CHAPTER V: Accumulation and depuration profiles of PSP in the clam fed with *G. catenatum*

1. Introduction

In the present chapter, attempts were made to clarify the accumulation and elimination profiles of PSP in the clam that had fed with *Gc*. Although the similar feeding experiment have carried out by using *Ac* in CHAPTER IV, the present trial is still worthwhile to conduct, since *Gc* is the only unarmored species known to produce PSP and has some different features from *Ac* as follows, 1) cell size of *Gc* much larger than that of *Ac*, 2) toxin content of *Gc* is roughly 10 times higher than that of *Ac*, 3) *Gc* posses only *N*-sulfocarbamoyl toxins whereas *Ac* produces carbamate toxin in addition to those analogues.

2. Identification of PSP in *Gc* cells used as feeding sources

2.1. Materials and Methods

2.1.1. Gc cells

A *Gc* cell used was the same strain as described in CHAPTER I. It was maintained in laboratory in a modified SWM-3 culture medium (pH 7.7–7.8, salinity 33‰) at 21℃ under a 12:12 LD cycle with fluorescent illumination (light intensity 35 μmol/m^2/s^1). Before feeding to the clams, the strain was mass cultured in a 3 L culture flask under the same conditions.

2.1.2. Toxin extraction

The toxin profiles of cultured cells of *Gc* used for the feeding experiment were determined similarly as *Ac* cells described in CHAPTER IV. The final filtrate thus obtained from the extraction procedure was subjected to HPLC-FLD analysis.

2.1.3. HPLC-FLD analysis

HPLC-FLD analyses to determine the PSP properties were operated on a Hitachi L-7100 HPLC system with similar conditions that was described in CHAPTER I. The Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries of Japan provided the reference materials. All toxins were quantified individually, but some of them are represented together in the Figs. 30-32.

2.2. Results

The original toxin profile of *Gc* culture at its early stationary phase is shown in Table 4. The toxin was found to consist only of *N*-sulfocarbamoyl toxins, C1, C2, GTX5 and GTX6, among which C2 was predominant. The total toxin content of *Gc* culture used to feed the short-necked clam was calculated to be 1164.7 nmol/100 ml.

Table 4. Toxin profile of *Gc* culture used to feed the short-necked clams

PSP analogues	Toxin content (nmol/100 ml)	Mol%
C1	68.0	5.9
C2	961.6	80.6
GTX5	65.3	5.7
GTX6	69.8	7.8
Total	1164.7	100.0

3. Accumulation and depuration profiles of PSP in the clam fed with *Gc* cells

3.1. Materials and methods

3.1.1. Experimental specimens

The sources of *Gc* cells were obtained from the mass cultured in a 3 L culture flask grown under similar conditions of *Ac* culture as described in CHAPTER IV. A total of 120 non-toxic specimens of the short-necked clam *T. japonica* with the same body size and same collection site as mentioned in CHAPTER IV were immediately transported to the laboratory.

3.1.2. Feeding experiment

The clam were supplied with 100 ml (4 x 10^6 cells) of *Gc* culture in early stationary phase once at the beginning of the experiment as the clam specimens were divided similarly with the *Ac*-feeding experiment described in CHAPTER IV. The 12 tanks (groups) were reared at 21℃ inside an incubator, and harvested at 0.5, 1, 3, 6, 12, 24, 48, 72, 96, 120, 144 or 168 hours after the supply of *Gc*. The whole edible tissues of 10 clams in each group were pooled, and their toxin profiles were analyzed as described below. On the other hand, the seawater remaining in each tank (total 1 L) was filtered through a 0.45 μm filter membrane under a negative pressure, and the residue (*Gc* cells and/or excrements) retained on the membrane was analyzed similarly.

3.1.3. Toxin extraction

The toxin of *Gc* cells was extracted and the test solution was prepared in the same manner as of *Ac* cells in CHAPTER IV. Meanwhile, the clam tissues were extracted according to the standard method recommended by the AOAC (2003) with slight modifications that illustrated in Fig. 25. The residue retained on the filter membrane was extracted in the same manner as for the dinoflagellate cell pellet (Fig. 24), and subjected to HPLC-FLD analysis.

3.1.4. HPLC-FLD analysis

HPLC-FLD analyses were operated and analyses as the same manner as was explained in CHAPTER I.

3.2. Results

3.2.1. Toxin accumulation and depuration profiles in clams

Changes in the toxin contents of clams and residue (remaining *Gc* cells and/or excrements) during the feeding experiment are shown in Fig. 30. The toxin content of clams rapidly increased from 0.5 hrs, and reached the maximum level (181 nmol/10 clams) 12 hrs after the supply of *Gc*. The value is equivalent to 16% of the total amount of toxin supplied originally as *Gc* cells. The toxin once accumulated in the clams sharply decreased thereafter, and only 6.4% of the maximum level remained in the clam tissues at the end of experiment (after 168 hrs). On the other hand, the residue was found to contain about 50% of the supplied toxin at 0.5 hrs, which rapidly decreased, and only a small amount or trace was detected at 12-168 hrs.

3.2.2. Toxin profiles of clams

Changes in the toxin composition of clams are shown in Fig. 31. The composition of clams varied clearly from that of *Gc*. Even 0.5 hrs after the supply of *Gc*, the ratio of C2 to C1 in clams was much lower than that in *Gc*, which further decreased up to 72 hrs. In addition, the proportion of C1+2 was also decreased gradually during the same period. On the other hand, dc toxins (dcSTX and dc GTX1, 2) first appeared at 3 hrs, and their proportion largely increased to attain the maximum level (about 50%) at 72 hrs. Small amounts of GTX2, 3 were also detected during 12-144 hrs. The proportion of GTX5+6 fluctuated throughout the rearing period.

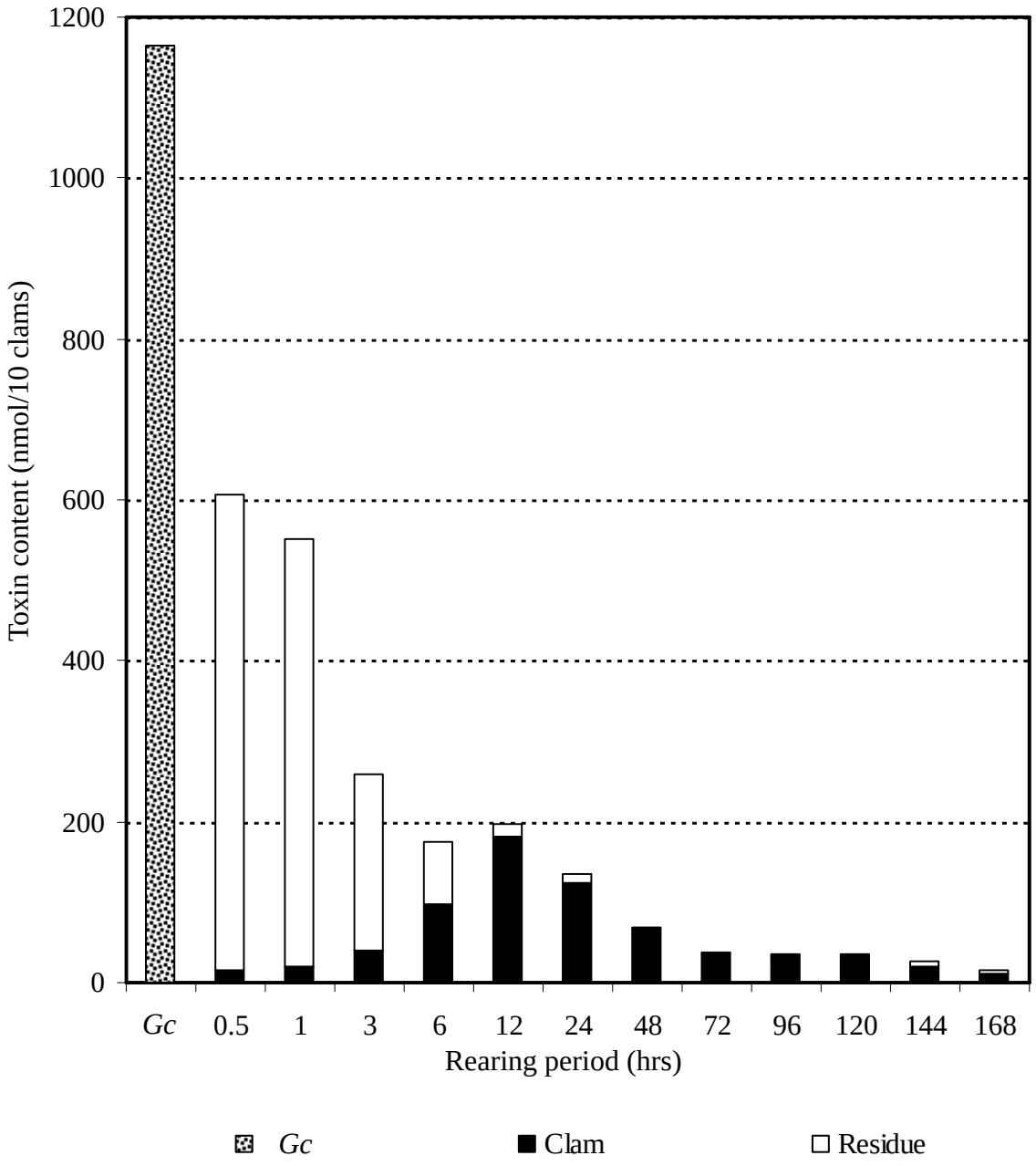

Fig. 30. Changes in the toxin contents of clams and tank residue (remaining *Gc* cells and/or excrements) during the feeding experiments.

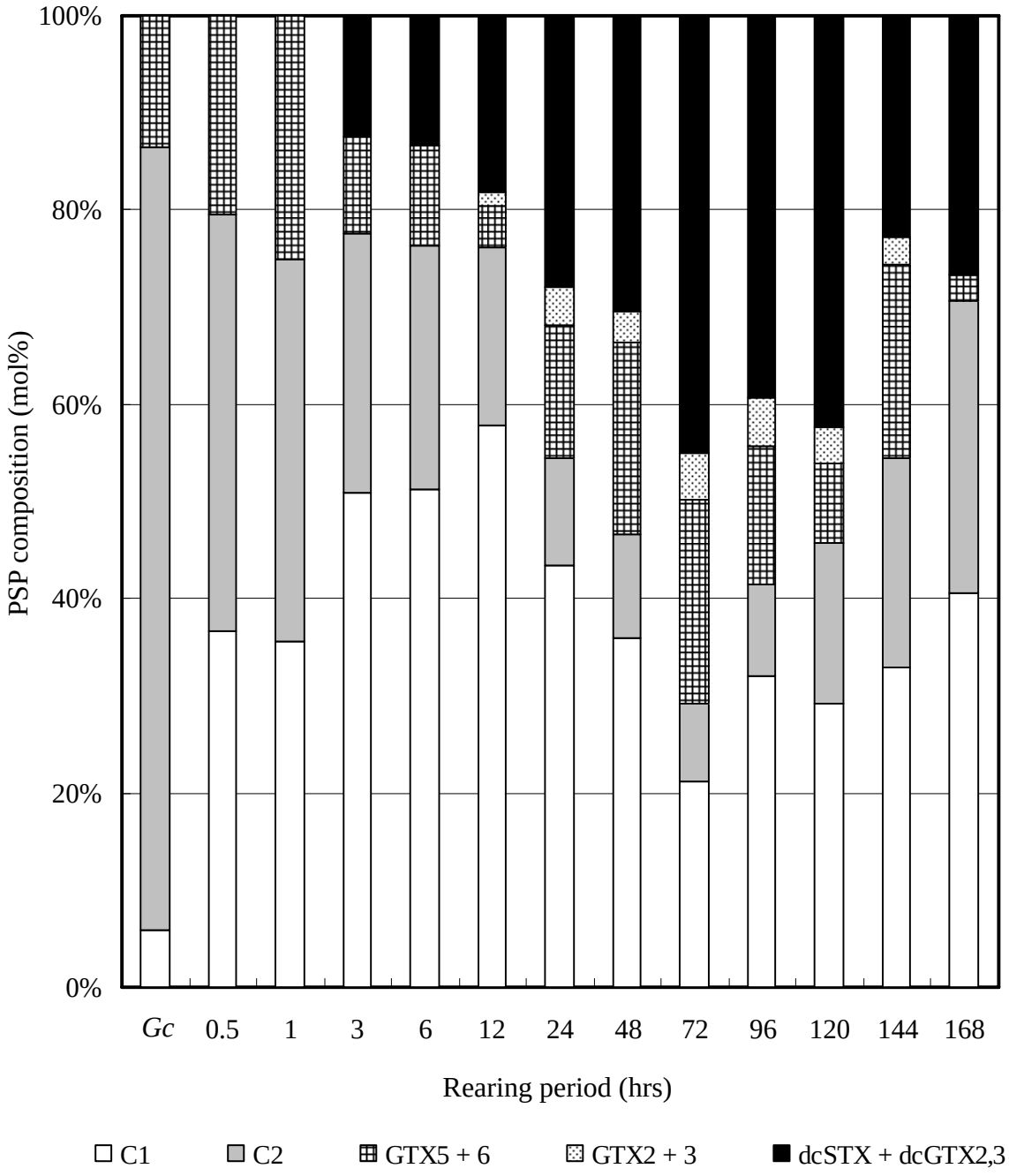

Fig. 31. Changes in the PSP composition of clams during the feeding experiments.

3.2.3. Toxin profiles of residue in rearing tanks

Changes in the toxin composition of residue during the feeding experiment are shown in Fig. 32. Up to 6 hrs, the composition of residue was similar to that of *Gc* except that a considerable amount of dc toxins were detected even after 0.5 hrs. From 6 until 12 hrs, however, a drastic change was observed, i.e., the proportions of dc toxins and C1+2 greatly increased and decreased, respectively, the ratio of C2 to C1 largely decreased, and GTX5, 6 disappeared. Thereafter, dc toxins diminished whereas C1+2 increased gradually, and GTX2, 3 suddenly appeared after 144 hrs.

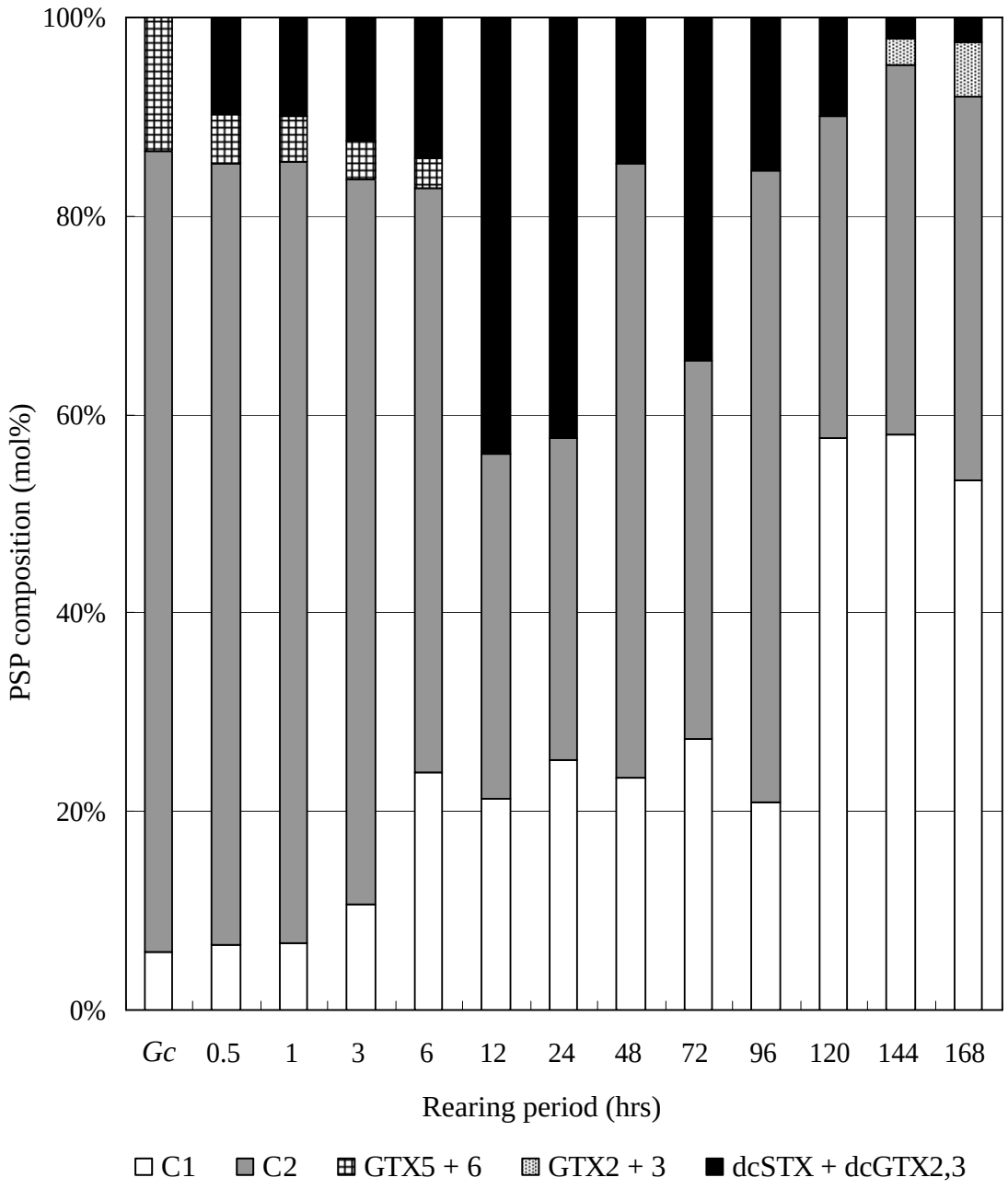

Fig. 32. Changes in the PSP composition of residue during the feeding experiments.

3.3. Discussion

In the present study, the short-necked clam accumulated a maximal amount of toxin after 12 hrs (Fig. 26). At this point of time, the residue contained little *Gc* cells, and retained only about 1% of the total amount of toxin supplied (Fig. 26), indicating that the clam had ingested most of the supplied *Gc* cells during the first 12 hrs. The rate of toxin accumulation at that time was 16%, which was almost the same level as that of the oyster fed with *A. tamarense* (maximum 19%) (Asakawa et al., 2006) but much lower than the mussel fed with *A. tamarense* (35%) (Ichimi et al., 2001) or the purple clam fed with *A. minutum* (50%) (Chen and Chou, 2001), and most of the toxin accumulated disappeared after 168 hrs (7 days).

The rate of intoxication in short-necked clams was reported to be generally low, and the acquired toxicity rapidly declined after disappearance of the causative dinoflagellate (Takatani et al., 1998a; Oshima et al., 1982). Hence, the present findings in this study seemed to be consistent with the intoxication profiles of clams under the natural conditions. In this connection, the short-necked clam, when fed with *A. catenella*, was also found to accumulate maximal amount of toxin 12 hrs after the supply of dinoflagellate, and eliminate most of it until 168 hrs (Samsur et al., 2006). The accumulation rate was 26%, which was somewhat higher than that in *Gc*-feeding.

The composition of PSP accumulated in the clams obviously varied from that of *Gc* even after 0.5 hrs, the ratio of C2 to C1 being much lower than in *Gc* (Fig. 31). In addition, dc (dcSTX and dcGTX2, 3) and carbamate toxins (GTX2, 3), which were undetectable in *Gc* cells, appeared from 3 and 12 hrs, respectively (Fig. 31). The

former could be attributable to the epimerization of C2 (11 β -epimer) to C1 (11 α -epimer) (Oshima, 1995; Oshima et al., 1990), and the latter to the enzymatic hydrolysis of *N*-sulfocarbamoyl toxins (C1, 2 and GTX5) (Oshima, 1995).

On the other hand, the proportion of C1+2 gradually lowered, instead that of dc toxins made a gradual ascent during the rearing period (Fig. 31). This may suggest that dc toxins could be retained longer in the bivalve tissues, whereas C1, 2 released more rapidly into seawater possibly through branchial respiration or by elution, since C toxins are the most water-soluble analogues of the PSP group.

The changes in composition described above were also observed in the naturally intoxicated short-necked clams (Takatani et al., 1998b; Oshima et al., 1990), or in case of *A. catenella*-feeding (Samsur et al., 2006). In the residue, dc toxins already appeared at 0.5 hrs (Fig. 32). Since these analogues were not yet recognized in the clams at that time (Fig. 31), it is unlikely that they were excreted from the bivalves.

Further studies are needed to elucidate this point, although they might come from the dead cells of *Gc*, which were often observed as precipitates at the bottom of the rearing tanks. The toxin composition of residue was drastically changed from 6 until 12 hrs, suggesting that the residue was mainly composed of *Gc* cells up to 6 hrs, while of excrement after 12 hrs.

The total amount of toxin detected in the clams and residue gradually declined, and only about 1% of supplied toxin was detected at the end of experiment (Fig. 31). A preliminary experiment suggested that a fairly large part of the lost toxin still existed,

without being decomposed, in the seawater of the tanks, as a toxin mostly consisting of C1, 2. However, further studies are needed to elaborate this point, since the methodology used in this experiment for recovering toxin from seawater was not sufficiently reliable for quantitative analyses.