry fillet (pg/g) between tilapia and yellow catfish were clearly alleviated (Fig. 2D–F), indicating the deposition of POPs in fatty fillets would be diluted by higher lipid content, as compared with the lean fillet.

We calculated the deposition rates of the POPs in the fillets of the two fish species using the RFO group as the control (Fig. 3). The deposition rates of dioxin, PCBs, and DDTs in fish fillets ranged from 1.3% to 5.2%. The PCB deposition rates between Nile tilapia and yellow catfish were similar (2.6% vs. 2.7%, respectively), but the dioxin deposition rate in Nile tilapia was higher than that in yellow catfish (4.8% vs. 1.8%, respectively), although the amounts of PCB and dioxin deposited in the yellow catfish fillet were significantly higher than those in Nile tilapia. These findings suggest that the pollutant deposition rates in organs were not only related to the pollutant concentrations. Notably, an additional Aroclor 1254 exposure experiment using the same experimental conditions in Nile tilapia resulted in a PCB deposition rate of 6.63% by using a different calculation equation (Table S4). Of note, the dietary PCB concentrations were different in the Aroclor 1254 experiment and the fish oil experiment (0.4 mg Aroclor 1254 per kg diet vs. 0.54–0.56 μg marine PCB/kg CFO diets), and the PCB types were also different between the two experiments (industrial Aroclor 1254 vs. natural marine PCBs); however, the PCB deposition rate values between the two experiments were the same magnitude (6.63% vs. 2.63%–2.77%), suggesting that the PCB DR in fish fillets is relatively stable. Therefore, the fillet DR values of the fish oil-containing pollutants in the present study have a high reference value.

3.4. The composition of POPs in fish fillet accords with that in dietary fish oil

We used dioxin as a representative pollutant to measure the composition of dioxin congeners in fish fillets and all potential POP suppliers in the present study to further explore the original source of the POPs in the fish fillets (Fig. 4A). The results indicate that the dioxin composition patterns in anchovy, fish oil, and fillets were similar, but were very different from those in water and the feed material, showing that the final POP deposits in fillets were sourced originally from anchovy through the fish oil. Moreover, the dioxin patterns of the two crude oils were more similar than that of refined oil (Fig. 4A). The RFO had less tetrachlorodibenzo-p-dioxin (TCDD) and penta-chlorodibenzofuran (PeCDF), but higher hexachlorodibenzo-p-dioxin, hexachlorodibenzofuran, and heptachmdibenzofuran (HpCDF) than CFOs, suggesting that TCDD and PeCDF of dioxin can be effectively removed during refining. The dioxin composition patterns in the fillets of the fish fed the different fish oils were also different from each other. Moreover, in general, the fillet dioxin composition in each fish group was similar to the pattern of the corresponding fish oil, particularly in the Nile tilapia groups.

We performed a PCA of dioxin according to the compositions of dioxin congeners in the fish fillets and three fish oils to further verify whether the different dioxin pattern in fish oil would affect

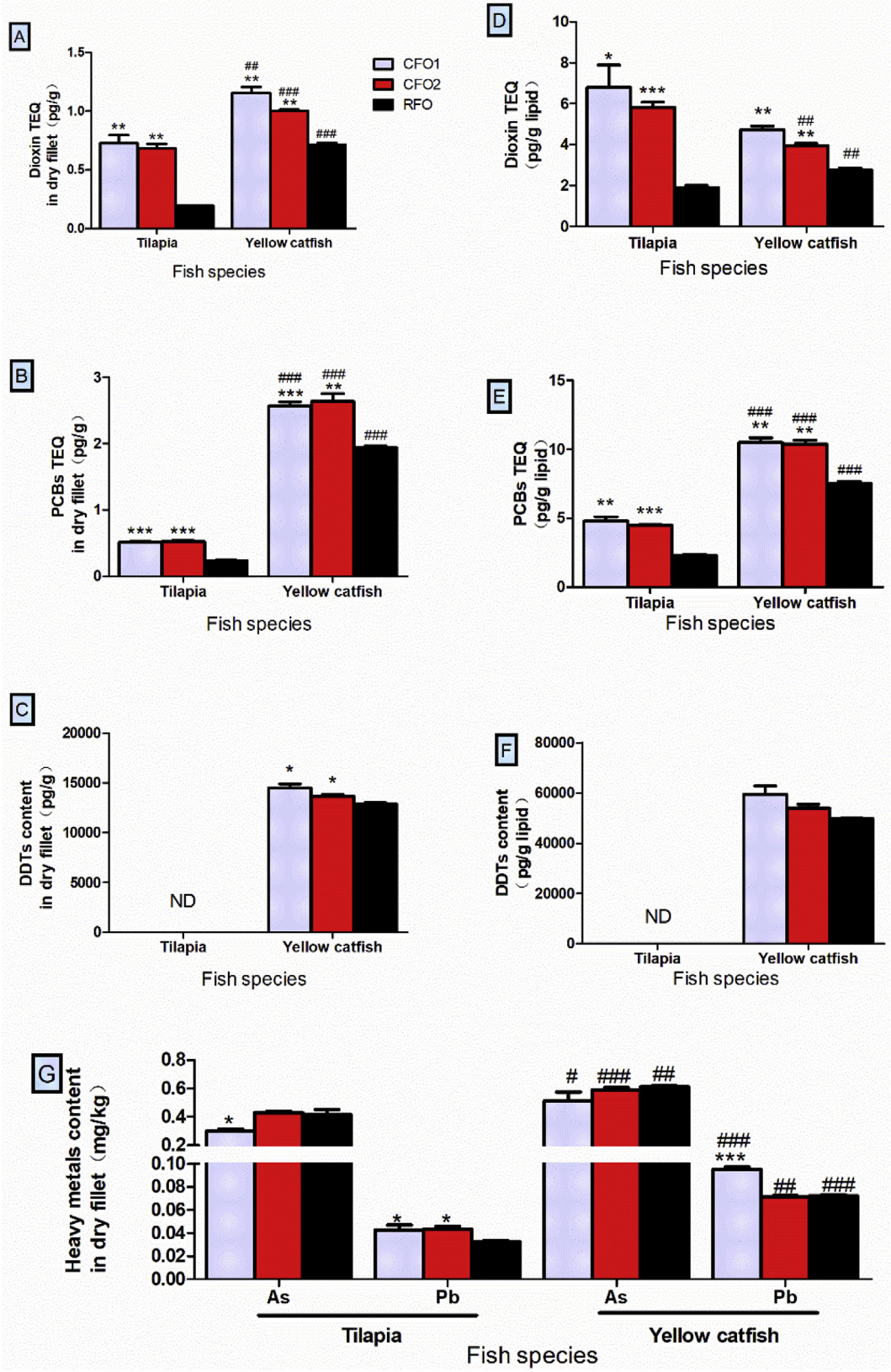


Fig. 2. The concentrations of dioxin (A), PCB (B), DDTs (C), As and Pb (G) in dry fillets and dioxin (D), PCB (E), DDTs (F) in lipid of two farmed fish. TEQ, toxic equivalence quantity; ND, not detected. '*' indicates statistical significance between CFO groups and RFO group in the same fish species. '#' indicates the statistical significance between two fish species in the same fish oil treatment. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$.

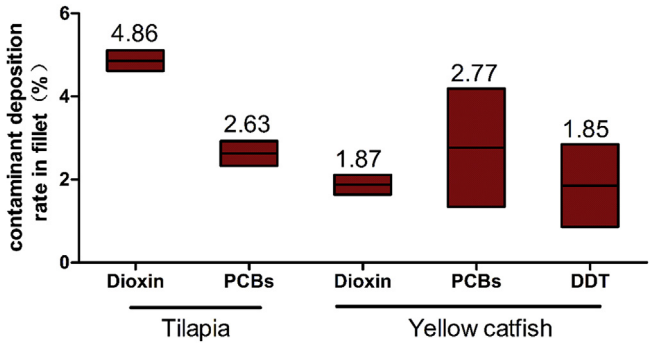


Fig. 3. The deposition rates of dioxin, PCBs and DDT in Nile tilapia and yellow catfish fillets. Each column was presented as mean ± SD (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the dioxin pattern in the fillets after feeding (Fig. 4B). The figure also indicates that among the three fish oils, the two crude oils had higher similarity than the refined oil. The same trend was also found in the fillets of the Nile tilapia and yellow catfish fed with the three fish oils. These results indicate that the differences in composition of the POPs between the RFO and CFO are also well retained in the farmed fish fillets after feeding fish oil-containing diets.

3.5. Health risk assessment of fish fillet pollutants to adults and children

The carcinogenic and non-carcinogenic risks of fish fillet pollutants to adults and children were assessed, respectively, to evaluate the potential health risks to consumers in the present study (Fig. 5A–E). For each POP, the HRs of the RFO fillets were lower than those of the CFO fillet in both fish species. For the two fishes, the HRs for dioxin and PCBs in yellow catfish fillets were higher than those in Nile tilapia fillets. Moreover, the HRs of PCBs were greater than those of dioxin, but less than those of the DDTs. The HR values in most of the groups were $\leq$1, except that the children carcinogenic HR of DDTs in yellow catfish fillets fed with the CFO diet reached 1 when DDTs are absorbed completely. These results reveal that fatty fish generate a higher risk to human health than lean fish, and that feeding fish with the RFO diet decreases the health risk caused by POPs.

4. Discussion

4.1. Fish oil is the main Carrier of marine POPs into aquaculture

Fish oil, as a main ingredient in aquafeeds, is extracted from wild marine fish. Marine fish accumulate large quantities of pollutants, including POPs and heavy metals through food chain-biomagnification. POPs are lipophilic pollutants that mainly accumulate in fat-enriched organs of fish, such as adipose tissue and the liver, rather than the edible fillets, which contain less fat (Cui et al., 2015; Smith and Gangolli, 2002; Uysal et al., 2009; Yang et al., 2007). The viscera of wild marine fish is actually the main contributor to dietary fish oil, considering that dietary fish oil is extracted from the whole body. Our present results indicate that the concentrations of pollutants in fish oil were significantly higher than those in the original wild fish (Table 1). Therefore, extracting fish oil from wild fish is regarded as a POP-specific selective and concentrating process, and fish oil is an artificial marine POP concentrate.

Fish oil normally contains higher contents of POPs compared with other oil sources used in aquafeeds, particularly vegetable oils (VO) (Berntssen et al., 2005). Because of decreases in the number of wild fish captured, replacing fish oil with VOs (such as soybean oil and rapeseed oil) in aquafeeds has become popular (Huang et al., 2017). In addition, VO in aquafeeds reduces the deposition of many pollutants in farmed fish (Bell et al., 2005; Berntssen et al. 2005, 2010; Drew et al., 2007). Therefore, replacing fish oil with VO not only helps to protect ocean fish resources, but also improves aquatic product safety (Albertgj and Marc, 2008). However, the dietary fish oil cannot be completely replaced by VO in many marine carnivorous fish, and the nutritional value (e.g., n-3 PUFA content) in farmed fish would also be reduced by using additional VO (Bell et al., 2001; Regost et al., 2003). Considering the largest market for fish oil is in aquaculture, which accounts for nearly 75% of total global fish oil production (Towers, 2014), and the continuous development of aquaculture in many developing countries, the large consumption of fish oil in aquaculture will continue in subsequent years. Therefore, fish oil remains the main carrier for transferring marine POPs into aquaculture and finally to human body.

Notably, aquafeed ingredients other than fish oil, such as fish meal which is an important dietary protein source for farmed fish, pig and poultry (Karimi, 2006), also contain pollutants, and this is also a huge contributor to the pollutants exposure to consumers. For example, imported fish meal in China maintained a steady 1.0–1.5 mmt per annum from 2000 to 2014 (Han et al., 2016). Therefore, the purified diets used in the present study actually revealed the minimum pollutant level as compared with practical aquafeeds which contain fish oil and fish meal. With the current tendency to use high-fat aquafeeds in aquaculture, the prohibition of use of crude fish oil is of great significance for human health.

4.2. Factors affecting the accumulation of POPs in farmed fish through fish oil

4.2.1. Species and size of farmed fish

Multipole factors affect the final accumulation of pollutants in farmed fish. A number of studies have indicated that the accumulation of pollutants varies in different fish species. It is also well known that POPs preferably accumulate in fatty fish rather than lean fish. Accordingly, higher concentrations of DDTs and PCBs were found in the fillets (wet weight) of salmon, pompano, and herring as fatty fish than those in silver carp and Japanese sea bass as lean fish (Du et al., 2012). Our present results also indicate that higher concentrations of POPs and heavy metals per weight of dry fillet were found in yellow catfish than those in Nile tilapia, even though the same diet contained the same amount of pollutants. Of note, the present study indicate that the POPs concentrations per weight of fillet lipid in fatty fish would be comparable with or even lower than those in lean fish (Fig. 2A–C vs 2D-F), however, the fish intake-causing health risk is mostly evaluated according to the amount of POPs intake, but not the POPs concentration in fillet lipid. Therefore, cultured fatty fish have higher POPs-causing health risks than lean fish.

The accumulation of pollutants in fish in their natural environment is tightly correlated with fish trophic level. Carnivorous species located in a higher trophic level will accumulate more pollutants through bioaccumulation and biomagnification than species at a lower trophic level (Borgå et al., 2004; Kong et al., 2005; Russell et al., 1999). Cultured fish are fed formulated aquafeeds directly, so the trophic level-dependent pollutant accumulation is weakened. However, the farmed carnivorous fish still consume more dietary fish meal and fish oil than omnivorous and herbivorous fishes (Albertgj and Marc, 2008). Thus, the health risks of

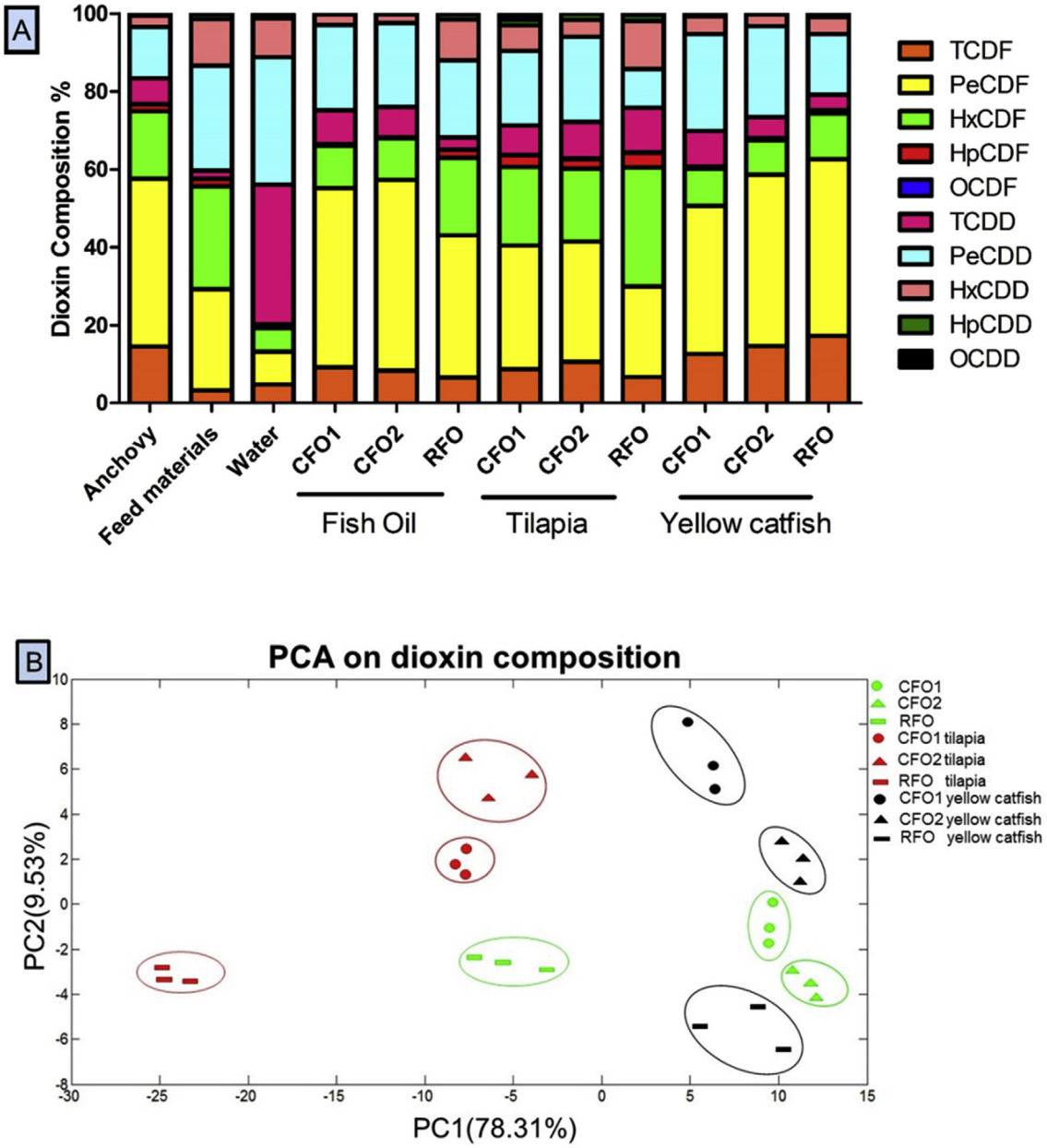


Fig. 4. The composition of dioxin congeners in anchovy, water, feed ingredients, fish oils and fish fillets. (A) The dioxin congeners compositions. Different colors mean different congeners of dioxins; (B) The PCA analysis of the dioxin congeners in three fish oils and the corresponding fish fillets. Fish oils, the fillets of tilapia and yellow catfish were distinguished by different colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

pollutants caused by feeding fish oil-containing diets are higher in carnivorous fish species at a higher trophic level.

The accumulation of pollutants in the fish body is positively correlated with fish size and age (Braune, 1987; Canpolat and Çalta, 2003; Farkas et al., 2003). The final body weights of the fish in the present study were lower than those of market sized-fish. However, we compared PCBs and DDTs concentrations in the fillets between the present study and our previous market survey in which commercial size farmed Nile tilapia and yellow catfish were obtained from big Chinese cities (Du et al., 2012) and found that the fillet pollutant levels in the two studies were very comparable. The DDT concentrations per wet weight of fillet were 3.21–3.63 μg/kg in the present study and 1.71–5.81 μg/kg in our previous study. Similarly, the PCB concentrations per wet weight of fillet were 0.052–0.260 μg/kg and 0.18–0.22 μg/kg in the two studies, respectively (Table S5). Therefore, despite the smaller fish size used in the present study than food-sized fish, the pollutants accumulations in the fillets were comparable; thus, our present results provide a valid reference study for food safety and human health.

4.2.2. Fish oil processing

As discussed previously, fish oil is the main delivery vehicle of pollutants from ocean to farmed fish. Previous studies have shown that pollutants contents in fish oil are associated with the species of the original material fish, ocean capture area, and fish oil processing type (Jacobs et al., 2002). Among these, fish oil processing affects largely pollutant contents in the final oil products. In the present study, anchovy was first delivered to two different factories using similar processing technologies to make crude oils, and similar pollutant compositions were obtained in both crude oils. However,

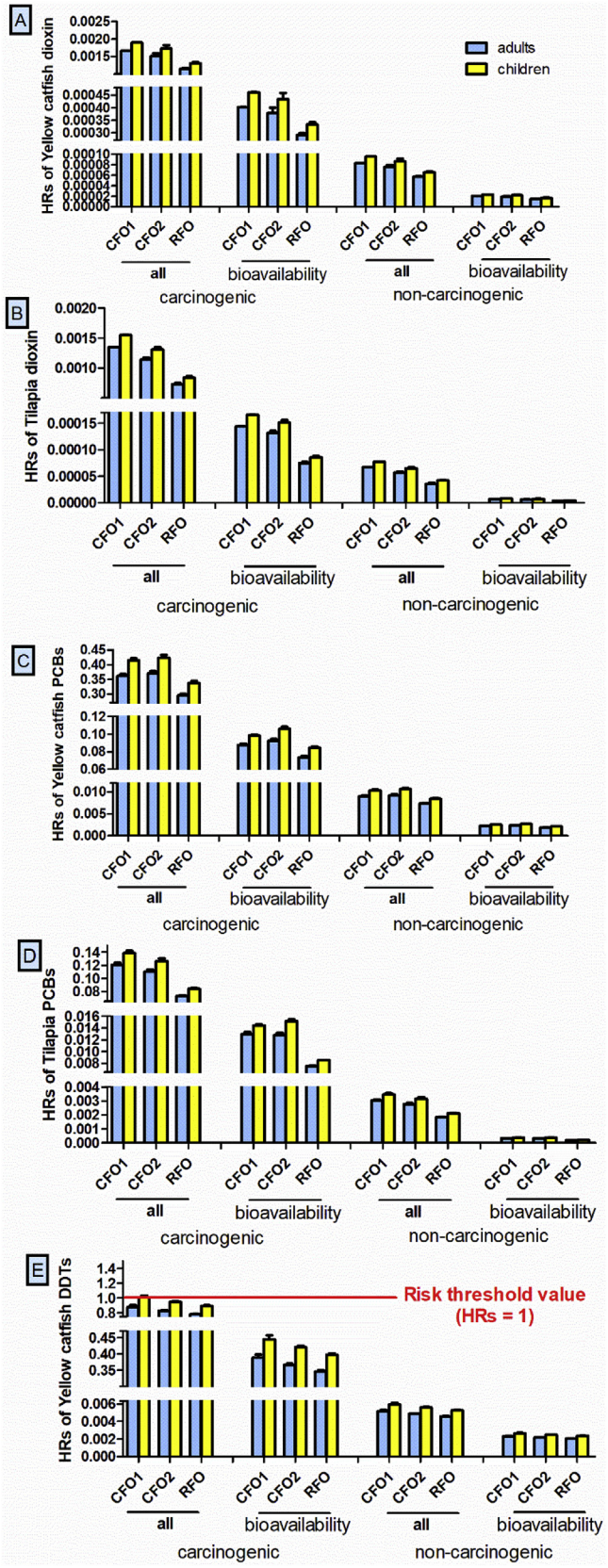


Fig. 5. The assessment of health risk of fish fillet consumption in adults and children. (A)–(B), HR of dioxin in yellow catfish and tilapia fillets; (C)–(D), HR of PCBs in yellow catfish and tilapia fillets; (E) HR of DDTs in yellow catfish fillet. 'CFO1, CFO2 and RFO' indicate the fillets of tilapia or yellow catfish fed with the corresponding fish oils. 'all' indicates the pollutants are absorbed entirely in human body. 'bioavailability' indicates the pollutants are absorbed partly, according to the absorption rate in human intestine. 'Carcinogenic' means the cancer risk to human and 'non-carcinogenic' means the health risks excluding cancer to human (U.S. EPA, 2000). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the refining process decreased significantly the concentrations of almost all pollutants, except DDTs. Moreover, the refining process also changed the composition of POPs (Fig. 4). All factories have the same "stewing", "squeezing", "alkali neutralization" and "vacuum drying" steps but refining has additional steps, such as "degumming", "bleaching", and "winterization". It could be concluded that the removal of pollutants is mainly attributed to the "degumming", and "bleaching" steps in the present study. However, we also noticed that the removal efficiencies among dioxin, PCBs and DDTs were different in the refined oil (Table 1). In fact, in the practical fish oil refining process, some absorbents are added in the bleaching and deodoring processes to absorb and remove organic impurity substrates, including some POPs. Different types of absorbents have different bonding efficiencies towards different POPs, thus the types and amount of these absorbents used in factories will largely affect the final removal efficiencies towards different POPs. This is likely to be the key reason to cause different removal efficiencies among dioxins, PCBs and DDTs in the refined fish oil in the present study. Till now, more advanced technologies, such as silicon and carbon-based solid adsorbents, countercurrent supercritical CO_2, and activated carbon treatments could remove POPs efficiently (Kawashima et al., 2009; Ortiz et al., 2011). However, these technologies are not normally included in dietary fish oil processing industries, presumably because of their cost, since the majority of aquaculture production occurs in developing countries. Nevertheless, considering that crude fish oil is still widely used in aquafeed in many countries, particularly in developing countries, the use of refined feed fish oil is strongly recommended to improve the safety of aquatic products.

4.2.3. Aquaculture technology

Except fish species (biochemical composition and trophic level) and fish oil, aquaculture technology affects the growth and carcass ratio, and thereby affects the accumulation of pollutants. However, this point has not been paid sufficient attention. Equation (7–12) can be used to calculate the fillet pollutant deposition rate:

$$\mathrm{DR} = \frac{FCL_{CFO} - FCL_{RFO}}{(\mathrm{OI} \times C)_{CFO} - (\mathrm{OI} \times C)_{RFO}} \times 100\% \tag{7}$$

$$= \frac{PMC_{end} - PMC_{start}}{PFC_{intake}} \tag{8}$$

$$= \frac{PMC_{end}}{PFC_{intake}} \tag{9}$$

$$= \frac{W_{body} \times \frac{W_{muscle}}{W_{body}} \times PC_{muscle}}{W_{feed} \times PC_{feed}} \tag{10}$$

$$= \frac{W_{body}}{W_{feed}} \times \frac{W_{muscle}}{W_{body}} \times \frac{PC_{muscle}}{PC_{feed}} \tag{11}$$

$$= \text{feed efficiency} \times \textit{carcass ratio} \times \frac{PC_{muscle}}{PC_{feed}} \tag{12}$$

In (7), DR is deposition rate; FCL_{CFO} and FCL_{RFO} are fillet pollutant levels in the CFO and RFO groups respectively; OI is oil intake of

each fish group; C is the pollutant content in fish oil; and $(OI \times C)_{CFO}$ and $(OI \times C)_{RFO}$ are pollutant intake levels through fish oil in the CFO and RFO groups, respectively. In (8), PMC_{end} and PMC_{start} are total muscle POP contents at the end and beginning of the feeding period, respectively and PFC_{intake} is total intake of POPs via feeding. Equation (8) was simplified to Eq. (9), because the pollutant contents in newborn fish (PMC_{start}) are very low, so ($PMC_{end} - PMC_{start}$) equated PMC_{end} and was further reformulated as Eqs. (10) and (11). In (10) and (11), W_{body}, W_{muscle}, and W_{feed} are the weights of the fish body, muscle, and feed intake and PC_{muscle} and PC_{feed} are the concentrations of pollutants in muscle and feed, respectively. From Eq. (11), W_{body}/W_{feed} is feed efficiency, and W_{muscle}/W_{body} is carcass ratio. Thus Eq (11) was retyped as Eq. (12).

Therefore, the DR of a given pollutant in a fish fillet is dependent on four factors: feed efficiency, carcass ratio, feed pollutant concentration, and fillet pollutant concentration. Feed pollutant concentration completely depends on the pollutant concentrations of dietary ingredients, such as fish oil. Fillet pollutant concentration is also correlated to fish species-specific characteristics, such as fillet lipid content. Equation (12) indicates that the feed efficiency and the ratio of fillet are two important factors affecting the deposition rate of a pollutant. Thus, higher feed utilization and growth increases the DR of a given pollutant. However, fillet pollutant concentration, not DR, is the main element affecting aquatic product safety. For example, in the present study, Nile tilapia had lower dioxin concentrations in fillets and a lower HR than yellow catfish; however, the higher growth and feeding efficiency of Nile tilapia caused a higher dioxin DR than that in yellow catfish. The correlations among feed efficiency, fillet ratio, and DR may seem contradictory. The high feed efficiency indicates high absorption of nutrients and pollutants, and the high fillet ratio provides more fillets for pollutant deposition. However, high feed utilization also means less feed and a shorter culture period are required for fast growth, and rapidly-growing muscle dilutes the concentrations of pollutants in fillets. From the point of risk evaluation, the lower fillet pollutant concentrations provide higher food safety, as the consumption portion of fish is relatively fixed for consumers. Thus, improving aquaculture technology to promote feed utilization and fish growth will help to reduce the human health risk caused by feed-originated environmental pollutants.

5. Conclusion

Dietary fish oil contains relative high concentrations of marine pollutants (particularly POPs) and is the main vector carrying marine pollutants to human consumers via farmed aquatic products. Furthermore, pollutant levels in farmed fish are influenced by comprehensive factors, such as fish species, intestinal morphology, lipid content, raw material selection, processing techniques of the dietary ingredients (e.g., fish oil), and aquaculture technology. Although the risk assessments of fillet-containing pollutants to human health did not reach a dangerous level in the present study, the risks to human health from aquatic products cannot be excluded. From a practical point of view, prohibiting use of crude fish oil in aquafeed industry and improving aquaculture technology to promote fish growth and feed efficiency are promising strategies to reduce the human health risks caused by pollutants in farmed fish.

Competing financial interests

The authors declare that they have no actual or potential competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.05.027.

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