Quantification of Waste Feed and Fish Feces using Stable Carbon and Nitrogen Isotopes

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Abstract：For improving the economic and environmental sustainability of fish farming, it is necessary to optimize feeding regimes and to decrease waste feed. We developed a method to quantify waste feed and fecal matter in sediment trap materials and sediments beside and beneath fish cages using stable carbon and nitrogen isotope ratios. The contribution ratio of three sources (waste feed, fecal matter and natural organic matter) in sedimentary organic matter in and around a red sea bream (*Pagrus major*) farm in Gokasho Bay, central Japan, was estimated using an isotopic mixing model. The result showed that waste feed (29% in the bulk organic matter) exceeded fecal matter (12%) in the fish farm area, suggesting the overfeeding in this farm. Then we monitored the growth and mortality of red sea bream, C and N fluxes and sediment chemistry at two commercial fish cages; cage 1 (conventional satiation feeding) and cage 2 (restricted feeding: same feeding frequency but 18% reduction in the feed amount) for 276 days. The restricted feeding achieved normal growth, increased feed conversion efficiency and reduced mortality of red sea bream and reduced contents of waste feed-derived organic matter in the sediment. Determining the relative amount of waste feed and fecal matter in settling and sedimentary organic matter is effective for the evaluation of the optimum ration level from the viewpoint of the minimization of waste feed.

Key words : fish farm, stable isotope, waste feed, fecal matter, feeding

Intensive fish farming in coastal waters generates large amounts of particulate organic wastes in the form of waste feed (WF) and fecal matter (FM). The particulate organic wastes settle onto the seabed and produce enriched sediments, which can result in the deoxygenation of the bottom water, the production of reduced compounds such as ammonium and sulfides and changes in the structure of benthic communities (reviewed by Pearson and Black, 2001). Such environmental deterioration often produces negative consequences for the management of the farm. The practical way to implement environmental management of fish farms is to reduce organic matter (OM) loads by selecting 'low-pollution' feed pellets such as extruded pellets (EP), which have high stability and appropriate buoyancy in water

(Beveridge, 1984), instead of trash fish and moist pellets. The feeding regime is also an important husbandry factor in efforts to reduce WF (Wang *et al.*, 2007). Overfeeding leads to a loss of feed, pollutes the environment and leads to a decrease in feed conversion efficiency, while underfeeding results in increased interfish competition for feed and reduced growth and decreased feed conversion efficiency (Talbot *et al.*, 1999). In this regard, it is necessary to determine the optimum ration level, which may be variable depending on the culture species, its body size and the environmental temperature (Azevedo *et al.*, 1998). Information on the optimum ration level is also effective for the farm management, because the feed represents a major cost in fish culture. From these viewpoints, Yokoyama *et al.* (2006) developed

a method to quantify waste feed and fecal matter in the sediment using stable carbon and nitrogen isotope ratios, and found that WF (29% in the bulk OM) exceeded FM (12%) in a fish farm in Gokasho Bay, central Japan. Based on this finding, we informed fish farmers of the cooperative in Gokasho Bay the possibility of overfeeding and proposed an investigation to the farmers to culture red sea bream (*Pagrus major*) under the conventional satiation feeding regime and a restricted feeding regime in the two neighboring fish cages in order to monitor and compare the growth and mortality of fish and environmental impacts over a period of 276 days (Yokoyama *et al.*, 2009). In this review, these results are summarized.

Method to quantify fish farm wastes in settling and sedimentary organic matter

In general, settling and sedimentary organic matter (OM) beside and beneath fish cages includes three main organic matter source, i.e., WF, FM, and natural settling or sedimentary OM derived from natural sources. Yokoyama *et al.* (2006) determined the average δ^{13}C and δ^{15}N values of feed supplied to the fish cages (as the WF value), feces excreted from the red sea bream, and natural settling or sedimentary OM as follows (Fig. 1).

WF: In the fish farm in Gokasho Bay, moist pellets (MP) and extruded pellets (EP) were used to culture red sea bream. The mean δ^{13}C and δ^{15}N values for moist pellets were -20.2‰ and 10.2‰, respectively, while those for extruded pellets (EP) were -20.3‰ and 9.1‰, respectively. Of feed supplied to the fish cages in 2002, MP and EP comprised 59% and 41% by dry weight, respectively. The overall mean values for feed supplied to the fish cages (= waste feed) were estimated as δ^{13}C = $-20.2 \times 0.59 + (-20.3 \times 0.41) = -20.2$‰, and δ^{15}N = $10.2 \times 0.59 + 9.1 \times 0.41 = 9.7$‰, respectively.

FM: The mean δ^{13}C and δ^{15}N for feces excreted from the red sea bream fed on MP were -24.7‰ and 5.6‰, which were 4.4‰ reduced in ^{13}C and 3.5‰ reduced in ^{15}N relative to the feed, respectively. The mean δ^{13}C and δ^{15}N values for feces excreted from the red sea bream fed on the dry pellets were -23.7‰ and 7.2‰, which were 3.5‰ reduced in

^{13}C and 3.3‰ reduced in ^{15}N relative to the feed, respectively. Assuming that the ^{13}C and ^{15}N shifts which were found between feed and feces in the laboratory experiment are applicable to the fish population in the fish farm in Gokasho Bay, mean isotopic compositions for feces that were loaded to the environment are estimated as δ^{13}C = $(-20.2 -4.4) \times 0.59 + (-20.3 - 3.5) \times 0.41 = -24.3$‰, and δ^{15}N = $(10.2 - 3.5) \times 0.59 + (9.1 - 3.3) \times 0.41 = 6.3$‰, respectively (Fig. 1).

Natural OM: Isotopic compositions of natural settling and sedimentary OM were determined by samples collected from reference stations. The δ^{13}C and δ^{15}N values of natural settling OM were -19.6‰ and 7.5‰, respectively, while those of natural sedimentary OM were -19.9‰ and 5.5‰ (Fig. 1).

The δ^{13}C and δ^{15}N values of sedimentary OM (SOM) at the 41 stations in and around the fish farm in Gokasho Bay are shown in Fig. 1. In this dual isotopic plot, most points of SOM are located in the area of a triangle which are formed by the isotopic compositions of WF, FM and SOM reference. This enables the calculation of the ratios of WF and FM in SOM based on the simple linear mixing model (Phillips, 2001). The ratios of WF and FM in sediment trap samples can be also determined based on the isotopic compositions of WF, FM and natural settling OM.
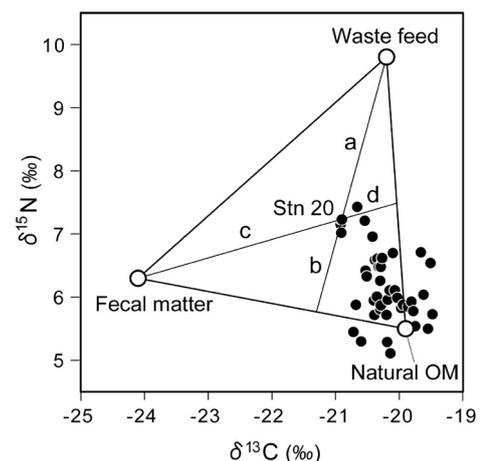

Fig. 1. Dual isotope plot for sedimentary organic matter in the fish farm (●) and the organic matter sources (○), which include feed, fish feces and natural sedimentary organic matter. For example, the ratios of waste feed and fecal matter in sedimentary organic matter at Stn 20 are calculated by {b/(a + b)} × 100%, and {d/(c + d)} × 100%, respectively (adapted from Yokoyama *et al.*, 2006).

Ratio of waste feed and fecal matter in sedimentary organic matter

Fig. 2 shows contours of the ratio of waste feed and fecal matter in SOM in and around the fish farm in Gokasho Bay. The WF ratio in the fish-farm area (within 30 m from the edge of cages) ranged from 10.3 to 43.2% (mean = 28.8%), whereas at most stations outside the fish-farm area the ratio was <10%. In the central part of the northern and southern cage rows, conspicuous high WF ratios >30% were found. The FM ratio in the fish-farm area ranged from 2.6 to 20.8% (mean = 11.9%), whereas at most stations outside the area the ratio was <10%. In the central part of the northern cage rows, high FM ratios of >20% were found. On average, the WF ratio was 2.4-fold larger than the FM ratio.

Effects of restricted feeding on fish culture and environments

The excess of WF over FM suggests the overfeeding in the fish farm in Gokasho Bay. We informed the fish farmers the possibility of overfeeding and proposed an investigation to culture red sea bream under two ration levels;

cage 1 (conventional satiation feeding, which was determined by a feed table that was provided by the manufacturer and by the fish farmers' experience) and cage 2 (restricted feeding: same feeding frequency but 18% reduction in the feed amount). Then, the experimental aquaculture was conducted in these 2 cages (initial stocking density = 11000 fish in each cage) from August 23, 2006 to May 26, 2007 (276 days) and we monitored the growth and mortality of fish, carbon and nitrogen fluxes immediately beside the cages and the sediment chemistry below the cages (Yokoyama *et al.*, 2009).

There was no significant difference in fish weight in each month between the 2 cages (two sample *t*-test, all p > 0.19) (Fig. 3a). As shown in the standard deviations of the fish weight in the 2 cages (Fig. 3a), there was no difference in the variability of the body size between them. The similar growth rate of fish in the 2 cages (i.e., 0.43% in cage 1 vs. 0.39% in cage 2) in spite of the difference in the total amount of feed resulted from a difference in feed conversion efficiency, 0.54 in cage 1 and 0.62 in cage 2.

Dead fish were found almost every day from August through October, however, they were rarely found after the beginning of November, when the water temperature fell below 21℃ and the ration decreased under 2.2%. Cumulative number of dead
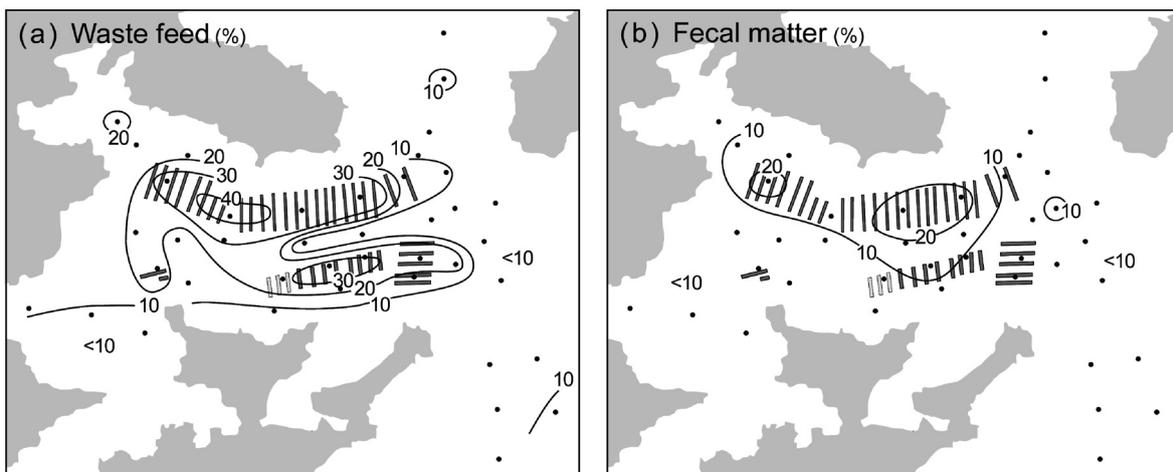

Fig. 2. Aquaculture wastes in the sediment. Isopleths of (a) waste feed ratio and (b) fecal matter ratio in sedimentary organic matter in and around a fish farm in Gokasho Bay (after Yokoyama *et al.*, 2006).

fish in cage 1 was 462, which was >2-times higher than in cage 2 (205) (Fig. 3b).

The sediment trap survey showed that the C and N fluxes beside the fish cages were several times higher than those at the control site and that the differences between the mean fluxes at the fish cages and the background ones were 1.1g C $m^{-2} d^{-1}$ and 0.11g N $m^{-2} d^{-1}$, respectively. However, there were no significant differences in the C and N fluxes between the 2 fish farm stations, probably due to the close vicinity of the 2 stations (distance = 13 m). The large differences in the isotopic composition between WF, FM and natural settling OM and the intermediate isotopic values of the fish-farm settling OM between these OM sources enabled the successful calculation of the contribution rates of WF and FM. As a result, we found that (1) aquaculture-derived OM (AOM), composed of WF and FM, accounted for most of the sedimentation at the fish-farm stations, excluding the period from January to April, when natural OM often accounted for half of the bulk OM, (2) there was a difference in the seasonal trend between the WF and FM fluxes,

and (3) there were no differences in the percentage compositions of WF and FM between the two fish-farm stations. Although we could not find the differences in the sedimentation rate and percentage compositions of WF and FM between the 2 fish-farm stations, there was a large variability in the relative amount of WF and FM (the WF:FM ratio, Fig. 4) both in the 2 feeding regimes. For instance, during November and December the amount of WF was scarce despite the large input of feed (low WF:FM ratios), whereas relatively large WF fluxes were found after January although there was only a small input of feed (high WF:FM ratios). This finding suggests that the WF fluxes depend not simply on the feed input but more strongly on the feed intake and digestibility of fish and that there is still scope for improvement of the feeding regime even in cage 2. The quantification of WF and FM in settling OM using the stable isotope ratios and the WF:FM ratio will serve for assessing the optimum ration size.

The stable isotope ratios were used to calculate the relative contributions of WF, FM and the natural source to the bulk sedimentary OM. The result
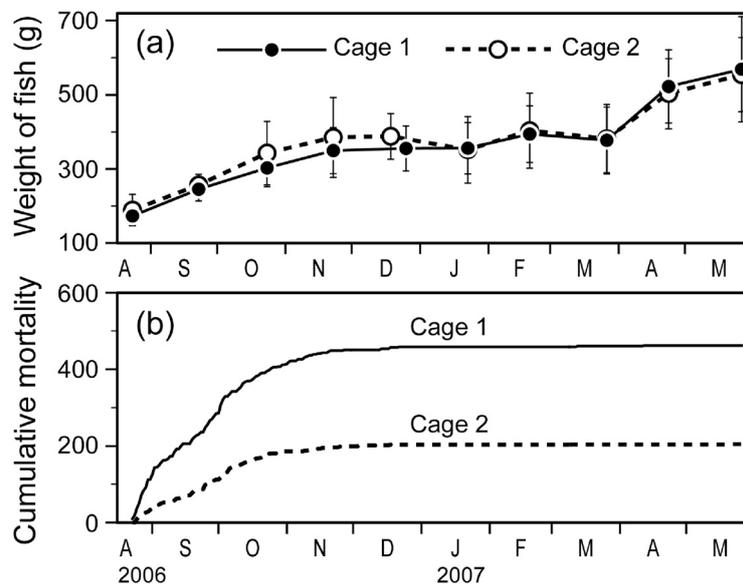

Fig. 3. Experimental aquaculture of red sea bream. (a) Growth of red sea bream and (b) cumulative number of dead fish in cage 1 (satiation feeding) and cage 2 (restricted feeding) (after Yokoyama *et al.*, 2009).

showed that on average WF contributed to 17%, FM contributed to 30% and the other source contributed to 53% of the bulk OM. Such values were distinct from those of the trap material (WF 43%, FM 43% and the natural source 14%), showing reduced contribution ratios in AOM, especially in WF in the sedimentary OM. The observed difference between them is probably due to highly labile compounds in AOM (Pearson and Black, 2001). AOM also could be incorporated into the food web through the digestion by macrobenthic animals in Gokasho Bay (Yokoyama and Ishihi, 2007) resulting in the decrease in the contribution ratios of AOM to the bulk OM.

WF-derived nitrogen contents in the sediment beneath cages 1 and 2 were calculated based on the total N contents in the sediment and the percentage compositions of WF and FM. The WF-derived N
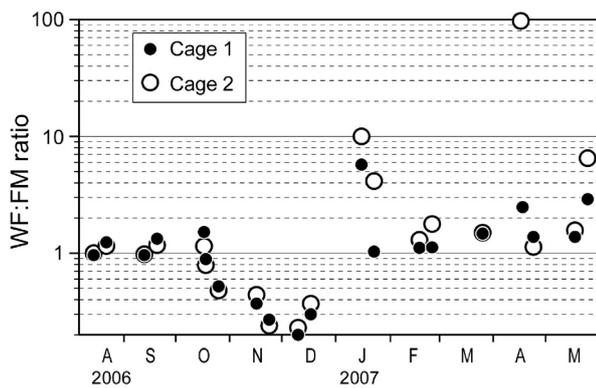

Fig. 4. Waste feed:fecal matter ratio in settling organic matter beside cage 1 (satiation feeding) and cage 2 (restricted feeding). (after Yokoyama *et al.*, 2009).
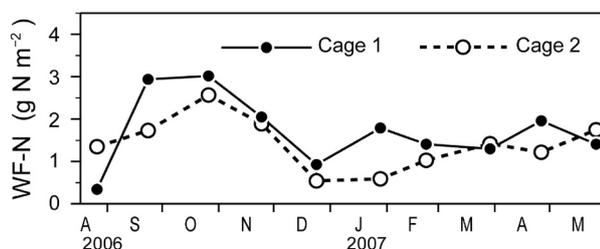

Fig. 5. Waste feed-derived nitrogen contents in the sediment below cage 1 (satiation feeding) and cage 2 (restricted feeding) (after Yokoyama *et al.*, 2009).

contents below cage 2 were significantly less than those below cage 1 (Fig. 5), whereas there were no significant differences in the FM contents between the two cages. This finding suggests that the effect of restricted feeding appeared in the sediment as a decrease of WF-derived OM.

In conclusion, the restricted feeding, in which the amount of feed was reduced by 18% relative to the conventional satiation feeding, achieved normal growth, increased feed conversion efficiency and reduced mortality of red sea bream and reduced contents of waste feed-derived organic matter in the sediment. The stable isotope analysis was shown to be a useful tool to quantify the amount of WF- and FM-derived C and N in the sediment trap material and the sediments. Determining the relative amount of WF and FM is effective when evaluating the optimum feeding regime from the viewpoint of the minimization of WF. The present study suggests that the conventional satiation feeding for the red sea bream aquaculture is overfeeding and that the optimum ration size should be examined further.

References

Azevedo, P. A., Cho, C. Y., Leeson, S., and Bureau, D. P., 1998: Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquat. Living Resour.*, **11**, 227–238.

Beveridge, M. C. M., 1984: Cage and Pen Fish Farming. Carrying Capacity Models and Environmental Impact. *FAO Fish. Tech. Pap.* **255**, FAO, Rome, 131pp.

Pearson, T. H., and Black, K. D., 2001: The environmental impacts of marine fish cage culture. *In* K. D. Black (ed.), Environmental Impacts of Aquaculture, Sheffield Academic Press, Sheffield, p. 1 – 31.

Phillips, D. L., 2001: Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia*, **127**, 166–170.

Talbot, C., Corneillie, S., and Korsøen, Ø., 1999: Pattern of feed intake in four species of fish under commercial farming conditions: implications for feeding management. *Aquacult.*

Res., **30**, 509–518.

Wang, Y., Kong, L. J., Li, K., and Bureau, D. P., 2007: Effects of feeding frequency and ration level on growth, feed utilization and nitrogen waste output of cuneate drum (*Nibea miichthioides*) reared in net pens. *Aquaculture,* **271**, 350–356.

Yokoyama, H., Abo, K., and Ishihi, Y., 2006: Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. *Aquaculture*, **254**, 411–425.

Yokoyama, H., and Ishihi, Y., 2007: Variation in food sources of the macrobenthos along a land-sea transect : a stable isotope study. *Mar. Ecol. Prog. Ser.,* **346**, 127–141.

Yokoyama, H., Takashi, T., Ishihi, Y., and Abo K., 2009: Effects of restricted feeding on growth of red sea bream and sedimentation of aquaculture wastes. *Aquaculture,* **286**, 80–88.