Radiocarbon Anomaly Found in Aquicultural Scallops Suspended in Coastal Sea

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The radiocarbon content of scallops aquiculturally suspended and grown in coastal sea, Funka Bay, were determined. While the Δ¹⁴C values of carbonate shells coincided with that of ambient seawater, those of soft tissues were smaller than the seawater value and varied rather widely. To clarify the cause of this variation, we further divided the soft tissues of composite scallops into each organ and determined their radiocarbon content. The results show that only the Δ¹⁴C of adductor (Kaibashira in Japanese) of scallops was significantly smaller than the others, those of gill and mantle coincided to that of seawater. This strange finding cannot be explained by the present knowledge on the dietary sources of scallops and the present theory of isotopic fractionation, unless any unknown physiological or oceanographical process determines the radiocarbon content of the organisms.

1. Introduction

Following the pioneering work of Williams et al. (1970) on radiocarbon in marine organisms, Percy and Stuiver (1983) used radiocarbon as a tracer for organic carbon in oceanic food webs and found a decrease with increasing depth from the surface to 500 m in the organic radiocarbon content of pelagic animals collected during the period from 1973 to 1976. Williams et al. (1987) confirmed the radiocarbon decrease down to 2 km depth, although the radiocarbon content varied widely (the Δ¹⁴C values defined later were 43 to 177‰ for fishes in the surface 1 km and around 0‰ at 2 km depth). Percy and Stuiver (1983) further revealed that the radiocarbon content of abyssobenthic organisms was variable and generally less or sometimes far less than those of pelagic organisms. The average Δ¹⁴C value of abyssobenthos was 6‰ and its 20‰ fraction was less than –40‰. They tried to explain the results by presenting some mechanisms, but failed to explain wholly the cause of their low radiocarbon contents, because nutritious organic particles contaminated with bomb radiocarbon sink rapidly into the deep sea as expected and found by the sediment trap experiments (Williams et al., 1970; Honjo, 1980; Tsunogai and Noriki, 1987).

Williams et al. (1987) postulated that the depletion was due to the low Δ¹⁴C values of dietary sources produced from DIC in the lower euphotic zone where the radiocarbon content decreased steeply with depth in 1970’s (Broecker and Peng, 1980; Druffel et al., 1986). They further presumed the low radiocarbon activities of pelagic organisms to be due to their prey formed with pre-bomb carbon in the past. They, however, excluded the extremely low radiocarbon content of abyssobenthic organisms, without quantitative discussion, describing only that the organisms feed on ¹⁴C-depleted sedimentary organic carbon or organisms that feed from this carbon source. As stated by Paul et al. (1989), if organisms use fossil methane from abyssal brine springs, their Δ¹⁴C values should be reduced. Heterotrophic bacteria can uptake dissolved inorganic carbon in
sea water (Karl and Knauer, 1984; Karl et al., 1984). Rau et al. (1986) have considered this as a possible cause of depletion of Δ^{14}C of deep-sea animals down to −160‰.

Strictly speaking, the above explanations are only suppositions. To avoid the complicated circumstances, we have determined the radiocarbon content of cultivated scallops suspended in surface water. Japanese fishermen succeeded in cultivating scallops with only natural prey in 1970s. Younger scallops are grown in cages of plastic net with multi-stair steel framework and afterward a few tens of larger scallops are roped in a row by making an eyelet in each shell. Thus the scallop samples simplify the conditions concerned. We, in consequence, have got a strange result, which may solve the above question, but is hard to explain using the present theory of chemistry.

2. Material and Methods

Scallops (Patinopecten yessoensis, Jay) samples of about 2 years of age were collected in Funka Bay, about 3 km off Mori, Hokkaido in October 1988. Both the shell length and width were about 10 cm and the dried weight of organic tissues was individually about 10 g. The scallops spent their whole life in Funka Bay. The scallops had been suspended in seawater of about 10 m deep (the total water depth, 50 m). The bay has an almost round plan (area, 2270 km^2) and its bottom is flat (maximum and mean depths, 100 and 60 m). There is no large river and no large city around the bay. Its oceanography has been studied fairly well, showing that the bay water is replaced mainly twice a year by the open sea water (Ohtani and Akiba, 1970; Ohtani and Kido, 1980).

Immediately after collection, the soft tissue of scallops was separated from shells and dried in vacuo at 60°C for 4 to 5 days. The weighed soft tissue samples were subjected to combustion in a stream of oxygen with catalysts of CuO and Pt. To get CO₂ quantitatively (more than 99%), the vaporized carbon in the stream gas was completely oxidized by passing through a chromic acid mixture (K₂Cr₂O₇–H₂SO₄) and CO₂ was absorbed in a KOH solution. BaCO₃ was precipitated from the KOH solution by adding BaCl₂. The carbonate shell samples were immersed in a NaClO solution for one month and their organic matter was completely removed in a muffle at 450°C for 6 hours after powdered in an iron mortar. Gaseous CO₂ evolved from the carbonate shells by pouring HCl was purified and precipitated as CaCO₃.

Carbonate and organic ^{14}C activities were measured by the liquid scintillation method principally of Noakes et al. (1963) and Togashi and Matsumoto (1983). The amount of CO₂ evolved from the precipitate of BaCO₃ or CaCO₃ determined precisely by reading its pressure in a volumetered flask after separating an aliquot for the determination of δ^{13}C. A definite amount of dead carbon was added to some samples of small amounts of CO₂. The CO₂ gas converted to Li₂C₂ with Li metal in an autoclave heating strongly with a gas burner for 4 hours. C₂H₂ gas was evolved from the Li₂C₂ with water and its amount was measured to check the efficiency. From the C₂H₂ gas, C₆H₆ (benzene) was synthesized on the surface of catalysts of activated Al₂O₃–V₂O₅ cooling with water. Afterwards the catalyst column was warmed slowly from 100°C to 250°C and the synthesized benzene was trapped in a column cooled with liquid nitrogen and weighed to calculate recoveries. The overall recoveries were 75 to 90%.

The β radioactivity of ^{14}C in the synthesized benzene was counted by a liquid scintillation counter after making a mixture of 4 ml of the synthesized benzene and 16 ml of 1% butyl-PBD in toluene. In a case of the amount of benzene smaller than 4 ml, benzene synthesized from dead carbon was added to make its volume 4 ml. To avoid false scintillation, the mixture in a low
potassium vial was stored for one week in the counter. The 30 minutes counting was repeated for a set of several samples, a standard, a blank and a background activity, until the total counts for each sample exceed 100,000. The blank was made from calcite containing no radiocarbon (dead carbon) collected at Garo Mine, Kamisso, Hokkaido. The working standard was made from the scallop shells collected in Funka Bay, which was standardized against worldwide standards in our laboratory, NBS Oxilic acid standard SRM 4990-C (18.36 dpm/gC) × 0.7459, and in Geological Survey of Japan by courtesy of Dr. E. Matsumoto.

We first calculated the \( \delta^{14}C \) values of samples from the specific activities of samples \( (R) \),

\[
\delta^{14}C\text{(in \%o)} = \frac{(R_{\text{sample}} - R_{\text{std}})}{R_{\text{std}}} \times 1000
\]

where the suffixes, sample and std, mean the sample and the standard, respectively. Correction for isotopic fractionation was made and \( \Delta^{14}C \) values were calculated according to the following equation (Broecker and Olson, 1961),

\[
\Delta^{14}C\text{(in \%o)} = \delta^{14}C - 2(\delta^{13}C + 25)(1 + \delta^{14}C / 1000)
\]

where \( \delta^{13}C \) is the permil difference in the isotopic ratio of \(^{13}C/^{12}C\) from that of the PDB standard, which was measured for CO\(_2\) evolved from the precipitate of BaCO\(_3\) or CaCO\(_3\) with a mass spectrometer.

3. Results and Discussion

3.1 Difference in the radiocarbon content between the soft tissue and carbonate shell of scallops

The \( \Delta^{14}C \) values of organic tissues and carbonate shells of 9 samples are given in Table 1. At the calculation of the \( \Delta^{14}C \), the \( \delta^{13}C \) values of some samples were assumed as written in Table 1, because of their small variation relative to the analytical errors in the radiocarbon determination. The \( \Delta^{14}C \) values of organic tissues \((11 \pm 14)\%o\) were significantly smaller than those of carbonate shells \((39 \pm 5)\%o\) and furthermore, the range or variation in the \( \Delta^{14}C \) values of organic tissues was wider than that of carbonate shells.

The variation (standard deviation = 5\%o) in the \( \Delta^{14}C \) values of carbonate shells was almost equal to that expected from the experimental errors \( (15/\sqrt{9} = 5)\%o \) and their mean \((39)\%o\) agreed to that of dissolved inorganic carbon (DIC) in seawater, which was determined by Nakajima (1988). He obtained a mean and a standard deviation of \((40 \pm 12)\%o \) with a range of 21--56\%o for 11 samples collected from the surface 30 m during four cruises in 1986. The \( \delta^{13}C \) values of carbonate shells nearly equaled that of DIC in seawater as well known (Keith et al., 1964). The coincidence of the \( \Delta^{14}C \) and \( \delta^{13}C \) values of carbonate shells with those of seawater suggests that carbonate shells are directly produced from DIC in seawater without isotopic fractionation. This agrees to the findings of Paul et al. (1989) and others.

On the other hand, the \( \Delta^{14}C \) values of soft tissues of scallops were smaller than those of carbonate shells, seawater and their prey, although we obtained only a few and highly uncertain \( \Delta^{14}C \) values of zooplankton and phytoplankton in the bay as given also in Table 1. To make clear the cause of this difference, we have further carried out a following experiment.
Table 1. $\delta^{14}$C, $\delta^{13}$C and $\Delta^{14}$C values (in %o) of carbonate shells and soft tissues of scallops, and planktons collected in Funka Bay. The fractions of dead carbon added during the benzene synthesis are also shown (in %).

<table>
<thead>
<tr>
<th>No.</th>
<th>Dead carbon</th>
<th>$\delta^{14}$C*1</th>
<th>$\delta^{13}$C*2</th>
<th>$\Delta^{14}$C*1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonate shells</td>
<td>1</td>
<td>0.0</td>
<td>95 ± 5</td>
<td>+1.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.0</td>
<td>106 ± 5</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0</td>
<td>96 ± 5</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0</td>
<td>91 ± 14</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.0</td>
<td>98 ± 14</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.0</td>
<td>101 ± 15</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>20.5</td>
<td>97 ± 15</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.0</td>
<td>93 ± 15</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>25.2</td>
<td>106 ± 15</td>
<td>n.d.</td>
</tr>
<tr>
<td>av.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft tissues</td>
<td>1</td>
<td>41.5</td>
<td>0 ± 12</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36.0</td>
<td>15 ± 1</td>
<td>−15.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.2</td>
<td>20 ± 11</td>
<td>−20.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>28.8</td>
<td>7 ± 12</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.7</td>
<td>27 ± 8</td>
<td>−19.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>23.5</td>
<td>26 ± 9</td>
<td>−21.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>33.2</td>
<td>32 ± 9</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24.3</td>
<td>41 ± 9</td>
<td>−19.7</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>23.2</td>
<td>28 ± 10</td>
<td>−20.8</td>
</tr>
<tr>
<td>av.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phytoplankton*6</td>
<td>1</td>
<td>92.0</td>
<td>43 ± 29</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94.8</td>
<td>50 ± 43</td>
<td>n.d.</td>
</tr>
<tr>
<td>zooplankton*6</td>
<td>1</td>
<td>81.5</td>
<td>47 ± 12</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*1σ values of the counting statistics.
*2n.d.: not determined.
*3The $\delta^{13}$C values are assumed to be +1.7%o for the calculation of $\Delta^{14}$C.
*4Standard deviations for the 9 determinations.
*5The $\delta^{13}$C values are assumed to be −20%o for animals and −25%o for phytoplanktons at the calculation of $\Delta^{14}$C.
*6Composite samples collected in Funka Bay.

3.2 Difference in radiocarbon content between parts of soft tissues of scallops

Next we took nine scallops to make a composite sample and separated their tissues into organs (Fig. 1), namely, adductor (Kaibashira in popular Japanese), gonad (Ko), gill (Era), mantle (Himo) and viscera (Wata or Uro), and determined $\Delta^{14}$C and $\delta^{13}$C values of the organs. Table 2 shows the results. The $\delta^{13}$C values were almost constant (−20.2 ± 0.6%). The $\Delta^{14}$C, however, varied widely from organ to organ of scallop tissues, but their weighted mean $^{14}$C value (+1%o)
Fig. 1. Illustration of scallop (*Patinopecten yessoensis*, Jay) and $\Delta^{14}C$ values (in $\%$) of scallops separated into organs. The value for carbonate shells written in parenthesis is a mean with a standard deviation for 9 determinations.

Table 2. $\delta^{14}C$, $\delta^{13}C$ and $\Delta^{14}C$ values of each organ separated from 9 scallops of soft tissues.

<table>
<thead>
<tr>
<th></th>
<th>Distribution of C (%)</th>
<th>Dead carbon added (%)</th>
<th>$\delta^{14}C$ ($%$)</th>
<th>$\delta^{13}C$ ($%$)</th>
<th>$\Delta^{14}C$ ($%$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adductor*¹</td>
<td>32.5</td>
<td>21.2</td>
<td>$-56 \pm 7$</td>
<td>$-19.9$</td>
<td>$-66 \pm 7$</td>
</tr>
<tr>
<td>Gonad</td>
<td>25.6</td>
<td>0.0</td>
<td>$-70 \pm 7$</td>
<td>$-20.4$</td>
<td>$-80 \pm 7$</td>
</tr>
<tr>
<td>Gill</td>
<td>7.7</td>
<td>62.4</td>
<td>$55 \pm 10$</td>
<td>$-20.2$</td>
<td>$45 \pm 10$</td>
</tr>
<tr>
<td>Mantle</td>
<td>14.7</td>
<td>27.2</td>
<td>$56 \pm 10$</td>
<td>$-20.6$</td>
<td>$46 \pm 8$</td>
</tr>
<tr>
<td>Viscera</td>
<td>19.5</td>
<td>0.0</td>
<td>$35 \pm 7$</td>
<td>$25 \pm 7$</td>
<td></td>
</tr>
</tbody>
</table>

Weighted mean $-20.2 \pm 0.3^{*2}$ $+1 \pm 58^{*2}$

*¹ The same sample was divided into two parts and determined duplicately.

*² The variations are the standard deviations of 6 determinations.

agreed substantially well with the $\Delta^{14}C$ values of whole soft tissue of individuals given in Table 1. The $\Delta^{14}C$ values of the adductor ($-66$ and $-80\%)$ were much lower than those of other organs. The $\Delta^{14}C$ values of the gill ($+45\%)$ and of the mantle ($+46\%)$ which was the organ producing CaCO$_3$ tests were nearly the same as those of hard shells and ambient seawater.

The low $\Delta^{14}C$ value of the whole tissue, therefore, is concluded to be due to the extremely low $\Delta^{14}C$ value of the adductor. The wider variation in the $\Delta^{14}C$ of soft tissue given in Table 1 is probably due to variation in the weight proportion of adductor. The adductor is the largest organ storing much energy and muscle. The depression in $\Delta^{14}C$ may have any special meaning for the scallops, but we have no information that the adductor concentrates specially some organic compounds in seawater.
3.3 Expected causes for the low radiocarbon content of soft tissue, especially the adductor of scallops

The scallops suspended in seawater cannot take organic carbon in sediments and even if the scallops take it up, organic matter on and in the surface sediments is not necessarily chronologically old. Their prey is mainly living planktons, of which \( \Delta^{14}C \) values are not significantly different from that of seawater (Table 1). Suspended organic detritus may contribute to some extent to the prey, but the proportion of resuspended old organic carbon in whole suspended organic carbon may be small. We have no information on the inflow of water containing low radiocarbon into the bay, such as the upwelling of deep water and the seepage of old carbon at the bottom. The \( \Delta^{14}C \) value of the surface water is reflected on that of the carbonate shells. Recently formed terrestrial organic matter, which may make some contribution to the prey of marine organisms, should have higher \( \Delta^{14}C \) values. The dietary sources of organic carbon depleted in radiocarbon, therefore, are hard to find for the cause of the low radiocarbon content, although we have ignored dissolved organic carbon and mineral oils containing low radiocarbon as the diet.

Furthermore, the variation of \( \Delta^{14}C \) values from organ to organ of soft tissue of scallops cannot be explained only by the isotopic composition of dietary sources, if they are formed from the same diet. We have used the equation of Broecker and Olsen (1961) for obtaining \( \Delta^{14}C \) by correcting the isotopic fractionation. At present, we have no evidence that the equation is not applicable to some physiological processes, although most of the isotopic fractionation processes induced by the biological activity can be corrected using the equation. It is usual to consider that the degree of isotope fractionation of \(^{14}C\) relative to \(^{12}C\) is twice that of \(^{13}C\), based on the thermodynamic properties of isotopic substances (Urey, 1947). We do not know whether kinetic processes at the assimilation of carbon (O’Leary, 1981; Wigley and Muller, 1981) is effective or not in this case. At any rate, the variation of \( \Delta^{14}C \) values found in this study is far beyond the expected one from the ordinary isotopic fractionation.

Contrary to the \( \Delta^{14}C \), the \( \delta^{13}C \) values show no significant variation from organ to organ coinciding with those published in literatures (Schwarz, 1969). The \( \delta^{13}C \) values were not determined for the synthesized benzene, the isotopic fractionation during the synthesis of benzene, however, seems to be small, because the recoveries were sufficiently high and fairly constant. Even if some isotopic fractionation arising during the synthesis, the effect should be almost common for all the organ’s \( \delta^{13}C \).

If some organs of pelagic and abyssobenthic animals are depleted in radiocarbon by an unknown metabolic or physiological processes, the previous discussion on the dietary sources (Percy and Suiver, 1983; Williams et al., 1987) may become meaningless and the questions in their discussion may be solved. Furthermore, the finding in the present study will give impacts on physiological and oceanographical problems. This study, however, is rather preliminary and further comprehensive studies including variation from chemical compounds to compounds will reveal the unknown process.

Acknowledgements

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References


