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Improvement of live coral shipping conditions using an illuminated box

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Abstract

The transportation of live coral nubbins is a major constraint of the coral trade. Corals must be transported within a timeframe of <20 hours using concentrated oxygen to avoid high post-transportation mortality. To understand the effects of transportation water quality on coral nubbin growth and post-transportation mortality, a series of transportation simulations were performed on *Seriatopora hystrix*. Different water volumes (dry method, 125 ml, 190 ml, and 325 ml) and oxygen concentrations in the gas phase (21 %, 40 %, and 85 %) were tested, and a 24 LED Handy Lamp was added to provide light irradiance of >100 µmol m^{-2} s^{-1} in the transportation box. During transportation nubbins extracted calcium carbonate for growth reducing water alkalinity and consequently pH to 6.67. Dissolved oxygen concentration also rapidly decreased to 0.19 mg L^{-1}. The concentration of inorganic nitrogen, particularly ammonium ions, increased after 24 hours and reached very high concentrations after 48 hours. Before 48 hours, the larger the water volume and oxygen concentration, the faster the nubbin growth after transportation. Beyond 48 hours, in all cases, reduction in water quality became critical for nubbins and resulted in partial mortality. Illuminating the transportation box improved water quality for 72 hours using normal air.

Keywords: coral transportation, *Seriatopora hystrix*, light irradiance, resistance

Introduction

Over the last few decades, research on coral reefs has mostly focused on coral biology, and the impact of global climate change and ocean acidification (e.g., Salvat and Allemand, 2009; Leblud *et al.*, 2014). This was due to the unprecedented decline of coral reef health status in the majority of coral ecoregions (Wilkinson, 1998, 2008). Coral reefs continue to be mainly exploited for fishery resources and tourism, but also for commercially valuable coral species.

Despite the significant volume of the coral trade, the live coral market remains poorly understood. Aquarium shops or public aquariums constitute the main market with an increasing demand from several countries, especially the USA and Europe (Smit, 1986; Green and Shirley, 1999). Since the 1990s, the harvesting and trade of wild live corals became regulated by the Convention on International Trade of Endangered Species of wild fauna and flora (CITES). All scleractinian corals are registered in Appendix II of CITES (CITES, 2016), and CITES certificates are required for their trade.

Coral aquaculture has been developing worldwide (Ellis and Sharron, 1999; Department of Fisheries of Western Australia, 2009; Albert *et al.,* 2012, Ng *et al.*, 2012; Leal *et al*., 2013; Todinanahary *et al.,* 2017). According to data from CITES (2016), from 1990 to 2014, total traded live scleractinians reached 156,252 pieces of live aquacultured corals. This is relatively low compared to wild harvesting that represents more

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than 80 % of the total trade, compared to only 1 % for aquaculture (the source of ~19 % being unknown). These results were calculated from importer-reported quantities (data from the CITES database). The principal importer countries are the USA, followed by Japan and France. Corals are mainly imported from Fiji, the Marshall Islands, and Indonesia. Considering the large distances between the importers and exporters, shipping is one of the main constraints of the live coral trade.

With the advent of modern air travel and an increase in flight connections to remote tropical locations, the transportation of live coral across the world has become common and rarely exceeds 30 hours travel time (Delbeek, 2008). However, the COVID19 pandemic has complicated the shipping process. The transportation techniques used are determined on a case-by-case basis, and depend on coral size, stage (larva, recruit, juvenile, or adult colony), and species. A short transportation duration can result in approximately >90 % post-transport survival, using mainly semi-dry (corals are packed in wet tissue or similar) and wet (corals are submerged in seawater) transportation techniques (Carlson, 1999; Petersen *et al.,* 2005). Corals can tolerate a wide range of environmental conditions, but they usually do not tolerate a sudden change in parameters such as temperature, salinity, pH, dissolved oxygen concentration, inorganic nitrogen, and phosphorus concentrations (Osinga *et al*., 2011; Wijgerde *et al*., 2014). Light irradiance is also an important factor; its intensity influences coral symbiotic algae photosynthesis and skeletal growth rate (Marubini *et al.*, 2001; Schutter *et al.*, 2008; Juillet-Leclerc and Reynaud, 2010).

Maintaining these parameters within the tolerated range for corals requires large financial investment and production costs in coral aquaculture, especially in *ex-situ* aquariums (Osinga *et al*., 2011). Moreover, the coral trade involves relocation into a new environment (either directly or after transportation) and requires adaptation to that new environment. The adaptation process generally requires acclimation either upstream (before transportation) or downstream (after transportation), and appropriate conditions must match those in the aquariums (Delbeek, 2008). The range of physicochemical characteristics of water allowing optimal coral growth within these aquariums has been widely studied (Osinga *et al.* 2011). However, both water conditions and coral ecophysiological processes during transportation remain unclear and poorly studied. To date, most studies have focussed on coral larvae (Petersen *et al*., 2005) or large colony transportation (Petersen *et al*., 2004). In the context of the aquarium coral market, it is important to understand the variations in the physical and chemical parameters (conditions) of the transportation water to identify the main limiting factors of successful coral nubbin transportation and ensuing husbandry.

The main aim of the present study was to improve coral shipping conditions in order to increase the resistance of small size coral nubbins and decrease post-shipping mortality. The objective was to identify the main water parameters affecting the post-transportation survival and growth of coral nubbins. Experiments were performed to simulate transportation and create well-defined conditions involving parameters such as water volume, dissolved oxygen concentration, and light irradiance.

Materials and methods

Studied species

The species *Seriatopora hystrix* (Dana, 1846) was used for all experiments. This species was chosen due to its availability at the laboratory facility, and because it is one of the most common species globally, and has been one of the most studied over the last few years (Leblud *et al*., 2014).

Experimental design

All simulation experiments were performed in a laboratory using 1-L borosilicate glass bottles with a wide mouth (GL80) as nubbin transportation containers. The bottles differ from soft plastic bags used for real transportations (lighter, cheaper, and more resistant to breakage than glass), but have the advantage of a perfectly constant shape and volume. The nubbin containers, the number of which depended on the replicates and the experiment, were packaged in cardboard boxes. To better standardize the simulated transportation conditions, the boxes were kept in the dark at ambient temperature in a thermoregulated room of the laboratory (25±1°C). The ratio of water/air (air was enriched in dissolved oxygen in Test 3) in each transportation container was maintained at 1/3, except for in the dry transportation method test.

At least 10 days prior to each transportation simulation, the required number of coral nubbins +30 % were fragmented from mother colonies derived from the ECONUM artificial reef mesocosm (Leblud *et al.*, 2014). Each fragmented nubbin of ~2 g buoyant weight was

attached with a nylon line and suspended in the mesocosm for acclimation. The buoyant weight of each nubbin was measured regularly before and after the transportation, and at each sampling period. In addition, each nubbin was photographed after each buoyant weighting for colour aspect observation, using a Canon EOS50D camera (Canon Inc., Ōta, Tokyo, Japan) with a Canon Macro Lens EF 100 mm (Canon Inc.).

The nubbin used for each transportation container was chosen randomly among the acclimated ones (function sample () in the R statistical software).

The first experiment (Test 1) was performed to determine whether the wet or dry transportation method improved the health status of the coral nubbins after transportation. The wet method consisted of transporting a nubbin immersed in a ~415 mL of water (V4 hereafter), while the dry method involved an imbibed strip of a plastic bag, which wrapped the nubbin. For the dry method, the total volume of water used in each transportation container was ~100 mL. The simulation lasted 36 hours and sampling was performed every 12 hours. Triplicate transportation containers were tested for both methods, at each sampling time. The containers of the same sampling time (for both V4 and the dry method) were packaged in the same box. Control containers (without coral nubbins) were also sampled after 36 hours.

In the second experiment (Test 2), the wet method was repeated to test the influence of water volume with an extended sampling time. This experiment aimed to extend the monitoring of the influence on coral nubbin physiology. Three volumes were tested: V1 = 125 mL; V2 = 190 mL; and V3 = 325 mL. 250 mL, 500 mL, and 1000 mL borosilicate glass laboratory bottles with a wide mouth (GL80) respectively were used to maintain the ratio of water/air of 1/3. The simulation lasted 72 hours and sampling was performed every 24 hours. Triplicate transportation containers were tested for both volumes, at each sampling time. Triplicates of control containers of V3 (without coral nubbins) were sampled after 72 hours.

In the third experiment (Test 3), a water volume of 325 mL in 1-L borosilicate containers was used. Three oxygen concentrations (OC) were added to the gas phase of the containers: OC1 = 21 %; OC2 = 40 %; and OC3 = 85 %. OC1 corresponded to the natural concentration of oxygen in the air. OC2 was obtained using oxygen enriched compressed gas mix (Nitrox 40) and OC3 was produced with a mobile oxygen concentrator (Weinmann OXYMAT 3, Hamburg, Germany). The simulation lasted 72 hours and sampling was performed every 24 hours. Five replicates of the transportation container were used for each OC at each sampling time. Five replicates of control containers for each OC were sampled after 72 hours.

The fourth experiment (Test 4) investigated how the addition of a light source in the transportation container affected the nubbins, and then consequently influenced water physicochemical parameters and

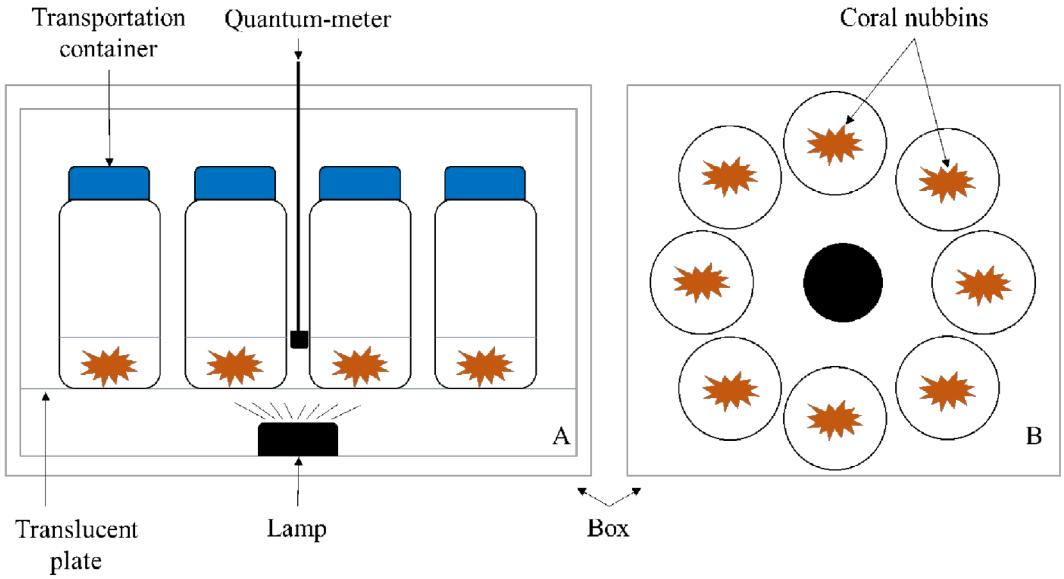

Figure 1. Schematic representation of Test 4. A. Profile view of the box; B. Location of the containers and the light, seen from the top.

post-transportation coral nubbin growth. A water volume of 325 mL and a duration of 72 hours was used. Five replicate transportation containers were established for both light (LB) and dark (DB) boxes, at each sampling time. Frigolite boxes were used to enhance the light irradiance (reflecting white walls). Five replicate control containers for both LB and DB were sampled after 72 hours. Each LB was illuminated with a 24 LED Handy Lamp (Hama GmbH, Monheim, Germany), which was powered by 3 Duracell Ultra alkaline LR20 batteries of 1.5 Volts. This light source was chosen as a representative, realistic, cheap, and single-use system that could be deployed in practice. The lamp was placed below the containers to better disperse the light in each container (Fig. 1). Light irradiance was measured from inside the boxes using a quantum meter (Apogee Quantum MQ-200, Apogee Instruments, Logan, Utah, USA). During the experiment, the intensity of the light irradiance varied from 380 at the beginning to 11 µmol m^{-2} s^{-1} after 72 hours due to battery drain. The irradiance decreased to almost 100 µmol m^{-2} s^{-1} after the first 12 hours before decreasing gradually until the end of the experiment (Fig. 2).

Water physico-chemical parameter monitoring

Salinity was measured with a Cond 340i WTW (Weilheim, Germany, 2002) salinometer. This meter was also used to measure temperature. Dissolved oxygen was measured with a pH/Oxi 340i WTW (Weilheim, Germany). Before each measurement, the CellOx 325 probe was calibrated in water-vapor-saturated air, supplied by the OxiCal®-SL air calibration vessel (WTW, Weilheim, Germany, 2002).

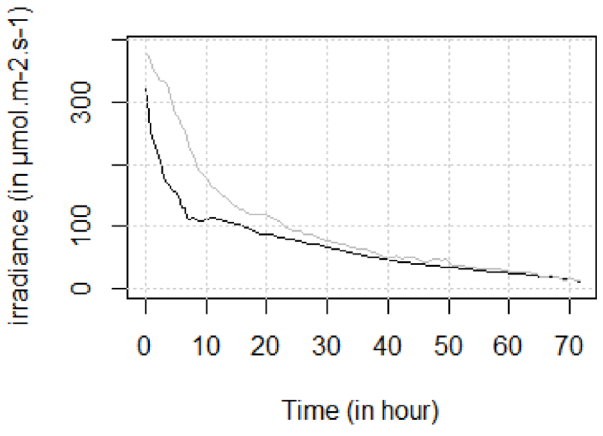

Figure 2. Irradiance variation during Test 4. Each line represents the variation from one box.

The pH_T (pH, total scale) was measured using a m-cresol pH-indicator, following a method adapted from SOP 6b (Dickson *et al.*, 2007). M-cresol colours the sample from yellow (acid) to purple (basic). pH_T is calculated from the absorbance values of the sample at three wavelengths: 548 nm; 434 nm; and 730 nm using the Spectroquant® Pharo100 spectrophotometer (Merck, Darmstadt, Germany; Merck, 2012). The wavelength 548 nm indicates the maximum absorbance for the basic form, while 434 nm applies to the acid form. The 730 nm wavelength allows for water-colour error correction. Absorbance was measured three times at both wavelengths: without m-cresol; with 50 µL; and with 100 µL m-cresol added to 5 mL of the sample. This enabled for the adjusting of the effect of the indicator addition to the real pH of the sample. Calculations were performed with R statistical software (R Core Team, 2015).

Total alkalinity was measured at each sampling time by potentiometric titration. The apparatus, measurement, and calculation methods were adapted from Dickson *et al.* (2007), and are described in Leblud *et al.* (2014).

The concentrations of nitrite (hereafter NO_2^-), nitrate ions (hereafter NO_3^+), ammonium ions (hereafter NH_4^+), and orthophosphates (hereafter PO_4^{3-}) were also determined using an automated colorimetric analysis: a Seal AA3 nutrient analyzer coupled to an XY-2 autosampler (Seal Analytical, Mequon, Wisconsin, USA). Calibrations were performed using standard solutions according to standard Seal procedures. During Test 1 and Test 2, NH_4^+ was dosed by fluorimetry adapted from Holmes *et al.* (1999), modified by Li *et al.* (2005), and refined by Stumpp *et al.* (2012). This method uses a reagent o-phtaldialdéhyde with a reducing agent (sodium sulphite) and a fluorimeter AquaFluor 8000-010 (Turner Designs, San Jose, CA, USA).

Coral nubbin growth monitoring

Weight measurements were performed by suspending the nubbins, which were immersed in seawater, to a hook connected to an electronic balance (Sartorius LE623P, precision = 1 mg; Sartorius Corporate, Goettingen, Germany). The salinity and temperature of the seawater the nubbins were immersed in during the measurements were quantified to calculate the density of the medium. The buoyant weight was converted into skeletal weight using the following equations (Jokiel *et al.*, 1978) that consider the density of the seawater and the skeleton (Davies, 1989):

$$Wstd = \frac{WB(\rho skeletal - \rho std)}{\rho skeletal - \rho sw}$$

$$WS = \frac{Wstd}{1 - \frac{\rho sw}{\rho skeletal}}$$

where, WB is the measured buoyant weight, WS is the skeletal weight, Wstd is the standard buoyant weight, ρskeletal is the skeletal density (the value used here is the density of aragonite [ρskeletal = 2930]), ρstd is the density of seawater at a temperature of 25°C and salinity of 35 psu, and ρsw is the density of seawater during the measurement.

The growth rate was calculated using the following equation:

$$k = \frac{100 \frac{WSt - WSt0}{WSt0}}{t}$$

where, k is the growth rate (% day^{-1}), WSt is the skeletal weight at time t (g), WSt0 is the skeletal weight at time t = 0 (g), and t is the time (day). Measurements were performed at the same time on each measurement day.

In addition to the growth rate measurements, the nubbins were photographed at each sampling time to evaluate their health status by comparing their colour and visual aspect. The following three main nubbin health statuses were recorded: healthy; bleached; and (partly) dead (Fig. 3).

Statistical analysis

All statistical analyses were performed using R software (R Core Team, 2015). Descriptive statistics were calculated first. The normality of the data was determined using a Shapiro-Wilk test, and the homogeneity of the variance was calculated using Levene's test. The significance of difference in means was determined using one-way ANOVA or t-test according to the number of the compared variables, with a level of 5 %. The Kruskal-Wallis test was used for non-normal data and/or data with non-homogeneity of the variances. For multiple comparison analysis, Tukey's test was used for pairwise comparisons between stations where the null hypothesis of equal means was rejected by ANOVA. The Pearson correlation was also calculated.

Results

Influence of volume, oxygen concentration, and light on water parameters

In the dry and wet method (V4), the concentration of dissolved oxygen, total alkalinity, and pH_T rapidly and significantly decreased compared to the initial values (Fig. 4A, B, C). Dissolved oxygen and pH_T decreased significantly more in the wet method within 24 hours ($p < 0.01$), while no difference was observed after 36 hours. There was a significant decrease in total alkalinity after 36 hours ($p < 0.05$), but no difference was observed between V4, the dry method, and the control containers.

The nutrient concentration results revealed a significant increase in total inorganic nitrogen (hereafter N_{ti}) in the dry method, compared to V4 and the control containers ($p = 0.0006$). This was strongly influenced by the high concentration of NH_4^+ and NO_2^- in the transportation water, which increased respectively from an initial concentration of 0.491 µmol L^{-1} (NH_4^+) and 0.160 µmol L^{-1} (NO_2^-) to 9.835 µmol L^{-1} (NH_4^+) and 0.355 µmol L^{-1} (NO_2^-) after 24 hours (Fig. 5A).

During Test 2, there was a significantly larger decrease in dissolved oxygen concentration and pH_T in V1, V2, and V3 containers compared to the initial

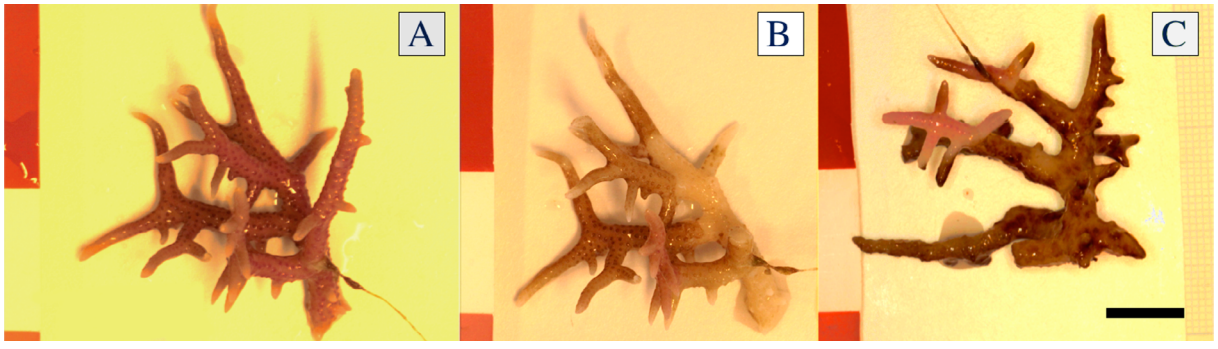

Figure 3. Colour and visual aspect of a nubbin before (A), at unpacking (B), and 2 weeks after transportation (C). A represents a healthy nubbin; B represents a bleaching/partly died nubbin; and C is a dead nubbin. Scale bar = 1 cm

concentration after 24 hours (respectively p = 0.002 and p < 0.0001) (Fig. 4D). These values remained stable for 72 hours, except in V3, in which the concentration of dissolved oxygen significantly decreased to 0.02 mg L^{-1} compared to V1, V2, and the control containers (p = 0.0003), with 3.34 ± 0.21 mg L^{-1}, 2.4 ± 0.57 mg L^{-1}, and 4.55 ± 0.62 mg L^{-1}, respectively. pH_T significantly (p = 0.0001) decreased to 7.03 ± 0.06, compared to 7.77 ± 0.01, 7.51 ± 0.03, and 7.92 ± 0.03 in V1, V2, and control containers, respectively (Fig. 4F).

In contrast to the dry method, total alkalinity gradually increased in V1 and V2 over 72 hours, while in V3, it decreased over 48 hours comparable to the dry method and in V4; however, it promptly increased after 72 hours (significant difference between 48 hours and 72 hours, with 2.24 ± 0 mmol $kg^{-soln-1}$ and 3.03 ± 0.17 mmol $kg^{-soln-1}$, respectively [p = 0.0008]).

In V1 and V2, N_{ti} significantly decreased over 72 hours (p = 0.0008), while an increasing concentration was observed in V3 from 24 hours (Fig. 5C). The overall concentration of $PO4^{3-}$ remained below 0.1 µmol L^{-1} before gradually increasing to 1.72 ± 0.16 µmol L^{-1} in V1 and V2 after 72 hours. It was sharply increased to 26.09 µmol L^{-1} (maximum) in V3 after 72 hours (Fig. 5D). This concentration significantly differed from the rest of the tested volumes and dry method (p < 0.0001).

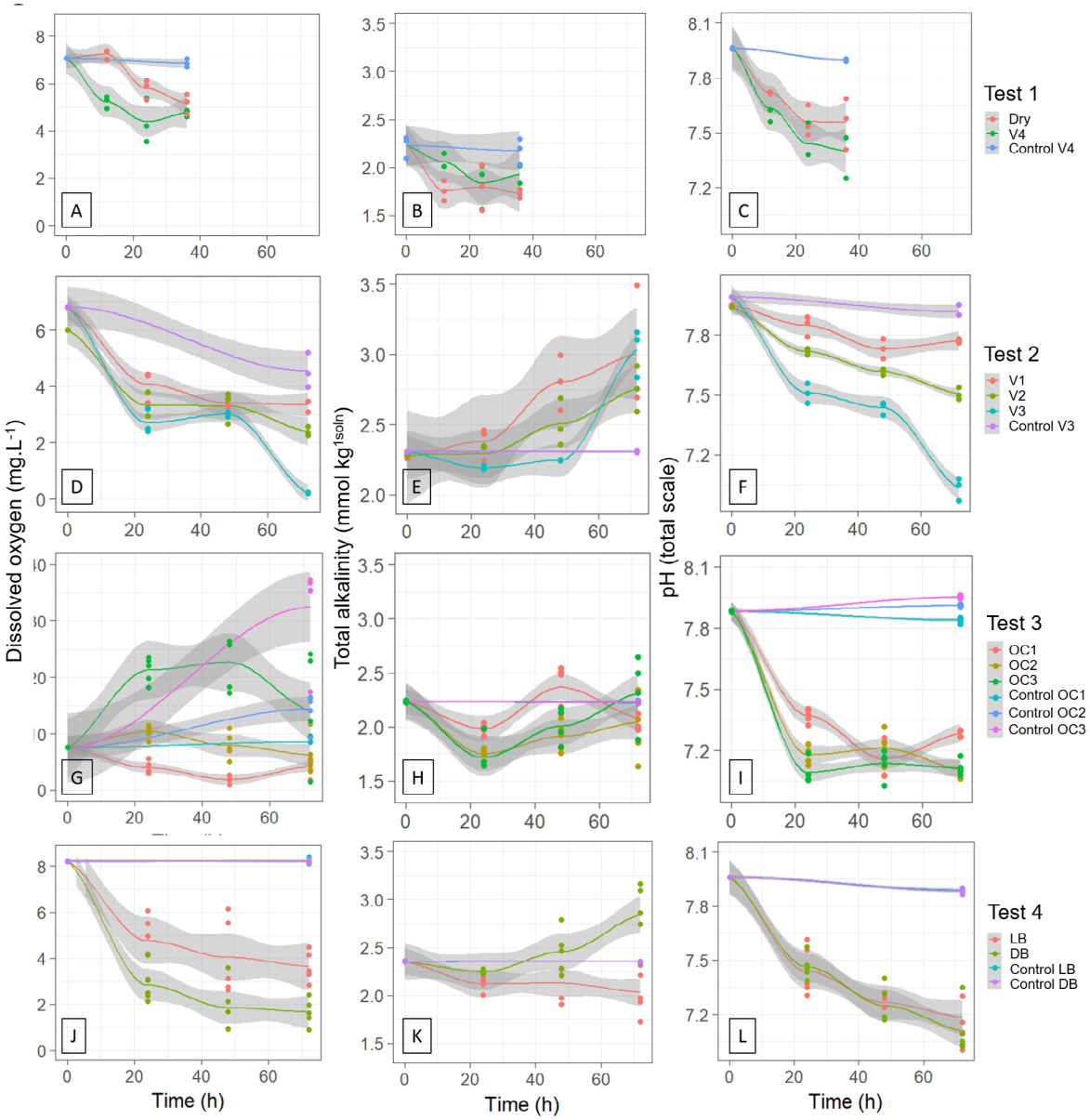

Figure 4. Changes in the dissolved oxygen concentration, total alkalinity, and pH of the transportation water. Grey shape: confidence interval; n=3 for Test 1 and Test 2; n=5 for Test 3 and Test 4. Note the different scale of the Y-axis on sub-figure G.

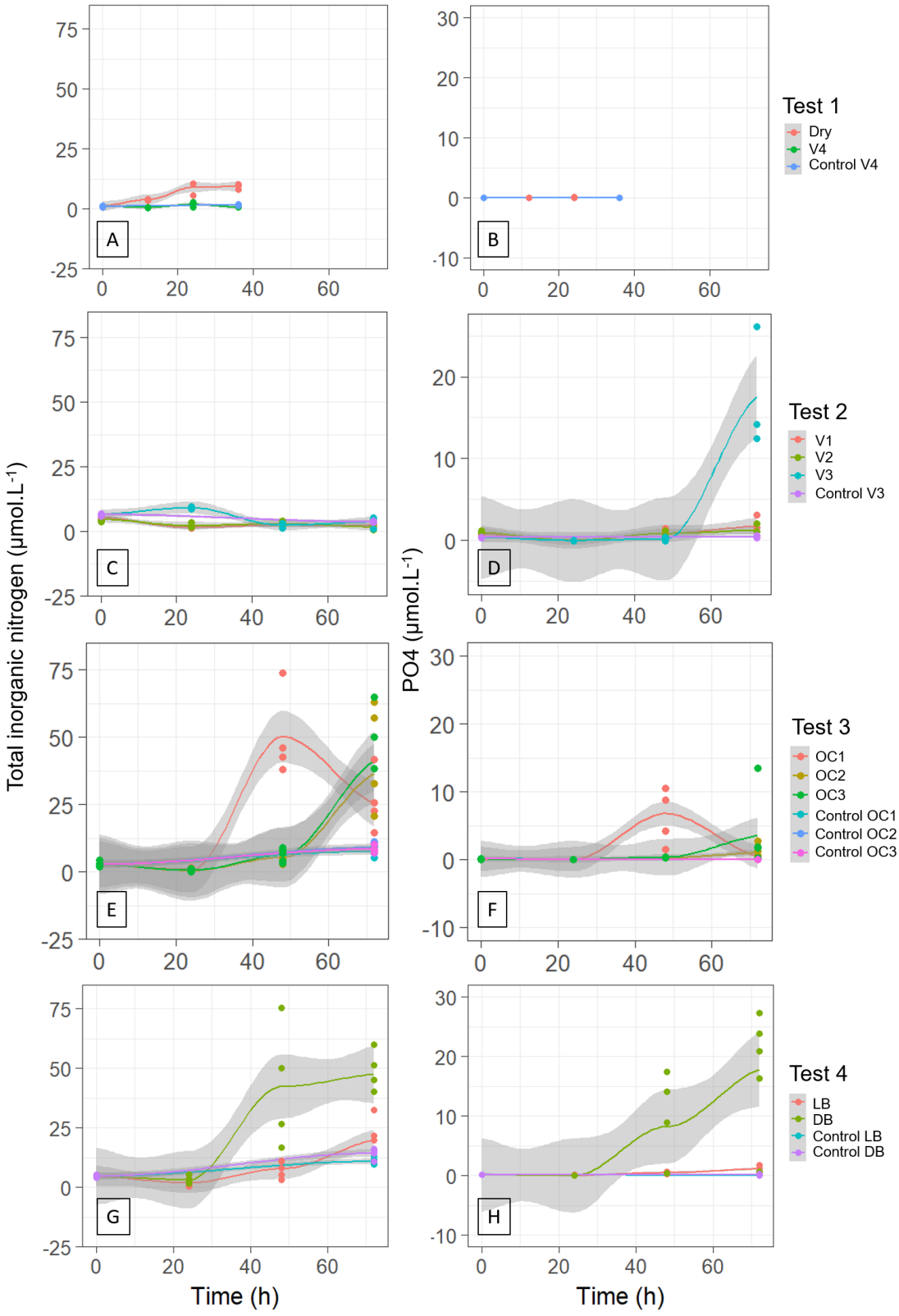

Figure 5. Variation in total inorganic nitrogen and PO_4^{3-} concentrations in the transportation water. Grey shape: confidence interval (method: loess, 95 %); n=3 for Test 1 and Test 2; n=5 for Test 3 and Test 4.

Adding a high concentration of oxygen in the gas phase to the transportation container resulted in an increase in the dissolved oxygen concentration of the water. In OC1, dissolved oxygen decreased to 1.85 ± 0.63 mg L^{-1} during transportation (Fig. 4G). A significant difference from the initial concentration was observed ($p = 0.0012$). In OC2 and OC3, a rapid and significantly increasing concentration was observed, reaching 10.4 ± 1.15 mg L^{-1} ($p = 0.0418$) and 21.29 ± 2.25 mg L^{-1}, ($p = 0.0109$), respectively after 24 hours compared to an initial concentration of 7.53 ± 0.03 mg L^{-1} in the transportation water. These concentrations stabilized until 72 hours.

Compared to an initial concentration of 2.23 ± 0.01 mmol $kg^{-soln-1}$, total alkalinity significantly decreased in OC1, OC2, and OC3, to 1.98 ± 0.05 mmol $kg^{-soln-1}$, 1.75 ± 0.05 mmol $kg^{-soln-1}$, and 1.71 ± 0.15 mmol $kg^{-soln-1}$, respectively, during the first 24 hours, before slightly increasing and matching the control values (Fig. 5H). There was no significant difference observed in the control containers at 48 and 72 hours. In OC1, OC2, and OC3, pH_T sharply decreased significantly after only 24 hours ($p < 0.0001$), but remained stable at 7.3 ± 0.19 until 72 hours (Fig. 4I).

N_{ti} also decreased during the first 24 hours, but increased to the level of the initial concentration at 48 hours, except in OC1, in which it increased to almost 20 times the initial values (from 2.85 ± 1.10 µmol L^{-1} to 56.97 ± 20.56 µmol L^{-1} ($p = 0.0002$) (Fig. 5E). A sharp increase was only observed in OC2 and OC3 at 72 hours, with 36.61 ± 23.20 µmol L^{-1} ($p = 0.0006$) and 50.40 ± 28.50 µmol L^{-1}, respectively ($p = 0.0002$).

N_{ti} were significantly influenced by NH_4^+; the Pearson correlation was $r = 0.9998$. In all containers, NO_3^+ decreased from the initial concentration while NO_2^- was stable below 0.02 µmol L^{-1}. PO_4^{3-} remained below 0.1 µmol L^{-1} before gradually increasing to 0.25 ± 0.01 µmol L^{-1} and to 0.33 ± 0.07 µmol L^{-1}, respectively in OC2 and OC3 after 48 hours (Fig. 5F). At 72 hours, the concentrations sharply increased to 13.52 µmol L^{-1} in some containers. PO_4^{3-} in OC1 showed a significant increase to 6.79 ± 3.74 µmol L^{-1} at 48 hours ($p = 0.001$), but at 72 hours it decreased to the same level as PO_4^{3-} in OC2 and OC3.

In both LB and DB, dissolved oxygen concentration rapidly and significantly decreased from the initial concentration of 8.22 ± 0.02 mg L^{-1} to 4.77 ± 1.17 mg L^{-1} ($p = 0.0424$) and 2.84 ± 0.80 mg L^{-1} ($p = 0.0144$), respectively, after 24 hours (Fig. 4J). It continued to decrease to 3.65 ± 0.66 mg L^{-1} and 1.67 ± 0.58 mg L^{-1}, respectively at 72 hours. There was no significant difference observed between the concentrations at 24 and 72 hours. There was a significant difference in total alkalinity between LB and DB ($p = 0.0148$). Both decreased during the first 24 hours ($p < 0.0001$), but then increased to 2.84 ± 0.33 mmol $kg^{-soln-1}$ after 72 hours in DB ($p = 0.0072$), and slightly decreased to 2.03 ± 0.23 mmol $kg^{-soln-1}$ ($p = 0.0259$) in LB (Fig. 4K). pH_T significantly decreased from 7.96 ± 0.0 ($p = 0.0001$) to 7.18 ± 0.14 and 7.10 ± 0.14 ($p = 0.0004$) in LB and DB, respectively (Fig. 4L).

N_{ti} was also highly influenced by NH_4^+; the Pearson correlation was $r = 0.993$. A slight decrease in NH_4^+ at 24 hours was observed in LB and DB, followed by an increase up to 19.77 ± 8.39 µmol L^{-1} and 47.46 ± 8.41 µmol L^{-1}, respectively at 72 hours. Compared to the control containers, there was only a significant increase in concentration in DB ($p = 0.0039$) (Fig. 5G). In addition, N_{ti} in DB started to significantly increase from 48 hours to 51.13 ± 29.97 µmol L^{-1} ($p = 0.0006$). There was a significant increase in PO_4^{3-} in DB containers, reaching 17.81 ± 10.44 µmol L^{-1} after 72 hours compared to an initial concentration of 0.15 ± 0.02 µmol L^{-1} ($p = 0.007$) (Fig. 5H).

Nubbin growth and survival

On the one hand, the nubbins transported during 36 hours with the wet method (V4) showed slow growth during the transportation and continued to grow thereafter, while those transported with the dry method did not completely recover to the initial growth rate (Fig. 6). Two weeks after transportation, the skeletal growth rate of the nubbins from dry transportation remained significantly lower than those transported with the wet method, with 0.42 ± 0.20 % day^{-1} and 0.62 ± 0.11% day^{-1}, respectively ($p = 0.0216$). On the other hand, during Test 2, within 48 hours of transportation, results showed that compared to the very small volume (V1), the larger water volumes (V2 and V3) allowed the nubbins to better tolerate the transportation, according to visual observation of their colour (Fig. 3B), even if their growth rate was not significantly different (Fig. 6). However, after 72 hours, nubbins from V3 were severely impacted and only a small portion of the colony survived (Fig. 3C). After transportation, the mean growth rate of the nubbins from V3 (0.27 ± 0.05 % day^{-1}) was significantly lower than those from V1 and V2 (0.50 ± 0.10 % day^{-1}) ($p = 0.017$) (Fig. 6).

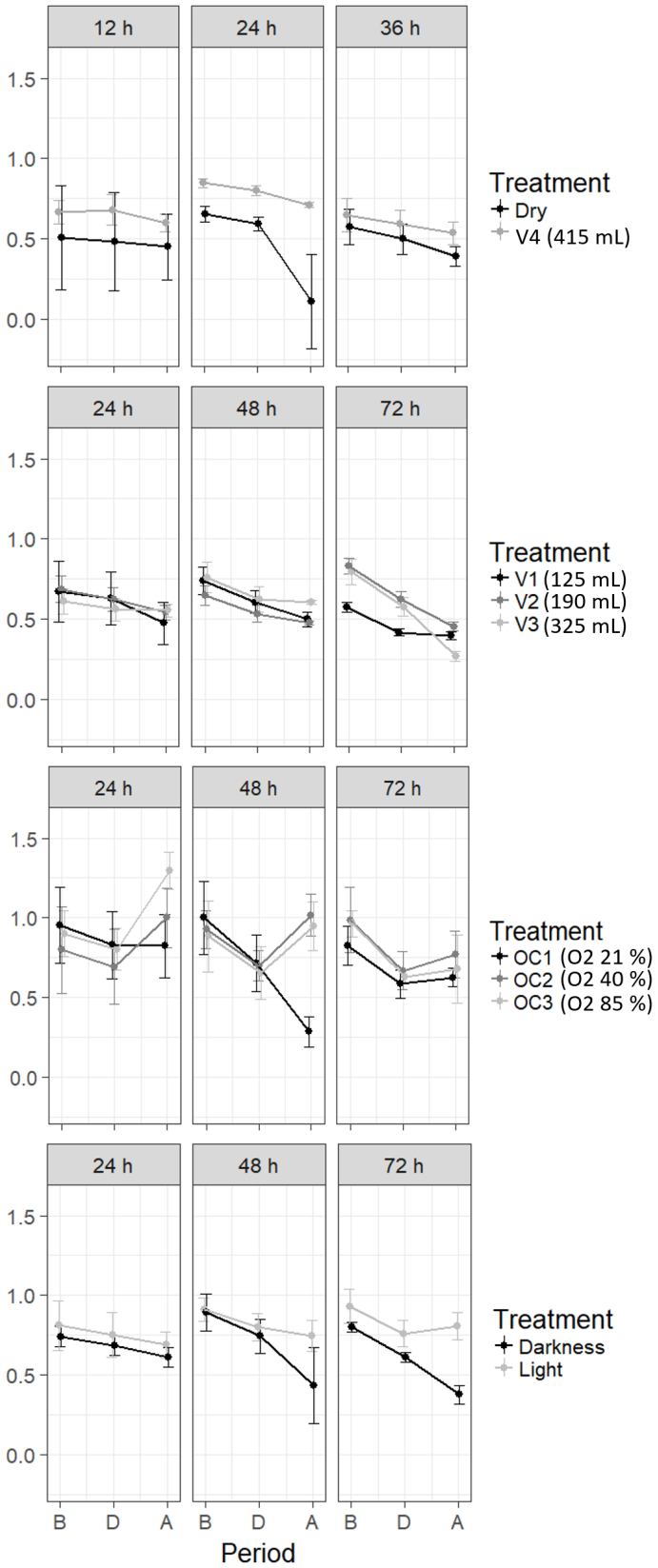

Figure 6. Skeletal growth rate (% day^{-1}, mean ± standard error, n=3 for Test 1 and 2, n=5 for Test 3 and 4) of the nubbins before (B), during (D), and after (A) transportation. Each subfigure corresponds to the nubbin transportation duration (12, 24, and 36 hours for Test 1; 24, 48, and 72 hours for Tests 2, 3, and 4).

Two weeks after transportation, nubbins transported in OC1 showed a significantly lower growth rate (0.58 ± 0.31 %day^{-1}, p = 0.0028) compared to those in OC2 (0.93 ± 0.28 % day^{-1}) and OC3 (0.97 ± 0.37 % day^{-1}) (Fig. 6). This low rate was highly influenced by the near-mortality of >50 % of the nubbins transported during 48 hour or longer. Sixty percent of the nubbins from both OC2 and OC3 presented some bleaching but recovered a few days after transportation and their growth rate remained higher than those from OC1.

Two weeks after transportation, the growth rate of the nubbins transported in LB (0.75 ± 0.15 % day^{-1}) was significantly higher than those transported in DB (0.47 ± 0.25 % day^{-1}) (p = 0.0013) (Fig. 6). This difference was particularly obvious when the duration of the transportation exceeded 48 hours. In LB, the longer the transportation duration, the higher the growth rate, while in DB the opposite effect occurred. Sixty percent of the nubbins transported during 48 hours in DB bleached, but had completely recovered 2 weeks later; however, 80 % of those transported for 72 hours showed part mortality (Fig. 3B, C; Fig. 6).

Discussion

The present results show that water volume and oxygen concentration, and the addition of a light source in the shipping box, differentially influence the health status and post-transportation growth of coral nubbins. They improve the understanding of how the coral uses the chemical and organic elements presents in the transportation water, and how these elements vary during transportation and effect coral holobiont physiology, as reported in previous studies (e.g., Osinga *et al*., 2011; Wijgerde *et al*., 2014). When using normal air, dissolved oxygen and pH rapidly decreased during the first 24 hours. The addition of a high oxygen concentration addressed this problem, but it did not prevent a decrease in pH in all tested conditions. These results support the findings of Wijgerde *et al.* (2014) highlighting the important role of oxygen in coral calcification. The authors suggested that within the current (natural seawater) oxygen and pH range, oxygen exerts a substantial control on coral growth, whereas the role of pH is limited (Wijgerde *et al*., 2014). Indeed, the results from the present study showed that, even without the light source, the transported corals continued to grow slowly during the first 24 hours, and steadily consumed the dissolved oxygen. This also led to a decrease in water alkalinity due to skeletogenesis. Without the possibility of zooxanthellae performing photosynthesis, the dissolved oxygen concentration continued to decrease, and the resulting modified carbonate system lowered the water pH (Kleypas *et al.,* 1999).

Coral colonies can survive and grow in a well-conditioned small volume of water such as a chemostat, but only if these conditions are maintained for a given period (Leblud, 2015). In the present study, except in the presence of light, the concentration of inorganic nitrogen, especially ammonium ions, increased dramatically beyond 24 hours. With the critically decreased pH, the level of inorganic nitrogen continued to increase due to degradation of parts of the coral tissue in some cases. The part mortality of the coral also implies skeleton dissolution and a subsequent increase in alkalinity. This mainly occurred after 48 hours of transportation, in dark containers; which confirms the conclusion by Gattuso *et al.* (1999), stating that coral calcification is, on average, three times higher in the light than in the dark.

The lower post-transportation growth rate of the coral nubbins with the dry method suggests that it was not appropriate for small nubbins compared to the wet method. Indeed, several real transportation experiences propose the use of the dry method only for large colonies (~18.3 cm), with respect to a particular preparation and packing system (Petersen *et al*., 2004). However, in contrast to the results of these experiences, in the present study, all small nubbins survived the transportation and changes in coral health status were only noticed when the duration exceeded 24 hours. This can be explained by the appropriate post-transportation conditions in the mesocosm that provided sufficient light irradiance to allow rapid recovery (Leblud *et al.,* 2014). It is important to note that, in these transportation experiments, the pre- and post-transportation conditions were identical (same as the mesocom). Thus, coral nubbins did not have to deal with, for example, different seawater chemistry or different pathogen strains when acclimatizing to the post-transportation conditions. While this increases post-transportation success, it also ensures that the changes observed in coral nubbin growth are due to the transportation itself, rather than other factors, which is what the present study aimed to investigate.

With the wet method, the use of a larger volume and normal air is suggested when the transportation duration does not exceed 48 hours, otherwise the sudden changes in parameters result in a markedly higher nubbin mortality (Osinga *et al*., 2011). Nubbins transported

in smaller volumes during 72 hours presented a lower post-transportation growth rate, but better health status compared to those in larger volumes, suggesting that using a small volume of water is much more profitable. This result is counterintuitive, and it is not easy to elaborate upon a hypothesis to explain such observations. Since both oxygen and pH decreased at a slower rate in the smaller volume, it seems that nubbin metabolism is rapidly limited, resulting in better oxygenation and pH values over a longer term in the transportation water, which would be overall less stressful to the coral. Further investigations are required to confirm these results and to determine the optimal water volume/nubbin size ratio. A previous study proposed to increase the volume of water to 70 % when packing corals for more than 20 hours and to reduce the amount of oxygen to decrease the risk of oxygen toxicity (Delbeek, 2008). The present results clearly do not support this hypothesis, except when the transportation time exceeds 48 hours. For shorter transportation, there was a marginal effect of water volume only. In addition, the results from this study suggest that the use of a high oxygen concentration with larger water volumes is only suitable for a transportation duration under 48 hours. The use of an oxygen concentration greater than 40 % may induce high mortality or reduce nubbin recovery capability post-transportation. However, the use of 100 % oxygen represents a huge financial investment in coral shipping (Osinga *et al.,* 2011).

This is thought to be the first study to propose the addition of a light source. Gattuso *et al.* (1999) reported a very strong correlation between photosynthesis and calcification at the organism level for corals. The results from the present study seem to support the hypothesis that calcification is dark-repressed rather than light-enhanced (Gattuso *et al.*, 1999). However, the addition of a light source may be logistically problematic in airplanes, where electronic devices cannot be transported switched on, although it should be possible to design certified systems that are safe for airplanes. The concept of adding a light source to the container is to trigger coral zooxanthellate photosynthesis during transportation to (partly) recycle oxygen, nitrogen, and phosphorus, and to attempt to stabilize the pH. This effect relates only to photoautotrophic organisms that can utilize light (Shi *et al.,* 2012).

Adding a light source in the transportation box greatly influenced the survival and growth of the nubbins. These results suggest that adding sufficient light in the transportation box prevents the need to use pure oxygen and instead permits the filling of the containers with much cheaper normal air. The cost of a single-use LED light in addition to a couple of cheap batteries is not much higher than the price of using pure oxygen in the bags. This technique also potentially allows an increase in the transportation duration for up to 72 hours, and may be useful for transportation to remote locations, or as a safety margin in more traditional transportations (for instance, to mitigate against flight delay, which can be a common issue). Indeed, it has been shown that with a relatively higher water volume and use of normal air, the post-transportation status of coral was better and the growth rates were higher compared to the other conditions. In this study, 24-LED Handy Lamps were used, one per box, which are readily available and cost less than 20 Euros (including batteries). However, the use of a supplementary accessory and a greater water volume will result in extra weight in the transport box. Several coral farming projects, especially in Madagascar, have stopped their production due to, among other reasons, frequent major loss occurring because of missed flight connections. This illuminated box method may represent a solution for such cases.

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