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Repeating rejuvenation in *Turritopsis*, an immortal hydrozoan (Cnidaria, Hydrozoa)

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Abstract. Repeating rejuvenation of *Turritopsis* sp. from medusa to polyp was observed 10 times, which is the most numerous number of such a reversal life cycle. Starting from a single immature medusa collected from Okinawa Island, southern Japan in May 2009 as a founder, every rejuvenated hydroid colony was continuously cultured for about two years, being kept separately in respective tank filled with fresh natural seawater (32 psu) at Shirahama, Wakayama Prefecture, Japan in a running seawater system in the laboratory at 21-29 ° C without strong natural sunlight which may induce algal growth on rearing vessels, and being fed mainly with *Artemia* nauplii.

Key words: rejuvenation, *Turritopsis*, culture, hydroid, clone, immortal.

Introduction

Among the multicellular animals, some cnidarian species such as *Turritopsis* spp., *Laodicea undulata*, *Chrysaora* sp., *Linuche unguiculata*, and *Nausithoe* spp. can be rejuvenated from medusa to polyp (Hadzi, 1912; Piraino et al., 1996; Jarms, 1997; De Vito et al., 2006; Kubota, 2005, 2006). In only one species such a rejuvenation was formerly observed repeatedly four times in a female colony of *Turritopis* sp. from southern Japan (Kubota, 2009), and the same colony still continues to repeat reversal life cycle as is reported here. In this paper ten times of such rejuvenation records are reported with a culture method to keep the colony in healthy conditions for eternal culture as a suitable material for any disciplines of biology and life science.

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Materials and Methods

First of all, for an adequate culture of *Turritopsis*, steady water flow of a fresh seawater (32 psu) in a running system is needful for polyp to produce many medusa buds after growing to a luxuriant hydroid colony on a small vessel (60 or 80 ml polystyrene vessel, each 60 mm in diameter and 15 or 30 mm high) . Secondly, in the initial rejuvenated stage, 1/2 body of the newly hatched *Artemia* nauplius is sufficient for a zooid for a few days fed by hand using a pair of needles (a body part of *Artemia* nauplius touched to the mouth of the zooid by needles, then it can engulf by itself). When a colony grows consisting of more or less seven zooids, it is the time that we can give many newly hatched *Artemia* nauplii as food, then each zooid fed by itself sufficiently (a few nauplii ingested per zooid as usual), and the colony grows quickly, extending stolons and producing many zooids.

The rejuvenation from immature medusa to polyp is naturally induced for the material that was collected at a coast on Okinawa Island, Japan on May 15, 2009 (Kubota, 2005, 2009). Many (maximally several tens) individuals of newly released medusae were kept in a small rearing vessel, then some of them can be rejuvenated within a few days possibly due to stress of crowd to swim freely with extending tentacles. Each case of a rejuvenation is followed in a shortest way, being continued throughout a year, keeping the sea water temperature at 21 - 29°C. In winter heating was needful since water temperature is low down below 20°C in near-natural conditions. Furthermore, strong sunlight in the culture room must be avoided to prevent growth of algae on the rearing vessel, and kept the colony in a dark room for a month or more. If algae grew on the bottom of rearing vessel, they should be removed by frequent cleaning by a wooden stick with a pointed tip.

Another solution to keep the healthy colony is transplantation of a part of the colony on a new rearing vessel, but it is no need to do so in the present culture. It is easily artificially to induce rejuvenation when the medusa body was stuck 50 or more times by a needle, but this method was not adopted in the shortest course of the present serial rejuvenation.

Results and Discussion

By the above-mentioned method *Turritopsis* hydroid colony has been kept in laboratory, and ten times of rejuvenations were continuously observed for about two years in the shortest course in near-natural conditions (Table 1). Each of the second to fourth rejuvantation took place for about one month one after another when the water temperature was at 25-29°C in summer and autumn (Table 1). The interval period of rejuvenation took longer when the water temperature was lowed below 22°C in winter and spring despite of heating seawater of the rearing tank (#5-6, 8-9 in Table 1).

From the smallest colony consisting of 36 zooids immature medusae began to release on the 43rd day after the first rejuvenation, while much more zooids are usually produced in a colony of the 2^{nd} to 7^{th} rejuvenation, spreading the stolon all over the bottom and side of the rearing vessel.

Table 1. Records of ten times of rejuvenation of *Turritopsis* sp. from southern Japan in the shortest course in laboratory at Shirahama, Wakayama Prefecture, Japan.

Times of Rejuvenation	Date rejuvenated earliest
#1	May 18, 2009
#2	July 7, 2009
#3	Aug. 7, 2009
#4	Late Sep., 2009
#5	Early Dec., 2009
#6	Mar. 23, 2010
#7	Aug. 8, 2010
#8	Sep. 25, 2010
#9	Jan. 5, 2011
#10	Mar. 31, 2011

A hydroid colony that rejuvenated seven times was transported to Porto Cesareo, Lecce, Italy and cultured it in a natural seawater (ca 39 psu) at a room temperature (ca 28°C) in a dark room without circulation of seawater (but changed at least three times a day). Irrespective of high salinity (c 7 psu higher), medusa bud formation was observed and occurred on the 11th day after it was carried to Italy from Japan despite most of the zooids degenerated during transportation for three days (no water change and no food in this period). This colony is still living after it carried back to Japan after keeping in the Mediterranean seawater for ten days, and again continued to culture under the above conditions. It is noteworthy that the medusa buds can be produced quickly in this colony. Among a total of 24 zooids, ten degenerated thier hydranths, while other 14 zooids are vivid and eight of them produced medusa buds just after came back to Japan from Italy. After the fifth day on this situation it became a smallest matured colony consisting of 13 zooids. Ten out of these 13 zooids in this dwarf colony produced medusa buds and maximally four medusa buds were produced per zooid.

Nine times of rejuvenation was succeeded in early January, 2011 after heating the seawater for a month from December 1, 2010 (Table 1), and ten times was at the end of March, 2011. However, most of these colonies died in April due to suffocation by many organic materials except several 5th and 6th rejuvenated colonies.

Turritopsis is an excellent living material, and we will be able to get innumerable clonal colonies from only one individual medusa through regenerated, transplanted as well as rejuvenated clones consisting of a great number of zooids and countless number of released medusae for application of any disciplines of biology and life science. *Turritopsis* will be kept forever by the present method and will be contribute to any study for everyone in the future.

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References

De Vito, D., Piraino, S., Schmich, J., Bouillon, J. and Boero, F. 2006. Evidence of reverse development in Leptomedusae (Cnidaria, Hydrozoa): the case of *Laodicea undulata* (Forbes and Goodsir, 1851). Mar. Biol., 49: 339-346.

Hadzi, J. 1912. Die Reduktion des Scyphopolypen und der Ephyla von *Chrysaora*. Verhandlungen des VIII. Internationalen Zoologen-Kongress zu Graz: 578-594.

Jarms, G. 1997. The polyps of Coronatae (Scyphozoa), a review and some new results. In: den Hartog, J. C. ed., Proceeding of the 6th International Conference on Coelenterate Biology, 1995, vol. Nationaal Natuurhistorisch Museum, Leiden: 271-278.

Kubota, S. 2005. Distinction of two morphotypes of *Turritopsis nutricula* medusae (Cnidaria, Hydrozoa, Anthomedusae) in Japan, with reference to their different abilities to revert to the hydroid stage and their distinct geographical distributions. Biogeography, 7: 41–50.

Kubota, S. 2006. Life cycle reversion of *Laodicea undulata* (Hydrozoa, Leptomedusae) from Japan. Bull. biogeogr. Soc. Japan, 61: 85-88. (In Japanese with English abstract)

Kubota, S. 2009. *Turritopsis* sp. (Hydrozoa, Anthomedusae) rejuvenated four times. Bull. biogeogr. Soc. Japan, 64: 97-99. (In Japanese with English abstract)

Piraino S., Boero, F., Aeschbach, B. and Schmid, V. 1996. Reversing the life cycle: medusae transforming into polyps and cell transdifferentiation in *Turritopsis nutricula* (Cnidaria, Hydrozoa). Biol. Bull., 190: 302-312.

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