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Research article

Maintaining natural spawning timing in *Acropora* corals following long distance inter-continental transportation

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Abstract

The majority of research focusing on coral reproductive biology (e.g. spawning timing and synchrony) is carried out in facilities adjacent to reefs that the corals originated from. This is in part because transporting corals over long distances by air leads to sub-lethal stress that may confound the results of any experimental study. However, these constraints often mean research associated with coral reproductive timing is restricted to relatively few locations. To assess the potential for studying environmental drivers of spawning timing in corals in captivity (defined here as ex-situ closed aquaria), 14 large (16–37 cm) *Acropora hyacinthus* colonies were transported from reefs in Singapore to a closed aquarium system in London (a journey time of ~34 hours). Collection was purposefully timed to occur just before the predicted annual mass spawning event and on the day of transportation it was noted that 12 of the 14 corals contained large visible oocytes. The 'inverted submersion method' was applied and the water used for transport was buffered to ensure the colonies remained healthy throughout their travel time. At the end location, all colonies were placed in a purpose-built aquarium research system which allowed for the approximation of the environmental conditions found on the fringing reefs south of Singapore (the original location). While three colonies appeared partially bleached (visibly pale) and one colony suffered from partial tissue loss, all colonies (i.e. 100% of those collected) were still alive at the time of writing (28 months post collection). More importantly, all corals that were gravid at the time of collection spawned ex situ within the same lunar month as those in the wild (within 3–4 nights of each other). This paper describes the procedures for carrying out long distance transportation of large gravid broadcast spawning coral colonies from reef sites to public aquariums or research facilities around the world for the purpose of ex-situ spawning research.

Introduction

Research on scleractinian coral reproduction is a prerequisite for the study of other life-history strategies associated with any given species, along with understanding the ecology and persistence of populations and communities and the management and preservation of reefs (Rapuano et al. 2017). Yet, despite over three decades of research into the reproductive biology of broadcast spawning coral, conflicting views remain regarding the putative cues that drive these annual events (Keith et al. 2016; van Woesik et al. 2006; Penland et al. 2004). Conducting in-depth research in controlled ex-situ environments (i.e. the use of mesocosms) is one approach to detangling the complex patterns seen in natural systems (Fordham 2015). However, conducting research in this way when exploring reproduction of broadcast corals has presented numerous challenges. The first challenge is driven by the fact that many of the coral species in question have long gametogenic cycles (Wallace 1985; Shikina 2012) and exhibit only single annual spawning events (Richmond and Hunter 1990; Penland et al. 2004; Kenyon 1995; Guest et al. 2002, 2005; Chelliah et al. 2014; Baird et al. 2001; Hayashibara et al. 1993; Vicentuan et al. 2008). As a result, the majority of research in this field is conducted at locations close to the reefs of origin of these corals (Harrison et al. 1984; Okubo et al. 2007; Negri and Heyward 2001).

The process of spawning has been shown to be affected by the physiological condition of the coral (Baird and Marshall 2002), in addition to exogenous timing cues which appear specific to any given location (Babcock et al. 1986; Kaniewska et al. 2016). However, little is known about how handling and transportation of the corals for use in ex-situ experiments

affect the synchrony and spawning success. If it were possible to show that corals could be successfully transported over both long and short distances, and without having any detrimental effect on the reproductive cycle, this would enable experiments to be conducted in a wider range of locations and for repeat experiments on the same colonies, thus expanding this field of coral biology. Here, researchers can turn to the field of coral aquaculture, zoos, aquariums and the hobbyist trade for guidance. However, even in these instances, the 'trade' appears to often rely on bulk transportation strategies rather than by ensuring a high level of fitness at the end point. Indeed, in certain instances issues associated with bacterial loading during transportation have resulted in massive disease outbreaks (Delbeek 2008). Traditionally, two packing techniques are used when transporting corals over long distances: the 'wet packing method' and the 'dry packing method' (Carlson 1999; Delbeek and Sprung 1994; Delbeek 2008; Petersen et al. 2002). The majority of shipments employ the wet method and, in many cases, corals shipped from coral farms and public aquariums are mounted onto bases which are suspended upside down in the transportation bag attached to a Styrofoam® float (Delbeek 2008). Referred to as 'inverted submersion' (Calfo 2001), the Styrofoam® raft is larger than the suspended coral, preventing contact with the sides of the transportation bag and preventing damage during transit. In this study, the potential of the inverted submersion method for transporting gravid colonies of *Acropora hyacinthus* over long distances (~34 hours travel time) is assessed. Transportation success is measured by comparing the health status and synchrony of the spawning events in both the ex-situ mesocosm and the parental colonies which remained on the reef.

Materials and methods

Species and study site

Acropora hyacinthus is a common reef-building species that is found on reef slopes or reef flats throughout the Indo Pacific. It forms flat wide plates (over 3 m in diameter) and/or tables or tiered aggregations of smaller plates (Veron 2000). For this study, all colonies were collected from two reefs south of mainland Singapore (Kusu reef and Pulau Satumu). In order to collect and ship live corals, the appropriate research permit was sought from the National Biodiversity Centre, National Parks Board Singapore (NP/RP14-115) and a CITES import (permit number: 532422/01) and export permit (permit number: 15SG006834AE) were also obtained for the shipment.

Stage 1: Local transportation and preparation for international transport

The collection of *A. hyacinthus* for this study was timed to be within 1 month of the actual spawning times in the wild (Guest et al. 2002, 2005), so that some of the corals would be gravid during transport and that the all corals would be in the late stage of oocyte development (Wallace 1985), to reduce the chance of oocytes being reabsorbed.

On 27th February 2015, 10 *A. hyacinthus* colony fragments were removed from parental colonies at Kusu reef using a hammer and chisel. No more than 10% of the total surface area was taken from any one colony, as this has been shown to have minimal impact on the parental colonies' health status (Epstein et al. 2001). Parental colonies were primarily selected based on the overall health of the colony (i.e. no tissue recession or bleaching). Furthermore, each colony sampled was separated by more than 8 m (horizontally along the reef), in order to minimise the chance of sampling genetically similar colonies (Ayre and Hughes 2000). The collected fragments were measured and photographed and the parental colonies were tagged (AH1–10). The fragments were moved to a temporary nursery located at approximately the same depth as the parental colonies (~3 m). The nursery was constructed with an aluminium angle bar and colony fragments were attached using cable ties. Fragments were left in the nursery for 18 days prior to transportation (Figure 1) to allow damage caused during fragmentation to heal.

One day prior to transportation, the water and packing materials were assembled. A reservoir for 'de-sliming' and a reservoir for packing water were prepared with 5 micron filtered seawater. The packing water was sterilised with a UV steriliser for 24 hours to reduce bacterial levels. Sodium bicarbonate was added to raise the alkalinity to 9 dKH (3.214 meq/l or 160.714 ppm CaCO3) to minimise pH changes during transport. On the day of shipment (17th March 2015), four additional colony fragments were removed from a further four parental colonies at Raffles Lighthouse Reef (Pulau Satumu) (AH11–14) using the method described above,

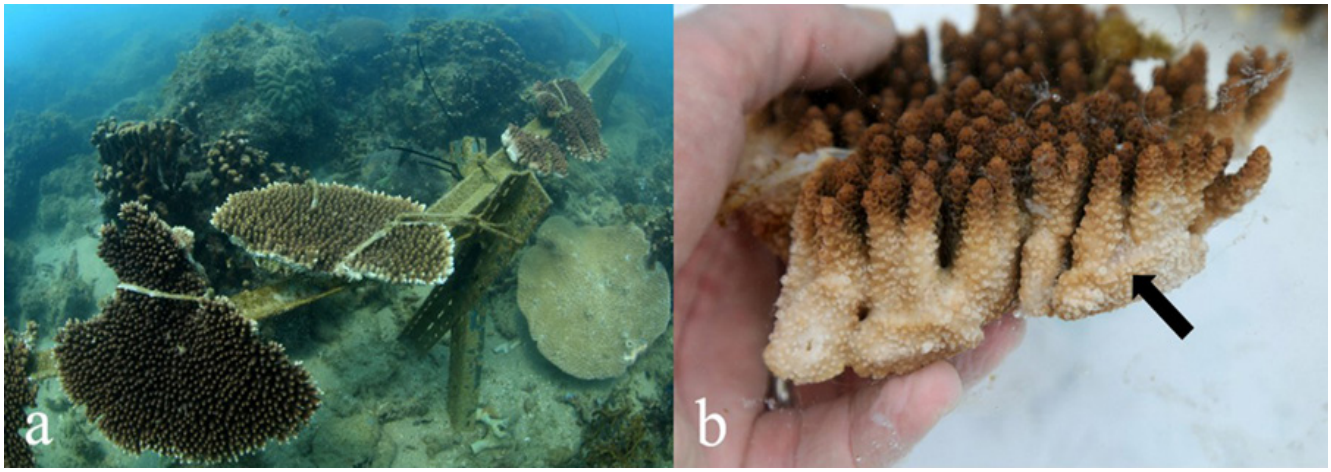

Figure 1. a) *Acropora hyacinthus* colony fragments attached with cable ties to temporary nursery and b) illustrating the new epithelial tissue (black arrow) which grew over all areas of exposed skeleton in the 18 days from initial removal from parental colony to date of transportation.

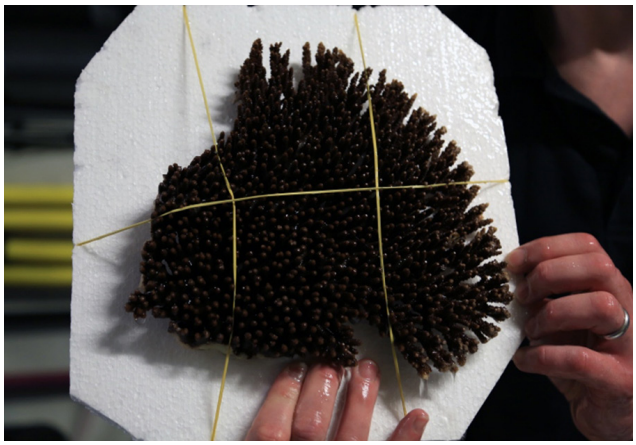

Figure 2. Large colony fragments are attached to Styrofoam® floatation rafts with elastic bands and floated upside down in the transportation bag.

and the 10 original colony fragments were collected from the temporary nursery site at Kusu reef. Any visible microfauna were removed from the coral colonies to prevent fouling of transport water. All colonies were transported back to the regional public aquarium (the S.E.A. Aquarium, Sentosa Island, Singapore) in insulated boxes fully submerged with seawater and covered with a lid. During this stage of the transportation, all fragments produced substantial amounts of mucus. Mucus was therefore removed from the containers and additional seawater added before the next stage of transportation. Temperature and pH were analysed during this period (using YSI Pro1030) and multiple water changes were also conducted based on the results and quantity of mucus being produced. Ice-filled plastic ziplock bags were floated inside the boxes in order to maintain a constant temperature during transit. The total transport time from collection to arrival at the local aquarium was 1 hour for AH 1–10 and 3 hours for AH 11–14. Upon arrival the transport water was slowly exchanged with filtered seawater to acclimate the colonies.

Stage 2: International long-distance transportation and acclimation procedure

Each colony fragment was attached, upside down, to a 25-mm thick Styrofoam® floatation raft with three or four large thin rubber bands (Figure 2). Floatation rafts were made larger (<5 cm diameter) than the coral itself to prevent the coral from touching the sides of the box, as this has been shown to cause damage that can result in secondary bacterial infections (Delbeek 2008). Attached corals were then suspended upside down in the 'de-sliming' reservoir for 20–30 min, allowing them to release the mucus produced as a result of handling, and thereby ultimately reducing the amount of mucus released during the next stage of transportation (Delbeek 2008).

Styrofoam® packing boxes were prepared with 15 l of buffered, sterilised seawater in a 61 cm (B) × 107 cm (L) plastic bag (note: corals are always double bagged in case of leakage). The initial water parameters in these bags were as follows: temperature 27.5°C; dissolved oxygen 96%; pH 7.97; and alkalinity 9 dKH (3.214 meq/l). Corals were packed separately and placed in the bag upside down, the Styrofoam® ensuring the corals float upside down. Pure oxygen (100%) was added before sealing in an oxygen-to-water ratio of between 1:2 and 1:3. Two heat packs were taped to the lid of the box to maintain temperature during the flight to the UK. Boxes were sealed and labelled with the appropriate parental colony tag number and sent for transport with a freight forwarder.

Table 1. Water chemistry post-transport from the S.E.A. Aquarium (Singapore) to the Horniman Museum and Gardens (London, UK).

Coral ID	Temperature (Celsius)	Salinity (ppt)	pH	Dissolved Oxygen (%)
AH1	24.6	32.2	6.94	304
AH2	25.6	32.2	7.03	293
AH3	24.5	32.2	7.08	283
AH4	27.0	32.2	7.02	313
AH5	26.1	32.2	7.07	208
AH6	26.2	32.1	6.93	258
AH7	24.8	32.2	6.93	260
AH8	26.7	32.2	6.95	214
AH9	26.0	32.1	7.40	336
AH10	25.7	32.1	7.05	250
AH11	-	-	-	-
AH12	26.3	32.2	7.16	288
AH13	28.2	31.3	7.16	123
AH14	25.7	32.1	6.97	271

Upon arrival in the UK (~34 hours of travel time), the corals were immediately unpacked and the water parameters, temperature, pH (Hach Lange HQ11d), and dissolved oxygen (OxyGuard, Handy Gamma) were tested (Table 1). Colony AH11 was transferred directly to a coral research system without acclimation, due to the amount of zooxanthellae released during transit and the subsequent dark brown water (Figure 3). The remaining corals (AH1–10, and 12–14) were acclimated to aquarium water for 2 hours, using 6 mm silicone tubing with system water being added at approximately 80 ml/min (0.5% of transportation water) via a gravity siphon. Acclimation was determined to be complete once transportation water closely matched system parameters (28.7 °C, NH^3 0 mg/l, NO^2 0 mg/l, NO^3 0.02 mg/l, PO^4 0.035 mg/l, 32 ppt salinity, pH 8.1 and alkalinity 7 dkh). Colonies were then attached to pieces of live rock using cable ties and transferred to the 1200 l coral research system. Internal water movement was provided by four internal flow pumps (Jebao RW-20), each rated to 20,000 l/hour. The coral research system was specifically designed for broadcast coral spawning research and has the ability to accurately replicate environmental parameters associated with broadcast coral spawning, including seasonal temperature change, photoperiod and lunar cycle and annual solar irradiation changes of Kusu reef (Craggs et al. 2017).

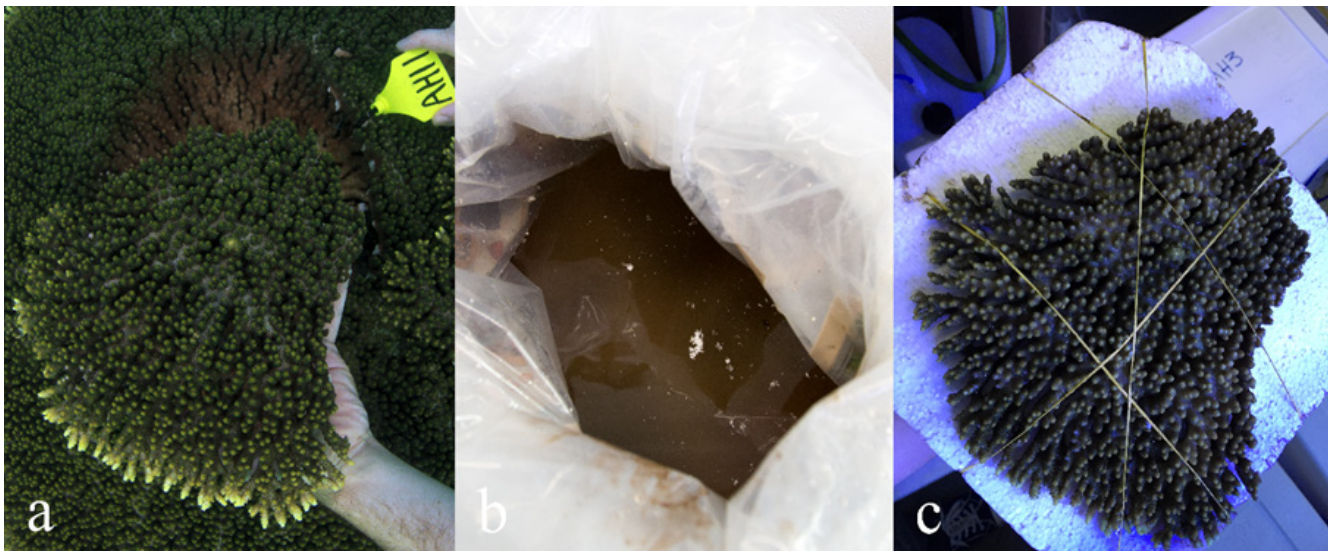

Figure 3. a) Colony fragment AH11 recently removed from the parental colony. Parental colony tag seen in top right. b) Packing water at the end of the 34 hour shipment showing high levels of zooxanthellae expulsion. c) Colony on arrived in UK with pigmentation loss during transportation.

Two days after arrival, daily heterotrophic feeds were presented consisting of yeast culture (16 ml/1,000 l), AcroPower by Two Little Fishes amino acid solution (16 ml/1,000 l), live phytoplankton Tetraselmis spp (200 ml/1,000 l), live Artemia salina nauplii (300 nauplii/l) and frozen Brachionus plicatilis (1,000–2,000 nauplii /l). During feeding the holding tank was isolated from the filtration for approximately 2 hours to aid feeding uptake.

Histological sampling to assess egg development

Three individual coral fragments (<3 cm in length) were taken from randomly selected areas of each colony on three occasions (25th March, 4th April and 23rd April 2015). Care was taken to avoid sterile zones on the periphery (Wallace 1985). To check for the presence of oocytes, cross sections of each sample were photographed using a Canon 5d MKIII with MP-E 65 mm lens set to ×5 magnification. Cross sections were illuminated using a Schott KL1500 LCD cold light source. Kelvin temperature of both light source and camera were calibrated to give true subject colour rendition. After imaging, the samples were then preserved in 10% formalin and histological sections were prepared and stained with haematoxylin and eosin following methods described by Chornesky and Peters (1987) (International Zoo Veterinary Group).

Results

Preparation for transport

All corals were prepared as planned, except one, AH7, which was too large to be floated in the bag as the coral touched the bottom when upside down. This colony was packed upright, fully submerged.

Transportation and acclimation procedure

The coral shipment arrived after a total transport time of 34 hours from the initial time of collection (reef) to when they were unpacked at the final destination (the Horniman Museum and Gardens, UK). The pH, temperature and dissolved oxygen varied

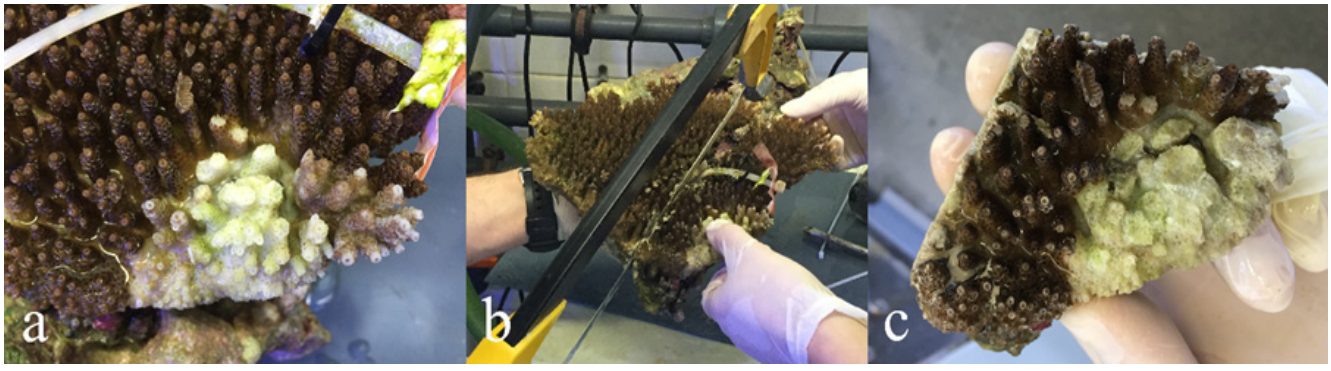

Figure 4. a) White syndrome on AH10 13 days post transportation. b) Infected area being removed with a hacksaw. c) Removed infected fragment including healthy band of tissue around the infection.

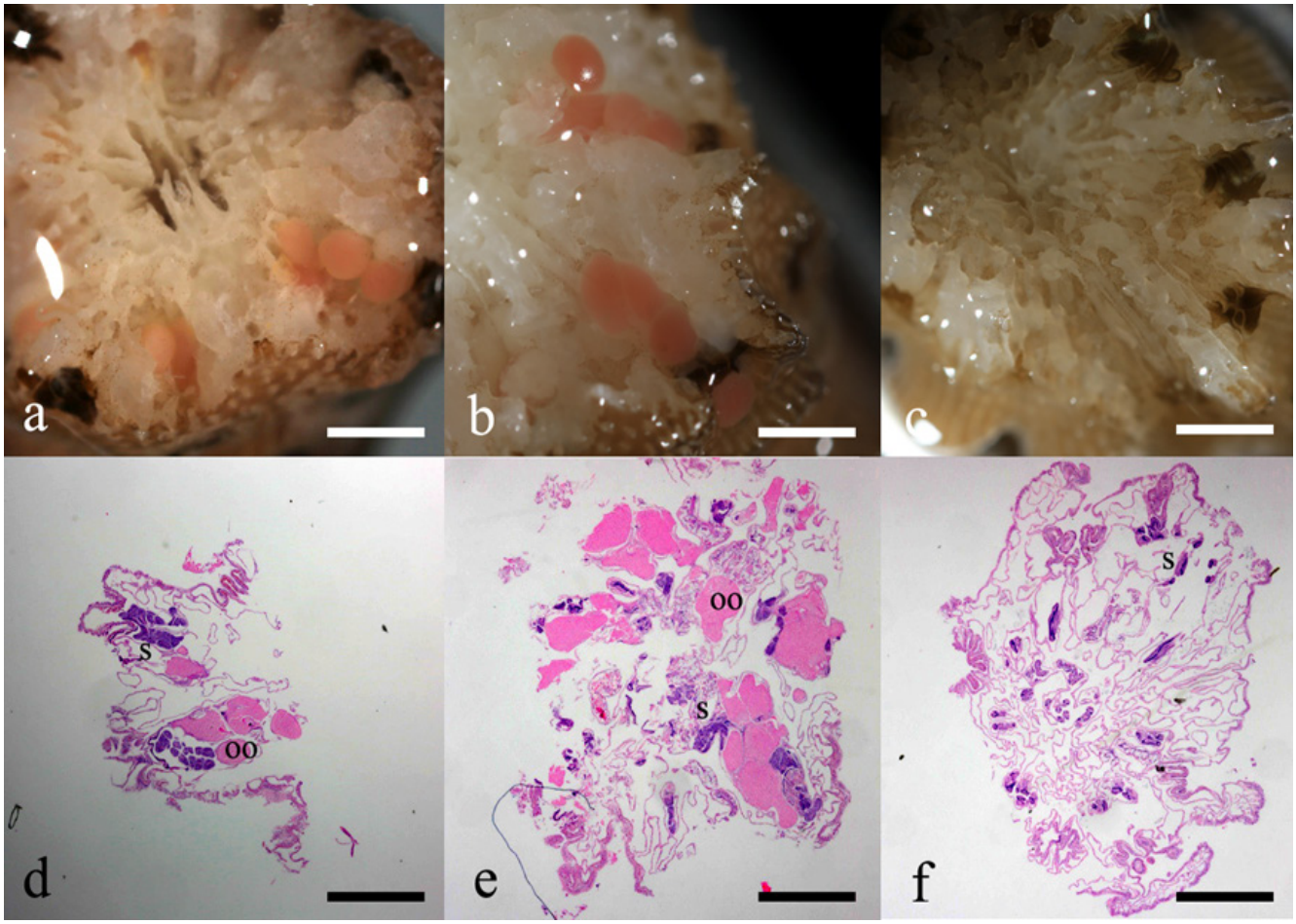

Figure 5. Three fragments were removed from each colony (AH1–14) on three separate dates: 25th March, oo - ooctyes in late stage of development (a and d), 4th April, oo - oocytes in late stage of development (b and e) and 23rd April 2015, oocytes absent (c and f). Here, colony AH9 is represented. Scale 1 mm.

between transportation boxes (Table 1). Temperature ranged from 24.8–28.2, pH 6.93–7.40, and dissolved oxygen 123–336%. All colonies released their algal symbionts (or Symbiodinium) to some degree however this varied from colony to colony. The most severe case was regarding AH11, whereby the packing water was visibly dark brown in colouration (Figure 3). As such, AH11 was immediately removed and placed in the holding tank due to concerns for colony health. Furthermore, one bag was punctured during transit (containing AH13); however, the colony did not appear to show any physical damage.

Post transportation survival rates and colony health

Pigmentation was deemed to have returned to 'normal' after approximately 2 months based on comparisons between photographs taken of parent colonies at the time of collection and the fragmented colonies in the tanks.

One day after arrival in the UK colonies started to exhibit the first signs of polyp extension, with full extension occurring within a further two weeks across all colonies. Colony AH10 suffered a disease outbreak, similar in appearance to white syndrome (Sweet et al. 2013), 13 days after transport. The infected area was cut away using a hacksaw, clearing the infected tissue by ~3 cm (Figure 4) and the infection was immediately halted with full healing occurring 3–4 weeks after the event.

A total of 13 hairy coral crab, Cymo andreossyi (Audouin 1826), were removed from the corals. Despite this species having previously been described as an obligate commensal of branching corals (Hogarth 1994), these crabs were seen feeding on the coral tissue, causing damage, manifested as denuded areas of skeleton. Despite partial mortality due to disease and predation (by the hairy coral crab, *Cymo andreossyi,* Audouin 1826), all colonies are still alive at the time of writing (28 months post transportation).

Histological sampling to assess egg development

Coral samples upon arrival showed numerous orange or pink pigmented oocytes (Figure 5), in 12 out of 14 colonies (Figures S1 and S2) which were in the late stages of oogenesis at time of transportation (Wallace 1985). Colony AH9 spawned ex situ at 2110 on 10th April 2015, six nights after full moon (NAFM) and colonies AH1, 2, 4–6, 8 and 10–14 spawned on 12th-13th April 2015, 8–9 NAFM. These latter instances of spawning were indirectly observed as evident by the turbid water within the research aquaria the morning after egg/sperm bundle release. This was later confirmed by checking the cross sections of the above colonies 10 days after spawning as there were no eggs seen in these corals indicating the corals had spawned (Figure S3).

In-situ observations from Raffles Lighthouse Reef on 3–6 NAFM (7th–10th April 2015) confirm 20 species of scleractinian coral spawned on 4 NAFM and Acropora species 6 NAFM (K. Tun pers. onal communication).

Discussion

This study was successful in the transportation of large gravid colonies of *Acropora hyacinthus* during a period of ~34 hours travel time from Singapore to the UK. All colonies were alive at the time of writing with only minor signs of compromised health recorded during the whole process. The conditioning of corals prior to shipment, involving the removal of excess mucus, has been suggested to be critical in reducing bacterial load during transport (Delbeek 2008). *A. hyacinthus*, as with all corals, produce a large amount of mucus when stressed (Brett personal observation). When being transported, this mucus remains closely associated with the coral surface most likely causing further stress and possibly exposing the corals to potential pathogenic organisms. Previous studies have documented dramatic shifts in the coral surface mucus (CSM) microbiome over short and long time periods (Sweet et al. 2017; Williams et al. 2015), and a change in the composition of the mucus when corals are stressed (Lee et al. 2016). Other studies have drawn the link with opportunistic coral pathogens associated with the CSM and this, together with the stressed state, may explain the onset of disease in one of the colonies post transportation (Banin et al. 2001; Rosenberg et al. 2007; Glasl et al. 2016). However, few studies have explored shifts in the microbiome regarding transportation of corals and this warrants further study to see if this part of the process could be improved in the future. For example, the use of beneficial coral microbes (BCM) has recently been suggested to maintain reef health (Peixoto et al. 2017), suggesting such inoculation during transportation to be a potentially interesting aspect to explore, although there are risks of such procedures in nature, which must be considered (Sweet et al. 2017). The study by Delbeek (2008) also suggested the idea of using a more extreme conditioning procedure before long distance transportation, whereby the corals are intentionally stressed in order to release their mucus reserves. This was not tested in this study as our aim was to successfully transport gravid corals to the UK; however, a smaller, targeted study could explore these options to improve the technique described in this study.

Another common cause of stress in scleractinian corals is physical damage (Chabanet et al. 2005). Here, the physical damage caused during transit was minimised by inverting the corals and attaching them to polystyrene floats. This allowed for the transportation of much larger colonies than previously documented, although Petersen and colleagues (2004) suggested this technique to be limited to corals under 1 kg. Furthermore, the method comes with some costs: namely, directly associated costs, such as the increase in freight costs, and indirect costs, such as the increased risk of damage through careless handling during transit (Delbeek 2008).

The overall goals of this study were to illustrate that large colonies could be transported significant distances with minimal effects on their health status, and to start a breeding stock of corals for a new ex-situ aquarium collection and for further associated reproduction studies. To achieve this, parental colonies which were gravid at the time of transportation were chosen. Not only did this permit another way to monitor health during transportation (i.e. the lack of reabsorption of eggs as witnessed in other studies; Okubo et al. 2005) but it also enabled (with careful husbandry) the induction of spawning in synchronisation with the spawning of parental colonies in the wild. This was achieved within a few days (3–4 nights) of the natural spawning event which was monitored at the original site. The ability to spawn corals ex situ can allow researchers the unique opportunity to explore reproductive biology anywhere in the world, providing care is taken with transportation and husbandry of the corals at the host site. This advances the previous capabilities of research in this area which was limited to the transportation of coral larvae over similar distances which had recorded success of >90% survivorship at densities of 4 larvae/ml-1 (Petersen et al. 2005). It is now our the intention to take these corals through a complete gametogenic cycle (Harrison et al. 1984), effectively closing the loop and offering researchers additional opportunities to study reproduction in scleractinian corals.

In conclusion, large gravid *Acropora hyacinthus* colonies can be successfully transported long distances (up to 34 hours in this instance), with no mortality and will spawn within the same lunar months as their natal reef. Such ability offers a suite of opportunities to coral reef scientists; from a greater understanding of the ecology and persistence of certain coral populations and communities to novel approaches to the management and preservation of reefs on a global scale.

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References

Ayre D.J., Hughes, T.P. (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *The Society for the Study of Evolution* 54(5): 1590–1605.

Babcock R.C., Bull G.D., Harrison P.L., Heyward A.J., Oliver J.K., Wallace C.C., Willis B.L. (1986) Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. *Marine Biology* 90: 379–394. http://doi.org/10.1007/bf00428562.

Baird A.H., Marshall P.A. (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. *Marine Ecology Progress Series* 237: 133–141. http://doi.org/10.3354/meps237133.

Baird A.H., Sadler C., Pitt M. (2001) Synchronous spawning of *Acropora* in the Solomon Islands. *Coral Reefs* 19: 286. doi:10.1007/s003380000116.

Banin, E., Israely, T., Fine, M., Loya, Y., & Rosenberg, E. (2001). Role of endosymbiotic zooxanthellae and coral mucus in the adhesion of the coral-bleaching pathogen Vibrio shiloi to its host, F*EMS Microbiology Letters* 199, 33–37.

Boch C.A., Ananthasubramaniam B., Sweeney A.M., Doyle F.J., Morse D.E. (2011) Effects of light dynamics on coral spawning synchrony. *Biological Bulletin* 220(3): 161–173. http://doi.org/220/3/161 [pii].

Calfo A. (2001) *The Book of Coral Propagation: Reef Gardening for Aquarists* vol 1, vers. 1.0. Monroeville, PA, USA: Reading Trees Publications, 450 pp.

Chabanet, P., Adjeroud, M., Andréfouët, S., Bozec, Y., Ferraris, J., & Schrimm, M. (2005). Human-induced physical disturbances and their indicators on coral reef habitats : A multi-scale approach, *Aquatic Living Resource* 18, 215–230.

Carlson B.A. (1999) Organism responses to rapid change: What aquaria tell us about nature. *American Journal of Zoology* 55: 44–55. http://doi.org/10.1093/icb/39.1.44.

Chelliah A., Hyde J., Yewdall K., Steinberg P.D., Guest J.R., Amar H. Bin. (2014). First record of multi-species synchronous coral spawning from Malaysia. *PeerJ* 3: e777. http://doi.org/DOI: 10.7287/peerj.preprints.645v1.

Chornesky E., Peters E. (1987) Sexual reproduction and colony growth in the scleractinian coral *Porites astreoides*. *The Biological Bulletin* 161–177. Retrieved from http://www.biolbull.org/content/172/2/161.short.

Craggs J., Guest J.R., Davis M., Simmons J., Dashti E., Sweet M. (2017) Inducing broadcast coral spawning ex situ: Closed system mesocosm design and husbandry protocol. *Ecology and Evolution* (May): 1–13. http://doi.org/10.1002/ece3.3538.

Delbeek J.C. (2008) Collecting and Shipping Live Coral: Techniques, Tips

and Headaches. Advances in Coral Husbandry in Public Aquariums. *Public Aquarium Husbandry Series* 2: 363–373.

Delbeek J.C., Sprung J. (1994) *The Reef Aquarium.* Ricordea Publishing, Coconut Grove, Florida.

Epstein N., Bak R.P.M., Rinkevich B. (2001) Strategies for gardening denuded coral reef areas: The applicability of using different types of coral material for reef restoration. *Restoration Ecology* 9(4): 432–442. http://doi.org/10.1046/j.1526-100X.2001.94012.x.

Fordham D.A. (2015) *Mesocosms Reveal Ecological Surprises from Climate Change* 1–7. http://doi.org/10.1371/journal.pbio.1002323.

Glasl B., Herndl G.J., Frade P.R. (2016) The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *The ISME Journal* 1–13. http://doi.org/10.1038/ismej.2016.9.

Guest J.R., Chou L.M., Baird A.H., Goh B.P.L. (2002) Multispecific, synchronous coral spawning in Singapore. *Reef sites Coral Reefs* 21: 422–423.

Guest J.R., Baird A.H., Goh B.P.L., Chou L.M. (2005) Seasonal reproduction in equatorial reef corals. *Invertebrate Reproduction & Development* 48(1–3): 207–218. http://doi.org/10.1080/07924259.2005.9652186.

Guest J.R. (2004) *Reproductive Patterns of Scleractinian Corals on Singapore's Reefs.* Department of Biological Sciences, Ph.D. Thesis, 192pp.

Harrison P.L., Babcock R.C., Bull G.D., Oliver J.K., Wallace C.C., Willis B.L. (1984) Mass spawning in tropical reef corals. *Science* 223: 1186–1189.

Hayashibara T., Shimoike K., Kimura T., Hosaka S., Heyward A., Harrison P., Omori M. (1993) Patterns of coral spawning at Akajima Island, Okinawa, Japan. *Marine Ecology Progress Series* 101(3): 253–262. http://doi.org/10.3354/meps101253.

Hogarth P. (1994). Brachyuran crabs (Xanthoidea: Xanthidae, Pilumnidae, Menippidae and Trapeziidae) of southern Oman. *Tropical Zoology* 6975(November). http://doi.org/10.1080/03946975.1994.10539244.

Kaniewska P., Alon S., Karako-Lampert S., Hoegh-Guldberg O., Levy O. (2015) Signaling cascades and the importance of moonlight in coral broadcast mass spawning. *eLife* 4(December): 1–14. http://doi.org/10.7554/eLife.09991.

Keith S.A., Maynard J.A., Edwards A.J., Guest J.R., Bauman A.G., van Hooidonk R., Baird A.H. (2016) Coral mass spawning predicted by rapid seasonal rise in ocean temperature. *Proceedings of the Royal Society B: Biological Sciences* 283: 2016011. http://dx.doi.org/10.1098/rspb.2016.0011.

Kenyon J.C. (1995) Latitudinal differences between Palau and Yap in coral reproductive synchrony. *Pacific Science* 49(2): 156–164. Retrieved from <Go to ISI>://BIOSIS:PREV199598236518.

Lee S.T.M., Davy S.K., Tang S.-L., Kench P.S. (2016) Mucus Sugar Content Shapes the Bacterial Community Structure in Thermally Stressed *Acropora muricata*. *Frontiers in Microbiology* 7: 371. doi: 10.3389/fmicb.2016.00371.

Negri A.P., Heyward A.J. (2001) Inhibition of coral fertilisation and larval metamorphosis by tributyltin and copper. *Marine Environmental Research* 51(1): 17–27. http://doi.org/10.1016/S0141-1136(00)00029-5.

Okubo N., Taniguchi H., Motokawa T. (2005) Successful methods for transplanting fragments of *Acropora formosa* and *Acropora hyacinthus*. *Coral Reefs* 24(2): 333–342. http://doi.org/10.1007/s00338-005-0496-0.

Okubo N., Motokawa T., Omori M. (2007) When fragmented coral spawn? Effect of size and timing on survivorship and fecundity of fragmentation in *Acropora formosa*. *Marine Biology* 151(1): 353–363. http://doi.org/10.1007/s00227-006-0490-2.

Peixoto R.S., Rosado P.M., Leite D.C.A., Rosado A.S., Bourne D.G. (2017) Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. *Frontiers in Microbiology* 8: 341. doi: 10.3389/fmicb.2017.00341

Penland, L., Kloulechad, J., Idip, D., & Van Woesik, R. (2004). Coral spawning in the western Pacific Ocean is related to solar insolation: Evidence of multiple spawning events in Palau. *Coral Reefs* 23(1), 133–140. http://doi.org/10.1007/s00338-003-0362-x.

Petersen D., Laterveer M., Van Bergen D., Kuenen M. (2004) Transportation techniques for massive scleractinian corals. *Zoo Biology* 23(2): 165–176. http://doi.org/10.1002/zoo.10127.

Petersen D., Hatta M., Laterveer M., Van Bergen D. (2005) Ex situ transportation of coral larvae for research, conservation, and aquaculture. *Coral Reefs* 24(3): 510–513. http://doi.org/10.1007/s00338-005-0498-y.

Rapuano H., Brickner I., Shlesinger T., Meroz-fine E., Tamir R. (2017) Reproductive strategies of the coral Turbinaria reniformis in the northern Gulf of Aqaba (Red Sea). *Nature Publishing Group* (October): 1–13. http://doi.org/10.1038/srep42670.

Richmond R., Hunter C. (1990). Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. *Marine Ecology Progress Series* 60: 185–203. http://doi.org/10.3354/meps060185.

Rosenberg, E., Koren, O., Reshef, L., & Efrony, R. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology* 5(May), 355–362. http://doi.org/10.1038/nrmicro1635.

Shikina S., Chen C.J., Liou J.Y., Shao Z.F., Chung Y.J., Lee Y.H., Chang C.F. (2012) Germ cell development in the scleractinian coral *Euphyllia ancora* (Cnidaria, anthozoa). *PLoS ONE* 7(7): 1–12. http://doi.org/10.1371/journal.pone.0041569.

Sweet M., Craggs J., Robson J., Bythell J. (2013) Assessment of the microbial communities associated with white syndrome and brown jelly syndrome in aquarium corals. *Journal of Zoo and Aquarium Research* 1(1): 20–27. http://www.jzar.org/jzar/article/view/21.

Sweet M.J. (2017) Evidence for rapid, tide-related shifts in the microbiome of the coral *Coelastrea aspera*. *Coral Reefs.* http://doi.org/10.1007/s00338-017-1572-y

Sweet M., Ramsey A., Bulling M., Sweet M., Ramsey A., Bulling M. (2017) Designer reefs and coral probiotics: great concepts but are they good practice? *Biodiversity* 8386(April): 1–4. http://doi.org/10.1080/14888386.2017.1307786.

Van Woesik R., Lacharmoise F., Köksal S. (2006) Annual cycles of solar insolation predict spawning times of Caribbean corals. *Ecology Letters* 9(4): 390–398. http://doi.org/10.1111/j.1461-0248.2006.00886.x.

Van Woesik R. (2010) Calm before the spawn: global coral spawning patterns are explained by regional wind fields. *Proceedings of the Royal Society* 277(1682): 715–722. http://doi.org/10.1098/rspb.2009.1524 .

Veron J.E.N. (2000) *Corals of the World. Vol 1.* Townsville: Australia Institute of Marine Science. 306–7 p.

Vicentuan K.C., Guest J.R., Baria M.V., Cabaitan P.C., Dizon R.M., Villanueva R.D., Heyward A.J. (2008) Multi-species spawning of corals in north-western Philippines. *Coral Reefs* 27(1): 83. http://doi.org/10.1007/s00338-007-0325-8.

Wallace C.C. (1985) Reproduction, recruitment and fragmentation in nine sympatric species of the coral genus *Acropora*. *Marine Biology* 88(3): 217–233. http://doi.org/10.1007/BF00392585.

Williams, A. D., Brown, B. E., Putchim, L., & Sweet, M. J. (2015). Age-Related Shifts in Bacterial Diversity in a Reef Coral. *PLOS ONE* 10(12) 1–16. http://doi.org/10.1371/journal.pone.0144902.