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Research article

The AutoSpawner system - Automated *ex situ* spawning and fertilisation of corals for reef restoration

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ABSTRACT

The restoration of reefs damaged by global and local pressures remains constrained by the scale of intervention currently feasible. Traditional methods for *ex situ* sexual propagation of corals produce limited materials, typically of limited genetic diversity and only sufficient for small field trials. The development and validation of new technologies to upscale and automate coral propagation is required to achieve logistically and financially feasible reef restoration at ecologically relevant scales. To address the need for upscaled production of genetically diverse material for use in reef restoration we designed an automated system (the AutoSpawner) for harvesting, fertilising and washing gametes from tropical broadcast-spawning corals. The system includes a novel high density dynamic fertilisation process, which enables the production of large numbers of fertilised coral eggs (>7 million per night for highly fecund species) without any downstream negative effects on larval quality. The functionality of the system and the quality of the produced larvae was assessed using multiple species from two coral families (Acroporidae and Merulinidae) across a range of spawning and gamete characteristics. We present the schematics and protocols required for automated sexual propagation of high-quality coral larvae using this novel system; and demonstrate that the time demands, and labour costs, associated with traditional manual-based sexual propagation of corals can be reduced by up to 113-fold using the AutoSpawner.

1. Introduction

Coral reefs are in decline worldwide due to global pressures, including climate change (Hughes et al., 2017), and local pressures, such as poor water quality, coastal development (Kroon et al., 2015; MacNeil et al., 2019) and harvesting for construction materials (Caras and Pasternak, 2009). Live reef-building corals are also in demand for scientific research, reef restoration programs and the aquarium trade (Barton et al., 2017; Prakash et al., 2017; Rhyne et al., 2014). Historically, live corals have been supplied through collection from wild populations, or as a mixture of wild harvest and local-scale culture of adult fragments (Rhyne et al., 2012; Wood et al., 2012). Husbandry and asexual propagation methods have also been developed for several genera and species, often by hobby aquarists. However, the number of species amenable to this form of domestication is limited, and the labour required for producing and maintaining corals *ex situ* is substantial (Banaszak et al., 2023; Randall et al., 2020). The sexual production of corals in aquaculture offers a promising approach for upscaling production, and can reduce harvesting pressures on local wild populations, delivering a more sustainable and reliable supply of live coral to meet growing demand.

Interventions to assist in restoring or rebuilding damaged coral reefs are being developed and employed to maintain functional and biological diversity of reef ecosystems, and are analogous to terrestrial restoration and conservation efforts (Horoszowski-Fridman and Rinkevich, 2017). Although gaining traction (Anthony et al., 2017), one of the greatest

Abbreviations: Aken, *Acropora kenti*; *Alor*, *Acropora loripes*; CASA, computer assisted sperm analysis; CCA, crustose coralline algae; *Dfav*, *Dipsastraea* cf. *favus*; FNU, formazin nephelometric units; FSW, filtered seawater; GBR, Great Barrier Reef; *Mele*, *Mycedium elephantotus*; SCADA, supervisory control and data acquisition.

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challenges for active coral reef restoration is the efficient production of selected corals that are able to cope with future climate scenarios at scale (Banaszak et al., 2023; Knowlton et al., 2021; Randall et al., 2020). The controlled propagation of corals for reef restoration remains heavily dependent on asexual methods, thus limiting the extent of restoration efforts due to harvesting restrictions and the time consuming and costly manual labour required (Boström-Einarsson et al., 2020; Randall et al., 2020). While harvest of wild larvae from surface slicks following major spawning events is an accepted method of delivering sexually produced larvae to the reef (Cruz and Harrison, 2017; Doropoulos et al., 2019a; Edwards et al., 2015; Heyward et al., 2002; Randall et al., 2020), it offers no control over species composition and limited options for enhancing larvae for heat tolerance. Alternatively, controlled breeding and *ex situ* propagation of corals provides similar benefits of maximising genetic diversity, while also providing opportunities to employ selective breeding or other interventions to produce corals better adapted to face future conditions (Humanes et al., 2021; Randall et al., 2020; van Oppen et al., 2015). Nevertheless, *ex situ* sexual propagation of corals continues to rely on a sequence of labour-intensive processes that remain derivative of original methods from fundamental studies in the 1980s and 1990s.

Broadcast spawning was identified as the dominant mode of sexual reproduction of scleractinian corals on the Great Barrier Reef (GBR; Australia) in the 1980s (Babcock et al., 1986; Harrison et al., 1984), with early breeding techniques developed through pioneering work by Babcock et al. (1986) and Babcock and Heyward (1986). These original methods have improved incrementally (Harrison et al., 2021; Heyward and Negri, 1999; Humanes et al., 2021; Pollock et al., 2017) and typically involve: (i) the collection of broodstock from wild populations; (ii) isolation of individual parent colonies; (iii) manual harvesting of gametes (as egg-sperm bundles); (iv) manual separation of sperm and eggs; (v) cross fertilisation of selected gametes under static conditions; (vi) manual washing of fertilised eggs and developing embryos; followed by (vii) transfer to culture tanks for larval rearing (Fig. 1; see Pollock et al. (2017)). These conventional protocols remain adequate for many experimental purposes (e.g. 1,000–100,000s of fertilised eggs). However, increased efficiencies in time, space and resources (including cost) are required if the mass culture of sexually produced coral larvae for application in restoration is to reach ecologically significant scales (Banaszak et al., 2023; Gibbs, 2021; Randall et al., 2020; Vardi et al., 2021), in particular when targeting high species diversity. For example, between 50 million and 5 billion fertilised eggs would be required to facilitate restoration across the GBR, ranging from key tourism sites to reef-wide interventions (i.e. 1 million to 100 million seeding devices with coral recruits deployed yearly) (Gibbs et al., 2019). Sexual propagation of corals for reef restoration has been explored both *in situ* (Doropoulos et al., 2019b; Sellares-Blasco et al., 2021; Suzuki et al., 2020) and *ex situ* (Harrison et al., 2021; Humanes et al., 2021; Ter Hofstede et al., 2019), again applying variations of the traditional, manual and static methods described above. However, applying the standard methods does not guarantee successful and efficient propagation of corals at larger scales. Reported issues with current methods include damage to gametes and embryos from manual handling during collection, fertilisation and embryo transfer (Guest et al., 2010; Omori, 2019); temporal delays over several hours due to logistical constraints (Chan et al., 2019; Humanes et al., 2021); and the static conditions during fertilisation (i.e. no active circulation or water movement) leading to poor water quality (e.g. oxygen depletion) as excess sperm degrade (Guest et al., 2010). Furthermore, the success of large-scale coral culturing efforts, under this current production model, requires a substantial number of trained personnel operating in synchrony across the multiple steps (Fig. 1), often with several species spawning simultaneously. The logistics of such operations are complex and can contribute to failure, further emphasising the need for a step change to streamline propagation methodologies and technologies (Banaszak et al., 2023; Randall et al., 2020).

Automation has the potential to overcome some of the obstacles to effective and efficient sexual propagation of corals at scale. Automation

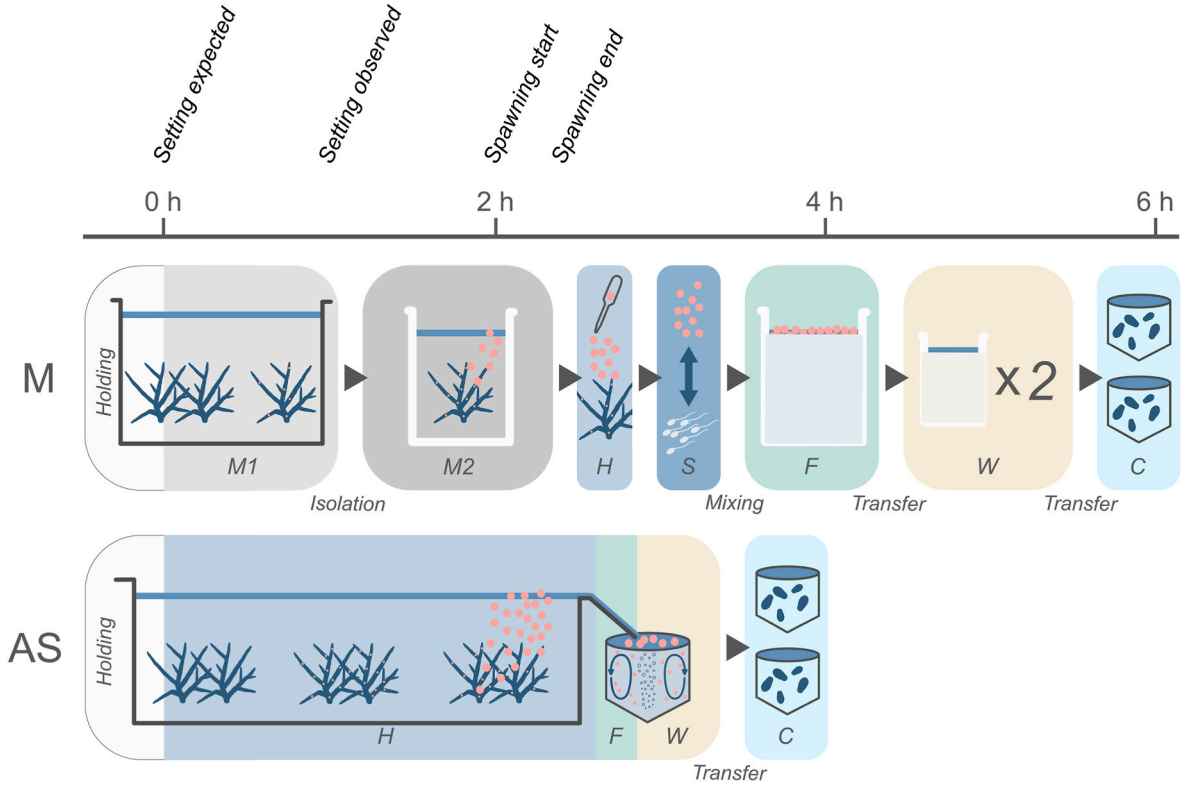


Fig. 1. Comparison of Manual (M) fertilisation and AutoSpawner (AS) workflows. M1 = Monitoring in holding tanks; M2 = Monitoring after isolation of individual colonies; H = Harvesting; S = Separation of gametes; F = Fertilisation; W = wash-down; C = culture.

has been successfully applied to upscale operations in industries such as agri- and aquaculture for decades (Balchen, 2002; Edan et al., 2009; Lee, 1995), including detection of shrimp spawning (Mueangdee et al., 2013), automated fish feeding (Burget and Pachner, 2005), seaweed propagation (Solvang et al., 2021) and fish stock assessments (Li et al., 2021). Here, we describe the design, performance and reproducibility of an automated system (named the 'AutoSpawner', this also incorporates the 'AutoFertiliser' which can be operated separately as a standalone fertilisation system; Fig. 2A and Fig. S1) for sexual propagation of scleractinian corals. The AutoSpawner aims to increase the throughput and efficiency of manual coral larval rearing systems (defined as the manual spawning work-flow where fertilisation is performed under static conditions), and consequently, its potential application to larger coral culture programs. This innovative system automatically collects, dynamically cross-fertilises and washes coral gametes from captive broodstock (Fig. 1). We assess the functionality of the system and compare its temporal and cost efficiency, and the quality of the resulting larvae, against the established manual method across four coral species from two taxonomic groupings: Acroporidae - *Acropora kenti (*formerly *A. tenuis* Bridge et al. (2023)*)* and *Acropora loripes*; Merulinidae: *Dipsastraea* cf. *favus* and *Mycedium elephantotus*.

2. Material & methods

2.1. AutoSpawner and AutoFertiliser system description

The AutoSpawner is a fully automated system for coral broodstock holding, gamete harvesting, dynamic cross-fertilising (i.e. fertilisation in a body of continuously moving water) and embryo wash-down for broadcast spawning corals with positively buoyant egg-sperm bundles (Fig. 2A). The system is managed by a Distributed Control System (DCS - SIMATIC PCS7, Siemens, Munich, Germany) and UR20 remote IO Profibus module (Weidmüller, Detmold, Germany) through a Supervisory Control and Data Acquisition system (SCADA; SIMATIC WinCC, Siemens, Munich, Germany; Fig. 2B) and follows a decision flow-chart (Supplementary materials 2). The system operates across 4 main modes: (1) *holding*, (2) *harvesting*, (3) *fertilisation* and (4) *wash-down* (refer to Figs. 1 and 2):

1. *Holding:* Multiple gravid coral colonies (up to 30 colonies depending on size) are held in a 1,150 L holding tank (2,800 × 900 × 450 mm; refer to Supplementary materials 2 and the associated data repository for full technical details (AIMS, 2024)). Temperature is controlled in this semi-open recirculating system by partial water replacement (100 L h^{-1}; 2 replacements day^{-1}) with filtered seawater (FSW; 0.04 μm). FSW exits the holding tank via a wide surface skimmer from where it is either diverted to waste or to the AutoFertiliser tank using automated ON/OFF valves (ER10.X0B.G00, Valpes, Moirans, France), depending on the operation mode (Fig. 2A).
2. *Harvesting*: The AutoSpawner is pre-programmed to commence *harvesting mode* 40–90 min prior to the expected start of spawning for the species of interest. Spawning, i.e. the synchronous release of buoyant egg-sperm bundles (Harrison and Wallace, 1990), is detected by the control system using turbidity as a proxy for sperm concentration. Turbidity is measured in formazin nephelometric units (FNU) by a turbidity transmitter (Turbimax CUS52D; Endress & Hauser AG, Reinach, Switzerland) fitted to the outflow of the AutoFertiliser (Fig. 2A). During *harvesting mode,* the system is switched to a full flow-through configuration and the water input into the holding tank is increased to 600 L h^{-1} using automated proportional valves (ER20.X3B.GP6, Valpes, Moirans, France), to allow for rapid collection of surfacing gamete bundles. Gamete bundles are collected by the surface skimmer (Video 1), and the outflow is re-directed to the AutoFertiliser by the engagement of automatic valves. Both ends of the holding tank are fitted with a water movement pump (Gyre XF330, Maxspect, Hong Kong), which run continuously during *holding mode*, and are programmed to intermittently pulse during this period (5 s on, 60 s off). The downstream pump (under the surface skimmer) is positioned 150 mm off the tank bottom, to help dislodge emerging bundles from excreted mucus, while the upstream pump is placed 20 mm underneath the water surface to facilitate the unidirectional transport of egg-sperm bundles across the surface of the tank to the skimmer for collection (Fig. 2).
3. *Fertilisation:* Once the turbidity sensor reads values above the pre-set trickle threshold (e.g. 10 FNU; Table S2), the AutoSpawner automatically transitions into *fertilisation mode.* The trickle threshold is the turbidity value which has been identified to indicate a large enough bundle release event to be in progress to warrant further processing, and initiates the transition of the AutoSpawner to fertilisation mode. In contrast to previous coral fertilisation approaches which combine eggs and sperm under static conditions, with buoyant eggs concentrated at the water surface (Guest et al., 2010; Negri and Heyward, 2000; Pollock et al., 2017), fertilisation takes place in the AutoFertiliser under turbulent, flow-through conditions (Video 2). The AutoFertiliser is an 85 L cylindroconical tank fitted with an upright cylindrical mesh screen filter (optimised to species gamete size, e.g. 212 μm for *Acropora* spp.), mounted from the bottom of the tank to prevent losses of harvested eggs (Fig. 2). An air curtain is generated around the filter by diffusing low pressure air from a hose ring (air ring; 50 L h^{-1}) positioned at the filter base. The air curtain keeps the eggs off the screen, and the mixture of eggs and sperm homogenous throughout the tank. A ring-shaped FSW sprinkler system (spray ring; 0.04 μm, 150 L h^{-1}) at the top circumference of the AutoFertiliser washes off the emersed tank walls to prevent egg entrapment in the water meniscus and reduce foam formation at the water surface (turned on using automated valves at the start of harvesting mode using automated ON/OFF valves; ER10.X0B.G00, Valpes, Moirans, France). Incoming water (total of ~750 L h^{-1}) continues to dilute the sperm concentration until the pre-selected FNU value (equivalent to the optimal ~$1 \bullet 10^6$ sperm mL^{-1}) has been reached. Fertilisation is allowed to proceed for an additional user-defined period (e.g. 10 min), with minimal introduction of additional FSW through the sprinkler system (150 L h^{-1}). After the fertilisation period is complete the AutoSpawner begins the *embryo wash-down procedure.* If no gamete release has been detected (i.e. no increase in turbidity observed that reached the trickle threshold) before the end of the predicted spawning window for the night, the system returns to *holding mode* to ensure optimal flow-through conditions for the broodstock.
4. *Wash-down:* Fertilised eggs are washed clear of remaining sperm through addition of FSW from both the holding tank and FSW sprinkler system (total of 750 L h^{-1}) until a target turbidity value is reached (typically ~1–2 FNU). The air curtain stops to allow the collection of fertilised eggs (e.g. for the transfer to larval culture tanks) by manually skimming from the water surface of the AutoFertiliser. The holding tank remains in flow-through configuration (similar to during *harvesting mode)* but with the outflowing water directed to waste. At the end of the programmed spawning window the AutoSpawner then returns to *holding mode* and a semi-recirculating configuration.

The AutoSpawner and AutoFertiliser can also be operated independently to accommodate specialised use cases, such as automatic harvesting from individual coral colonies, as well as automatic bulk fertilisation of gametes collected using other methods. For additional technical specifications and schematics for the full AutoSpawner system, and use of the AutoFertiliser independently, refer to Supplementary materials 2 and the associated repository (AIMS, 2024).

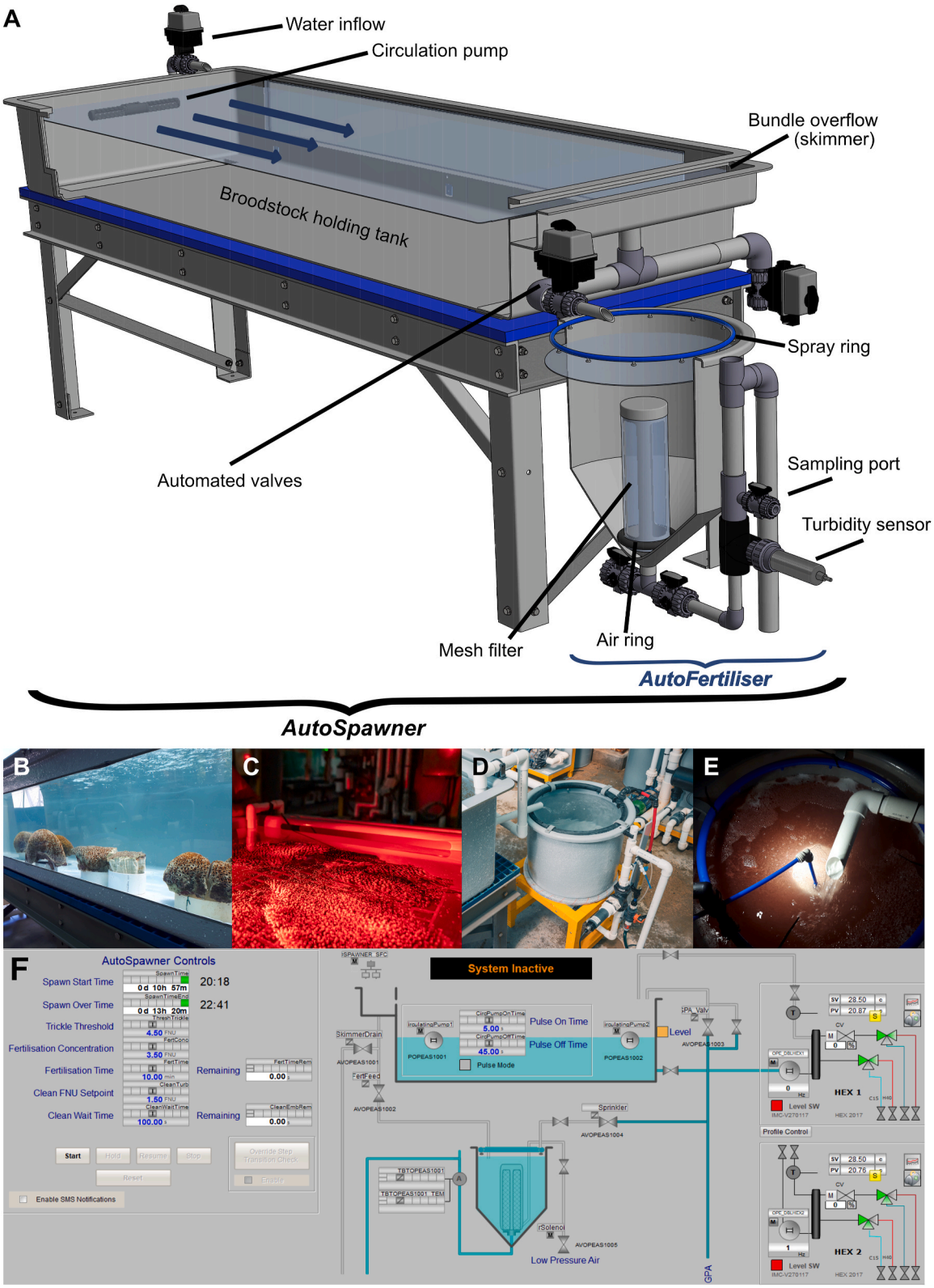


Fig. 2. Schematic of the AutoSpawner system with key components (A), images showing the AutoSpawner system (B, C) and AutoFertiliser (D, E) in use and the Supervisory Control And Data Acquisition (SCADA) system used for programming and automated use of the AutoSpawner (F). The AutoSpawner system operates across four main modes in accordance with user selected pre-programming (F): 1. Holding – Gravid broodstock colonies are held in the broodstock holding tank under semi-recirculating conditions (B); 2. Harvesting – The system is switched to full flow-through conditions and water collected in the skimmer is redirected from waste to the AutoFertiliser tank (C); 3. Fertilisation – Once the preset turbidity threshold value is reached the system automatically enters fertilisation mode and continues to dilute the sperm concentration in the AutoFertiliser towards the optimal sperm concentration for fertilisation ($1 \bullet 10^6$ sperm mL^{-1}) and then allows fertilisation to occur during dynamic conditions for a pre-set period of time (D, E): Wash-down – Following completion of fertilisation the water flow is increased again and remaining sperm washed out of the AutoFertiliser until a target turbidity value is reached (e.g. 1–2 FNU). Image C provided by D. Tsai. For technical drawing and the associated programming decision flow-chart refer to Supplementary material 2.

2.2. Comparison of AutoSpawner to manual fertilisation method

2.2.1. Broodstock origin

The four species tested in the AutoSpawner are simultaneous hermaphrodites, which produce positively buoyant gamete bundles, and can be considered representative of the majority of broadcast-spawning coral species on the GBR (Australia; Babcock et al. (1986); Babcock and Heyward (1986)). Gravid colonies of *Acropora kenti (Aken;* Palm Islands, 18°45′54.8"S 146°31′36.1"E), *Dipsastraea* cf. *favus* (*Dfav*; Palm Islands, 18°45′54.8"S 146°31′36.1"E) and *Acropora loripes* (*Alor*; Davies reef, 18°49′12.2"S 147°38′39.4"E) were collected by hand on SCUBA in the weeks leading up to the 2022 November (*A. kenti* and *D. favus*) and December (*A. loripes*) spawning events on the Central GBR (Australia), under GBR marine Park Authority Permit G21/45348.1 (refer to Table S1 for further details). Field collected gravid corals were transported in flow-through seawater tanks to the National Sea Simulator (Australian Institute of Marine Science, Townsville Australia) shortly before the respective spawning events. In addition to field collections, gravid broodstock colonies of *Mycedium elephantotus* (*Mele*; originating from Davies Reef, Central GBR), maintained *ex situ* since 2018 at the National Sea Simulator, were used during the December coral spawning event. All corals were transferred to the AutoSpawner holding tanks and maintained in flow-through filtered seawater (0.04 μm) at ambient temperature (Table S2) and shaded natural light (maximum intensity of photosynthetically active radiation ~100 μmol quanta m^{-2} s^{-1}; LI-250A Light meter with a LI-190R Quantum sensor, Li-Cor Biosciences, Lincoln, USA).

2.2.2. AutoSpawner functionality

The functionality of the AutoSpawner was assessed in a series of tests to optimise the operation and performance for the four selected coral species, including: i) the evaluation of sperm concentration and homogeneity within the AutoFertiliser; ii) validation of the use of turbidity as a proxy for sperm concentration in the AutoFertiliser; and iii) fertilisation time windows in the AutoFertiliser across species.

2.2.2.1. Sperm concentration and homogeneity evaluation within the AutoFertiliser. To assess outflow turbidity as an appropriate proxy for sperm concentration within the AutoFertiliser, and to assess whether the sperm concentration reached homogeneity prior to the start of the *fertilisation mode*, paired samples from the top and bottom of the AutoFertiliser were collected during AutoSpawner operation for *A. kenti*. A sperm-free blank (1 mL) was collected shortly after the fertilisation tank filled with FSW. Samples (1 mL) were then collected every 2–3 min following the commencement of spawning, or more frequently if rapid changes in turbidity were observed (changes >0.2 FNU), and processed within 2 h for a combined sperm motility and quantity analysis as described below. Additional samples were preserved (for counts only) by adding 0.5 mL of sample to 0.1 mL of sodium-β-glycerophosphate-buffered formaldehyde (final concentration of 1% formaldehyde and 0.28 mg sodium-β-glycerophosphate mL^{-1} in fixed samples) and stored at 4 °C until analysed as described below (within 3 days). The coral sperm quantity and motility analyses were performed using a CEROS II computer assisted sperm analysis (CASA) system (version 1.11.9) by Hamilton Thorne Inc (Beverly, USA), in combination with a Zeiss AXIO Lab.A1 phase contrast microscope (10 × /0.25 Ph1 A-Plan objective, Germany), and four-chamber 20-μm microscopy slides by Leja Products B.V. (Nieuw-Vennep, Netherlands). The CASA requires 3 μL of fresh or activated sperm per processed replicate ($\geq 3.5 \bullet 10^6$ sperm cells mL^{-1} captured in 15 frames) to precisely measure cell counts and motility. Analysis was performed as per the manufactureres recommendations (Hamilton, 2018) using a protocol addapted for coral sperm (Zuchowicz et al., 2021), refer to Supplementary Materials 1 for further details.

2.2.2.2. Species-specific correlations between sperm concentration and turbidity. To assess whether species-specific turbidity programming of the AutoSpawner would be required to initiate the *fertilisation mode*, the paired sperm concentration samples and turbidity readings collected for each species were assessed (refer to section 2.1.2.1 for information on collection methods). Sperm samples were extracted from a collection port adjacent to the turbidity sensor on the outflow line of the AutoFertiliser (Fig. 2). Samples and one sperm-free blank were collected, preserved and processed as described above.

2.2.2.3. Fertilisation time windows in the AutoSpawner system. Most broadcast spawning corals are simultaneous hermaphrodites that combine both eggs and sperm into tightly packaged, buoyant bundles for release (Babcock and Heyward, 1986). At or near the surface the bundles separate into individual eggs and clouds of sperm, a process that typically takes around 30 min, but can occur faster under turbulent conditions (e.g. Padilla-Gamiño et al. (2011)). It takes a few minutes for most egg-sperm bundles to reach the AutoFertiliser following release in the holding tank and fertilisation requires enough time for the bundles to break apart under turbulence and for sufficient egg-sperm encounters until maximum fertilisation success is achieved (i.e. due to potential species-specific differences in the fertilisation window; Oliver and Babcock (1992); Omori et al. (2001)). To assess the effective fertilisation time windows for each species, egg samples were collected at multiple time points from the AutoFertiliser during the *harvesting* and *fertilisation modes*. Egg samples (10 mL) were randomly collected from the top 10 cm every 5–10 min after first bundle separation was observed. The eggs were then gently washed free of sperm in consecutive baths of clean FSW and eggs from each time point were maintained in 10 mL of clean FSW. Fertilisation success in washed eggs was assessed ~2.5–4 h after the beginning of spawning using a dissecting microscope (MS5; Leica Microsystems GmbH, Wetzlar, Germany).

2.2.3. Comparison of automated and manual fertilisation workflows

To validate the use of the AutoSpawner in sexual coral propagation, the workflow efficiency and biological quality of coral spawn fertilised using the AutoSpawner was compared to widely used manual, static fertilisation methods. Parent colonies in the holding tank were monitored on expected spawning nights and the timings of setting and release of egg-sperm bundles recorded. For details on the programming and presets used for each coral species refer to Table S2. Once spawning was observed and detected by the AutoSpawner, a subset of bundles were gently collected by hand from each spawning colony in the holding tank (Guest et al., 2010; Pollock et al., 2017). This subsample of released spawn (~0.6–1.4 million eggs per species) was fertilised manually in parallel to the spawn automatically harvested and fertilised by the AutoSpawner system (~1.6–6.0 million eggs per species). Briefly, manually collected egg-sperm bundles were transferred to a 70 L plastic tank with minimal FSW. Bundles were gently agitated (using a clean plastic pipe) until the majority of eggs and sperm were separated. The sperm concentration was then diluted through gentle addition of FSW until an approximate concentration of $1 \bullet 10^6$ sperm mL^{-1} was reached (estimated against a colour chart of measured sperm mL^{-1}). Gametes were allowed to fertilise for ~45 min under static conditions and the eggs and developing embryos washed by gentle, manual transfers to two consecutive 70 L tanks containing clean FSW (Guest et al., 2010; Negri et al., 2011). Samples for sperm concentration measurements by CASA (1 mL; n = 5 per time point) were collected from the centre of the fertilisation tanks at the start and end of the incubation periods.

Following completion of the automatic and manual fertilisation and wash-down procedures, the fertilised eggs and developing embryos were transferred to cylindroconical 85 L flow-through (0.04 μm, 17 L h^{-1}) culture tanks ($n_{Technical}$ = 2 per fertilisation method) fitted with mesh screen filters (106 or 212 μm, depending on larval size) at a density of 0.5 embryos mL^{-1}. The cultures were maintained at a temperature

appropriate for the reef of origin and spawning month (Table S3) under a ~12:12 h light:dark regime (<30 μmol quanta m^{-2} s^{-1}). Approximately 24 h post-fertilisation, gentle air bubbling was initiated to provide mixing and aeration. Water quality parameters (temperature, dissolved oxygen, salinity and pH) were assessed prior to culture start and following termination of larval culturing (Table S3).

To enable the comparison of the automatic and manual fertilisation methods, data on the quality of the sexually produced corals, and the effort required per coral produced, was collected across multiple time scales and parameters. On spawning nights, the *time and personnel required* to complete each task was recorded for the respective methods. Additionally, *fertilisation success* was assessed (n = 6 samples per fertilisation method and species; >100 eggs and embryos counted per sample) through manual counts using dissecting microscopes. Unfertilised eggs and developing embryos were photographed (14 MP, ToupCam L3CMOS14000KPA camera; ToupTek Photonics, Zheijang, China) for morphological reference and qualitative estimation of size distribution. The survival of developing embryos and larvae during rearing (from day one post-fertilisation until settlement) was assessed once every 24 h. Larval competency to settle was monitored starting 24–48 h after active swimming was first observed. The species-specific cues to induce larval settlement were selected through repeated, small-scale assessments of larval responses to multiple species of live crustose coralline algae (CCA) or reef rubble for each coral species. Once fully competent, the metamorphosis success of larvae was assessed for each culture tank (n = 24) by exposing larvae (n_{Larvae} = 12 per replicate) to ground live *Porolithon onkodes* (*A. kenti*, *D. favus* and *A. loripes* (Heyward and Negri, 1999)) or 5 × 5 mm pieces of reef rubble (*M. elephantotus*) in 10 mL of FSW (Abdul Wahab et al., 2023). Metamorphosis success was assessed using bright (*A. kenti* and *A. loripes*) or fluorescence (*D. favus* and *M. elephantotus*; SFA-RB, NightSea, Lexington, USA) microscopy (MS5; Leica Microsystems GmbH, Wetzlar, Germany) following incubation for 17 h (*A. kenti*, *A. loripes*, *M. elephantotus*) or 48 h (*D. favus*).

2.3. Statistical analysis

Statistical analyses were performed using the *brms* package (Bürkner, 2017) and *rstan* (Stan Development Team, 2023) using *R* (version 4.2.2; R Core Team (2022)) and *RTools42* (version 42; RTools Team (2022)) through *RStudio* (version 2023.03.0; RStudio Team (2023)). Model fits were assessed using the inbuilt functions of *brms* and graphical results were produced using *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2020). For full analyses details refer to the corresponding sections in the Supplementary materials 1, as well as the analysis scripts and data sheets for further details on excluded samples (9% of total for sperm concentration-turbidity data, 0–3% of total for all other data sets), model specification and assessment of model fits (AIMS, 2024; Nordborg, 2024).

3. Results

3.1. AutoSpawner and AutoFertiliser: functioning and parameters

3.1.1. Gamete harvesting in the AutoSpawner

Gamete harvesting was highly efficient and the combination of the AutoSpawner flow-settings and tank design successfully harvested the gamete bundles of all four species tested. Egg-sperm bundles rose to the water surface after release (Fig. S3) and moved slowly (~2–3 cm s^{-1}; Video 3) with the water flow to the skimmer from where they gently entered the AutoFertiliser. Minimal losses of gamete bundles occurred (qualitatively estimated to <5%, typically due to the formation of eddies in the corners of the holding tank or from bundles getting stuck in mucus on broodstock colonies), with 1.6–6 million eggs collected over the course of 40–90 min, depending on the species and the synchrony of spawning of individual colonies (Table 1 and Table S4).

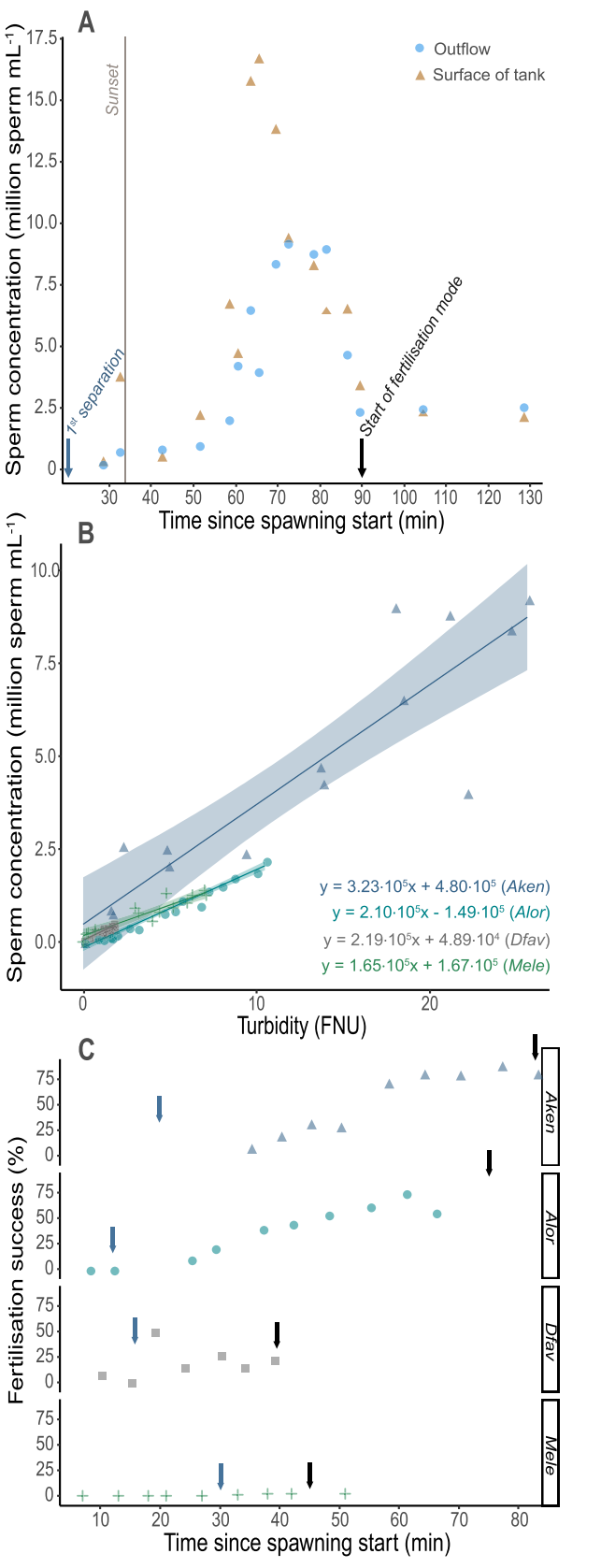
3.1.2. Homogeneity of sperm density in the AutoFertiliser

The homogeneity of sperm density within the AutoFertiliser was assessed using the *A. kenti* spawning. Sperm concentration increased throughout the AutoFertiliser following the first observation of gamete bundle separation (~18:10, ~20 min after spawning start; Fig. 3A) and peaked in both the surface and outflow of the tank ~45 min after initial bundle separation. Sperm concentration varied more in the top part of the tank than in the outflow during *harvesting mode* (briefly peaking at ~17•10^6 cells mL^{-1}), but followed the same general trend. Following the initialisation of *fertilisation mode* (~19:20, ~90 min since spawning start), the sperm concentration became homogenous throughout the tank, and remained stable at ~2.5•10^6 cells mL^{-1} for the duration of the fertilisation period (Fig. 3A).

Table 1
Summary of quality for spawn dynamically fertilised using the AutoSpawner system (AS) compared to spawn statically fertilised using the manual method (M) and differences in labour and cost required to produce fertilised eggs. Larval survival at induction of settlement calculated as the percent alive out of total larvae present 1-day post-fertilisation. Cost calculated assuming an hourly rate of $45 AUD for a trained operator. For details on calculation of spatial footprint of the respective fertilisation methods and species refer to Table S5. For updated programming parameters based on results refer to Table S6.

	A. kenti		*D. favus*		*A. loripes*		*M. elephantotus*	
	AS	*M*	*AS*	*M*	*AS*	*M*	*AS*	*M*
No. of parent colonies	9		5		10		9	
Eggs harvested (millions)	6.0	1.4	3.5	0.6	1.6	1.2	2.2	0.9
Sperm concentration (million cells mL^{-1})	3.2	2.2	0.5	2.4	1.4[a]	2.8	0.7	5.6
Fertilisation success (%)	89 (87–90)	92 (90–93)	46 (43–50)	54 (51–57)	94 (93–95)	95 (94–96)	48 (46–50)	56 (53–59)
Cell division observed pre-transfer to culture tanks?	No	Yes	N/A	N/A	No	Yes	No	Yes
Median larval survival at induction of settlement (%)	89 (58–127)	87 (59–127)	98 (61–145)	100[b] (73–156)	19 (11–32)	20 (12–34)	100[b] (56–209)	100[b] (72–232)
Metamorphosis success (%)	98 (97–99)	98 (97–98)	58 (55–62)	52 (49–56)	62 (58–66)	56 (52–60)	93 (91–94)	91 (89–93)
Spatial system footprint for parent colonies used (m^2)	6.0	13.2	4.0	9.4	4.0	13.2	6.0	14.4
Total labour required on spawning nights (h fertilisation $batch^{-1}$)	2	5.2	1.5	3.5	1.9	6.3	1.4	5.5
Labour required per fertilised egg (ms)	1.4	74	3.4	378	4.6	198	1.3	40
Labour cost per 1 million fertilised eggs ($AUD)	$18	$927	$42	$4,720	$58	$2,477	$59	$494
X-fold difference in labour cost	52		113		43		8	

[a] Only samples from start of the *fertilisation mode*.
[b] Median larval survival in cultures at induction of settlement (relative to 1-day post-fertilisation) may be higher than 100% due to the variability in the count estimates for collected subsamples.



(caption on next column)

Fig. 3. Functional assessment of the AutoSpawner and AutoFertiliser systems. Comparison of sperm concentration at the surface (orange triangles) and outflow (blue circles) of the AutoFertiliser during spawning of A. kenti (A); species-specific sperm concentration-turbidity correlations (B) and relationship between fertilisation success and time passed since first observation of spawning (C) for *A. kenti* (blue triangles; Aken), *A. loripes* (teal circles, Alor), *D. favus* (grey squares; Dfav) and *M. elephantotus* (green crosses, Mele). Blue arrows indicate when bundle separation was first observed in the AutoFertiliser while black arrows indicate the start of the fertilisation mode (A and C). Linear regressions fitted using Bayesian methods in the software R, model median (solid line) and 95% credible intervals (shaded area) shown for each species (B) FNU = formazin nephelometric units. For references to colour please refer to the online version of the publication. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1.3. Sperm density-turbidity correlation in the AutoFertiliser

The turbidity of the outflowing water from the AutoFertiliser increased linearly with increasing sperm concentration for all four species assessed. The fitted regressions were similar for *A. loripes*, *D. favus* and *M. elephantotus* (m = 0.21, 0.22 and 0.17 million sperm mL^{-1}, respectively), but the range of concentrations and FNUs varied for each species (Fig. 3B). In contrast, the regression fitted for *A. kenti* was significantly different from regressions fit for the other three investigated species for turbidities >5 FNU (i.e. no overlap of 95% credible intervals; Fig. 3B). The slope differed (m = 0.32 million sperm mL^{-1}) and the median intercept (b) with the y-axis was higher, with higher median sperm concentrations expected for *A. kenti* than for the other species at any given turbidity above 5 FNU (Fig. 3B).

3.1.4. Fertilisation delay

A time delay between initial release of bundles and the successful fertilisation of eggs was observed for all four species assessed. Following this delay, fertilisation success gradually increased with increasing time from spawning initiation. This initial delay varied across species and was ~15 min for *A. kenti* and *D. favus,* but 20 min and 25 min for *M. elephantotus* and *A. loripes*, respectively. The upper asymptote of the fertilisation success for the washed samples varied between species and was reached 10–50 min after the first fertilisations occurred (Fig. 3C).

3.2. Comparison of fertilisation workflows

3.2.1. Gamete morphology

In the lead up to spawning, the eggs and sperm of hermaphroditic broadcast-spawning corals are tightly packaged into bundles before being released (Babcock and Heyward, 1986; Padilla-Gamiño et al., 2011). This tight packing causes some temporary deformation of gamete morphology, in particular for oocytes. Following release into the water column the gamete bundles dissociate and eggs gradually become more ovoid within ~30 min after dissociation (Padilla-Gamiño et al., 2011). No differences were observed in egg or sperm morphology between gametes collected from the automated (dynamic) and manual (static) systems (Fig. S4). However, egg sizes and colouration varied between the four species, showing some within species variation. A more pronounced within species variation in egg size was observed for *M. elephantotus*, and some eggs did not fully round out for *D. favus* (regardless of fertilisation method; Fig. S4). Sperm morphology was consistent within species regardless of fertilisation method and no indications of damage were observed (e.g. separation of tail and head).

3.2.2. Fertilisation success

Successful fertilisation was achieved for all four coral species using both the AutoSpawner and the manual, static fertilisation methods. Median fertilisation success was very high (≥89%) for both *A. kenti* and *A. loripes*, with no significant statistical differences detected between fertilisation methods (Fig. 4A and Table 1). Median fertilisation success

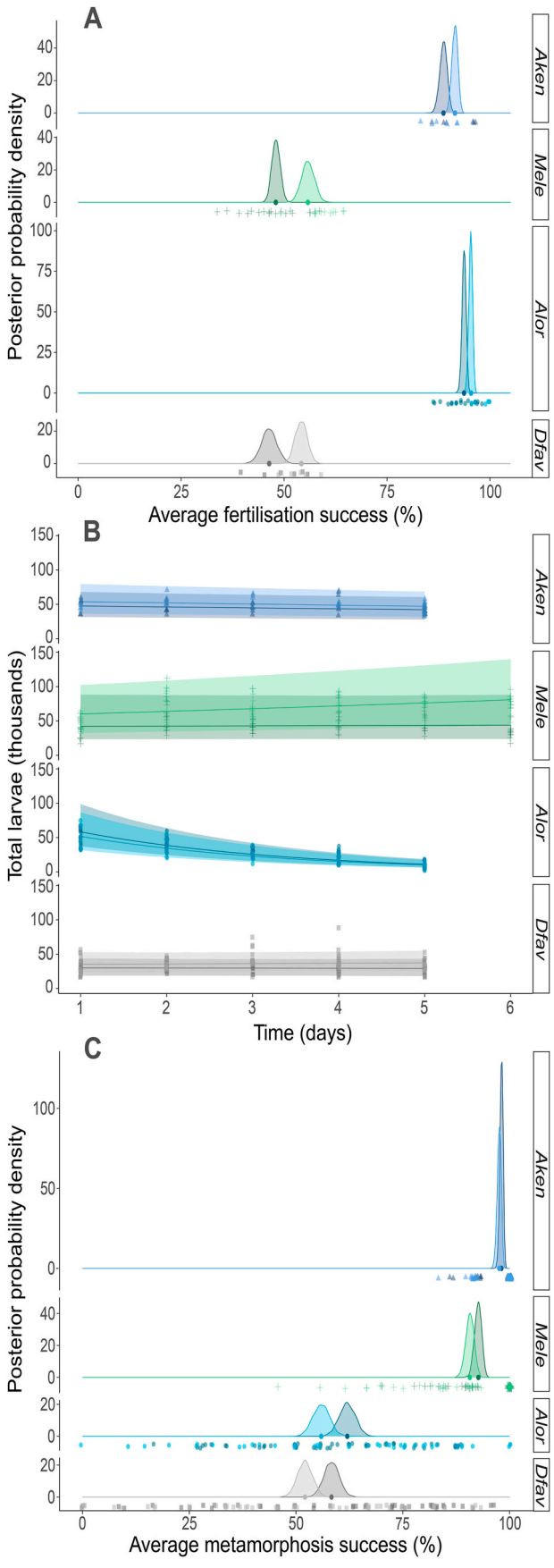


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Fig. 4. Comparison of fertilisation success (A), survival of larvae in culture (B) and metamorphosis success (C) for corals fertilised using the AutoSpawner (dark colours) or traditional, static methods (light colours) for *A. kenti* (blue triangles; Aken), *M. elephantotus* (green crosses; Mele), *A. loripes* (turquoise circles; Alor) and *D. favus* (grey rectangles; Dfav). Model fitting performed using Bayesian methods in the software R. Solid circles (A and C) and lines (B) show model median while shaded areas (A–C) show 95% credible intervals for each species. Individual replicates shown as opaque symbols (A–C). For references to colour refer to online version of manuscript. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was lower for *M. elephantotus* and *D. favus* than for the two acroporid species (46–56%; Table 1) and a statistical difference in fertilisation success between fertilisation methods could not be excluded for *D. favus* and *M. elephantotus*. However, the median fertilisation success was similar across methods for both species (<10% difference; Fig. 4A and Table 1), despite sperm concentrations in the AutoFertiliser being 5–8-fold lower than for the manual, static method (Table 1). Early embryonic development progressed as expected regardless of fertilisation method used (Fig. S5).

3.2.3. Larval survival

Survival during larval rearing was high (>85%) for *A. kenti*, *M. elephantotus* and *D. favus*, with no statistically significant differences in larval survival observed between fertilisation methods for any species (Fig. 4B and Table 1). Larvae remained actively swimming throughout the rearing period, with no auto-settlement (i.e. settlement of larvae on the surface of culture tanks without any settlement cues) observed in culture tanks prior to the induction of settlement for these species. Larval survival of *A. loripes* was low (~20%) regardless of the fertilisation method. No differences in larval morphology were observed within species across fertilisation methods (Fig. S6).

3.2.4. Larval metamorphosis success

Larvae from all four species successfully settled when presented with settlement cues (CCA or rubble), with no statistical differences in metamorphosis success observed between fertilisation methods for any species (Fig. 4C). Larvae from *A. kenti* (98%) had the highest median metamorphosis success, followed by *M. elephantotus* (~92%), *A. loripes* (~59%) and *D. favus* (~55%; Fig. 4C and Table 1). Onset of settlement competency occurred within 1–4 days of fertilisation, depending on species, with no differences observed between fertilisation methods. No morphological differences were observed in early recruits, regardless of fertilisation method used (Fig. S7).

3.3. Differences in required time, labour and cost

The use of the AutoSpawner system resulted in substantial increases in efficiency on spawning nights, with significant cost and time savings compared to manual fertilisation workflows. 1.3–5.8-fold more eggs were collected and fertilised using the AutoSpawner than what was collected by hand, while requiring on average 54 times less labour than the manual method across the four species (Table 1, Fig. 1 and Table S4). As a result, the labour cost per million fertilised eggs produced was reduced to as little as $18 (AUD) for the species with the largest spawning event (*A. kenti*; Table 1). The average workflow from the onset of spawning to the transfer of embryos to culture tanks was substantially shorter for gametes from the automated systems (100 min) compared to manually fertilised gametes (160 min; Fig. 1 and Table S4) and the total labour required per produced fertilised egg was reduced (Table 1 and Table S4). Finally, the spatial footprint required in the experimental facility was smaller for the automated workflow than for the manual fertilisation method (Table 1 and Table S5), as a result of the elimination of the need to isolate individual parent colonies and manually separate the gametes during large batch culturing (Table S5), although these

needs may change with experimental design or the intended use of larvae.

4. Discussion

Here we described the design, operation and testing of the highly automated AutoSpawner system which is able to efficiently produce large numbers of high-quality coral embryos. The automation was effective for all four coral species assessed, with no negative impacts on larval culture and quality through to settlement. The use of the AutoSpawner system increased the efficiency of coral larval culturing and reduced both the labour-associated costs and spatial footprint required for the first steps of the upscaled production process. In addition, the fertilisation of coral gametes under dynamic conditions was performed for the first time (using the AutoFertiliser), without any observed negative effects downstream. Further development and application of automated systems such as the AutoSpawner is necessary to undertake reef restoration with sexually propagated corals at more ecologically relevant scales.

4.1. Broodstock holding and gamete collection

Automated gamete harvesting from a broodstock holding tank offers several advantages over manual sexual propagation methods for broadcast-spawning corals. In the context of large-scale coral propagation, whether for reef restoration, research or commercial purposes, the quality of the produced material, the cost-effectiveness of the processes used and the successful maintenance of healthy broodstock are all important considerations in sustainable facility design and operation (Banaszak et al., 2023; Guest et al., 2010; Omori, 2019; Randall et al., 2020). Manual sexual propagation typically involves a number of separate steps or processes (Fig. 1), which can lead to increased stress for broodstock (e.g. repeated manual handling during isolation and boundary layer hypoxia during isolation under static conditions) and reduced production of fertilised eggs (e.g. due to spawning starting prior to isolation, bundles separating prior to collection or extended time-delays before fertilisation commences due to staff shortages). The use of the AutoSpawner reduced the manual handling of broodstock colonies by eliminating the need for physical isolation, as well as the risk of gamete bundle losses due to uncertainty about when bundles might be released from the polyps (which differs between species). For example, the isolation of colonies and manual collection of gametes can be challenging when 'setting' is difficult to identify, or the release of the bundles happens immediately after setting can be detected (as observed for *D. favus* and *M. elephantotus*). These systems can also facilitate the use of larger numbers of parent colonies, resulting in a larger pool of genetic diversity in the produced material, without increasing the labour and effort required. Additionally, established husbandry protocols for broodstock can easily be implemented to the AutoSpawner system outside of expected spawning time-windows, to ensure optimal broodstock health during extended holding *ex situ* (e.g. as required for *D. favus* in the present study; Table S1), as well as during expected spawning windows (by allowing for continued water replacement and circulation in holding tanks). The AutoSpawner also maximised the potential number of gamete bundles collected, as well as eggs fertilised, by reducing the manual labour required for bundle collection and the time to fertilisation start (i.e. immediately following the start of the fertilisation window of the species in question; Table S4). This process more closely simulates the natural timings for bundle separation and fertilisation in the wild. While we applied protocols for bulk culturing (i.e. holding and mixing of gametes from several genotypes in a single process), the AutoSpawner and AutoFertiliser systems could also be easily adapted for separate collection of gamete bundles from individual colonies if required (by e.g. varying the holding tank sizes and configurations).

The use of fully automated systems (including the AutoSpawner and AutoFertiliser) will likely require species-specific programming and system configurations to account for differences in for example turbidity-sperm concentration relationships, synchronicity of spawning and fecundity. This was exemplified by the differences in turbidity observed at the AutoFertiliser outflow (*A. kenti*), by extended spawning windows (*A. loripes* and *M. elephantotus*) and by a high gamete output (*A. kenti*) for the species assessed here. To account for species-specific reproductive characteristics (e.g. fecundity, synchrony, sperm characteristics etc.), the use of systems of differing sizes (e.g. smaller holding tanks) or configuration (e.g. multiple AutoFertilisers used in each AutoSpawner to allow for secondary harvesting) may be required. For example, successful fertilisation of eggs from the most fecund species tested, *A. kenti*, could conceivably be doubled to 12 million by the addition of a second AutoFertiliser unit to the described AutoSpawner system. Conversely, later application of the system in 2023 revealed that even very small spawning batches, as low as 40,000 Acroporidae eggs, can be successfully fertilised with operator adjustments to maximise retention of sperm in the AutoFertiliser (AIMS, *unpublished data*). In addition, the assessment of spawning behaviour of species not previously used in the system is necessary to develop a database of fundamental reproductive processes and to account for species-specific differences (e.g. time from bundle release until bundle separation to release sperm, resulting in a detectable turbidity increase; Table S4). The gains in efficiency during subsequent spawning events, and associated potential to upscale sexual propagation processes within and across species, would ultimately outweigh the initial time and cost investment to gather the required information.

4.2. Dynamic fertilisation

Successful fertilisation of eggs is a key step in the sexual propagation of corals and optimisation of methods to successfully fertilise large numbers of eggs are necessary for upscaled production of corals for reef restoration. Historically, the fertilisation of eggs from broadcast-spawning corals have been performed under static (*ex situ*; Babcock et al. (1986); Guest et al. (2010)) or semi-static (e.g. floating oceanic ponds; Cruz and Harrison (2017); Edwards et al. (2015); Heyward et al. (2002)) conditions to avoid damage to developing embryos, due to the lack of a protective membrane (Heyward and Negri, 2012). However, the use of static conditions during fertilisation presents some substantial risks, in particular losses of fertilised eggs due to hypoxia as excess sperm starts to degrade (Guest et al., 2010), lowered fertilisation rate due to reduced chance of encountering compatible sperm (if mixture is not homogenous) and that eggs may undergo cleavage prior to transfer to culture tanks as a result of time-consuming, sequential washes performed by hand (e.g. Pollock et al. (2017)). Additionally, the continuing degradation of unfertilised, damaged or dead eggs and excess sperm transferred into larval cultures may lead to unwanted downstream mortality of larvae due to poor water quality (Guest et al., 2010; Pollock et al., 2017). These issues may be further compounded as egg stocking densities and culture sizes continue to increase in upscaled production processes to meet demand for restoration programs.

Here, and for the first time, we successfully fertilised coral eggs under dynamic conditions (using the AutoFertiliser) and showed that the vigorous mixing of the body of water during fertilisation did not negatively impact fertilisation success, egg morphology or larval quality (Table 1, Fig. 4 and Fig. S4). This demonstrates that the eggs (pre- and post-fertilisation) are more robust than previously assumed and that the 'gentle or careful' handling of coral spawn recommended previously (Babcock and Heyward, 1986; Guest et al., 2010; Heyward and Negri, 1999; Pollock et al., 2017) is not applicable for the time period prior to first cleavage. Dynamic fertilisation may instead lead to fewer damaged embryos and lowered early mortality in larval cultures by shortening the time from the start of spawning to transfer of fertilised eggs to culture tanks, and by ensuring that transfers are complete prior to the first occurrences of cell cleavage (Table 1). Dynamic fertilisation also resembles the natural turbulence (e.g. wave action) encountered by wild

populations, which occurs as often as calm conditions (Heyward and Negri, 2012). Additionally, dynamic fertilisation may increase the rate of interactions between genetically compatible sperm and eggs by ensuring continuous mixing and an even distribution of sperm throughout the tank (Fig. 3A).

As the system is modular, the AutoFertiliser could be operated as a stand-alone unit (refer to the AutoFertiliser section of the Supplementary Materials 1 for details) across a range of applications. For example, in a coral breeding program to enhance heat tolerance, gamete bundles could be collected in isolation from specific coral genotypes and rapidly fertilised in several AutoFertilisers to generate selected lines of interest (Chan et al., 2019; Humanes et al., 2021; van Oppen et al., 2015). This highlights the flexibility of the system, which can also be operated in part (or full) manually if desired.

4.3. Quality of embryos and larvae from AutoSpawner

Larvae produced from the AutoSpawner were of the same quality (morphology, survival and metamorphosis success) as those produced using the manual collection and fertilisation method (Fig. 4 and Fig. S6). There were no apparent morphological differences in the embryos produced using the two fertilisation methods (Fig. S5) with consistent embryogenesis and development 24 h post-fertilisation, suggesting no damage was sustained by embryos when fertilised using the AutoFertiliser. Up to 80% larval mortality was observed in the *A. loripes* cultures; however, this was consistent across the cultures generated from both the automated and manual workflows. This *A. loripes* mortality was higher than expected, and higher than the other species investigated here, but is comparable to that reported for *in situ* rearing pools over a similar period of time (95% mortality (Heyward et al., 2002)) and 400 L *ex situ* rearing tanks (55–88% mortality (Pollock et al., 2017)). Nevertheless, this unexplained mortality provided the opportunity to challenge the AutoSpawner workflow with sub-optimal gametes; and the survival and performance of produced larvae was no different to those produced using the manual method (see below). The number of larvae maintained in culture for *A. kenti*, *D. favus* and *M. elephantotus* was consistent across the study period and methodology (automated vs. manual). The variability in the collected count data among technical replicates was substantial (e.g. *M. elephantotus*), but comparable to the variability for larval culture count data reported elsewhere (Heyward et al., 2002). This variability could be reduced in future studies by taking larger subsamples or increasing the number of technical replicates at each assessment timepoint.

Metamorphosis success ranged from 52 to 98% across all four species assessed and was consistent within species across the two fertilisation methods tested. Metamorphosis success for *A. kenti* and *M. elephantotus* were both within the range expected (i.e. >90%) for healthy and competent larvae exposed to an appropriate settlement cue (Abdul Wahab et al., 2023). In contrast, fewer *A. loripes* larvae successfully completed metamorphosis than expected for a healthy batch culture (56–62% compared to 80–100%; Whitman et al. (2020)). This was not surprising given the low larval survival for this species, and may indicate low gamete quality. *D. favus* had the lowest metamorphosis success out of all species tested (52–58%); however, this is within the range previously reported for this species (Abdul Wahab et al., 2023).

4.4. Timings, labour and spatial footprint

The successful development of automated systems such as the AutoSpawner is crucial to upscaling coral aquaculture and coral recruit seeding approaches to 10s of millions of corals per spawning event (Anthony et al., 2019). Costing estimates have been made for complementary large scale restoration approaches, such as *in situ* embryo capture, transport and deployment (Doropoulos et al., 2019a,b). However, different coral life stages are addressed and the processes, infrastructure and required permitting are very different to those described here, and will also differ markedly between operations and locations. Therefore, a direct cost comparison between *in situ* and *ex situ* approaches is beyond the scope of the present study. Instead, we focussed on comparing the financial and spatial costs between the automated and manual systems in coral aquaculture that share comparable facilities and workforces.

The sexual propagation of corals is labour intensive and has been a manual process to date, which will require technological innovation and automation to facilitate the upscaled production required for ecologically meaningful reef restoration efforts. Labour in particular has been identified as the main cost associated with the sexual propagation of corals (both during spawning and the following rearing of recruits; Baria-Rodriguez et al. (2019)). The AutoSpawner significantly increases the efficiency of the sexual propagation of corals by reducing: i) the labour required to generate a unit of fertilised eggs; ii)the time required for the transfer of fertilised eggs to culture tanks from spawning; and iii) the spatial footprint of systems (Table 1). For example, a facility producing 4 million recruits for each of 20 broadcast-spawning species in a single spawning event might require the capacity to collect and fertilise 10 million eggs per species (assuming an average ~38% of collected eggs will successfully complete development and undergo settlement, as reported here). This would require the facility to hold at least 20 broodstock colonies per species (depending on colony size and the synchronicity of spawning each species *ex situ*), requiring approximately 100 staff to manually collect and fertilise the gametes (assuming one staff member is needed for every two colonies spawning at any given time, and that the spawning windows for 10 species overlap). Additionally, the facility space required for spawning systems would be ~440 m^2 to accommodate the separate steps involved in the manual workflow, and the holding of broodstock. In contrast, the same targets could be achieved using around 15 staff (depending on the level of automation) and a facility with ~230 m^2 of spawning systems if utilising AutoSpawners with two AutoFertilisers per system (i.e. a total of 40 AutoSpawner systems).

This cost-benefit analysis (financial and spatial) for facility design and workflow selection will; however, vary depending on several factors, including the location of the facility and the cost of local labour compared to initial outlay for building the manual and automatic systems. In addition to the biological advantages (discussed above) and cost efficiencies, the AutoSpawner can benefit work health and safety by reducing the likelihood of staff fatigue (decreasing the nighttime working hours) and reduced occurrences of manual handling injuries. While the initial outlay for a fully automated system may be inhibitory for some stakeholders and practitioners (e.g. non-government organisations), a more affordable version of the system can be acquired, while retaining the majority of benefits of the AutoSpawner, by operating the system in a semi-automated or manual mode (i.e. a staff member monitors the broodstock and manually adjusts valve settings). This may also be beneficial in locations where power is not reliable, or where automated components may be difficult to source. Operating these systems manually would however require staff with higher biological and technical expertise.

4.5. Future directions & further development

The AutoSpawner performed well across the four broadcast-spawning species assessed here and provides a pathway for further optimisations and developments for application across a broader diversity of corals. Adaptation of the system to allow for the semi-automatic collection of bundles separately for individual colonies would be useful for certain commercial and research applications. Similarly, the development of an analogous system for gonochoric species, or for species with negatively or neutrally buoyant bundles/gametes, as well as for rearing of recruits would be highly beneficial for reef restoration initiatives. However, substantial knowledge gaps remain with regards to the sexual propagation of many coral species, with several bottlenecks

preventing their use in reef restoration. This includes a broader understanding of species spawning times and behaviours, gamete collection (e.g. for dioecious species), fertilisation success, husbandry required for successful embryogenesis and larval rearing, induction of settlement and early recruit survival (Banaszak et al., 2023; Randall et al., 2020). Application of the AutoSpawner in reef restoration projects, or research and development programs, could free up existing expertise and personnel for exploring the early-life ecology of additional coral species, thereby increasing the rate of knowledge acquisition. Finally, the mobilisation of these systems could facilitate the set-up of production facilities for reef restoration projects in closer proximity to selected restoration sites where other infrastructure may currently be lacking. For example, the AutoSpawner could be readily adapted for mobility through containerisation (Craggs et al., 2023) or installation onboard vessels (AIMS and Chadwick, 2020; AIMS, 2022).

5. Conclusions

Reliable production of high-quality coral larvae and recruits in sufficient quantities is vital to significantly upscale reef restoration programs. Automated systems for coral propagation are therefore likely to become critical infrastructure as reef restoration efforts are ramped up worldwide to meet demand in coming years. All technological and methodological solutions for upscaling sexual propagation of corals must efficiently produce large amounts of high-quality material. Here we have demonstrated that systems such as the AutoSpawner can resolve, or minimise, several barriers identified for upscaling the production of corals for reef restoration. The AutoSpawner is a highly versatile system and can be operated fully automated or semi-manually and can be readily combined with other reef restoration interventions and systems. The AutoSpawner is expected to be applicable to most broadcast spawning species with buoyant egg-sperm bundles once appropriate, species-specific operational parameters have been determined. The application of automated systems enhances our ability to perform reef restoration at more ecologically relevant scales and offers a means to apply interventions for increased resilience using large numbers of selectively bred offspring. Increased efficiencies in reef restoration research and development programs, and increased opportunities to collaborate or build on existing designs and systems (e.g. through open access to blueprints), will become ever more important as the impacts of climate change on coral reef environments become more severe. However, rapid progress on potential interventions and methods for reef restoration must be combined with actions to reduce emissions from fossil fuels to ensure the survival of reef ecosystems in the medium to long-term (Hughes et al., 2023; Randall et al., 2020).

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CRediT authorship contribution statement

Andrea Severati: Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **F. Mikaela Nordborg:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Andrew Heyward:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Muhammad A. Abdul Wahab:** Writing – review & editing, Supervision, Methodology. **Christopher A. Brunner:** Writing – review & editing, Investigation, Data curation. **Jose Montalvo-Proano:** Writing – review & editing, Investigation. **Andrew P. Negri:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data, technical drawings and analysis scripts are available through the associated script (Nordborg, 2024) and data (AIMS, 2024) repositories.

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Appendix A. Supplementary data

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