Estimation of carbon released by mesopelagic fish in the global open ocean using a carbon release model and model fish-derived parameters

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Key Points:

- We propose a new conceptual model for quantifying the dissolved organic carbon (DOC), CO_2, and particulate carbon (PC) released by fish
- We quantified a detailed carbon budget for a marine model zooplantivorous fish by feeding the fish radiocarbon-labeled living zooplankton
- Using the model and model fish- and literature-derived parameters, mesopelagic fish were estimated vital sources of DOC and fast-sinking PC

Abstract

The role of zooplanktivorous mesopelagic fish in the ocean carbon cycle is attracting increasing attention. However, little information is available regarding the carbon budget of marine zooplanktivorous fish, let alone that of mesopelagic fish. Here, we propose a carbon release model that divides fish-released carbon into two parts (based on the source: ingested food and the fish body) and three forms (as dissolved organic carbon (DOC), CO_2, and particulate carbon (PC)). By feeding a model marine zooplanktivorous fish, marine medaka (*Oryzias melastigma*), a radiocarbon-labeled living rotifer, *Brachionus plicatilis*, we quantified a detailed carbon budget for the fish. The results indicate that 53%–75% of the ingested food carbon was not assimilated but was released mainly as DOC (48%–59%), followed by CO_2 (30%–40%) and PC (11%–13%). The release (/efflux) rates of fish body carbon changed from 0.12 to 0.053 d^{-1} when daily food rations shifted from 2.2% to 4.3% of the fish biomass. DOC, CO_2, and PC accounted for 39%–42%, 40%–45%, and 16%–18% of the carbon released from the fish body, respectively. By using the carbon release model and the parameters derived from the model fish and from the literature, we estimate that mesopelagic fish in the global open ocean produce 1.34–15.2, 0.95–10.8, and 0.35–3.97 Pg C/y of DOC, CO_2, and PC, respectively. Our results show that marine zooplanktivorous fish can transform substantial fractions of their daily ingested food and released body carbon into DOC and that mesopelagic fish may be important sources of DOC and fast-sinking PC in the ocean.

1 Introduction

Increasing attention is being paid to the roles played by fish in the ocean carbon cycle. Small-sized (< 6 cm) mesopelagic fishes (most of which are zooplanktivorous fish) are very abundant in the mesopelagic layer (from 200 to 1000 m in depth) of the open ocean and dominate the world's total fish biomass. Recent surveys indicate that the biomass of these mesopelagic fish could be one order of magnitude higher than a previous estimate of ~1000 million tons in the global open ocean (Davison et al., 2013, 2015; Irigoien et al., 2014; Proud et al., 2019). Increasing evidence indicates that these fish can mediate carbon export to deep waters by performing diel vertical migration and producing fast-sinking fecal pellets (Boyd et al., 2019; Pershing et al., 2010; Saba & Steinberg, 2012; Trueman et al., 2014). In addition, mesopelagic fish, as a globally important source of marine calcium carbonate, may play a key role in the marine inorganic carbon cycle (Salter et al., 2017; Wilson et al., 2009).

Nevertheless, the contribution of zooplanktivorous fish to the ocean carbon cycle is still poorly quantified. Bioenergetics is the study of the balance between the energy supply from food and energy expenditure (Cho et al., 1982); it can describe the fate or allocation of consumed food to growth, respiration, and waste products (e.g., exudates and feces) (Ney, 1993 and reference therein). Thus bioenergetics has been used for the study of fish contributions to the ocean carbon cycle (Davison et al., 2013). Much effort has been invested in examining and analyzing the allocation of consumed food to growth and respiration. In contrast, less attention has been paid to the "waste carbon" released by fish (Ney, 1993 and reference therein), although waste carbon is key for understanding the roles of fish in the ocean carbon cycle. In fact, the bioenergetics model cannot exactly describe all the carbon dioxide (CO_2) and waste carbon released by fish, especially at small time scales such as the daily scale, because part of the CO_2 and waste carbon may come from the fish body rather than ingested food in fish gut (e.g., a fish without any food in its gut will continue to release CO_2 and waste carbon come from only the fish body). In addition, the allocation of fish food carbon to CO_2 through respiration and to dissolved organic carbon (DOC) from the excretion and leakage of fish feces has seldom been measured directly. To the best of our knowledge, the proportion of food carbon released as DOC and the release of fish body carbon as CO_2, DOC, and feces have not yet been quantified. The lack of such data concerning the carbon budget of zooplanktivorous fish impedes our understanding of the roles of small fish, such as mesopelagic fish, in the ocean carbon cycle (Davison et al., 2013; Saba & Steinberg, 2012). For example, due to the lack of data, fish-released DOC was not considered in a pioneering estimation of carbon export mediated by mesopelagic fish in the northeastern Pacific Ocean (Davison et al., 2013), and piscivorous or freshwater fish-derived variables have to be used in the models for marine fish (Bachiller et al., 2018; Ney, 1993).

The previous studies inspired us to propose that the daily carbon released by a fish could be divided into two parts on the basis of its source, from either ingested food or the fish body. It is possible to extrapolate from the carbon release parameters of a model fish in order to estimate the carbon released by mesopelagic fish. Power-law scaling functions have been reported to describe the relationship of carbon turnover rates of fish with fish mass and temperature (Weidel et al., 2011), and the daily food rations and metabolic rate of mesopelagic fish are also fish mass- and temperature-dependent (Davison et al., 2013; Gillooly et al., 2001). The fate of the ingested food can be simply considered to be either assimilated by the fish or released as CO_2, DOC, and particulate carbon (PC), and the lost fish body carbon will be released as CO_2, DOC, and PC. Theoretically, if we know the allocation of ingested food and the body carbon released from a mesopelagic fish to CO_2, DOC, and PC at a certain temperature, we could estimate the total carbon released by the fish at any temperature or by another fish of a different size. In fact, as discussed above, such data are lacking. It is difficult to obtain such data for wild mesopelagic fish in situ, and as well as in the laboratory, as rearing mesopelagic fish in the laboratory is still a technical challenge (Martin et al. 2020). This leads us to consider estimating the carbon released by mesopelagic fish by extrapolating it from the carbon release parameters of a model fish with a similar feeding habitat and body size as the mesopelagic fish. Marine medaka *Oryzias melastigma* may be a good choice for such a model fish. It has been widely used as a model fish in ecological and ecotoxicological studies (Bo et al., 2011; Kong et al., 2008; Mu et al., 2015). More importantly, marine medaka resembles mesopelagic fish ecologically, as it feeds on zooplankton and has a body size (in centimeters) comparable to that of zooplanktivorous mesopelagic fish (Davison et al., 2013; Irigoien et al., 2014).

Therefore, to estimate the contribution of mesopelagic fish to the ocean carbon cycle, we first proposed a conceptual model dividing fish-released carbon into two parts, i.e., food carbon release and body carbon release, based on the source (from either ingested food in the fish gut or tissues in the fish body), and into three forms, DOC, CO_2 and PC. Second, by feeding the model fish a radiocarbon (^{14}C)-labeled living rotifer *Brachionus plicatilis*, the three forms of carbon released from ^{14}C-labeled ingested food and ^{14}C-labeled fish body were quantified. Finally, on the basis of the conceptual model and by using carbon release parameters derived from the model fish and parameters (e.g., mesopelagic fish biomass and daily food rations of mesopelagic fish) from the literature, we estimated the carbon release from mesopelagic fish in the global open ocean. Our results indicate that mesopelagic fish play an important role in the active export of not only PC but also DOC and CO_2 to the depths of the ocean.

2 Materials and Methods

2.1 Conceptual model

The conceptual model for the estimation of the carbon released from fish (Figure 1) is derived from the bioenergetics model for fish (Warren & Davis, 1967) and a carbon flow model for zooplankton (He & Wang, 2006). Theoretically, the carbon released from a fish can originate either from ingested food or from the fish body during short-term (e.g., daily) observations. Fish absorb carbon from ingested food; generally, most of the carbon in the ingested food will be released and lost through respiration, excretion and defecation, and only the carbon remaining is assimilated and used for fish growth or reproduction. Carbon in the fish body is renewed continuously, and the "old" carbon is released and is lost from the body. All the released carbon is released in the form of DOC, CO_2, and PC. Therefore, the fish-released carbon derives from either ingested food or from the fish body and can be divided into the three forms, DOC, CO_2, and PC.

2.1.1 Released carbon from ingested food

According to the bioenergetics model for fish (Warren & Davis, 1967), ingested food carbon will be allocated to physiological compartments such as defecation, respiration, excretion and assimilation (Figure 1). Specifically, part of the ingested carbon is absorbed across the fish gut wall after digestion. The unabsorbed food carbon is transformed and defecated as feces. The absorbed carbon is first allocated to the metabolic pool (e.g., blood and liver), which has a fast turnover rate. Then part of the absorbed carbon is further assimilated and incorporated into the fish body (e.g., white muscle), i.e., the structural carbon pool, which has a slow turnover rate. The unassimilated part of the absorbed carbon in the metabolic pool is directly catabolized and excreted. Thus, the assimilation efficiency (AE) is the fraction of ingested food that is incorporated into the body. The unassimilated food carbon is released into water as DOC, CO_2, and PC.

Therefore, the carbon budget model for ingested food is simplified to $C = FC_{\text{release}} + \text{A}$, where C is the ingested food carbon, A represents the assimilated carbon, and FC_{release} represents the carbon released from ingested food, i.e., the food carbon release.

2.1.2 Turnover and release of fish body carbon

The assimilated carbon in the structural pool supports animal body maintenance, growth and reproduction (He & Wang, 2006). First, part of the structural carbon is replaced by newly assimilated carbon, used and transformed during metabolism, and then released into the environment as CO_2 (by respiration), DOC (by excretion or feces leakage), and PC (by defecation). Second, some of the structural carbon is used directly for reproduction (breeding) and released as PC (e.g., spawn). The carbon released from the fish body is called the body carbon release (BC_{release}) (Figure 1).

2.1.3 Carbon release model for fish

In short, all the fish-released carbon, C_{release}, can be divided into FC_{release} and BC_{release}, based on its original source, and classified into DOC, CO_2, and PC, based on its final form. Therefore, a carbon release model is proposed: $C_{\text{release}} = FC_{\text{release}} + BC_{\text{release}}$ (Figure 1). This model enables us to calculate the amount of DOC, CO_2, and PC released by a fish if the carbon release parameters of the fish are known.

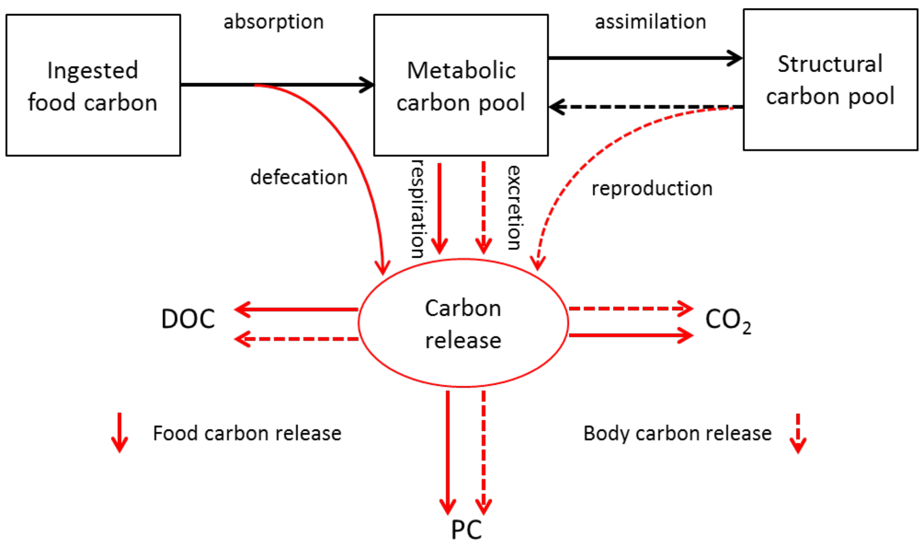

Figure 1. Schematic diagram of the carbon release model. Ingested food carbon is absorbed after digestion. Unabsorbed food carbon is released as feces through defecation. The absorbed carbon is first allocated to the metabolic carbon pool (e.g., blood and liver). Part of the absorbed carbon in the metabolic carbon pool is released from the fish through respiration and excretion. The remaining absorbed carbon is further assimilated into the structural carbon pool (e.g., white muscle). Meanwhile, part of the structural carbon is replaced, transformed through catabolism and reproduction, and released from the fish. The solid and dotted lines show the carbon flows starting from the ingested food and from the structural carbon in the fish body, respectively. The released carbon originating from ingested food and the fish body are called the food carbon release (red solid arrow lines) and the body carbon release (red dotted arrow lines), respectively. All the released carbon is in the form of dissolved organic carbon (DOC), CO_2, and particulate carbon (PC).

To develop the conceptual model, we examined the food carbon release and body carbon release parameters of a model marine zooplankivorous fish, marine medaka (*O. melastigma*), through short-term (36 h) depuration (the process of releasing ^{14}C from the fish) experiments performed by labeling fish food with ^{14}C, and through long-term (8 days) depuration experiments performed by labeling the bodies of the fish with ^{14}C. The lab experiments were also designed to be extrapolated to mesopelagic fish. By using carbon release parameters derived from the model fish and parameters from the literature, we composed the model scenarios for estimating the carbon release of mesopelagic fish in the global open ocean (Figure 2).

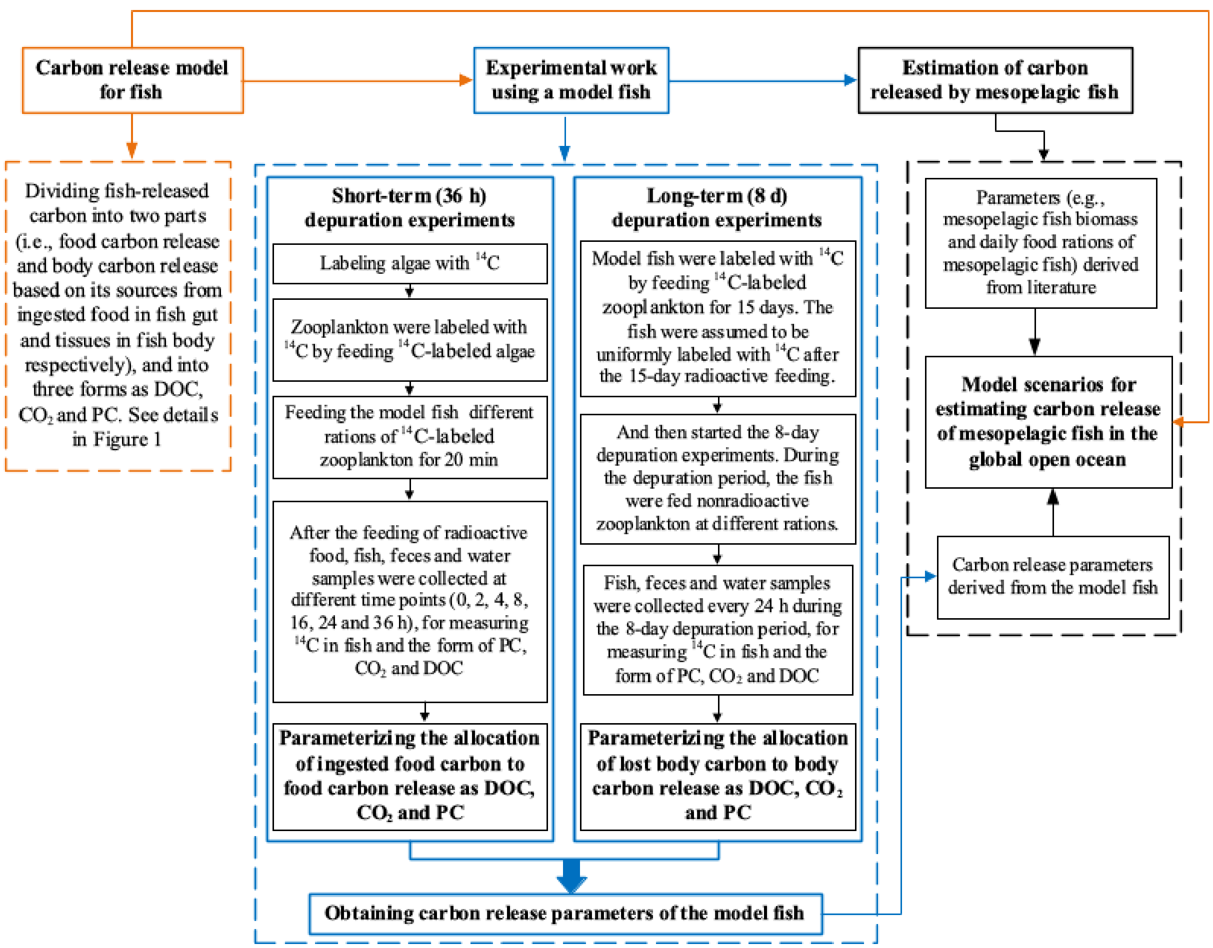

Figure 2. A flow chart showing the relationships among the conceptual model, the experimental work parameterizing model fish carbon release, and the model scenarios for estimating the carbon release of mesopelagic fish. DOC, CO_2, and PC indicate dissolved organic carbon, carbon dioxide, and particulate carbon, respectively.

2.2 Lab experimental designs

2.2.1 Cultures and radiocarbon labeling of zooplankton

The zooplankton rotifer *B. plicatilis* was raised in a 30-L polyethylene tank containing 15 L 0.22-μm filtered seawater (FSW) at a temperature of 25 ± 1°C under illumination of 120 μmol photons/m/s with a 14:10 h light/dark cycle. *Chlorella* sp. algal cells (with a final density of 10^5–10^6 cells/mL) at the exponential growth phase were used to feed the rotifer on a daily basis. One-third of the seawater in the tank was renewed each day.

The rotifers were fed ^{14}C-labeled algae *Chlorella* sp. (see details in Text S1) once per day as described above. One week later, the rotifers were considered to be uniformly labeled with ^{14}C (12.9–28.4 Bq/μg C).

2.2.2 Fish rearing

Marine medaka (*O. melastigma*) were reared under the same conditions as the rotifer. Four-month-old fish that had been acclimated to feed on the rotifer (*B. plicatilis*) were used for the experiments. None of the fish reached sexual maturity during the experiment. The average fish weight was 77.7 ± 11.2 mg in wet weight (WW) and 23.5 ± 3.7 mg in dry weight (DW). The ratio of DW to WW for the experimental fish was 30.3% ± 2.7% (n = 4). The carbon content of the fish was 46.7% ± 8.4% (n = 6) of the DW (see details in Text S2).

2.2.3 Short-term depuration experiments: examining the allocation of ingested food carbon by labeling fish food with ^{14}C

Prior to the experiments, the experimental fish were placed in aerated FSW without the presence of food for 24 h for evacuation. Fish were then transferred individually into 50-mL feeding beakers with 25 mL aerated FSW. The three rations of ^{14}C-labeled rotifers were 1000, 1500 and 4000 ind./fish, corresponding to 2.2%, 3.2% and 8.6% of the fish DW, respectively (assuming that each rotifer contained 226 ng C, and its carbon content was a factor of 0.444 of its DW) (Hansen et al., 1997; Øie et al., 1997). The fish were fed different rations of rotifers for 20 min, a period shorter than the fish gut passage time (approximately 30–60 min) (see detailed methods in Text S3; data not shown). All rotifers were consumed during the feeding period, except in the ration of 4000 ind./fish, in which only 2650 rotifers (equal to 5.7% of the fish DW) on average were eaten by each fish.

After being fed the radioactive food, the fish were collected, rinsed with FSW, and immediately transferred to beakers with 25 mL of new FSW for depuration for 36 h. The seawater in the beaker was changed at 2, 4, 8, 16, 24, and 36 h. Three to five fish were collected at 0, 2, 4, 8, 16, 24, and 36 h for measuring ^{14}C in fish. Feces and water samples were collected from the depuration system for measuring ^{14}C in the form of PC, CO_2, DOC, and colloidal organic carbon (COC) at 2, 4, 8, 16, 24, and 36 h. Fish feces were collected by filtering all seawater in each beaker through a polycarbonate membrane (0.2 µm pore size, Millipore). $^{14}CO_2$ in 15 mL of the filtrate was collected into 5 mL of 1 M NaOH according to a method described by Lee and Fisher (1992). Two portions of 3-mL aliquots of the filtrate were used for measuring $DO^{14}C$ and $CO^{14}C$ in the seawater according to the method described by Zhang and Wang (2004). The fish and feces were digested in 1 mL of 2 mol/L NaOH at 80°C overnight for ^{14}C measurement according to the method described by He and Wang (2006). The detailed procedures for collecting and processing the samples are provided in Text S4.

To determine the radioactivity of ^{14}C in all the above samples, a 4-mL liquid scintillation cocktail (OptiPhase Hisafe 3, Perkin-Elmer Life Science) was added to each sample. The sample was thoroughly mixed by vortexing and placed in the dark for more than 12 h.The sample was then was revortexed before being measured by a liquid scintillation counter (Beckman LS 1801). The ^{14}C recovery rates in the fish (41% ± 5%, n = 3), feces (41% ± 5%, n = 3), DOC (75% ± 1%, n = 3) and CO_2 (46% ± 0.0%, n = 3) were used to calculate the actual ^{14}C in the samples.

The decreasing rate of ^{14}C retained in fish (h^{-1}) was calculated as the slope of the linear regression of the natural logarithms of ^{14}C in fish with the time of depuration. The carbon AE was operationally calculated as the percentage of ingested ^{14}C retained in the fish after depuration. The release rates of $DO^{14}C$, $^{14}CO_2$ and $P^{14}C$ (µg C/mg DW/h) at each stage during the depuration were calculated by dividing the measured ^{14}C in each form by the time interval of the stage and then normalizing this result to the DW of the fish. According to the mass balance, the apparent food carbon release ($F^{14}C_{release}$) was calculated as the difference between the ^{14}C ingested at the beginning and the ^{14}C retained in fish at the end of the depuration. The sum of the actually collected $^{14}CO_2$, $DO^{14}C$ and $P^{14}C$ during depuration was taken as the measured food carbon release ($F^{14}C'_{release}$). The ratio of $F^{14}C'_{release}/F^{14}C_{release}$ was used to indicate the ^{14}C recovery of the total released ^{14}C in seawater.

2.2.4 Long-term depuration experiments: examining the allocation of released body carbon by labeling fish bodies with ^{14}C

To examine the release (/efflux) rate of fish body carbon and the allocation of released fish body carbon as DOC, CO_2, and PC, the marine medaka were ^{14}C-labeled by feeding the fish ^{14}C-labeled rotifers for 15 days (0.7–1.1 Bq/μg C). Then, three to five fish were collected for ^{14}C measurement in fish at time zero, and other fish were individually transferred into 50-mL feeding beakers containing 25 mL clean FSW for 8-day depuration. During the depuration period, the fish were fed nonradioactive rotifers at two rations of1000 and 2000 ind./fish/day, corresponding to 2.2% and 4.3% of the fish DW, respectively. These rations are within the typical range of daily food consumption for wild mesopelagic fish (Davison et al., 2013). During depuration, fish, feces and water samples were collected every 24 h for the measurement of the ^{14}C retained in fish and released into seawater in the 2000 ind./fish/day ration treatment. The samples were collected on days 1, 3, 6, and 8 in 1000 ind./fish/day ration treatment. All the samples were handled and measured by the same methods as described for the short-term depuration experiments.

The release (/efflux) rate of fish body carbon was calculated as the slope of the linear regression of the natural logarithms of the ^{14}C retained in fish with the time of depuration. Only the data on the second day and afterward were used for the regression. Based on the mass balance, the apparent body carbon release ($B^{14}C_{release}$) was calculated as the difference between the amount of ^{14}C in fish at the beginning and the ^{14}C retained in the fish at the end of depuration. The sum of the collected $^{14}CO_2$, $DO^{14}C$ and $P^{14}C$ during depuration was taken as the measured body carbon release ($B^{14}C'_{release}$). The ratio of $B^{14}C'_{release}/B^{14}C_{release}$ was used to indicate the ^{14}C recovery of the total released body ^{14}C in seawater.

2.3 Scenarios for estimating carbon release from mesopelagic fish in the global open ocean

Carbon release model scenarios were separately established for four groups of mesopelagic fish classified on the basis of their ocean latitudes (40°N–40°S vs. 40–70°N/S) and fish behavior (diel vertically migrating (DVM) vs. nonmigrating (NM)) (Table 1). The carbon release parameters of marine medaka were extrapolated to wild mesopelagic fish. Other parameters, such as the mesopelagic fish biomass and the daily food intake of mesopelagic fish, were derived from the literature.

We assumed that the wild fish would release the same proportions of their daily food carbon to seawater in the forms of DOC, CO_2, and PC as marine medaka. The food carbon release parameters measured after 24-h depuration were used in the model scenarios for two reasons. First, the release rates of DOC, CO_2, and PC from food decreased to relatively constant low values during the 16–24 h depuration phase, indicating that 24 h or less was enough for the fish to completely allocate the ingested food carbon to assimilation or food carbon release. Second, the feeding behavior of DVM mesopelagic fish usually has a diel rhythm. Therefore, the mean proportions of ingested food allocated to AE (38.9%) and released as DOC (32.7%), CO_2 (20.9%), and PC (7.5%) during 24-h depuration were used in the model scenarios (Table 1).

The body carbon release (/turnover) rate (K_e) of mesopelagic fish was extrapolated from the carbon release (/efflux) rate of marine medaka by using the power-law function reported in Gillooly et al. (2001) and Davison et al. (2013). That is, K_e is proportional to $1924 \times WW^{0.75} \times e^{(-5020/K)}$, where WW is the wet weight of fish (g), and K is the absolute temperature. Based on this formula, the K_es of 0.5-g mesopelagic fish living at different temperatures could be derived from the K_e of marine medaka. The K_e (0.053 d^{-1}) of marine medaka at the daily food ration of 4.3% fish DW was used as the basis for estimating the K_es of mesopelagic fishes (0.5 g in WW) (Table 1). Following the pattern of marine medaka, 40.4%, 42.6% and 16.9% of the released body carbon of wild mesopelagic fish was assumed to be in the forms of DOC, CO_2, and PC, respectively. Carbon released through reproduction was not considered in the current estimation.

Variations in the body carbon release rate during different activities, such as swimming, feeding and resting, were not considered in the present estimation. As a simplification, DVM mesopelagic fish were assumed to feed and live in surface waters at night (for 12 h) and inhabit mesopelagic depths during the daytime (for 12 h). NM mesopelagic fishes were assumed to feed and live at mesopelagic depths all day. That is, the DVM mesopelagic fish have two different K_es, depending on the mean temperatures of the surface waters and of the mesopelagic waters. In contrast, the NM mesopelagic fish have only one K_e, depending on the mean temperature of the mesopelagic waters.

The biomass of the mesopelagic fish in the open ocean was assumed to be constant over time. The total WW of the mesopelagic fish in the open ocean between 40°N and 40°S was assumed to be 10^9–10^{10} t, and the WW of the mesopelagic fish in other regions between 40°N–70°N and 40°S–70°S was assumed to be 0.3×10^9–10^{10} t (Lam & Pauly, 2005; Irigoien et al., 2014). Furthermore, 30%–50% of the mesopelagic fish were assumed to undergo diel vertical migration (Davison et al., 2015; Klevjer et al., 2016).

For mesopelagic fishes living in the open ocean, the ratio of fish DW to WW was 19.1%, and the carbon content was 43.8% of the fish DW (Childress & Nygaard, 1973), which is similar to that (46.7%) of marine medaka. The mean WW of individual fish was assumed to be 0.5 g (Davison et al., 2013, 2015).

The mean temperatures in the surface and mesopelagic waters in the open ocean between 40°N and 40°S were assumed to be 25°C and 9°C, respectively (Davison et al., 2013; Irigoien et al., 2014), whereas the mean temperatures in the surface and mesopelagic waters at high latitudes, 40°N–70°N and 40°S–70°S, were assumed to be 8°C and 3°C, respectively (Kaeriyama & Ikeda, 2004; Max et al., 2012). The daily food ration for a 0.5-g mesopelagic fish was assumed to be temperature-dependent according to Davison et al. (2013), i.e., the daily food ration was 10%, 5%, 5%, and 4% of the fish WW at 25°C, 9°C, 8°C, and 3°C, respectively. All mesopelagic fishes were assumed to be zooplanktivorous, and the carbon content of zooplankton was assumed to be 0.12 mg C/mg WW (Harris et al., 2000).

The annual rate of carbon release in each form was calculated by multiplying the daily rate by 365.

2.4 Data analysis

All statistical analyses were conducted in SPSS 17.0. Specifically, for the short-term depuration experiments, a paired t-test was used to compare the mean values of the food carbon AE measured under the different depuration times (24 h and 36 h), and an independent t-test was used to compare mean values among different depuration times in the same experiment or among experiments with different food rations at the same time. For the long-term depuration experiments, analysis of covariance (ANCOVA) was used to compare the release rates of fish body carbon under different daily food rations. Bivariate correlation with the Pearson correlation coefficient was used to examine the correlation of the proportions of DOC, CO_2, and PC with depuration time in the long-term depuration experiments.

3 Results

3.1 Recovery of ^{14}C released in seawater

The results showed that the ^{14}C released from the food and fish body sources could be sufficiently recovered. For the food carbon, $F^{14}C'_{release}/F^{14}C_{release}$ were 105%, 108% and 112% at food rations of 2.2%, 3.2% and 5.7% of the fish DW, respectively. For the fish body carbon, $B^{14}C'_{release}/B^{14}C_{release}$ were 89% and 105% at the daily food rations of 2.2% and 4.3% of the fish DW, respectively.

3.2 Food carbon assimilation efficiency and release

The amount of ingested carbon retained in the marine medaka decreased quickly (0.07–0.15 h^{-1}) during the first 4 h, and then the decrease slowed to lower rates (0.02–0.03 h^{-1}) until the end of the depuration (Figure 3). During the depuration from 24 h to 36 h, there were no significant changes in the carbon retained in fish at any of the three food rations (t-test, $p > 0.10$) (Figure 3). The food carbon AE decreased with increasing food rations (Figure S1). Based on the experiments with different food rations, the carbon AEs in the fish were 30%–49% (38.9% on average) and 25%–47% (33.7% on average) corresponding to depuration times of 24 h and 36 h, respectively; the difference was not statistically significant (paired t-test, $p > 0.05$).

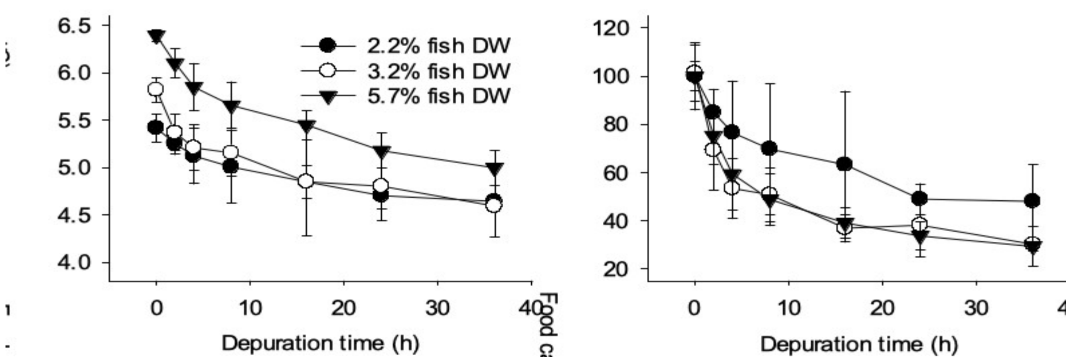

Figure 3. Retention of ingested carbon in marine medaka during the 36-h depuration. Ingested food rations are expressed in percentages of fish dry weight (DW). Data are the mean ± SD (n = 3–5). The error bars represent the standard deviations. Note the natural logarithm scale in the left subfigure.

The proportions of carbon released as DOC, CO_2, and PC varied during the depuration

(Figure 4). During the first 2 h, most of the released carbon was DOC (55%–60%), and the proportion of DOC decreased during the first 16 h (to 39%–55%). In contrast, the proportion of CO_2 increased (from 25%–32% to 40%–54%) during the first 16 h. The contribution of PC peaked during the first 4 h (up to 15%–25%); thereafter, it decreased quickly to the lowest values (to less than 8%) during the depuration from 8–16 h, and remained constant until the end of the depuration (Figure 4a, b, c).

The release rates of DOC, CO_2, and PC from ingested food decreased with the depuration time. With the food ration of 2.2% fish DW, the release rates of DOC, CO_2, and PC decreased from 0.61, 0.32, and 0.13 μg C/mg DW/h at the beginning to 0.03, 0.05 and < 0.01 μg C/mg DW/h during the depuration from 8–16 h, respectively. With the increase in food rations, all release rates of DOC, CO_2, and PC were increased proportionally (Figure 4d, e, f). However, no significant differences in the release rates of PC among the different food rations were observed after 8 h of depuration (t-test, $p > 0.1$), and the release rates of PC at all three food rations remained constant (< 0.01 μg C/mg DW/h) after 16 h of depuration (Figure 4d, e, f) (t-test, $p >$ 0.1).

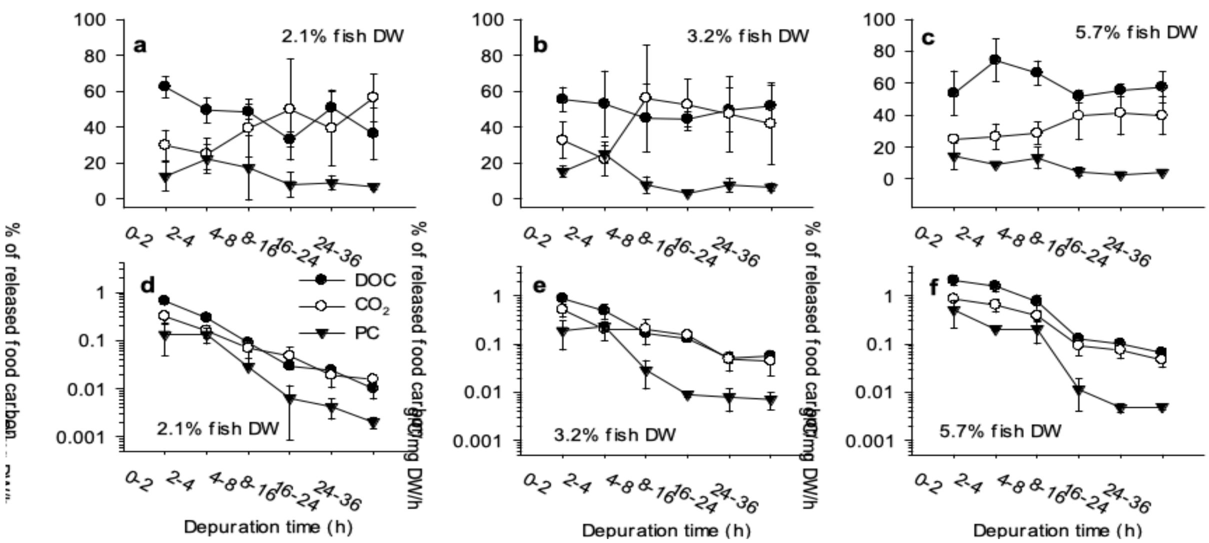

Figure 4. Relative contributions (**a, b, c)** and release rates (**d, e, f**) of different forms of carbon at different stages of the 36-h depuration under different food rations. Data are the mean ± SD (n =3–5). The error bars represent the standard deviations. DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon. Ingested food rations are expressed in percentages of fish dry weight (DW)

During the whole depuration period, most of the unassimilated food carbon was released into seawater in the forms of DOC (48%–59%) and CO_2 (30%–40%), and only 11%–13% of the released carbon was PC in fecal pellets (Figure 5a).

Taking the ingested food carbon as 100%, for the 36-h depuration, 25%–45% (34.8% on average), 18%–29% (22.9% on average), and 7%–8% (7.6% on average) of the food carbon were released as DOC, CO_2, and PC, respectively (Figure 5b). For the 24-h depuration, the released DOC, CO_2, and PC accounted for 26%–42% (32.7% on average), 18%–25% (20.9% on average), and 7%–8% (7.5% on average) of the food carbon, respectively.

A substantial proportion (46%–49%) of the released DOC was COC. The ratio did not vary significantly at different stages of the 36-h depuration, or with different food rations (Figure S2).

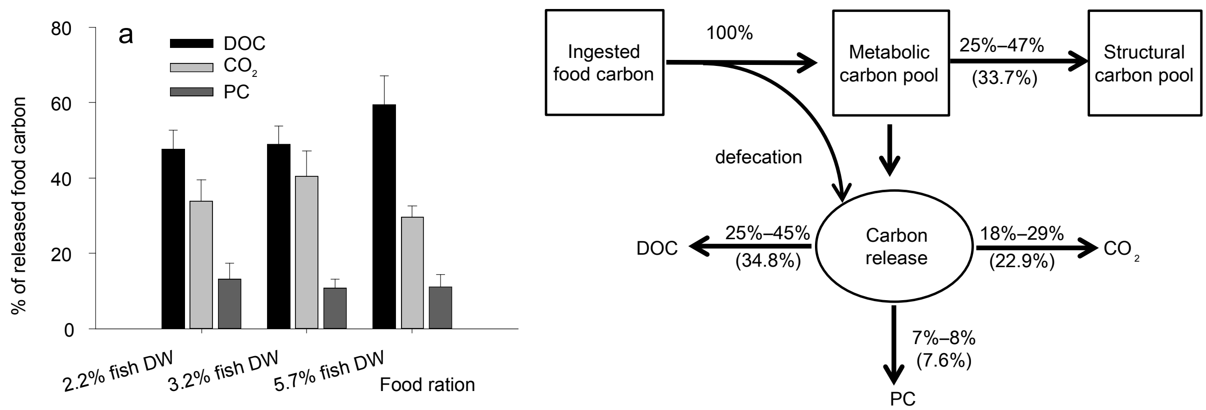

Figure 5. Relative contributions of different forms of carbon to the released food carbon over the entire 36-h depuration at different food rations (**a**) and the allocation of the ingested food carbon of marine medaka (**b**). Data are the mean ± SD (n =3–5). The error bars represent the standard deviations. DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon. The food rations are expressed as a percentage of the fish dry weight (DW). The values in brackets in subfigure (**b)** indicate the means of the above-noted ranges.

3.3 Release and turnover of fish body carbon

The body carbon of the fish was replaced and released at relatively high rates. The K_e was 0.053 d^{-1} at the daily food ration of 4.3% fish DW, whereas the rate was significantly increased (0.12 d^{-1}) at the daily food ration of 2.2% fish DW (ANCOVA, $p < 0.05$) (Figure 6a).

The proportions of DOC, CO_2, and PC for both daily food rations did not show clear change trends with depuration time (least-squares regression, $p > 0.1$) (Figure 6b, c). For the entire 8-d depuration, the proportions of DOC, CO_2, and PC did not vary significantly between the two daily food rations (Figure 7a). DOC, CO_2, and PC accounted for 39%–42% (40.4% on average), 40%–45% (42.6% on average), and 16%–18% (16.9% on average) of the released body carbon, respectively (Figure 7b).

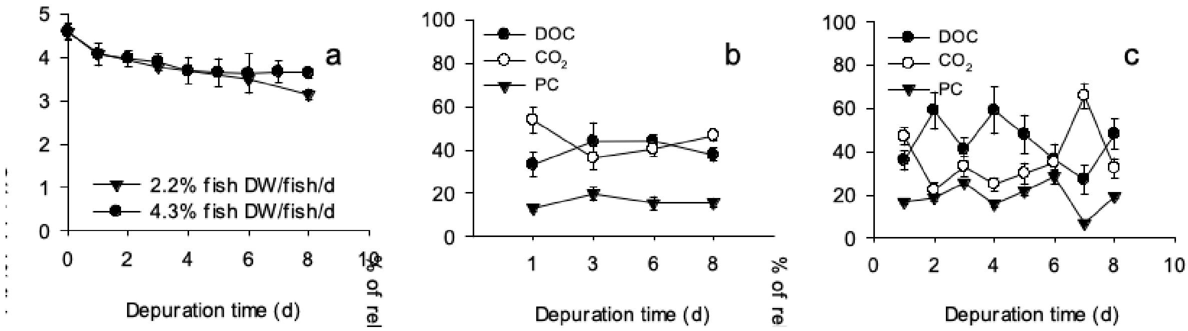

Figure 6. Retention of [14]C-labeled structural carbon in marine medaka during the 8-d depuration (**a**) and the relative contribution of different forms of carbon to the released fish body carbon at daily food rations of 2.2% (**b**) and 4.3% (**c**) of the fish dry weight (DW). Data are the mean ± SD (n =3–5). The error bars represent the standard deviations. DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon. Note the natural logarithm scale in subfigure (**a**)

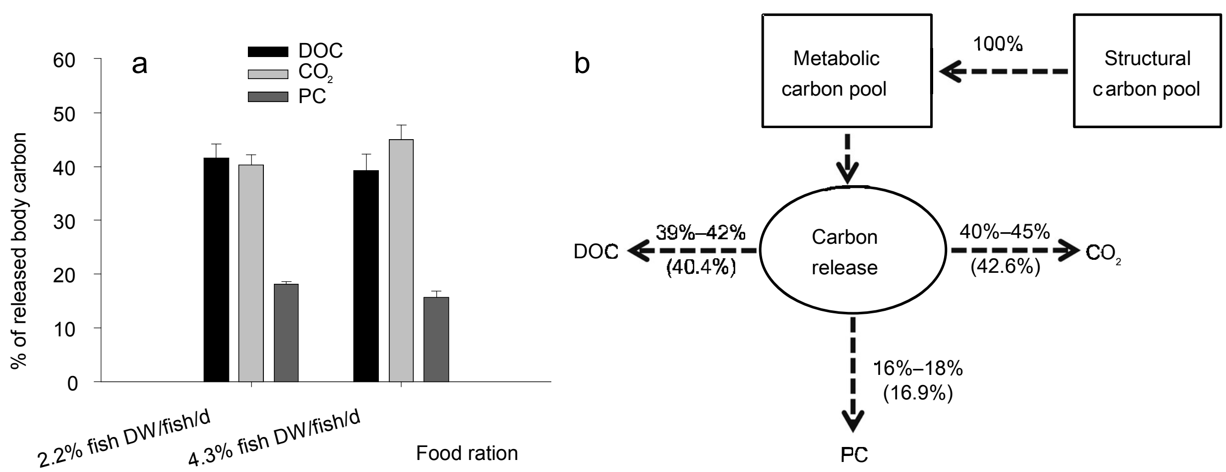

Figure 7. Relative contributions of different forms of carbon to the released body carbon over the 8-d depuration period with different daily food rations (**a**) and the allocation of the released body carbon of marine medaka (**b**). Data are the mean ± SD (n =3–5). The error bars represent the standard deviations. DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon. The food rations are expressed as a percentage of the fish dry weight (DW). The values in brackets in subfigure (**b)** indicate the means of the above-noted ranges.

3.4 Carbon release from mesopelagic fish estimated using the carbon release model

The results showed that DVM mesopelagic fish ate 1.51–25.2 Pg zooplankton carbon and released 0.59–9.84 Pg C DOC, 0.42–6.95 Pg C CO_2, and 0.15–2.56 Pg C PC annually in the global open ocean (Figure 8a). NM mesopelagic fish ingested and released similar amounts of carbon annually (Figure 8b, and see details in Text S5).

In total, of the 3.41–38.8 Pg zooplankton carbon ingested by all mesopelagic fishes per year in the global open ocean, 1.33–15.1 Pg of the ingested carbon was assimilated in the fish body, whereas the remaining unassimilated carbon was released as DOC (1.12–12.7 Pg C/y), CO_2 (0.71–8.10 Pg C/y), and PC (0.26–2.91 Pg C/y) in seawater. Meanwhile, 0.57–6.30 Pg body carbon of the mesopelagic fish was lost annually as DOC (0.23–2.55 Pg C/y), CO_2 (0.24–2.69 Pg C/y), and PC (0.10–1.07 Pg C/y). That is, mesopelagic fish in the global open ocean annually released 1.34–15.2, 0.95–10.8, and 0.35–3.97 Pg C in the forms of DOC, CO_2, and PC, respectively.

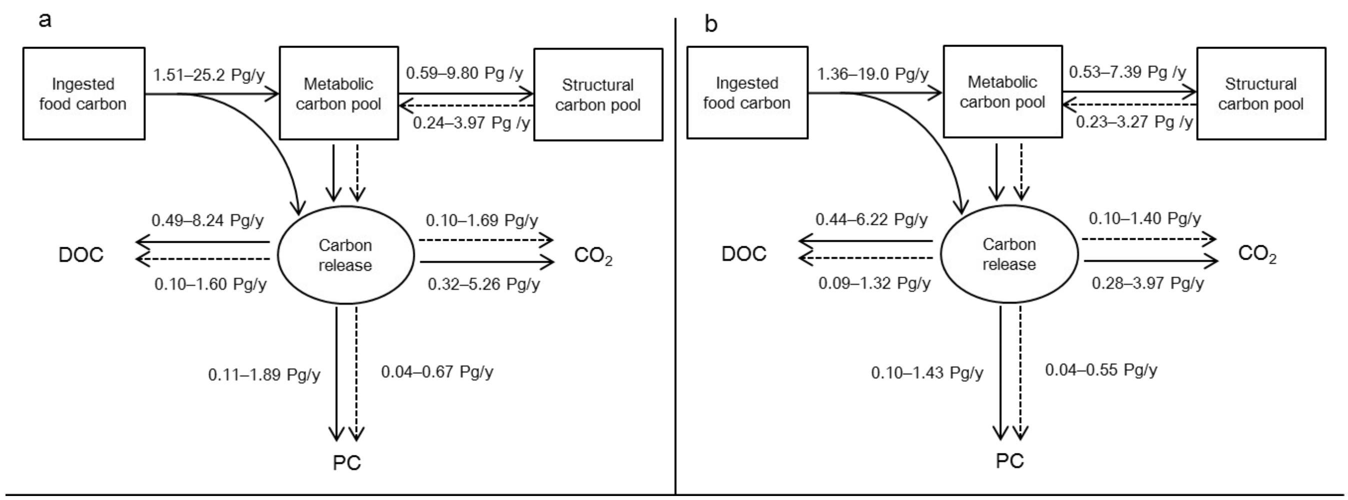

Figure 8. Carbon flow in and release from mesopelagic fish in the global open ocean. The solid and dotted lines show the carbon flows from the ingested food and the structural body carbon (fish body), respectively. **a**, diel vertically-migrating mesopelagic fish, and **b**, nonmigrating mesopelagic fish. DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon.

Our results showed that the vertical migration of mesopelagic fish contributes greatly to the active export of carbon. Assuming that half of the daily food carbon release by the DVM mesopelagic fish occurs in deep waters, the global active exports of DOC, CO_2, and PC mediated by the DVM mesopelagic fish are 0.28–4.59, 0.19–3.13, and 0.07–1.14 Pg C/y, respectively (Text S5; Table S1).

The results also showed that $FC_{release}$ was approximately 3.7 times as much as $BC_{release}$ (Table 1). For all the DVM and NM mesopelagic fishes at both low and high latitudes, the DOC released from food was more than 4 times higher than that released from the fish body, and the CO_2 and PC released from food were more than 2 times higher than those released from the fish body (Table 1).

Table 1. Carbon release model scenarios for mesopelagic fish in the global open ocean. The mesopelagic fishes were divided into diel vertically migrating (DVM) and nonmigrating (NM) groups, and the global open ocean was divided into two regions: open oceans in 40°N–40°S, and other regions in high latitudes up to 70°N/S.

	Unit	DVM mesopelagic fish		NM mesopelagic fish	
Parametes/Area		40°N–40°S	Other regions	40°N–40°S	Other regions
Fish biomass[a]	10^9–10^{10} t WW	0.30–0.50	0.09–0.15	0.50–0.70	0.15–0.21
Individual fish WW	g	0.5[b]	0.5[b]	0.5[b]	0.5[b]
P_{SW}	h	12	12	0	0
P_{MW}	h	12	12	24	24
T_{SW}	°C	25[c]	8[d]	–	–
T_{MW}	°C	9[c]	3[d]	9[c]	3[d]
R_{SW}	% fish WW	10[e]	5[e]	0	0
R_{MW}	% fish WW	0	0	5[e]	4[e]
Carbon in food	mg C/ mg WW	0.12[f]	0.12[f]	0.12[f]	0.12[f]
Daily ingested carbon	10^6–10^7 t C/d	3.6–6.0	0.54–0.90	3.0–4.2	0.72–1.01
Assimilation efficiency		38.9% (30%–49%)	38.9% (30%–49%)	38.9% (30%–49%)	38.9% (30%–49%)
Daily assimilated carbon	10^6–10^7 t C/d	1.40–2.33	0.21–0.35	1.17–1.63	0.28–0.39
% *FC* released as DOC		32.7% (26%–42%)	32.7% (26%–42%)	32.7% (26%–42%)	32.7% (26%–42%)
% *FC* released as CO_2		20.9% (18%–25%)	20.9% (18%–25%)	20.9% (18%–25%)	20.9% (18%–25%)
% *FC* released as PC		7.5% (7%–8%)	7.5% (7%–8%)	7.5% (7%–8%)	7.5% (7%–8%)
K_e at 25°C	d^{-1}	0.0331	0.0331	0.0331	0.0331
K_e at 9°C	d^{-1}	0.0127	0.0127	0.0127	0.0127
K_e at 8°C	d^{-1}	0.0120	0.0120	0.0120	0.0120
K_e at 3°C	d^{-1}	0.0087	0.0087	0.0087	0.0087
Carbon in fish	% fish WW	8.37[g]	8.37[g]	8.37[g]	8.37[g]
% released *BC* as DOC		40.4% (39%–42%)	40.4% (39%–42%)	40.4% (39%–42%)	40.4% (39%–42%)
% released *BC* as CO_2		42.6% (40%–45%)	42.6% (40%–45%)	42.6% (40%–45%)	42.6% (40%–45%)
% released *BC* as PC		16.9% (16%–18%)	16.9% (16%–18%)	16.9% (16%–18%)	16.9% (16%–18%)
DOC released from food	10^6–10^7 t C/d	1.18–1.96	0.18–0.29	0.98–1.37	0.24–0.33
CO_2 released from food	10^6–10^7 t C/d	0.75–1.25	0.11–0.19	0.63–0.88	0.15–0.21
PC released from food	10^5–10^6 t C/d	2.70–4.50	0.41–0.68	2.25–3.15	0.54–0.76
Total food carbon release	10^6–10^7 t C/d	2.20–3.67	0.33–0.55	1.83–2.57	0.44–0.62
DOC released from body	10^5–10^6 t C/d	2.32-3.87	0.31-0.52	2.15-3.01	0.44-0.61
CO_2 released from body	10^5–10^6 t C/d	2.45-4.08	0.33-0.55	2.27-3.18	0.46-0.65
PC released from body	10^5–10^6 t C/d	0.97-1.62	0.13-0.22	0.90-1.26	0.18-0.26
Total body carbon release	10^5–10^6 t C/d	5.74–9.57	0.77–1.29	5.32–7.45	1.08–1.52

Notes: WW, wet weight; P_{SW}, time spent in surface waters; P_{MW}, time spent in mesopelagic waters; T_{SW}, mean temperature in surface waters; T_{MW}, mean temperature in mesopelagic waters; R_{SW}, daily food ration in surface waters; R_{MW}, daily food ration in mesopelagic waters; *FC*, food carbon; *BC*, body carbon; K_e, body carbon release rate; DOC, dissolved organic carbon; CO_2, carbon dioxide; PC, particulate carbon. Data in brackets are ranges corresponding to the above mean values.

a, The total WW of the mesopelagic fish in the open ocean between 40°N and 40°S was assumed to be 10^9–10^{10} t, and the WW of the mesopelagic fish in other regions between 40°N–70°N and 40°S–70°S was assumed to be 0.3×10^9–10^{10} t (Lam & Pauly, 2005; Irigoien et al., 2014). The biomass of DVM and NM mesopelagic fishes were calculated by assuming 30%–50% of the mesopelagic fish undergo diel vertical migration (Davison et al., 2015; Klevjer et al., 2016).

b, Davison et al., 2015; Davison et al., 2013

c, Davison et al., 2013; Irigoien et al., 2014
d, Kaeriyama & Ikeda, 2004; Max et al., 2012
e, Davison et al., 2013
f, Harris et al., 2000
g, Childress & Nygaard, 1973

4 Discussion

4.1 Carbon release from marine fish

Existing knowledge regarding the food carbon allocation and body carbon release (/turnover) of marine fish is limited. The carbon AE of marine fish has seldom been reported.

The proportion (7%–8%) of PC released from the food carbon of marine medaka was in the range (0.8%–9.7%) of those of seven carnivorous marine fishes fed fish (*Ammodytes personatus*) pieces (Tang et al., 2003) and was comparable to that (8.2%–9.7%) of the detritivorous fish *Liza haematocheila* (Kang et al., 2007, 2010).

The K_e (0.053–0.12 d^{-1}) of marine medaka measured in this study was within the range (0.0044–0.14 d^{-1}) of reported carbon turnover rates for fish muscle tissue (Weidel et al., 2011). Our results showed that most (40%–45%) of the replaced and released body carbon was released in the form of CO_2, indicating that respiration is the largest loss route for the released body carbon. The proportion was at the lower end of the reported values (44.3%–79.4%) for carnivorous marine fishes (Tang et al., 2003). The measured CO_2 from body carbon in our study may be only part of the total carbon used for respiration because some catabolized carbon (in the form of bicarbonate) is excreted in fish intestines, forms precipitated carbonates, and is finally released as fecal pellets (Salter et al., 2017; Wilson et al., 2009). Providing that the entire measured PC released from the body carbon was precipitated carbonates in fish feces, we could estimate that up to 58%–61% (59.5% on average) of the released body carbon was used for respiration. In fact, according to our conceptual model, the carbon used for marine medaka respiration was from not only replaced (and released) fish body carbon but also from ingested food. In other words, the daily respiration rate of mesopelagic fish could be derived from the daily release rate of CO_2 from food and that from released body carbon (Text S6).

The mesopelagic fish respiration rates derived from the model fish marine medaka are consistent with recent understanding about the power-law relationship of the mesopelagic fish respiration rate to the fish wet mass and habitat temperature (Text S6; Figure S3). Using 74 data points (each of which includes the respiration rate of myctophids, one of the most biomass dominant groups of mesopelagic fish, the temperature and the fish WW) from five studies, a power-law equation was developed to calculate the WW-specific respiration rate from fish WW and ambient temperature (Belcher et al., 2019). By using this equation, we calculated the daily respiration rates of 0.5-g mesopelagic fish at the different ambient temperatures (3, 8, 9, and 25°C) used in our estimation. The daily respiration rates calculated by using the equation were not different from the daily CO_2 release rates derived from the model fish (paired t-test, $p > 0.1$; Figure S3).In addition, significant power-law relationships exist between the calculated daily respiration rates, and the model fish-derived daily CO_2 release rates (Figure S3).This consistency justifies our use of carbon release parameters derived from marine medaka to extrapolate the carbon release of wild mesopelagic fish.

Our results showed that substantial proportions of the ingested food carbon and the lost body carbon of the model fish are released as DOC. To our knowledge, the DOC excreted by marine fish has not yet been directly measured in previous studies.

In addition, the release of DOC from fish feces has not yet been reported. The contribution of fecal leakage to the measured $DO^{14}C$ was not examined in the present study. Contradictory results have been reported about the contribution of fecal pellets to DOC released from zooplankton. Substantial DOC released from fecal pellets of zooplankton has been reported (Thor et al., 2003; Urban-Rich, 1999), but some studies show that the leaching of DOC from fecal pellets of zooplankton was insignificant compared to the DOC released through excretion (Steinberg et al., 2000). We do not think that the leakage of fish feces (if it exists) would contribute much to the $DO^{14}C$ measured in the present study; PC in feces only accounted for a small proportion (7%–8%) of the food carbon release (Figure 5), and the release rates of the DOC did not peak during the first 2–4 h of the depuration, when the release rates of feces peaked (Figure 4d, e). However, why a substantial proportion (46%–49%) of the released DOC was COC (Figure S2) is still open for discussion.

4.2 Estimated carbon release from mesopelagic fish in the open ocean

Assuming a mean global primary production of 59.2 Pg C/y (41–77 Pg C/y) as the scaling basis (del Giorgio and Duarte 2002), our estimation shows that the DOC (1.34–15.2Pg C/y), CO_2 (0.95–10.8 Pg C/y), and PC (0.35–3.97 Pg C/y) released by mesopelagic fish in the open ocean were 2.3%–25.7%, 1.6%–18.2%, and 0.6%–6.7% of the global primary production, respectively. Our estimation of the global CO_2 released by mesopelagic fish was comparable to an estimation that the carbon consumed in by mesopelagic fish respiration was approximately 10.5% ± 7.8% of the primary production along a global investigation transect (Irigoien et al., 2014).The upper limit of the estimated CO_2 released by mesopelagic fish was comparable to the amount of carbon consumed by mesozooplankton respiration (13.0 Pg C/y) in global oceans (Hernández-León & Ikeda, 2005). The amount of PC in fecal pellets released by mesopelagic fish was approximately 1/20 to 1/2 of the amount of fecal carbon (with upper limits of 6.2–6.8 Pg C/y) released by mesozooplankton in epipelagic oceans (Steinberg & Landry, 2017). By assuming that half of the daily food carbon release of DVM mesopelagic fish occurred in deep waters, the active carbon export mediated by the DVM mesopelagic fish (0.54–8.86 Pg C/y) was estimated to be comparable to or even higher than the carbon export mediated by DVM zooplankton (1.04 ± 0.26 Pg C/y) (Archibald et al., 2019). The contribution of DVM micronekton (mainly dominated by fish) to respiratory flux has been reported to be similar to that of DVM zooplankton in the northeastern Atlantic Ocean (Ariza et al., 2015). The high biomass of mesopelagic fish, which is comparable to or even higher than the biomass of mesozooplankton in global oceans, may explain the high carbon release from mesopelagic fish. The global mesopelagic fish biomass (1.3–13 Pg WW or 0.11–1.1 Pg C) used in our estimation is comparable to or even higher than the global mesozooplankton biomass (0.26 Pg C) in the epipelagic ocean (0–200 m in depth) (Hernández-León & Ikeda, 2005), where most zooplankton are distributed. In fact, mesopelagic fish biomass is likely higher than mesozooplankton biomass at low latitudes. For example, recent studies show that the biomass of mesopelagic fish is approximately 1.51–29.38 g C/m^2 in the open oceans between 40°N and 40°S, and 2.09–3.10 g C/m^2 in the southern California current ecosystem, whereas the epipelagic mesozooplankton biomass is only 0.15–1.3 g C/m^2 at the same latitudes (Davison et al., 2015; Hernández-León & Ikeda, 2005; Irigoien et al., 2014).

Our estimation of the active export of DOC by mesopelagic fish (0.28–4.59 Pg C/y) was comparable to the estimates of the global export of DOC below 74 m by mixing (2.31 ± 0.60 Pg C/y) (Roshan & DeVries, 2018) and indicates that DVM mesopelagic fish are an important source of not only ammonium (Bianchi et al. 2014) but also DOC for the mesopelagic layer. This may explain the dissolved organic matter anomalies concurrent with migrating animals (mainly fish) in the mesopelagic layer (Boyd et al., 2019) and support the argument that the supply of significant amounts of labile DOC from mesopelagic fish sustains a microbial growth efficiency in the mesopelagic layer that is twice as high as that at the surface of the Red Sea (Calleja et al. 2018).

The present estimation of the active export of PC by mesopelagic fish (0.07–1.14 Pg C/y) is lower than a recent estimation of the global magnitude of carbon export by the mesopelagic migrant pump (0.9–3.6 Pg C/y), which is mediated mainly by mesopelagic fish (Boyd et al., 2019), but is comparable to an estimation of the active carbon export by vertically migrating marine fish (0.19 Pg C/year) (Aumont et al. 2018).

4.3 Uncertainties in the estimation of carbon released from mesopelagic fish

Our estimation of the carbon released from mesopelagic fish is undeniably still far from precise. This is an opportunity to thoughtfully examine the values coming from lab experiments and the other inputs to the extrapolation analysis and to offer specific advice on future research topics. Uncertainties may come from the use of parameters derived from marine medaka and from the literature, including the estimation of mesopelagic fish biomass, the vertical migration behavior of mesopelagic fish, the use of only a single food for the model fish, the allocation of ingested food carbon to release, and the exclusion of varying metabolic rates and K_es of fish during different activities. Better information about these factors will help to improve the estimations.

Our estimation of the carbon released from mesopelagic fish is strongly dependent on the mesopelagic fish biomass. The substantially varying estimates of global mesopelagic fish biomass lead to much uncertainty. The mesopelagic fish biomass used in our estimation (1.3–13 Pg) is similar to a recent estimate of global mesopelagic fish biomass of 1.8–16 Pg (Proud et al., 2019). The large variation in the estimates of mesopelagic fish biomass could be the most important factor accounting for the large ranges (covering one order of magnitude) in the estimated DOC, CO_2, and PC released by mesopelagic fish in the present study (Figure 8). In fact, the estimates of mesopelagic fish biomass used in our estimation and in Proud et al. (2019) are based on the acoustic method. Methodological uncertainties from the acoustic method (e.g., interference from siphonophores) are the major cause of variation in the estimation of mesopelagic fish biomass (Proud et al. 2019). In addition, the lack of acoustic data about mesopelagic fish at high latitudes may further undermine the estimation of mesopelagic fish biomass in the global open ocean. Early studies based on trawling document that the density of mesopelagic fish at high latitudes could be several fold higher than that at low latitudes (Lam & Pauly, 2005). A recent study based on global observations from a satellite-mounted lidar also shows that the total DVM animal biomass is higher in the more-productive high-latitude oceans (Behrenfeld et al., 2019). Therefore, constraining the uncertainty of the acoustic method and performing more surveys based on multiple methods and covering a broader area, especially those at high latitudes, are needed to more precisely determine the biomass of global mesopelagic fish.

The ratio of DVM mesopelagic fish to total mesopelagic fish was based on two recent studies at low latitudes, from 40 °N to 40 °S, and in the southern California current system (Davison et al., 2015; Klevjer et al., 2016). Little is known about the proportion of DVM mesopelagic fish at high latitudes. It may be reasonable to assume that the DVM mesopelagic fish at low latitudes spend 12 h of diurnal time in the upper ocean and another 12 h of nocturnal time at mesopelagic depths. However, diurnal and nocturnal times at high latitudes vary significantly in different seasons. Little is known about seasonal variations in vertical-migration behavior or about mesopelagic fish biomass at high latitudes. More observations at high latitudes are needed, and recent progress in satellite observations of DVM animal biomass may facilitate such work (Proud et al. 2019).

The use of parameters derived from the model zooplanktivorous fish marine medaka to extrapolate the allocation of ingested food and the released body carbon of mesopelagic fish may lead to uncertainties. However, the extrapolation is reasonable, at least for the moment. First, the model fish marine medaka ecologically resembles mesopelagic fish, especially because both the model fish and most mesopelagic fish live on zooplankton, and they have similar body sizes (in centimeters). Second, it is still a technical challenge to catch and rear living mesopelagic fish in the lab (Belcher et al., 2019), making it difficult (if not impossible) to measure the "actual" carbon release parameters of mesopelagic fish. Third, very little (if any) data on the carbon release of marine zooplanktivorous fish (let alone mesopelagic fish) were available before our study, making it difficult to find appropriate data in the literature to fit our model. As discussed above, the consistency of the model fish-derived respiration rates of mesopelagic fish with those from the literature provides strong support for our extrapolation (Text S6; Figure S3). Undoubtedly, more experimental work is needed to examine the carbon release parameters of other zooplanktivorous fish, especially mesopelagic fish, if possible.

The use of only rotifers as the food for the fish may lead to the underestimation of the fraction of ingested food allocated to fish feces. Only rotifers were used as living zooplankton food for the marine medaka, and no other zooplankton, such as copepods, the main natural food for mesopelagic fish, was used to feed the fish. One of the main reasons for this is that it is still a challenge to rear enough living copepods or other zooplankton to feed fish and complete experiments. The fish may allocate the carbon of ingested food differently depending on the zooplankton types in their diet. For example, the exoskeletons of copepods may lead to an increased fraction of feces, as the chitin in the exoskeletons cannot be digested by most fish (Durbin & Durbin, 1981, Pinnegar & Polunin, 2006). Therefore, more lab experiments that feed zooplanktivorous fish (including marine medaka) copepods and other zooplankton are needed to examine the carbon release parameters of zooplanktivorous fish.

Although our model fish-derived respiration rates for mesopelagic fish are consistent with those from the literature (Text S6; Figure S3), the estimated allocation of ingested food carbon and lost body carbon to respiration and release as CO_2 by mesopelagic fish might be underestimated because metabolism during feeding and diel swimming between the upper ocean and mesopelagic depths were not considered in the present estimation. According to our experimental designs, CO_2 release from food was counted only after the feeding; no intense swimming occurred after the feeding because the individual fish had been placed in small beakers for the depuration. The active, feeding metabolic rate can be four times the standard metabolic rate of resting, inactive fish (Davison et al., 2013; Smith & Laver, 1981); therefore, our estimation, which did not consider the fish metabolism during feeding and swimming, may underestimate the CO_2 released from the ingested food of the mesopelagic fish. For the same reason, the derived K_es and related CO_2 release rates of mesopelagic fish might also be underestimated. Thus, future work is needed to examine the release rates for the model fish during different activities, such as swimming.

In contrast, the decrease in K_e with the increase in the daily food ration indicates that the K_e of marine medaka used for the estimation might be overestimated. According to our pilot studies, 1000 and 2000 rotifers were enough to fill the experimental fish stomachs 38% and 75% full, respectively. However, as a daily food ration for a marine medaka with WW of approximately 0.08 g and living at 25°C, 1000 rotifers is below the maintenance level for fish growth, and the high K_e (0.12 d^{-1}) indicates that substantial body carbon was used for catabolism. The decreased K_e (0.053 d^{-1}) following the doubling of the daily food ration indicates that, as the supply of food increased, the body carbon used for metabolic turnover decreased. We expect that K_e would continue to decrease if we further increased the daily food ration. From this perspective, the K_e of 0.053 d^{-1} used to extrapolate the K_es of mesopelagic fish might overestimate the body carbon release from the mesopelagic fish. However, the consistency of our model fish-derived respiration rates of mesopelagic fish with those from the literature (Text S6; Figure S3) indicates that the uncertainties from the two factors discussed above may offset each other.

Other factors, such as the simplification of the mean temperature of seawater in the upper and mesopelagic depths, the use of a temperature-dependent daily food ration, the exclusion of fish mortality and reproduction, and the assumption that all mesopelagic fish to be zooplanktivorous, may also have led to uncertainties in the present estimation. Further efforts to minimize the negative influences of the factors discussed above are needed to improve the accuracy of the estimates of carbon releases from mesopelagic fish in the global open ocean.

4.4 Implications for the importance of the contribution of mesopelagic fish to the ocean carbon cycle

By providing the first quantitative estimates of DOC, CO_2, and PC released by mesopelagic fish in the global open ocean, our results strengthen the argument that mesopelagic fish may play important roles in the ocean carbon cycle by mediating carbon export in the ocean. First, our results show that the DOC released by mesopelagic fish could be an important organic carbon source for heterotrophic biota in the ocean. Substantial amounts of released DOC, as well as of CO_2 and PC, may be actively transported to mesopelagic depths through the vertical migration of mesopelagic fish. The DOC influx to mesopelagic oceans through DVM mesopelagic fish may narrow the carbon imbalance between the estimated organic carbon influxes and the measured heterotrophic carbon consumption, which is significantly higher than the former in mesopelagic oceans (Burd et al., 2010; Giering et al., 2014; Steinberg et al., 2008).

Second, the PC in the fecal pellets produced by mesopelagic fish could contribute greatly to carbon export through the biological carbon pump. As noted above, the amount of PC in fecal pellets (0.35–3.97 Pg C/y) released by mesopelagic fish is nonnegligible, even substantial. The contribution of mesopelagic fish to carbon export may be even more important, if we consider that the sinking rates of fish fecal pellets are much (even one order of magnitude) greater than those of zooplankton fecal pellets (Saba & Steinberg, 2012).

5 Conclusions

We propose a carbon release model that divides fish-released carbon into two parts, i.e., food carbon release and body carbon release (on the basis of the source: ingested food or the fish body, respectively), and three forms, DOC, CO_2, and PC, which enable the quantification of the release of carbon by fish. By using ^{14}C-labeled living zooplankton to feed a model marine zooplanktivorous fish, this study provided a detailed methodology for precisely quantifying the carbon budget and carbon release of marine fish. By using the carbon release model and parameters derived from the model fish and the literature, we estimated the DOC, CO_2, and PC released by mesopelagic fish in the global open ocean. Our results demonstrated that marine zooplanktivorous fish such as marine medaka can convert substantial fractions of their daily ingested food carbon (26%–42%) and released (/replaced) body carbon (39%–42%) into seawater as DOC. Mesopelagic fish in the global open ocean were estimated to produce 1.34–15.2, 0.95–10.8, and 0.35–3.97 Pg C/y of DOC, CO_2, and PC, respectively. The conceptual model, the laboratory experiments with model fish, and the extrapolation to mesopelagic fish generated a complete solution for estimating the carbon released by fish, especially by global mesopelagic fish. Our estimation is undeniably still far from precise, and factors bringing about uncertainties were discussed. More experimental work is needed to examine the carbon release parameters of marine zooplanktivorous fish, and further observations based on multiple methods are suggested to cover broader areas, especially those at high latitudes, to more precisely determine the mesopelagic fish biomass and their vertical migration behaviors at different latitudes. Our study indicates that mesopelagic fish could be an important source of DOC in the ocean and play critical roles in the biological pump by producing substantial amounts of DOC and fast-sinking fecal pellets and by the active export of DOC, CO_2, and PC into deep waters through their diel vertical migration.

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Author contributions

Q. Liu played a key role in designing and implementing the experiments, analyzing results and preparing the manuscript. L. Zhou played a key role in forging the model scenarios, interpreting results, preparing the manuscript, and helped the implementation of the experiments. Y. Wu contributed to the data analysis and paper writing. X. He contributed to the data analysis and modeling, and paper writing. N. Gao contributed to implementation of the experiments. Professor L. Zhang supervised the whole research design, experimental process, data interpretation, and the manuscript composition

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