Expulsion of zooxanthellae (*Symbiodinium*) from several species of scleractinian corals: comparison under non-stress conditions and thermal stress conditions

Lisa FUJISE¹, Hiroshi YAMASHITA², Go SUZUKI², and Kazuhiko KOIKE^{1,*}

¹ Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan

² Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency, 148-446 Fukai-Ohta, Ishigaki, Okinawa 907-0451, Japan

* Corresponding author: K. Koike

E-mail: kazukoike@hiroshima-u.ac.jp

Communicated by Michio Hidaka (Editorial Board Member)

Abstract Corals expel zooxanthellae (*Symbiodinium*) not only under thermal stress conditions, but also under non-stress conditions. To better understand the significance of this, we compared cell numbers, morphologies and photosynthetic activities of the expelled *Symbiodinium* under different temperatures for six species of scleractinian corals; *Acropora selago*, *Acropora muricata*, *Heliofungia actiniformis*, *Ctenactis echinata*, *Oxypora lacera* and *Pocillopora eydouxi*, kept in aquaria. During the eight day aquarium experiment, every coral expelled two types of *Symbiodinium* morphologies, normal and degraded form, and the ratios of these forms differed depending on the temperature. Under a non-stress, initial temperature condition of 27°C, mainly degraded cells were expelled. In contrast, when the water temperature was increased and reached to 32°C, the expulsion rates drastically increased, except *P. eydouxi*, and almost all the expelled populations exhibited normal cell morphology. Under this thermal stress conditions, photosystem II maximum quantum yield (F_v/F_m) of expelled *Symbiodinium* from two Acroporidae and two Fungiidae was 35% lower than those retained in the tissue, but was not significantly different in the cases of *O. lacera* and *P. eydouxi*. These results in-

dicate that, under the non-stress conditions, the corals functionally regulate *Symbiodinium* density by expelling degraded cells. This function may collapse under high-temperature stress, resulting in the extensive loss of healthy-looking but photosynthetically damaged *Symbiodinium*, probably because of host cell detachment. Moreover, susceptibilities toward temperature increase might be different in coral species or symbiont types and result in the differences of expulsion amounts or photosynthesis damages.

Keywords *Symbiodinium*, Degraded cell, Expulsion mechanism, Coral bleaching

Introduction

Corals and some other marine invertebrates form symbioses with unicellular microalgae called zooxanthellae (dinoflagellate genus *Symbiodinium*). *Symbiodinium* inhabit coral tissues and provide photosynthetic products necessary for corals to survive in oligotrophic tropical

waters (e.g. Muscatine 1967; Muscatine and Cernichiaro 1969). Under stressful conditions, the relationship can collapse, causing corals to expel large amounts of *Symbiodinium* and often resulting in “coral bleaching” (Goreau 1964; Jaap 1979; Egaña and DiSalvo 1982; Hoegh-Guldberg and Smith 1989; Gates et al. 1992; Brown et al. 1995; Brown 1997). Corals can also expel *Symbiodinium* under non-stressful conditions (Hoegh-Guldberg et al. 1987; Titlyanov et al. 1996; 1998; Jones and Yellowlees 1997; Baghdasarian and Muscatine 2000; Koike et al. 2007; Dimond and Carrington 2008; Yamashita et al. 2011). These “expulsion” phenomena do not share the same mechanism: the former instance seems to be a breakdown of the *Symbiodinium* maintenance system in corals, while the latter case is the steady-state healthy function in corals. In the reports dealing with *Symbiodinium* expulsion under stressful conditions, there were seemingly healthy cells in the expelled populations (e.g. Gates et al. 1992; Ralph et al. 2001; Bhagooli and Hidaka 2004; Ralph et al. 2005; Hill and Ralph 2007), which would reduce photosynthetic capacities in corals-symbionts relationship. Titlyanov et al. (1996; 1998) reported corals under non-stress conditions actively expelled “degraded” *Symbiodinium* cells from the polyp mouth and concluded that the mechanism is to regulate *Symbiodinium* density in the corals. Understanding these functional differences between different types of expulsion would lead to a better understanding of *Symbiodinium* losses in corals. There are few studies focusing on the expulsion of degraded cells under non-stress conditions contrasted with the expulsion of healthy-looking cells under stressful conditions and quantitatively comparing these cells for a variety of corals.

In this study, we compared the abundance of degraded cells and healthy-looking *Symbiodinium* cells in the expelled materials from six species of scleractinian corals in aquaria, under non-stress (27°C) and stressful conditions (32°C), to understand the maintenance and collapse of symbiosis between corals and *Symbiodinium*.

Materials and methods

Collection of corals and aquarium conditions

In October 2011, six species of scleractinian corals

were collected at the Urasoko Bay, Ishigaki Island, Okinawa, Japan (24°27'N, 124°13'E). These were *Acropora selago* (Studer, 1878) (collected 1 m depth, 594 g of total skeletal weight), *Acropora muricata* (Linnaeus, 1758) (2 m, 383 g), *Heliofungia actiniformis* (Quoy and Gaimard, 1833) (19 m, 1,012 g), *Ctenactis echinata* (Pallas, 1766) (18 m, 1,003 g), *Oxypora lacera* (Verrill, 1864) (24 m, 268 g), and *Pocillopora eydouxi* (Edwards and Haime, 1860) (7 m, 1,080 g). *A. selago* and *A. muricata* were selected as representatives of bleaching sensitive species in Acroporidae, and *H. actiniformis* and *C. echinata* were in Fungiidae. *O. lacera* and *P. eydouxi* were representatives of a deep-water species and a bleaching tolerant species, respectively. We should note that the aim of this study was to cover expulsion trends in wide species ranges from bleaching sensitive to insensitive and thus the experiment was not employed in multiplicate, rather one or two colonies for each coral species (total skeletal weights were mentioned above) were put into a single 12 L aquarium by species. These six 12 L aquaria were situated in a large water bath to maintain the water temperature at 27°C. The aquaria were aerated with air-stones and maintained with temperature-regulated flowing seawater (provided by a GA7500-ODHT-E, Gunji, Osaka, Japan) filtered by MEMCOR Ultra-filtration unit (0.2 µm pore size membrane module, JFE Engineering, Tokyo, Japan) at a flow-rate of 1 L min⁻¹ for each aquarium. The light was provided by three 500 W metal halide lamps and six 12 W LED lights, hanging over the entire aquaria, at a photon-flux density of 220 µmol photons m⁻² s⁻¹ with a 12:12 h light/dark cycle. The experiment was performed at the Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, in Okinawa, Japan.

Temperature treatments

After a two-week period of acclimation, the experiment was started as follows: for the first two days, temperature was maintained at 27°C. The seawater temperature was raised stepwise (0.5°C increase for every 8 h) for the following three days, until it reached 32°C, where it was maintained for three days.

Collection of expelled *Symbiodinium* from the corals

Expelled *Symbiodinium* from the corals were collected

in the initial two days at 27°C and in the three days at 32°C. To determine expulsion during the temperature increase, samples were also collected for two more days after the initial two days for *A. selago* and *H. actiniformis*. According to our previous report (Koike et al. 2007), corals have a daily rhythmicity of *Symbiodinium* expulsion, with a peak at noon. Therefore, from 12:00 to 14:00, the supplies of seawater to the aquaria were stopped, and expelled *Symbiodinium* during these two hours accumulated in the aquaria. To prevent the water temperature from increasing, the seawater was kept running into the water bath. Four L of seawater (including expelled *Symbiodinium*) in each aquarium was collected after mixing it well and sieved through a 20- μ m mesh to remove large particles, then concentrated into 50 ml using a 5- μ m mesh. Microscopy confirmed absence of *Symbiodinium* in the filtrate.

Counting the expelled *Symbiodinium*

Symbiodinium cells in 1 ml of the concentrated sample were trapped onto a polycarbonate filter (Isopore membrane filters, 0.8 μ m ATTP, ATTP01300, Millipore, Billerica, MA, USA) by gentle vacuuming. The filter was then put onto a slide glass, immersed in a drop of mineral oil to prevent dehydration and oxidation, and covered with a cover slip. Samples were kept in the dark and stored in a freezer (−20°C) until observation. The cells on the filter were counted by observing chlorophyll *a* auto-fluorescence under an epi-fluorescent microscope (BX51, Olympus,

Tokyo, Japan) with blue light excitation. Fluorescence and transmitted-light micrographs were taken of twenty randomly selected areas using a microscopy camera (Cool Snap ES, Photometrics, Tucson, AZ, USA) under a 20 $\times$ objective lens. The cell numbers of *Symbiodinium* were counted by the morphologies in the micrographs: a normal form in which the size was around 10 μ m and possessed bright auto-fluorescence, and a degraded form around half the normal form size with a weak auto-fluorescence and a condensed form (Fig. 1). The cell numbers per each observation field were averaged for the twenty areas, and total cell numbers were determined per 1 ml of concentrated sample. Expelled *Symbiodinium* cell numbers per an aquarium were determined and converted to hourly expelled cells per 100 g of coral skeletal weight.

Measurement of PSII maximum quantum yield of *Symbiodinium*

A dark-adapted maximum quantum yield of photosystem II (*Fv/Fm*) was measured using a pulse-amplitude modulation (PAM) fluorometer (WATER-PAM, Walz, Effeltrich, Germany) (PM gain 10) for both expelled *Symbiodinium* and freshly isolated *Symbiodinium* from the host tissue. The latter *Symbiodinium* were obtained using a water-pik, and the concentrated samples of expelled *Symbiodinium* were used for this measurement. The measurements were conducted every day; however, the fluorescence during the initial two days was too low to determine *Fv/Fm* and was therefore removed from the data.

Fig. 1 Micrographs of *Symbiodinium* cells expelled from corals. **a** Micrograph of degraded forms of *Symbiodinium* expelled under non-stress conditions. **b** Micrograph of normal forms of *Symbiodinium* expelled under thermal stress conditions. Scale bars are 10 μ m

During the third day after reaching 32°C, diatoms increased in the aquaria and the data obtained from these waters were also rejected.

Results

Numbers of expelled *Symbiodinium* from corals

Fig. 2 shows the numbers of expelled *Symbiodinium* cells from the corals (hourly expulsion per 100 g of coral skeletal weight) grouped by morphologies: normal and degraded forms (Fig. 1). Total number of expulsions drastically increased under the thermal stress conditions compared with those under the non-stress conditions, except for *P. eydouxi*. The numbers of expelled *Symbiodinium* cells after reaching 32°C (average for the three days at the temperature) were higher than the average for the initial two days at 27°C, for *A. muricata* (4 times), *A. selago* (34 times), *H. actiniformis* (7 times), *C. echinata* (7 times), and *O. lacera* (39 times). *P. eydouxi* expelled almost the same amount during the non-stress and the thermal stress conditions, and the values were one or two orders lower than the remaining five species.

Morphologies of expelled *Symbiodinium* from corals

All six corals expelled more degraded forms under the non-stress conditions and more normal forms under the thermal stress conditions (Fig. 2 and 3). The average ratios of the degraded forms under the non-stress conditions (for the initial two days) were 60, 56, 41, 58, 41 and 85% for *A. muricata*, *A. selago*, *H. actiniformis*, *C. echinata*, *O. lacera* and *P. eydouxi*, respectively. Under the thermal stress conditions, however, the average ratios for three days at 32°C were 8, 7, 1, 0, 0 and 5%, respectively. This indicates that the remaining 92 to 100% of the *Symbiodinium* biomass was composed of the normal form under the thermal stress conditions. The expulsion under the non-stress conditions was mainly due to the relatively small numbers of degraded *Symbiodinium*, while under the thermal stress conditions, the expulsion was composed of a large number of normal *Symbiodinium* and few degraded *Symbiodinium* form.

PSII maximum quantum yield (F_v/F_m) of freshly isolated *Symbiodinium* and expelled *Symbiodinium* from corals

Fig. 4 shows F_v/F_m values of freshly isolated *Symbio-*

Fig. 2 Cell numbers of expelled *Symbiodinium* from corals. The black bar graph shows the number of normal forms of *Symbiodinium* and the white bar graph shows the number of degraded forms of *Symbiodinium*. The scales of cell numbers are different in each coral. **a** *A. selago*. **b** *A. muricata*. **c** *H. actiniformis*. **d** *C. echinata*. **e** *O. lacera*. **f** *P. eydouxi*

Fig. 3 Ratio of morphologies of expelled *Symbiodinium* from corals. The black bar graph shows the percentage of normal forms of *Symbiodinium* and the white bar graph shows the percentage of degraded forms of *Symbiodinium*. **a** *A. selago*. **b** *A. muricata*. **c** *H. actiniformis*. **d** *C. echinata*. **e** *O. lacera*. **f** *P. eydouxi*

Fig. 4 F_v/F_m of freshly isolated *Symbiodinium* (black bar) and expelled *Symbiodinium* from corals (white bar) under thermal stress conditions. **a** *A. selago*. **b** *A. muricata*. **c** *H. actiniformis*. **d** *C. echinata*. **e** *O. lacera*. **f** *P. eydouxi*

dinium (black bars) and of the expelled cells (white bars). Even under the thermal stress conditions, the values of freshly isolated *Symbiodinium* were high: on the initial day after reaching 32°C, they were greater than 0.65, and on the second day were still as high as 0.63, except in *O. lacera* (0.59). This implies that the *Symbiodinium* resident in the coral tissue were slightly damaged under the high temperature but were still photosynthetically competent. In contrast, regardless of the healthy-looking natures of the expelled *Symbiodinium*, *Fv/Fm* values of the expelled *Symbiodinium* were lower than those in the coral tissue. Two Acroporidae and two Fungiidae were 0.45 on average for two days and 35% lower than in the tissue. Interestingly, the expelled cells from *O. lacera* and *P. eydouxi* did not exhibit such significant drops in *Fv/Fm*, and still maintained competent values of 0.60 and 0.62 for two days.

Discussion

These six corals expelled more degraded *Symbiodinium* cells under the non-stress steady conditions and more healthy-looking, but photosynthetically damaged cells, (especially in Acroporidae and Fungiidae) under the thermal stress conditions. The expulsion under the non-stress conditions is considered to be a functional mechanism to regulate *Symbiodinium* density within corals. Such a mechanism has been proposed by previous studies in many coral species (Titlyanov et al. 1996, 1998; Jones and Yellowlees 1997; Baghdasarian and Muscatine 2000; Dimond and Carrington 2008). Titlyanov et al. (1996; 1998) reported that the number of degraded *Symbiodinium* in corals was similar to the number of dividing *Symbiodinium*, concluding that the digestion and expulsion of *Symbiodinium* might be a functional regulation for *Symbiodinium* densities in corals. Our current results agreed with these findings, and we can conclude that the expulsion of degraded (digested) cells is a mechanism for corals to regulate the symbionts. In contrast, under the thermal stress conditions, large numbers of the normal form *Symbiodinium* were expelled from the corals, and the phenomenon apparently results in coral discoloration. Under these circumstances, few degraded *Symbiodinium* were expelled in contrast to the report by Downs et al. (2009) in which

the digestion of *Symbiodinium* in the coral tissue was promoted by heat stress. In another study, degraded *Symbiodinium* accumulated in coral tissues in naturally bleached corals (Brown et al. 1995). Consequently, under such stress conditions, digestion of *Symbiodinium* may occur, but disposal from the polyp mouth (Titlyanov et al. 1996; 1998) is disabled and results in the accumulation of waste cells. Compared with such an active dumping mechanism, expulsion under thermal stress conditions seems to be a negative action for corals, probably because of host cell detachment, as observed by Gates et al. (1992). Even though such cell detachment occurs, degraded *Symbiodinium* would not be expelled at the early stage of the stressful conditions since they mainly inhabit mesenterial filaments (Brown et al. 1995; Titlyanov et al. 1996), which are probably not influenced by host cell detachment. However, when corals are exposed to thermal stress conditions over a longer time, degraded *Symbiodinium* might increase (Brown et al. 1995), and be eventually expelled as reported by Franklin et al. (2004), in which they observed only degraded *Symbiodinium* were expelled from *Stylophora pistillata* exposed to harsh thermal and light conditions.

Two Acroporidae (*A. muricata* and *A. selago*) and Fungiidae species (*H. actiniformis* and *C. echinata*) expelled *Symbiodinium* that were more photosynthetically damaged than those that resided in the tissue. If the expulsion under thermal stress conditions is indeed caused by host cell detachment, such “selective” releases for damaged cells are not feasible. Alternatively, we considered that *Symbiodinium* inhabiting the outer layer of the endodermal tissues adjacent to environment were more subjected to environment stresses. On the other hand, those in the inner layer of the endoderm across the gastrovascular cavity and facing the coral skeleton were less affected (inner layer and outer layer of endoderm were showed in diagrams of Brown et al. 1995 and Titlyanov et al. 1996 studies). More damaged *Symbiodinium* were present in the outer layer, and therefore expelled *Symbiodinium* due to host cell detachment might result in the expulsion of photosynthetically damaged cells, while the cells extracted by the water-pik were also derived from the inner layer of the endoderm and thus gave higher *Fv/Fm* values. Interestingly, *O. lacera* and *P. eydouxi* expelled *Symbiodinium* having almost the same

competency as the freshly isolated cells. Bhagooli and Hidaka (2004), Ralph et al. (2001; 2005) and Hill and Ralph (2007) reported that photosynthetically active *Symbiodinium* could be expelled from *Galaxea fascicularis*, *Cyphastrea serailia* and *Pocillopora damicornis* even under high water temperatures. These reports are congruent with our results from *O. lacera* and *P. eydouxi*. Nevertheless, we assume that the consequences of the *Symbiodinium* expulsion are different between these two species because the numbers of expelled *Symbiodinium* were different. *O. lacera* is a rather deep-water species and seems to be less tolerant of increased temperature. *O. lacera* expelled extremely large numbers of *Symbiodinium* under the thermal stress conditions with many cnidocysts, indicating that extensive host cell detachment, probably with the inner parts of coral tissue, might occur. On the contrary, the numbers of expelled *Symbiodinium* from *P. eydouxi* were not different between the non-stress and thermal stress conditions. *P. eydouxi* has been reported to be a thermal tolerant species (Baird and Marshall 1998; Paulay and Benayahu 1999), probably because of a relationship with clade D *Symbiodinium*, which is a thermally tolerable genotype (Glynn et al. 2001; Rowan 2004). *P. eydouxi* in the study area Urasoko Bay, also harbored clade D *Symbiodinium* (Yamashita et al. 2011). From these results, it is assumed that *P. eydouxi* expelled photosynthetically competent *Symbiodinium*, but in small numbers.

In conclusion, the expulsion mechanisms for corals are divided into two types. One is a regulation mechanism for corals to maintain constant *Symbiodinium* density. In this mechanism, they digest excess *Symbiodinium* and expel the degraded cells. This is a normal function for corals to maintain symbionts. However, when this function collapses under thermal stress, interaction between corals and *Symbiodinium* breaks down, and corals lose a large number of “healthy-looking” *Symbiodinium* through host cell detachment, which may result in “coral bleaching”. Expulsion is a key mechanism for maintenance and collapse of symbiosis between corals and *Symbiodinium*.

The study was conducted only for a single batch for each species, and thus the results are still preliminary. However, trends of expulsion patterns were common among the coral species, in which expulsion under the non-stress conditions was mainly due to the relatively

small numbers of degraded *Symbiodinium*, while under the thermal stress conditions, the expulsion was composed of a large number of normal cells. The strength of reactions, however, differed between the coral species, and quantitative experiments and statistical analysis will be needed in replicate experiments, including subjecting other stress factors than merely temperature stress. Moreover, observing expulsion patterns in wild corals in the field is necessary to elucidate whether the trends that we found in this study also happen in nature.

Acknowledgments

This work was supported by a Grant-in-Aid (No. 24570028) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to KK. The authors express their sincere thanks to the Research Center for Subtropical Fisheries, Seikai National Fisheries Research Institute, Fisheries Research Agency for support during this investigation.

References

- Baghdasarian G, Muscatine L (2000) Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *Biol Bull* 199: 278–286
- Baird AH, Marshall PA (1998) Mass bleaching of corals on the Great Barrier Reef. *Coral Reefs* 17: 376
- Bhagooli R, Hidaka M (2004) Release of zooxanthellae with intact photosynthetic activity by the coral *Galaxea fascicularis* in response to high temperature stress. *Mar Biol* 145: 329–337
- Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs* 16: S129–S138
- Brown BE, Le Tissier MDA, Bythell JC (1995) Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. *Mar Biol* 122: 655–663
- Dimond J, Carrington E (2008) Symbiosis regulation in a facultatively symbiotic temperate coral: zooxanthellae division and expulsion. *Coral Reefs* 27: 601–604
- Downs CA, Kramarsky-Winter E, Martinez J, Kushmaro A, Woodley CM, Loya Y, Ostrander GK (2009) Symbiophagy

- as a cellular mechanism for coral bleaching. *Autophagy* 5: 211–216
- Egaña AC, DiSalvo LH (1982) Mass expulsion of zooxanthellae by Easter Island corals. *Pac Sci* 36: 61–63
- Franklin DJ, Hoegh-Guldberg P, Jones RJ, Berges JA (2004) Cell death and degeneration in the symbiotic dinoflagellates of the coral *Stylophora pistillata* during bleaching. *Mar Ecol Prog Ser* 272: 117–130
- Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biol Bull* 182: 324–332
- Glynn PW, Maté JL, Baker AC, Calderón MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997–1998 El Niño–Southern Oscillation event: spatial/temporal patterns and comparisons with the 1982–1983 event. *Bull Mar Sci* 69: 79–109
- Goreau TF (1964) Mass expulsion of zooxanthellae from Jamaican reef communities after hurricane Flora. *Science* 145: 383–386
- Hill R, Ralph PJ (2007) Post-bleaching viability of expelled zooxanthellae from the scleractinian coral *Pocillopora damicornis*. *Mar Ecol Prog Ser* 352: 137–144
- Hoegh-Guldberg O, McCloskey LR, Muscatine L (1987) Expulsion of zooxanthellae by symbiotic cnidarians from the Red Sea. *Coral Reefs* 5: 201–204
- Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *J Exp Mar Biol Ecol* 129: 279–303
- Jaap WC (1979) Observations on zooxanthellae expulsion at Middle Sambo Reef, Florida Keys. *Bull Mar Sci* 29: 412–422
- Jones RJ, Yellowlees D (1997) Regulation and control of intracellular algae (=zooxanthellae) in hard corals. *Phil Trans R Soc Lond B* 352: 457–468
- Koike K, Yamashita H, Oh-Uchi A, Tamaki M, Hayashibara T (2007) A quantitative real-time PCR method for monitoring *Symbiodinium* in the water column. *Galaxea* 9: 1–12
- Muscatine L (1967) Glycerol excretion by symbiotic algae from corals and *Tridacna* and its control by the host. *Science* 156: 516–519
- Muscatine L, Cernichiaro E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol Bull* 137: 506–523
- Paulay G, Benayahu Y (1999) Patterns and consequences of coral bleaching in Micronesia (Majuro and Guam) in 1992–1994. *Micronesica* 31: 109–124
- Ralph PJ, Gademann R, Larkum AWD (2001) Zooxanthellae expelled from bleached corals at 33°C are photosynthetically competent. *Mar Ecol Prog Ser* 220: 163–168
- Ralph PJ, Larkum AWD, Kühl M (2005) Temporal patterns in effective quantum yield of individual zooxanthellae expelled during bleaching. *J Exp Mar Biol Ecol* 316: 17–28
- Rowan R (2004) Thermal adaptation in reef coral symbionts. *Nature* 430: 742
- Titlyanov EA, Titlyanova TV, Leletkin VA, Tsukahara J, van Woesik R, Yamazato K (1996) Degradation of zooxanthellae and regulation of their density in hermatypic corals. *Mar Ecol Prog Ser* 139: 167–178
- Titlyanov EA, Titlyanova TV, Loya Y, Yamazato K (1998) Degradation and proliferation of zooxanthellae in planulae of the hermatypic coral *Stylophora pistillata*. *Mar Biol* 130: 471–477
- Yamashita H, Suzuki G, Hayashibara T, Koike K (2011) Do corals select zooxanthellae by alternative discharge? *Mar Biol* 158: 87–100

Received: 3 June 2013

Accepted: 27 July 2013

© Japanese Coral Reef Society