La mer 45 : 81-90, 2007
Société franco-japonaise d'océanographie, Tokyo

Fluorescent labelling of cultivated corals as a sustainable management tool in coral trade and reefs conservation

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Abstract: Scleractinian corals are part of an important growing lucrative market trade, which is primarily focused on wild-caught corals. Improving trade regulations and developing asexual/sexual reproduction programmes in aquaria to decrease the pressure exerted on wild populations may require labelling systems to certify coral origin (cultured *vs.* wild-caught). We investigated a simple labelling method based on calcein incubation using 81 coral fragments of six cultivated coral species of two different growth forms (branched and foliaceous). We tested two calcein concentrations (0.01 and 0.02 g l^{-1}) and three incubation times (12, 24 and 36 hours) to determine optimal labelling conditions. The labelling visibility on fragments was assessed 8, 12 and 16 weeks following the incubations. Respectively 59, 61, 79, 96, and 98 % of the calcein incubated-fragments were successfully labelled for *S. caliendrum, Echinopora* sp., *T. reniformis, P. damicornis*, and *S. pistillata and Montipora* sp. While the quality and the durability of the label varied between species, both were significantly improved at the longest incubations for both calcein concentration tested. The relevance of the calcein technique in labelling cultivated corals is discussed in relation to other potential labelling methods and as a sustainable management in coral trade and reef conservation.

Keywords: *Scleractinian, calcein, coral labelling, coral trade, CITES*

1. Introduction

The impacts of anthropogenic activities on coral reefs have been widely reported in the literature and include processes such as eutrophication, oil pollution, tourism expansion, trampling, dredging, overfishing, and cyanide fishing (e.g. SHUMAN *et al.*, 2004 : WILKINSON, 2004 : FABRICIUS, 2005).

Scleractinian corals have also been the focus of a lucrative and constantly growing trade (GREEN and SHIRLEY, 1999 : GREEN and HENDRY, 1999 : FOLKE *et al.*, 2000 : BRUCKNER, 2000, 2001 : DELBEEK, 2001 : WABNITZ *et al.*, 2003). Despite the development and improvement of maintenance and husbandry techniques, less than one percent of the total trade in hard corals is derived from cultured corals (GREEN and SHIRLEY, 1999). Since 1983, international trade of more than 2000 species of corals has been monitored and regulated under the Convention on International Trade in Endangered Species (CITES). All traded coral species are now listed in Appendix II of CITES and then require an export permit from the country of origin, along with proofs that a specimen was legally obtained and that the export will not harm the survival of that species. Countries are required to publish export quotas showing the amount of coral that can be collected and traded each year (GREEN and HENDRY, 1999 : BRUCKNER, 2001 : http://www.cites.org, July 2005). Different environmental agreements, programmes, partnerships, networks, non governmental and governmental organisations have been working to protect and conserve coral reefs (UNEP, 2003) with for instance, the creation of Marine Protected Areas (MPA) where fishing or collecting activities are strictly banned.

Although CITES legislation is strict (GREEN and HENDRY, 1999 : BRUCKNER, 2001), illegal or unreported fishing and coral collecting

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activities (e.g. HANFEE, 1997 : GREEN and SHIRLEY, 1999 : ISHIHARA, 2000 : TEO, 2005) as well as unreported export/import of live corals are still current. The illegal trade of corals on the black market is one of the greatest concerns in the conservation and protection of coral reefs. In addition, intensive coral collecting activities greatly reduce the percentage of coral cover (e.g. HARRIOTT, 2002 : BRUCKNER and BORNEMAN, 2005) hence affecting the entire reef ecosystem; e.g. up to 70 % of the total reef cover have been reduced in only one decade in the Philippines (GREEN and SHIRLEY, 1999). It is therefore necessary to improve trade regulations in order to minimize coral reef decline. Development of aquaculture facilities for coral propagation in aquarium by both asexual and sexual reproduction (e.g. DELBEEK, 2001 : PETERSON *et al.*, 2006) could allow pressure to be reduced on wild populations, but should require labelling systems which may guarantee coral proveniences (cultivated vs. wild–caught) and help tracking coral in trade. In March 2004, the Permanent Comity of the CITES raised the question regarding the identification of a labelling system for hard corals, which would help to differentiate cultivated from wild–caught corals (SC50 Doc. 10.1. Convention of the International Trade on Endangered Species, 50th session of the Permanent Comity, Geneva, 2004). Furthermore, the Marine Aquarium Council (MAC) has launched an international certification scheme providing security on the traded organisms with the idea of a sustainable management of the reef and the market trade (SHUMAN *et al.*, 2004 : http://www.aquariumcouncil.org, December 2006). In particular, the assessment of labelling methods to distinguish wild–caught from cultured corals requires further investigations as it could lead to the development of a sustainable tool for more consistent monitoring of the coral trade market.

Internal fluorescent markers such as calcein (2, 4 – bis – [N, N' – di (carboxymethyl) – aminomethyl]– fluorescein) are easy to apply, cost effective (i.e. a large number of individuals can be marked in a short time with minimum handling) and can last for several weeks (LEIPS *et al.*, 2001 : THORROLD *et al.*, 2002). Calcein has been used as an efficient marker for both identification and growth measurements (BERNHARD *et al.*, 2004) in various invertebrates such as sponges (ILAN *et al.*, 1996), sclerosponges (WILLENZ and HARTMAN, 1999), gastropods (MORAN, 2000), bivalves (DAY *et al.* 1995, KAEHLER and MCQUAID , 1999) and echinoderms (RUSSEL and MEREDITH , 2000 : RUSSEL and URBANIAK , 2004). Recently, MARSCHAL *et al.* (2004) used calcein as a new method to measure the growth and age of the Mediterranean gorgonian, *Corallium rubrum* (commonly referred to as 'red coral'). Calcein is a fluorescein complex which binds to calcium and is therefore incorporated into growing calcium carbonate structures (BERNHARD *et al.*, 2004) without affecting the growth of the stained individual. Furthermore, when compare to other stains such as alizarin red S or tetracycline calcein appears to be more suitable for staining invertebrates (e.g. DODGE *et al.*, 1984 : DAY *et al.*, 1995). Once bound to calcium, calcein fluoresces and becomes detectable when exposed under ultraviolet light.

In this context, the objectives of this work was to develop a simple calcein–based method for labelling cultivated coral species, thus introducing the idea of a 'conservation label' for traded hard corals. More specifically, using 6 species of branched and foliaceous corals we investigated (i) the optimal labelling conditions necessary to obtain a visible and long lasting mark, (ii) the potential effect of calcein on fragment growth, and (iii) the inter–specific variability in the labelling efficiency.

2. Material and Methods

2.1. Species

The two main families of stony corals traded internationally are Acroporidae and Pocilloporidae (GOMEZ *et al.*, 1985 : WABNITZ *et al.*, 2003). The six species of scleractinian corals considered in the present work have been specifically chosen as they are listed in Appendix II of CITES, known to grow well in aquarium and to reach rapidly a commercial size (GREEN and SHIRLEY, 1999 : DELAHAYE , 2003) and as such can be thought as being representative species for aquarium trade. Three branched Pocilloporidae species (Stylophora pistillata,

Seriatopora caliendrum, and Pocillopora damicornis) and one foliaceous Acroporidae (Montipora sp.) were considered. Two other foliaceous species of Faviidae (Echinopora sp.) and Dendrophylliidae (Turbinaria reniformis) were investigated in order to ensure the generality and relevance of the present work.

2.2. Cutting and handling

For each species, small coral fragments of about 5 cm in length for the branched species and a diameter of about 5 cm for the foliaceous species were obtained from cultivated colonies using a pair of pliers. Tags attached by a thin plastic cable were used to identify each fragment. Fragments of the each species were placed on separate PVC plates and separated from each other to avoid any interaction. Holding plates were then transferred in four 800 l aquaria filled with biologically filtered seawater and fitted with a circulating pump (Eheim 1060, 1200 l h–1), allowing sufficient water flow to support coral growth. Light (300 μE cm–2 s–1) was provided by two m*et al* halide lamps (HQI) located one meter above each aquarium. The temperature was maintained at 26.5–27.5 °C during the entire study.

2.3. Calcein labelling

The labelling experiment consisted in six different incubating conditions carried out in order to infer an optimal condition for obtaining a visible and long lasting mark on coral fragments. Calcein concentrations of 0.01 g l^{-1} to more than 0.60 g l^{-1} (e.g. KAELHER and MCQUAID , 1999 : RUSSELL and MEREDITH, 2000) have been used to stain various invertebrates without affecting their survival. Given the calcein concentrations used by MARSCHAL *et al.* (2004) to stain a gorgonian coral, two calcein solutions (0.01 g l^{-1} and 0.02 g l^{-1}) were prepared according to MORAN (2000). Coral fragments of each species were then removed from the 800 l aquaria and incubated in 50 l aquaria without calcein (control), and with calcein at 0.01 g l^{-1} and 0.02 g l^{-1} for 12, 24, or 36 hours. After incubation, fragments were returned to the 800 l culture aquaria. The 50 l glass aquaria were filled with the same seawater of the 800 l aquaria and their temperature maintained at 26.5–27.5 °C for the duration of the incubations.

Calcein–incubated fragments were subsequently observed one by one under ultraviolet light (UV lamp: 365 nm) for less than one minute. Four mark quality indexes were defined according to the different calcein fluorescence intensity levels (FIL) observed (1 = no mark or absence of calcein : 2 = detectable but faint mark : 3 = bright mark : 4 = very bright mark). The remaining in the visibility of the label on the fragment skeleton was assessed by repeating the observation under the UV lamp 8, 12, and 16 weeks after the first incubations.

2.4. Coral growth

The fragment growth or increase in weight (g) was estimated to the nearest 0.1 g at each time interval of the study (after 12, 24, and 36 hours of calcein incubation, and 8, 12, and 16 weeks later). Coral fragments were taken out of the aquariums and put on a tray for five minutes before weighing them to allow excess water to drain away (DELAHAYE , 2003).

2.5. Statistical analysis

The effects of calcein concentration (g l^{-1}), incubation time (hours), durability of the label over time (weeks), and the inter–species variation on calcein mark readability observed in fragments were tested using ordinal logistic regressions (SAS Enterprise Guide® V2). In order to test for the effect of calcein concentration on fragment growth, we performed for each species a one–way analysis of variance (ANOVA) on the relative weigh increase (in %) of fragments over time. Parametric testing was possible as both the normality and the Levene and Bartlett' s tests for homogeneity of variance were satisfied ($p > 0.05$).

3. Results

After 12, 24, or 36 hours of incubation in calcein concentration of 0.01 or 0.02 g l^{-1}, more than 59 % of all incubated–fragments of each studied species showed a faint, bright or very bright yellow–green fluorescent mark when observed under the UV lamp. The mark was readily distinguished from naturally occurring

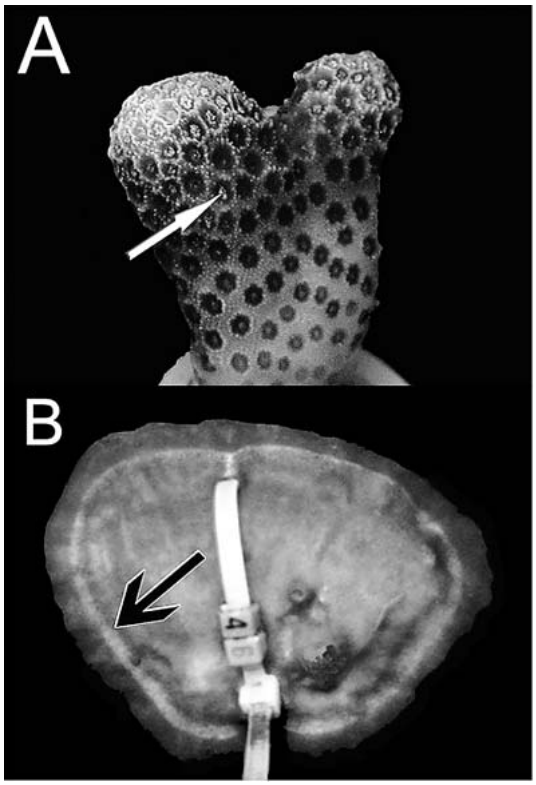

Fig. 1. Illustration of fluorescent labelled-fragments observed under ultraviolet-light. A: Calcein fluorescence observed immediately after incubation on a fragment of S. pistillata and easily differentiated from the polyps auto-fluorescence (white arrow). B: A calcein fluorescent band (black arrow) easily observed on the shaded part of a fragment of T. reniformis 8 weeks after incubation.

auto fluorescence by comparing calcein–incubated fragments with the controls (Fig. 1). The mean FIL obtained for both experiments are given in Table 1. They give information on the amount but especially on the quality of the marks obtained (DAY *et al.*, 1995). In addition, the evolution of the percentage of labelled–fragments obtained from incubations is represented for each species in Fig. 2. This percentage is considered to be easier to picture and it provides a better idea of the reliability of the method; i.e. < 50 % of labelled–fragments will mean that the conditions used in this study are thus not reliable for a labelling system, 50 to 80 %: the method can potentially be reliable but the conditions need to be reviewed, > 80 %: the method is reliable but may need to be improved.

3.1. Optimal conditions after incubations and inter–species variation

Firstly, we determined whether a condition of incubation would be more suitable (i.e. higher marking scores obtained) for each species separately. While an effect of the incubation time was significantly showed for fragments of *S. caliendrum* ($p < 0.05$) with higher scores obtained from 36 hours of incubation, no effect of the incubation time was showed for the other species ($p > 0.05$). For each species, both the effect of the incubation time on the FIL and the mean FIL obtained were not influenced by the calcein concentrations ($p > 0.05$). Secondly, we found that decreasing the incubation time (e.g. 24 or 12 hours) led to significant variations in the obtained FIL between species ($p < 0.05$) for both calcein concentrations. While *P. damicornis, Montipora* sp. and *S. pistillata* did not show any significant difference in their FIL, they were all significantly higher than those of *T. reniformis* ($p < 0.05$), and which ones were found significantly higher than *S. caliendrum* and *Echinopora* sp. ($p < 0.05$). These trends are well reflected by the percentage of labelled–fragments obtained after all incubations. Respectively 59, 61, 79, 96, and 98 % of the calcein incubated–fragments were successfully labelled for *S. caliendrum, Echinopora* sp., *T. reniformis, P. damicornis*, and *S. pistillata* and *Montipora* sp.

3.2. Persistence of the label and inter–species variation

The percentage of labelled–fragments of each species significantly decreased over the course of the study ($p < 0.05$), in particular between the first observation following the incubations and the second observation performed 8 weeks later (Fig. 2). However, some species were observed to "lose" the brightness of their label faster than others. For example, while 8 weeks after all conditions of incubation no more marks were visible on the skeleton of the fragments of *S. caliendrum* (Table 1), there were

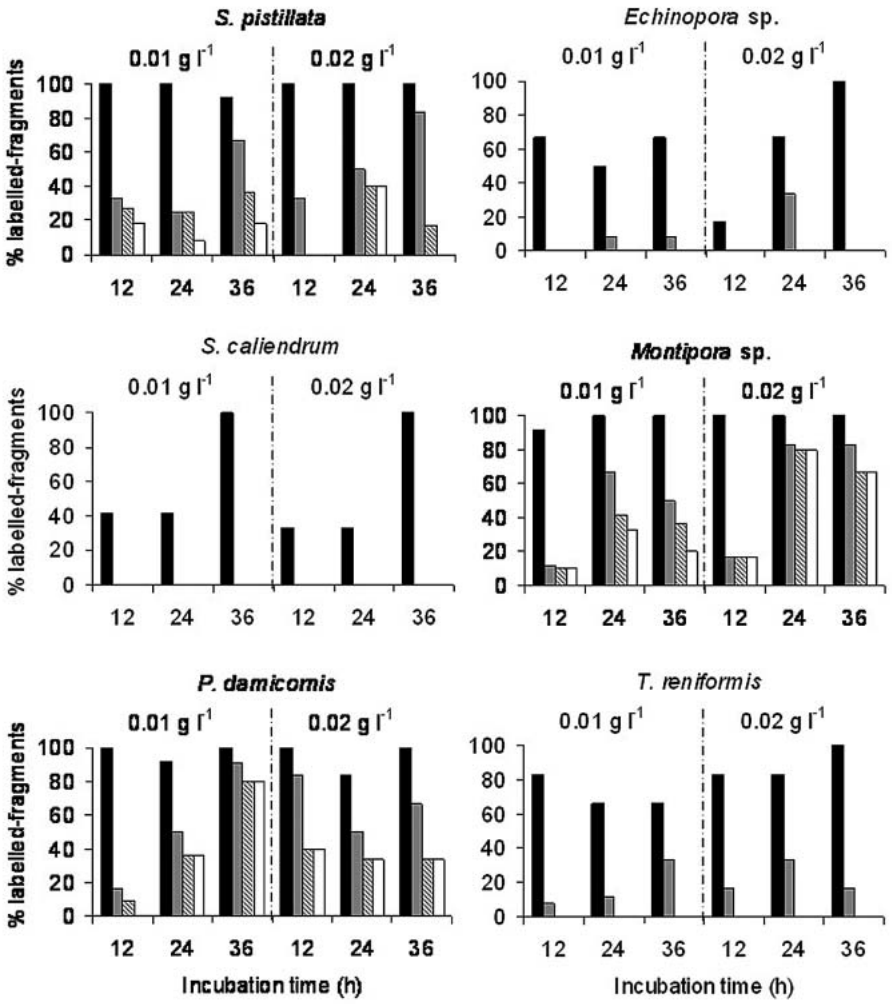

Fig. 2. Temporal evolution of the percentage of labelled-fragments obtained after incubation of each species in calcein at different concentrations (0.01 and 0.02 g l^{-1}) and different incubation times (12, 24, and 36 h). Black: immediately after incubation, grey: 8 weeks after incubation, grey stripes: 12 weeks after incubation, white: 16 weeks after incubation.

still more than 50 % (up to 90 %) of labelled–fragments of *P. damicornis* for most of the condition tested (Table 1). Again, 8 weeks following all incubations, the FIL were not significantly different between *P. damicornis, Montipora* sp. and *S. pistillata*, but were significantly different between these three species and *T. reniformis* and *Echinopora* sp ($p < 0.05$). *T. reniformis* significantly showed higher FIL than *Echinopora* sp. ($p < 0.05$). Twelve weeks after the incubations no marks could be detectable in all fragments skeleton of *Echinopora* sp. and *T. reniformis*. In contrast, the three other species commonly remained a weak percentage (less than 40 %) of labelled–fragments that gradually decreased over the next weeks (Fig. 2). No significant difference in their FIL was showed 12 and 16 weeks after incubations ($p > 0.05$).

Apart from *S. caliendrum, Echinopora* sp. and *T. reniformis*, fragments of the other species studied significantly showed a higher probability to keep a visible mark with higher FIL if incubated for 24 and/or 36 hours ($p > 0.05$).

Table 1. Fluorescent intensity levels (FIL) obtained for different coral species immediately after calcein incubations and 8, 12 and 16 weeks after incubations for each concentration and incubation time tested. Mean ± Standard deviation; n: sample size.

Species	Cone. (g. 1^{-1})	Time (weeks)	12h	n	24h	n	36h	n
S. pistillata	0.01	0	3.3±0.9	12	3.9±0.3	12	3.7±0.9	12
		8	1.3±0.5	12	1.5±1.0	12	1.8±0.7	12
		12	1.3±0.5	11	1.4±0.8	12	1.4±0.5	11
		16	1.2±0.4	11	1.1±0.3	12	1.2±0.4	11
	0.02	0	3.5±0.8	6	3.5±0.8	6	4.0±0.0	6
		8	1.3±0.5	6	1.7±0.8	6	2.3±0.8	6
		12	1.0±0.0	6	1.6±0.9	5	1.2±0.4	6
		16			1.6±0.9	5	1.0±0.0	6
S. caliendrum	0.01	0	1.6±0.9	12	1.9±1.2	12	3.9±0.3	12
		8	1.0±0.0	6	1.0±0.0	11	1.0±0.0	12
	0.02	0	1.7±1.2	6	2.0±1.6	6	3.5±0.8	6
		8	1.0±0.0	6	1.0±0.0	5	1.0±0.0	6
P. damicornis	0.01	0	3.6±0.5	12	3.6±0.9	12	4.0±0.0	12
		8	1.3±0.6	12	1.7±0.8	12	3.3±1.1	11
		12	1.1±0.3	11	1.4±0.5	11	2.2±0.8	10
		16	1.0±0.0	11	1.4±0.5	11	2.1±0.7	10
	0.02	0	3.8±0.4	6	3.3±1.2	6	3.8±0.4	6
		8	2.3±1.0	6	1.7±0.8	6	1.8±0.8	6
		12	1.4±0.6	5	1.3±0.5	6	1.3±0.5	6
		16	1.4±0.6	5	1.3±0.5	6	1.3±0.5	6
Echinopora sp.	0.01	0	1.8±0.6	12	2.2±1.4	12	2.2±1.2	12
		8	1.0±0.0	12	1.3±0.6	12	1.1±0.3	12
		12			1.0±0.0	12	1.0±0.0	11
	0.02	0	1.3±0.8	6	2.3±1.4	6	2.7±1.0	6
		8	1.0±0.0	6	1.3±0.5	6	1.0±0.0	6
		12			1.0±0.0	5		
Montipora sp.	0.01	0	3.7±0.9	12	3.8±0.4	12	3.7±0.5	12
		8	1.1±0.3	12	2.2±1.2	12	2.2±1.3	12
		12	1.1±0.3	10	1.4±0.5	12	1.6±1.0	11
		16	1.1±0.3	10	1.3±0.5	12	1.4±1.0	10
	0.02	0	3.5±0.8	6	3.3±1.0	6	4.0±0.0	6
		8	1.3±0.8	6	2.5±1.4	6	3.0±1.3	6
		12	1.2±0.4	6	2.6±1.1	5	2.3±1.2	6
		16	1.2±0.4	6	2.2±0.8	5	2.3±1.2	6
T. reniformis	0.01	0	3.2±1.2	12	2.7±1.4	9	2.7±1.4	9
		8	1.0±0.0	11	1.1±0.3	9	1.6±1.0	9
		12			1.0±0.0	9	1.0±0.0	8
	0.02	0	3.5±1.2	6	3.2±1.3	6	3.7±0.5	6
		8	1.2±0.4	6	1.3±0.5	6	1.2±0.4	6
		12	1.0±0.0	5	1.0±0.0	6	1.0±0.0	6

Those results suggest that longer period of incubation might help remaining a higher percentage of labelled–fragments over time. The concentration effect was only significant for fragments of *Montipora* sp. and was higher for clacein concentration of 0.02 g l^{-1} . Furthermore, for fragments of *P. damicornis* increasing the incubation time at lower calcein

Table 2. Growth rates (% of relative weight increase) of each species obtained 8, 12, and 16 weeks after incubations at each calcein concentration (C0: unlabelled control, C1: 0.01 g l^{-1} , C2: 0.02 g l^{-1}). Mean ± Standard deviation; n: sample size.

Species	Calcein groups	Initial weight (g)	n	% weight increase					
				8 weeks	n	12 weeks	n	16 weeks	n
S. pistillata	C0	3.2±0.6	6	25.0±11.8	6	66.3±25.7	6	111.6±40.8	6
	C1	5.2±1.6	36	31.7±13.0	36	65.9±23.3	34	116.4±46.1	34
	C2	6.2±3.0	18	24.7±13.4	18	54.7±29.1	17	92.9±52.9	17
S. caliendrum	C0	2.6±0.5	6	36.9±15.1	6	85.9±23.2	6	147.5±58.9	6
	C1	2.5±0.7	36	24.0±14.6	29	62.4±34.5	29	118.6±69.6	29
	C2	2.8±0.8	18	23.1±15.7	17	58.0±31.4	17	103.4±58.0	17
P. damicornis	C0	4.5±1.4	6	44.8±10.9	6	108.7±31.0	6	199.5±68.0	6
	C1	3.8±1.0	36	47.2±20.1	35	127.0±49.1	32	218.9±85.5	32
	C2	4.0±1.3	18	48.4±22.8	18	134.1±51.6	17	245.0±92.9	17
Echinopora sp.	C0	4.0±2.2	6	33.2±20.6	6	60.2±21.9	6	106.1±38.6	6
	C1	3.7±1.4	36	38.4±16.9	36	59.8±46.9	35	101.0±61.8	35
	C2	3.5±1.2	18	36.2±22.5	18	52.9±50.5	17	100.3±67.0	17
Montipora sp.	C0	4.5±1.3	6	47.6±16.6	6	89.9±17.4	6	156.8±12.8	6
	C1	3.3±1.2	36	52.3±31.7	36	91.3±28.6	33	142.0±64.0	33
	C2	3.3±1.5	18	49.0±21.4	18	69.4±49.9	17	129.7±76.7	17
T. reniformis	C0	8.7±2.1	6	19.6±11.9	6	26.5±13.9	6	42.7±12.4	6
	C1	7.0±3.9	30	13.7±8.7	29	29.1±15.3	27	47.1±21.1	27
	C2	6.4±3.2	18	11.7±8.0	18	24.4±14.5	17	39.3±22.0	17

concentration significantly helped in keeping the label visible over time ($p < 0.05$).

3.3. Effect of calcein on fragment growth

Fragments of each species showed an exponential growth over time, and their growth rates (% relative weight increase) obtained at the end of the study are reported in Table 2. Although the relative growth rates were observed to vary between species from 40 % (*T. reniformis*) to 200 % (*P. damicornis*), incubating fragments in calcein concentration of 0.01 g l^{-1} or 0.02 g l^{-1} did not significantly affect the growth rates of the fragments 8, 12, and 16 weeks following incubations (one–way ANOVA, $df = 2$, $p > 0.05$).

4. Discussion

4.1. On the importance of labelling in cultured corals

The live coral trade is worth about US$ 7,000 per tonne (WABNITZ *et al.*, 2003) and have mainly been focusing on fast growing branched species such as species of the genus Acropora, Pocillopora, Seriatopora, and Stylophora, (YATES and CARLSON, 1992). Improving culturing traded species in both *in situ* farms and *ex situ* aquarium and integrating standardised labelling methods for captive–bred or cultivated corals are likely to improve the conservation of coral reefs. Furthermore, cultivated corals would be more adapted to "aquarium conditions" compared to wild–caught corals (BORNEMAN and LOWRIE, 2001). The trial to test a practical protocol for which all scleractinian corals could be traded is one of the major and relevant issues in coral trade and represents a fair objective. Physical supports, plastic bud vases, and recycled plastic bottle lids fixed underneath a support have been used to trade cultivated coral colonies between aquarium centres (Van Dongen–Vogels, personal observations). Although these techniques appeared to be sensible enough for trading corals, to our knowledge, they still need to be standardised.

The method used in the present work

involves incubation or immersion of fragments of different scleractinian species into a calcein solution, hence the integration of a fluorescent complex during calcification of the fragments. The use of fluorochromes represents a relatively inexpensive and non detrimental method. For example, the estimate cost to label one coral fragment is less than 0.2 to 0.4 euros (in 2005). In addition, the ability to easily observe the fluorescent label in coral fragments (i.e. as easier as checking money notes under a UV lamp) and the fact that the calcein labelling method does not require unusual, specialized equipment nor timely analysis adds to the appeal of the approach

4.2. Optimal conditions and inter–species variation

In order to obtain labelled–fragments of six different cultivated scleractinian species, six different conditions of incubation were tested during this study. Although incubation at a calcein concentration of 0.01 g l^{-1} for at least 24 hours was sufficient to obtain 100 % of labelled –fragments of *S. pistillata, Montipora* sp., *S. caliendrum* and *P. damicornis*, incubation at a higher calcein concentration of 0.02 g l^{-1} for 36 hours resulted in 100 % of labelled–fragments of all species. MARSCHAL *et al.* (2004) showed that a 0.01 g l^{-1} calcein concentration were sufficient to stain octocoral skeleton, but in other taxa such as molluscs, higher calcein concentrations were required to obtain consistent fluorescent marks, e.g. 0.10 g l^{-1} (MORAN, 2000), 0.20 g l^{-1} (RUSSEL L and MEREDITH, 2000) and 0.50 g l^{-1} (KAELHER and MCQUAID, 1999). In any case, as shown here, longer incubation times (e.g. 24 or 36 hours compared to 12 hours) improved the efficiency and the durability of the label. Similar results were observed in BARNES (1970) who incubated corals into a 20 mg l^{-1} alizarin solution for 3 to 24 hours. In previous invertebrates studies, calcein incubation times tested varied on average from 3 to 55 hours (ILAN *et al.*, 1996), but a 24 hours period of incubation was appropriate for successful labelling (MORAN, 2000 : RUSSELL and MEREDITH, 2000 : RUSSELL and URBANIAK , 2004 : MARSCHAL *et al.*, 2004).

As calcein is incorporated in the aragonite skeleton during calcification, our results are reflecting the difference in calcification rates between coral species (GOREAU *et al.*, 1996 : GATTUSO *et al.*, 1999). The absence of a visible mark on some fragments immediately after calcein incubation also suggests that no or very little calcification occurred for those fragments during incubation (WILLENZ and HARTMAN, 1999). A potential weakness of using calcein in coral trade would be its relative lack of robustness over time. BASHEY (2004) observed that in *Poecilia reticulata* calcein marks can fade within 14 days when exposed to high temperatures or sunlight. Yet the observed decrease in the ability of detecting the marks within 8 to 16 weeks is believed to result from the addition of skeletal material on top of the calcein marks (BARNES, 1972). This would suggest that while the coral is incubated, the extension of the skeleton would result in the integration of the calcein and consequently in a visible mark on the skeleton of the fragment. However, as the thickening of the skeleton is occurring after its extension, the label will then appear undetectable. Therefore increasing the growth rate of the fragments during incubation (i.e. allowing both the growth and thickening of the coral skeleton) can be suggested in order to improve both the durability and efficiency of the labelling (DAY *et al.*, 1995 : DUVIVIER, 2006).

5. Conclusion and perspectives

We showed that the use of calcein can be a potential short–term, non–destructive tool in cultivated corals in trade over wild–caught ones. However, unsuccessful calcein labelling of *Lythophyton* sp. suggests that this method is inappropriate for soft corals (Van Dongen–Vogels, unpublished observations). An alternative labelling method may rely on the use of microchips which has proved to withstand in saline water up to 6 m depth. Moreover, once incorporated in the coral fragments of *S. caliendrum, Acropora* sp. and *Montipora* sp., the microchips was still easily read by a scanner after 3 months (DUVIVIER, 2006). This technique could be interesting in the long term but because of its relatively high cost, it is still difficult to implement it on a global scale. Further studies are then needed (i) to extend the

application of the calcein labelling method to a larger number of cultivated species, and (ii) to compare different potential labelling systems as well as evaluating the cost of these systems in the live coral trade market. International standardisation of the most efficient labelling system should ultimately be made and decided through proper international regulations.

Acknowledgements

We gratefully thank the Nausicaä Aquarium (Boulogne–sur–mer, France) for the use of their infrastructure and their coral species. We also thank L. Seuront for his constructive comments and criticism of this manuscript as well as one anonymous reviewer. This research was part of a Master thesis which was partially funded by the Catholic University of Louvain (Belgium).

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Received June 6, 2007
Accepted August 6, 2007