 Laboratory observations of sexual and asexual reproduction of *Trochammina hadai* Uchio

HIROSHI KITAZATO and SATOSHI MATSUSHITA

Institute of Geosciences, Shizuoka University, Oya 836, Shizuoka, 422 Japan
Shin-Nippon Meteorological and Oceanographical Consultant Co. Ltd., 2-2-2 Hayabuchi, Tsuzuki Yokohama, 224 Japan

Received 30 November 1995; Revised manuscript accepted 23 February 1996

Abstract. *Trochammina hadai* Uchio is abundant in brackish water bays around the Japanese Islands and exhibits a biphasic life cycle in natural environments. In the laboratory sexual reproduction, characterized by the release of thousands of gametes, occurred in spring. The release of gametes continued for 1.5 hours. After gametogenesis, parts of the cytoplasm still remained in the mother test. Asexual reproduction took place in the laboratory during autumn and continued for 6 hours. The mother test was not destroyed during asexual reproduction. The geographical distribution of *T. hadai* in Hamana Lake differs from season to season. This may be explained by the differential dispersal of juveniles during the sexual and asexual phases, which take place at different times of the year.

Key words: Reproduction, biphasic life cycle, microspheric and megalospheric generations, geographic distribution, *Trochammina hadai*

Introduction

Recently, agglutinated foraminifers have become one of the important groups in the study of extreme environments such as the deep sea and brackish waters (Hessler and Jumars, 1974; Bernstein et al., 1978; Bernstein and Meador, 1979; Gooday, 1986, 1988, 1990; Gooday and Lambsehead, 1989; Schroeder et al., 1988). Foraminiferal biomass may be significant in such environments, e.g., accounting for up to 50% of the total biomass in the deep sea (Snider et al., 1984). Foraminifers also may act as an important factor in the deep-sea carbon budget (Gooday et al., 1992). The foraminiferal fauna below the calcite compensation depth (CCD) is composed entirely of agglutinated species. Thus, biological information about agglutinated foraminifers is required for understanding deep-sea biology, ecology and biogeochemical cycles.

Agglutinated foraminifers are also widely distributed in coastal regions. They are good indicators for monitoring polluted environments (Alve, 1991). In this case as well, study of the biology of agglutinated foraminifers is important for an understanding of modern ecological processes. However, investigations of the biology and ecology of agglutinated foraminifers are scarce even for shallow marine environments in comparison to calcareous foraminifera (Boltovskoy, 1966; Matera and Lee, 1972; Salami, 1976; Sliter, 1986; Angell, 1980, 1990).

*Trochammina hadai* Uchio is an agglutinated foraminifer that is abundant in brackish waters of Japan (Ishiwada, 1958; Uchio, 1962a, b; Ikeya, 1970, 1977; Matoba, 1970). This species, together with *Ammonia beccarii* (Linné), accounts for the greater portion of the foraminiferal population in brackish waters.

To understand the life history of *T. hadai*, we observed almost continually the seasonal changes of populations at a fixed station in Hamana Lake, Central Japan, from 1988 through 1990. The biphasic life cycle was inferred from the seasonal occurrences of the natural populations (Matsushita and Kitazato, 1990). To examine our investigations under natural conditions we cultured *T. hadai* in the laboratory. During the course of this culture experiment, both sexual and asexual reproduction were observed. Here, we describe the reproductive processes of *T. hadai* in detail.

Method of study

Culture studies were carried out at the Fisheries Laboratory of the Faculty of Agriculture, University of Tokyo at Maisaka, which faces Hamana Lake in Shizuoka Prefecture, Japan. Sediment samples for culture were collected by SCUBA diving from a fixed station in the Shonai inlet of Hamana Lake, where we observed almost continually the seasonal occurrences of natural populations of *T. hadai* (Matsushita and Kitazato, 1990). Sediments were transported with ambient sea water to the laboratory. Living individuals were picked from the sediments and transferred to a petri dish.
Figure 1. Successive photographs of sexual reproduction. Time of photographic exposure is given in the upper left corner of each photograph. All photographs were taken on March 20, 1980. 1. Release of gametes. \( \times 25.4 \). 2. Clusters of gametes rising up from aperture. \( \times 50.8 \). 3. Clusters of gametes. \( \times 38.1 \). 4. Clusters of gametes. \( \times 38.1 \). 5. Clusters of gametes. \( \times 25.4 \). 6. Clusters of gametes. \( \times 38.1 \).
Figure 2. Successive photographs of sexual reproduction. Time of photographic exposure is given in the upper left corner of each photograph. All photographs were taken on March 20, 1990. 1. Release of gametes. \( \times 25.4 \). 2. Release of gametes. \( \times 38.1 \). 3. Release of gametes mostly completed. \( \times 50.8 \). 4. End of gametogenesis. \( \times 25.4 \). 5. Beginning of extrusion of rhizopodia. The end of rhizopodia are rounded and creep shaped. \( \times 76.2 \). 6. Extrusion of many rhizopodia and movement of foraminifer. \( \times 38.1 \)
using a pipette. Individuals were cleaned with filtered sea water by transferring from one dish to another repeatedly. Each individual was put in a petri dish for culture. Both dried green algae of the genus Chlorella and living diatoms of the genus Navicula served as foods. Diatoms were supplied by the fisheries laboratory. Small amounts of carbonadium particles were added to a petri dish instead of using natural sediments. All petri dishes were placed in a laboratory at room temperature (15-18°C) and were exposed to natural light through laboratory windows. Bottom waters (salinity: 28‰) collected at the fixed station was used for culture. Cultures for observing sexual reproduction were carried out from March 14 through 24, 1990. Cultures for asexual reproduction were performed during the period from October 26 to November 9, 1990. The periods for observations were determined from the population dynamics data of natural populations of *T. hadai*, which were continually monitored at a fixed station in the Shonai Inlet (Matsushita and Kitazato, 1990).

The reproductive processes were observed with a phase contrast apparatus attached to an inverted microscope (Nikon Diaphot TMD and Olympus IMT-2). Both gamogony and agamogony were recorded on Kodak Plus-X film and video tapes respectively.

**Reproductive process**

Sexual reproduction: Sexual reproduction occurred only in spring. Seven of the 30 adult individuals reproduced sexually in March. One individual reproduced in May. Sexual reproduction took place around half moon in March, and just one day after full moon in May. No asexual reproduction occurred in culture during the spring season.

Sexual reproduction progressed as follows (Figures 1 and 2).

1) Prior to reproduction, mature individuals that exhibited dark orange cytoplasm withdrew their rhizopodia except for rhizopodia needed to anchor the test at the bottom. During sexual reproduction no reproductive cyst was constructed around the mother tests.

2) Release of gametes took place twice. At first, gametes were released from the aperture (Figures 1-1 and 2). Then, several clusters of gametes were extruded (Figures 1-5 and 6), and the second release of gametes occurred explosively 15 minutes later (Figure 2-1). The morphology of each gamete is droplet-like in outline and consists of two flagellae, based on light microscope observations. A sketch of gametes is drawn in Figure 8. However, morphologic details of the gamete have not yet been elucidated under an electron microscope.

3) Release of gametes continued for 1.5 hours. The mother test did not move during reproduction and the gametes dispersed quickly. We did not observe zygote formation.

4) After the release of gametes a part of the cytoplasm still remained within the mother test and slowly rhizopodia

![Sexual reproduction](Spring)

![Asexual reproduction](Autumn)

**Figure 3.** Proloculus diameters of mother tests that reproduced in laboratory cultures.
extruded again from the aperture, and the test moved away (Figure 2-6).

All individuals that released gametes had a large proloculus, ranging from 40 to 55 μm in diameter (Figure 3). Test diameters of mother individuals ranged from 383 to 540 μm (Table 1a).

In order to obtain zygotes we mixed gametes of two different mother individuals. However, they did not form a zygote within the culture. The gametes were very difficult to maintain under culture conditions, as they died within a day.

Asexual reproduction: Asexual reproduction took place in autumn. Eleven individuals among 30 adults reproduced asexually within our culture. Reproduction took place within three days after full moon. No sexual reproduction was observed in autumn.

The reproductive process progressed as follows.

1) Reproductive cyst formation: A reproductive cyst, formed from chlorella cells and/or diatom frustules, was constructed around the mother test, leaving vacant spaces (Figure 4). During construction of the cyst, the individuals extruded radially many rhizopodia along the bottom of the petri dishes to collect material to form the cyst. This rhizopodial pattern used during construction of the cyst is different from the usual pattern. Usually, T. hadai extrudes rhizopodia mainly in front of (i.e., in the direction of movement) and behind the test (Figure 5).

2) Cytoplasm invades the reproductive cyst: One or two hours after the reproductive cyst was constructed, the cytoplasm spread slowly into the cyst and the space was filled with cytoplasm.

3) Multiple division: Multiple division occurred synchronously within the cyst cavity and within the mother test (Figure 6-1). First, the velocity of cytoplasmic streaming

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Test diameter (μm)</th>
<th>Proloculus diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>478</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>420</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>447</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>393</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>505</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>435</td>
<td>42</td>
</tr>
<tr>
<td>7</td>
<td>457</td>
<td>?</td>
</tr>
<tr>
<td>8</td>
<td>540</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 1a. Individuals participating in sexual reproduction

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Test diameter (μm)</th>
<th>Proloculus diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>407</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>459</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>425</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>507</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>459</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>533</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>451</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>422</td>
<td>?</td>
</tr>
<tr>
<td>9</td>
<td>486</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>441</td>
<td>20</td>
</tr>
<tr>
<td>11</td>
<td>425</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 1b. Individuals participating in asexual reproduction

Figure 4. Reproductive cysts were constructed around a mother test during asexual reproduction. The photograph was taken on November 3, 1990. ×25.4.
accelerated and then multiple division occurred within a few minutes. Fifty to one hundred gamont juveniles were visible both in the reproductive cyst and in the mother test 15 minutes after the division.

4) Spreading of juvenile individuals: Mostly one or two hours after multiple division, juvenile gamonts started to leave the mother test (Figure 6-2, -3 and -4). Juvenile individuals are spherical without an arenaceous wall (Figure 6-5 and -6). The diameters of juvenile gamonts ranged from 45 to 65 μm. This is the same as the proloculus diameters of megalospheric adults (Figure 3). Immediately after juveniles left the mother test, they started to collect both Chlorella cells and diatom frustules.

5) Formation of an agglutinated test: One or two hours after juveniles had left the mother test, most individuals started to collect carborundum grains to form an arenaceous wall. At first, the individuals gathered any particles around the cell and fixed them on the first chamber wall (proloculus wall), after which the foraminifers grabbed selectively carborundum grains that had been placed to one side. This shows that Trochammina hadai definitely discriminates carborundum grains from other biogenic particles. Carborundum grains were cemented first on one side of the cell and then their use gradually extended around to the other side (Figure 7). This means that the test formation of agglutinated foraminifera probably starts from one point. This observation of the initiation of chamber formation is similar to the description by Bender (1992) for Textularia candelana d'Orbigny. Nearly the same chamber formation process was described by Angell (1990) for Trochammina inflata.

Asexual reproduction continued for approximately 6 hours. The mother test was not destroyed during asexual reproduction, as observed by Salami (1976) for T. cf. T. quadriloba Hoeglund. However, the mother test was weakened and fragile after reproduction.

The proloculus diameters of mother tests that released gamonts were smaller than those of the gamonts. They ranged from 20 to 33 μm (Figure 3). Test diameters of the mother tests ranged from 407 to 533 μm (Table 1b).

Discussion

Both sexual and asexual reproduction took place during specific seasons (Figure 8). Sexual reproduction occurred only in spring and asexual reproduction during autumn. No exceptions have been observed. The observations support our previous observations that the life cycle of T. hadai is biphasic and annual (Matsushita and Kitazato, 1990).

What fundamental factors control seasonal timing of reproduction? Dissolved oxygen and temperature of Hamana Lake bottom waters are roughly the same in spring and autumn during the two types of reproduction, suggesting there may be some connection between these variables and physiological cues or limits to reproduction. Water temperature at Hamana Lake fluctuates seasonally between about 6 and 27°C, but the spring and autumn periods of reproduction both fall within the limited temperature range of 13–18°C (Figure 9). The dissolved oxygen content of the bottom water both in spring and autumn is about 5–8 ml/l, restricted compared with the annual range of <2 to >10 ml/l. Thus, reproduction may take place under conditions of approximately 15°C and 6 ml/l in dissolved oxygen. We could not find a direct relationship between water salinity and reproductive season, as seasonal salinity changes can fluctuate from year to year, largely due to variable precipitation rates.

Nutrient conditions also do not seem to be one of the controlling factors of reproduction. Seasonal chlorophyll-a concentration in the Shonai Inlet shows that phytoplankton production was high throughout the year, although activity was slightly lower in spring and autumn (Anil et al., 1990).
Figure 6. Successive photographs of asexual reproduction. Time of photographic exposure is given in the upper left corner of each photograph. All photographs were taken on November 4, 1990.
1. Construction of blood cyst. ×32. 2. Gamont individuals. ×20. 3. Gamonts depart from cyst under phase contrast. ×50. 4. Enlargement of gamonts showing rhizopodia extruding from each gamont under phase contrast. ×90. 5. Gamonts depart from cyst. ×50. 6. Gamont collecting Chlorella cells and diatom frustules for food. ×96.
Nutrient levels are thus likely sufficient at all times.

Figure 7. Gamont juveniles collecting carborundum grains for constructing a test wall. The upper hemisphere of each cell is covered by carborundum grains. Time of photographic exposure is given in the upper left corner of each photograph. Prior to this stage, multiple division had occurred at 9:30 on November 5, 1990.

In some organisms there is a temporal relationship between reproduction dates and the lunar cycle. Sexual reproduction mainly took place around half moon. Asexual reproduction occurred within three days after full moon, which may be comparable to the reproduction cycle of some planktonic foraminifers (Spindler et al., 1979; Bijma et al., 1990). Further research is needed, including experimental...
Megalospheric generation

![Diagram of life cycle with seasons and reproduction stages]

Microspheric generation

Figure 8. Integrated life cycle of *T. hadai* Uchino in relation to seasons. The figure is revised from figure 15 of Matsushita and Kitazato (1990).

cultures, in order to clarify the nature of ambient factors that induce or control reproduction.

The asexual reproduction process of *T. hadai* is mostly the same as that in *T. inflata* (Angell, 1990). However, the duration of reproduction is different in the two species. *Trochammina hadai* took about 6 hours, whereas *Trochammina inflata* required ca. 24 hours (Angell, 1990).

Juvenile individuals of *T. hadai* consist of only one chamber when they leave the cyst. However, both *T. inflata* (Angell, 1990) and *T. cf. T. quadriloba* Hoeglund (Salami, 1976) have 2-3 chambers at the time when the juveniles left the mother test, the same as most investigated calcareous foraminifera such as *Ammonia beccarii* (Linné), *Elphidium crispum* (Linné), *Pararotalia nipponica* (Asano), *Glabrata* spp., *Bolvina* spp.,
Figure 9. Seasonal changes of water temperature, salinity, dissolved oxygen and thickness of oxygenated layer in the sediments at a fixed station at Hamana Lake during 1988 through 1990. Solid lines show the values for bottom water just above the sediment-water interface. Broken lines show surface conditions. Shaded area shows the main reproductive seasons.
and others (Kitazato, unpublished observations).

We observed that *Trochammina hadai* collected carborundum grains and cemented the grains, using an organic cement, to the surface of gamont cells to construct an agglutinated test. Bender (1992) proposed six constructional stages during chamber formation for agglutinated foraminifera that secret calcitic cement. We probably observed only initial parts of the chamber formation process. It is probable that chamber wall construction by gamont juveniles is a mixture of stages 1 through 3 of Bender (1992), though in our observations gamont juveniles simply construct an agglutinated wall on the cell surface without actually forming a new chamber. Further studies are needed for correlating correctly our observations, including constructional stages of chamber formation, with those of Bender (1992).

The seasonal distribution of this species may reflect the mode of reproduction in *T. hadai*. Microspheric agamont generations of *T. hadai* are spread widely throughout the inlet in June. The megalospheric generation was dispersed in November. However, the distributional ranges of the megalospheric generation was narrower than those of the microspheric generation (Figure 10). These variations in the seasonal distribution of *T. hadai* might be explained by the difference in modes of dispersion in the sexual and asexual progeny of this species. Agamont juveniles can disperse widely during sexual reproduction because the gametes are able to swim of float in the water. In contrast, gamont individuals tend to be concentrated in a rather narrow area in the lake, because gamont juveniles disperse only by rhizopodia in and on sediments. The average moving velocity of *T. hadai* is 14 µm/minute (Kitazato, 1981). This means that *T. hadai* is able to migrate only 2 cm per day.

A widespread distribution may be advantageous for keeping high survival rates under stressful summer abiotic conditions, which include bottom anoxia due to strong water column stratification (Matsushita and Kitazato, 1990). During summer the density of living *T. hadai* individuals decreases drastically throughout the inlet. Surviving agamonts reproduce asexually in autumn. This means that a relatively small numbers of agamonts found the succeeding gamont generation.

Figure 10. Geographical distribution of *T. hadai* in June and November, 1990, at the Shonai Inlet, Hamana Lake. Open circles show the stations where *T. hadai* was not found. Closed circles indicate the stations where *T. hadai* were found. Histograms show size distribution of *T. hadai* at each station. Open bars of the histograms beside the stations show the number of dead individuals; closed bars show the number of living individuals. L is the number of living individuals per 10 cm².
Acknowledgments
We are grateful to K. Chinzei, Ch. Hemleben and D.B. Scott for constructive suggestions and warm encouragement during the course of this study. Ch. Hemleben and R.M. Ross provided valuable comments on the manuscript. We are also indebted to the staff of the Fisheries Laboratory, Faculty of Agriculture, the University of Tokyo for their kind help during our use of their laboratory. An anonymous reviewer provided valuable comments on the manuscript. This research was supported by Grants in Aid from the Ministry of Education, Science, and Culture of Japan (nos. 61480027 and 02454026).

References cited