Culturing the sea cucumber *Holothuria poli* in open-water Integrated Multi-Trophic Aquaculture at a coastal Mediterranean fish farm.

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Abstract

The survival and growth of the sea cucumber *Holothuria poli* were assessed during a 12-month field study when cultured at a commercial fish farm in Malta as part of an integrated multi-trophic aquaculture (IMTA) system. Sea cucumbers were cultured directly below a fish cage at 0 m, E0, at 10 m (E10) and 25 m (E25) distances from the cage and at two reference sites (R1 and R2) located over 800 m away from the fish farm. Mass mortalities were recorded at E0 within the first month of the study due to smothering by settled wastes. All individuals died at one of the reference sites, R1, by the end of the study. After deducting missing sea cucumbers, survival rates at E10 (23%) and E25 (33%) from the fish cage were similar to the remaining reference site (R2) (27%). Stocking density and physical disturbances to the sea cucumber cage setup were also probable cause for the low survival rates. The relative weight gain (RWG) and specific growth rates (SGR) of *H. poli* varied significantly between sites close to the fish farm and the reference site. The SGR of *H. poli* at E10 (0.18 ±0.02% day^{-1}) and E25 (0.20 ±0.01% day^{-1}) was positive over the whole study period while no average growth was recorded at the reference site (-0.04 ±0.07% day^{-1}) over the same period. Differences in RWG and SGR were recorded throughout the study. The overall growth observed in *H. poli* by January was followed by a drop in growth rate across all sites and an increase in SGR at E25 in July. Slower growth rates were observed as water temperature approached 15 ^{o}C. The results indicated that the sediments near the commercial fish cage provided an enriched source of food that supported significantly better growth in *H. poli*. This suggests that *H. poli* in IMTA might have the potential to uptake organic farm waste and increase aquaculture production, albeit with important considerations for setup design and stocking density.

Keywords:

Sea cucumber, IMTA, sustainability, biodeposition, circular economy

1. Introduction

The sustainable development of aquaculture requires consideration of carrying capacity and environmental impact (Ross et al., 2013). In coastal areas where competition for space is a limiting factor, existing production sites may need to be optimised through innovative and novel approaches for further aquaculture expansion (Barrington et al., 2009). These strategies should address the widely documented effects of waste deposition from intensive marine fish farming that include loss of benthic biodiversity (Mazzola et al., 2000; Kalantzi and Karakassis, 2006; Tomassetti et al., 2016) and changes in sediment chemistry, such as increased organic carbon and oxygen depletion (Karakassis et al., 2000; Porello et al., 2005; Papageorgiou et al., 2010).

The uptake and conversion of fish farm waste into marketable biomass by extractive organisms co-cultured with fed species through integrated multi-trophic aquaculture (IMTA) is often recommended as a way to diversify and maximise production while reducing the environmental impact of intensive farming (Troell et al., 2009; Chopin et al., 2012). While previous studies have shown the viability and efficiency of IMTA in land-based systems or laboratory trials, the fewer reported cases of open-water IMTA have reported contradictory growth performances for different fed-extractive species combinations (Mazzola and Sarà, 2001; Cheshuk et al., 2003; Handå et al., 2012; Irisarri et al., 2013, 2014; Jiang et al., 2013; Giangrande et al., 2020). This demonstrates the challenges of coastal and nearshore IMTA in the real-world environment.

Deposit-feeding sea cucumbers have been proposed as potential candidates to take up and reduce the excess accumulation of organic nutrients in seafloor sediment from farm production (Cubillo et al., 2016; Zamora et al., 2016). The potential of deposit-feeders to mitigate organic pollution emerges from their capacity to rework as much as 10,590 kg dry sediment m^{-2} $year^{-1}$, while feeding selectively on enriched sediments (Lee et al., 2018). Sea cucumbers are not only recommended for use in IMTA for their waste biomitigation potential but also for their economic value (Toral-Granda et al., 2008; Purcell, 2015). High value sea cucumber species are sold as premium products to meet the increasing demands of luxury seafood markets in Asia. In the Mediterranean region, active fisheries exist for commercially important sea cucumber species with *Holothuria poli* and the similar shallow water holothurian, *Holothuria tubulosa* becoming increasingly popular target species (González-Wangüemert et al., 2018) and favourable candidates for aquaculture (Rakaj et al., 2019). Recently, these species have been increasingly harvested along the Italian coast leading to a moratorium on sea cucumber fishing in Italy.

The little data available in literature on the growth and natural density of this species for populations present in other regions of the Mediterranean is limited by variation in geographic and bathymetric distribution (Francour, 1989). Similarly, the population density of the related *H. tubulosa* varies across the Mediterranean within the range of 0.1 – 3.77 individuals m^{-2} (Coulon and Jangoux, 1993; Kazanidis et al., 2010). In the wild, *H. poli* is found in soft marine sediment where naturally occurring organic content is low. Consequently, the foraging behaviour and digestive capacity of *H. poli* allow this sea cucumber to effectively select and assimilate organic-rich particles (Mezali and Soualili, 2013; Belbachir et al., 2014). This underlies the potential importance of this species for nutrient recycling. *H. poli* is also considered a benthic indicator sensitive to pollution (Harmelin et al., 1981; Mezali, 2008). While high organic inputs may be an opportunity for value-added production, additional nutrient inputs from a fish farm could have detrimental effects on the physiological performance of the selected species.

In recent studies in laboratory settings and pilot-scale field experiments, sea cucumbers were able to survive and grow on organic waste from finfish culture (Nelson et al., 2012; Yu et al., 2012; Hannah et al., 2013; MacDonald et al., 2013; Yokoyama, 2013; Yu et al., 2014a, b). In the Mediterranean, the potential of *H. tubulosa* to reduce organic waste in fish farm biodeposits has been shown by the three-fold growth difference when placed under an open-water commercial monoculture farm in sediment with organic carbon content 30 times higher than that present in natural environment (Tolon et al., 2017a). The capacity of individual holothurians to ingest up to 15.25 kg dry weight sediment m^{-2} yr^{-1}, and reduce as much as 74.09% of organic carbon per mean total drained weight of 196 g, should lead to further research into the suitability of deposit-feeding sea cucumbers to recycle and remediate organically rich sediments in nearshore IMTA (Neofitou, 2019). Studies on the reduction rate and absorption efficiency of organic carbon and *in situ* growth rates of *H. poli* are lacking. Nonetheless, the proposed species exhibited similar growth rates as *H. tubulosa* under laboratory conditions (Tolon, 2017) with comparable capacities for particle selectivity (Mezali and Soualili, 2013) and higher adaptability to elevated salinities (Tolon, 2017). This suggests *H. poli* could also be a good candidate for IMTA.

This study introduces *H. poli* as an alternative candidate to open-water IMTA in the Mediterranean and aims to provide baseline information on the survival and growth performance of this sea cucumber when cultured in a commercial fish farm.

2. Materials and methods

2.1. Study site

The study was set up at Malta Fish Farming Ltd., a nearshore commercial fish farm in Marsaxlokk Port (35°49'39.90"N, 14°32'30.73"E), southeast Malta (Figure 1A), situated at the centre of the Mediterranean. The fish species that are cultured at this nursery site include the gilthead sea bream (*Sparus aurata*)*,* the European sea bass (*Dicentrarchus labrax*), and the greater amberjack (*Seriola dumerili*). Gilthead sea bream and European sea bass are produced using formulated commercial feed whilst the amberjacks are fed baitfish. The farm had a total annual production of 719 t and a feed conversion ratio (FCR) of 1.7 during the study period.

The nursery site is in shallow water (depth 8-12 m) and on a seafloor characterised by heterogeneous soft sediments (very fine to medium grain size). The farm is surrounded by patchy distributions of seagrass, *Posidonia oceanica*. Dredged navigation channels are present on the south eastern side to accommodate transhipment traffic. The reference sites, R1 (35°49'55.1"N, 14°32'59.3"E) and R2 (35°49'53.5"N, 14°32'54.5"E), were located >800 m north east of the fish farm in 8 m water depth. No aquaculture activity was present within a 600 m radius from the nursery site (Figure 1B). The simulated maximum velocity of the dominant wind-driven currents from the prevailing northerly winds inside the bay never exceed 0.4 m s^{-1} (Eikema and van den Boomgaard, 2007).

2.2. Sea cucumber cage siting

The spatial arrangement of the IMTA setup at the farm site (Figure 1C) was based on the output of a particulate depositional model (Telfer et al., 2006; Baltadakis et al., 2020). The spreadsheet-based model uses production information, hydrography, and empirically derived measurements to determine the amount of organic carbon and the form of waste that is dispersed from the fish farm to the environment.

The horizontal dispersion of unconsumed food and fish waste was modelled using site-specific water current speed and directions. Production data was obtained from the farm management and hydrographic data was collected at 8 m and 12 m depths over 6 weeks using a current meter (Aquadopp Profiler 400Hz; Nortek, Norway). The profiler was deployed on the seabed between May and June 2018, at two different locations both approximately 10-15 m from the fish cages (Figure 1C). Stocking biomass, feed input, water depth, and cage dimensions were incorporated into the particle depositional model to predict nutrient dispersion using a grid resolution of 5 m x 5 m. Input data on food losses and settling velocities for feed and faecal particles for the different cohorts of cultured fish species on the farm was obtained from literature (Chen, 2000; Vassallo et al., 2006; Magill et al., 2006; Piedecausa et al., 2009; Cromey et al., 2012; Brigolin et al., 2014; Ballester-Moltó et al., 2017).

Three cylindrical sea cucumber cages (1 m x 0.2 m (*d* x *h*)) made of 0.8 cm galvanised mesh wiring and a synthetic rope mesh bottom were set at 8 m depth directly below a commercial fish cage at 0 m (E0), another three cages at 10 m (E10) and then at 25 m (E25) from the fish cage. The same cage setup was used at the reference sites, R1 and R2 (Figure 1B). At each site, sea cucumber cages were spaced out evenly every 2 m, weighted at either side of each cage and moored to the seafloor in parallel to the fish cages.

2.3. Sea cucumbers

Juvenile *H. poli* specimens were collected in September 2018 by SCUBA diving Palermo, Sicily and shipped to a land-based facility in Malta. Sea cucumbers were acclimated in 500 L tanks that were supplied with flow-through ambient water from the nearshore study site. The juveniles were fed an artificial microalgal diet (Algamac Protein Plus, Pacific Trading, Ireland) for two weeks prior to the start of the experiment. Faeces and accumulated uneaten feed were removed every two days, and physicochemical parameters of water quality in the tanks were assessed daily. No evisceration or mortalities were recorded during the acclimation period. At the end of this period, specimens that showed no evident signs of disease and were of similar initial mean weight (± standard deviation, SD) (24.6 ± 2.1 g) were selected.

The field trials started in October 2018 and ran until September 2019. 150 sea cucumbers were selected for the experiment and each cage randomly stocked with 10 sea cucumber specimens with an initial mean stocking biomass of 313 ± 6.6g m^{-2}. The initial coefficient of variation (CV) for sea cucumber weight was <12% and without significant differences (p= 0.183) between sites.

2.4. Sea cucumber survival and growth

Every two months, SCUBA divers retrieved all the sea cucumbers from their cages to assess survival and growth performance. Missing sea cucumber individuals were considered as mortalities and deducted. In addition, apparent diseased sea cucumbers present in each cage were removed and considered dead. Any water, sediment, and detritus on the sea cucumbers' integument was removed and individuals were weighed to the nearest ± 0.1g at 30 s of removal from seawater to allow sea cucumbers to drain. After weighing, the surviving sea cucumbers were redeployed to their respective cage.

The mean relative weight gain (RWG), growth rate (GR), specific growth rate (SGR) and survival rate of *H. poli* for each experimental cage were determined according to Equations 1 to 4 respectively.

$$\text{RWG (\%)} = 100 \times (W_2 - W_1)/ W_1 \qquad \text{[Equation 1]}$$

$$\text{GR (g day}^{-1}) = W_2 - W_1/ t \qquad \text{[Equation 2]}$$

$$\text{SGR (\% day}^{-1}) = 100 \times (\ln W_2 - \ln W_1)/ t \qquad \text{[Equation 3]}$$

$$\text{Survival rate (\%)} = 100 \times (n_2/ n_1) \qquad \text{[Equation 4]}$$

where W_2 and W_1 are final and initial mean wet weight (g); t is culture duration (days); and n_2 and n_1 are final and initial number of *H. poli* individuals in each cage.

2.5. Environmental parameters

Measurements of water quality were recorded at each experimental site. Temperature (°C), dissolved oxygen (DO; mg L^{-1}), and pH were measured on site from near-bottom water samples collected in Niskin bottles and measured by an HQD Intellical meter (HQ30d; HACH, US), over 12 months between October 2018 and September 2019. Duplicate 2 L water samples were collected and transferred to the laboratory in a cool box for the quantitative analysis of nutrients and total suspended solids (TSS). These water quality parameters were assessed monthly from the fourth month of the study (February 2019) when suitable equipment and method detection limits were applied. Samples for the analysis of N-NH_4^+ were preserved with sulphuric acid. The parameters and methods of analyses, together with their limits of detection (LOD), are presented in Table 1.

Seafloor sediments were collected in triplicates using sediment cores (5 cm diameter) at the start of the experiment in October 2018, then in February, May and at the end of the study, in September 2019, from each individual sea cucumber cage site. The top 3 cm of sediment samples were sliced, extracted and rinsed with distilled water. Dried samples were ground and homogenised prior to the analysis of total organic carbon (TOC) and total nitrogen (TN). Sub-samples were weighed in tin capsules and then analysed using a FlashSmart NC ORG elemental analyser. TOC was determined by deduction after ashing samples at 600°C for 12h, and analysing total inorganic carbon. TOC and TN contents were calculated by comparison to standard samples. Elemental C: N ratios reported as weight ratios and expressed as mg mg^{-1}.

2.6. Data analyses

Statistical analysis was performed using SPSS v26.0 for Windows (SPSS Inc., Chicago, USA). Shapiro-Wilk's test was used to assess normality of variables whereas homogeneity of variances was determined using Levene's test. A significance level of 0.05 was assumed. Environmental parameters were assessed using a General Linear Model (GLM) (*site* x *time*) followed by pairwise comparisons with Bonferroni adjusted significance levels.

Survival and growth parameter data were analysed using a GLM (site x time) with pairwise comparisons with Bonferroni correction. Differences between sites at the same sampling time were assessed using one-way ANOVA followed by Tukey's *post hoc* test. A generalised linear model was used to assess data that violated the assumption of normality. Data on growth rates were represented by the average value for each cage for the different experimental sites. Zero values for growth parameter data were omitted from the analysis.

3. Results

3.1. Hydrography and waste dispersion

The hydrography collected from the two sites near the fish farm showed average near-seabed flows of 0.091 m s^{-1} in May and 0.149 m s^{-1} in June 2018, with slow residual current flows and a south westerly to westerly direction during these periods.

The dispersion of particulate waste from the fish cages was highest directly below the fish cage, 74.3 gC m^{-2} day^{-1} in May and 23.3 gC m^{-2} day^{-1}in June (Figure 2). The predictions of sedimentation show that the deposition of organic carbon was local to the fish cage area with flux decreasing with increasing distance from the edge of the fish cage. Model predictions showed similar estimated values for organic carbon at E10 in May (5.6 gC m^{-2}) day^{-1} and June (5.1 gC m^{-2} day^{-1}). The model shows that sea cucumbers were placed in an area of high organic waste deposition of at E0, whereas low amounts of organic carbon reached the experimental site at 25 m (E25) (May: 0.02 gC m^{-2} day^{-1}; June: 0.4 gC m^{-2} day^{-1}).

3.2. Environmental parameters

Throughout the trial period, temperature ranged between 14.9 ± 0.1 ºC and 28.3 ± 0.1 ºC with similar readings recorded across sites (p= 0.186) (Figure 3A). The lowest water temperature was recorded in February, and then increased steadily to reach peak readings in August. DO levels varied significantly over time (p< 0.001) in the range between 6.3 ± 0.3 mg L^{-1} and 10.5 ± 0.1 mg L^{-1} (Figure 3B). The lowest readings were recorded in proximity to the fish cages (E0 and E10) whereas the highest values were recorded at the reference sites (p< 0.001). DO levels were always above 5 mg L^{-1}. The water pH was consistent until March (8.18 ± 0.06 to 8.32 ± 0.01), and then levels decreased to reach the lowest recorded values (7.90 ± 0.03) at E0 in June (Figure 3C).

TSS and nutrients levels varied significantly across sampling sites (p< 0.05), with the exception of ammonia (p= 0.186). Levels of ammonia changed significantly over time (p< 0.001), particularly apparent were the peak level recorded in August (p< 0.05) (Figure4A). Similarly, peak nitrate levels were recorded towards the end of summer, notably at E0 (2.89 ± 0.92 µmol L^{-1}) (Figure 4B). The nitrite levels were consistently below 0.05 µmol/L and similar across all sites (Figure 4C); however, concentrations increased significantly in April and September at these sites (p< 0.05). TP levels dropped rapidly after February with significant differences across most sampling periods (p< 0.05) (Figure 4D). TSS levels increased to a peak concentration in late spring (33.38 ± 0.35 mg L^{-1}). Pairwise comparisons revealed marked variation in TSS levels across most sampling periods (p< 0.05).

Differences in nitrate concentration were significant between E0 and the sites close to the fish cage, E10 and E25, and the reference sites, R1 and R2 (p< 0.05). On the other hand, marked differences in nitrite levels were only recorded between sites E0 and R1, and R1 and R2 ($p < 0.05$). TSS levels under the fish cage (E0) were significantly higher than those recorded at E10 and R1 ($p < 0.05$).

The content of TN, TOC and C/N in surface sediments varied significantly between the different sites and over time ($p < 0.05$), except TN levels between the different sampling periods ($p = 0.437$). Sediments near the fish cages were generally more enriched in TN content than at the reference sites, with distinct spatial patterns (Figure 5A). TOC content in surface sediments did not show consistent spatial patterns between sites near fish cages and those at the reference location during the study (Figure 5B). Measured sedimentary organic carbon levels were lowest at all the sampled sites in May ($p < 0.001$), with the weight ratios of C/N showing similar temporal trends ($p < 0.001$). The C/N ratios in sediments were significantly lower near fish cages in October (Figure 5C).

3.3. Survival and growth

Within the first month of the study, all experimental cages on the seafloor directly below the fish cage (E0) were completely smothered with sediment consequently leading to mass mortality of the sea cucumbers (Figure 6). At the other IMTA and reference sites, survival rates were relatively high until March, followed by a marked drop across all groups by May. Episodic storm events between March and May disturbed the sea cucumber cage setup and contributed to escapees and mortalities. At R1, all sea cucumbers were either missing or dead by September. The survival rates remained stable in the remaining groups until the end of the study. As a result of loss or mortality, the final survival rates at E10 and E25 were 23 % and 33 % respectively, similar to that of *H. poli* at R2 which was 27 % ($p = 0.706$).

The final weight of *H. poli* juveniles at E10 ranged between 38.5 g and 61.0 g (mean ± SD, 47.0 ± 7.0 g, *n*= 7), whereas that at E25 was between 42.5 g and 58.5 g (mean ± SD, 48.6 ± 5.0 g, *n*= 10). The final weight range of *H. poli* at R2 was between 11.4 g and 36 g with a mean of 20.5 ± 8.0 g (*n*= 8) (Figure 7). Juvenile *H. poli* cultured at E10 and E25 showed an overall positive RWG with the greatest increase being the approximate two-fold increase at E25 over the culture period (Figure 6), though not statistically different from that of *H. poli* at E10 (87%). On the other hand, *H. poli* at R2 had suffered a loss in biomass by the end of the experiment (-12 %). The RWG differed significantly between sites (p= 0.008) with biomass gains in juvenile *H. poli* at E10 and E25 being significantly higher than those at R2.

The SGR of *H. poli* at sites E10 and E25 was positive over the whole study period (E10= 0.18 ± 0.02 % day^{-1}; E25= 0.20 ± 0.01 % day^{-1}) whereas that at R2, was negative (-0.04 ± 0.07 % day^{-1}). The most marked differences in growth rates were between E25 and R2 (p= 0.037) (Figure 8). The growth rates were influenced by site during the study with multiple comparisons showing differences in SGR of *H. poli* between R1 and the IMTA sites, E10 and E25 (p< 0.001). Differences in growth rates were recorded with peak growth at E25 in July (SGR= 0.64 % day^{-1}; GR= 0.24 ± 0.05 g day^{-1}) and E10 in November (SGR= 0.58 ± 0.1% day^{-1}; GR= 0.20 ± 0.01 g day^{-1}). The SGR of *H. poli* dropped significantly by March with little to no growth reported at the IMTA sites and negative growth rates at the references sites in the same period (p< 0.05), though not necessarily correlated with the temporal variability in the water quality parameters. The SGR of *H. poli* increased again towards the end of the experiment (p> 0.05).

4. Discussion

This work aimed to assess the survival and growth of *H. poli* on seafloor sediment that receives organic waste from uneaten fish feed and faecal pellets. Findings show that with proper siting the deposit-feeding *H. poli* can survive and grow better on seafloor sediment near commercial fish farms compared to the natural environment.

The mass mortalities reported for sea cucumbers directly below the fish cage indicate unfavourable conditions for bottom culture of *H. poli*. The lower DO levels recorded under the fish cage may be attributed to a higher flux and accumulation of organic matter, but they were not at levels considered lethal in other species. Similar events of mass mortality were reported for *Holothuria leucospilota* cultured directly below the fish cage when DO levels were <2.5 mg L^{-1} but survived when DO levels were above 3.2 mg L^{-1} (Yu et al., 2012). In this study, the concentrations of DO in near-bottom water were consistently above 5 mg L^{-1}, similar to conditions maintained in the laboratory culture of *H. poli* (Tolon, 2017). This sea cucumber species has been reported to survive and grow in laboratory conditions (Tolon, 2017) at similar water temperature experienced during the mass mortality at E0. Moreover, sea cucumbers were able to grow in similar temperature conditions at the other IMTA and references sites therefore this is not expected to have contributed to the mass mortality at E0. Other water quality parameters measured would not be expected to bring about this level of mortality, which implies that the mortalities could be attributed to the conditions that prevailed in seafloor sediment below the fish cage and possibly farming activity that was only relevant to this area. Conversely, the species may have a high natural mortality in the wild but there is little information on this at present. Further work is therefore needed to investigate this mortality and to understand how this will effect commercial sea cucumber IMTA.

The particulate depositional modelling conducted prior to the present study showed that the estimated deposition of organic carbon was highest directly below the sea bream cage, at 2302.3 gC m^{-2} in May and 721.4 gC m^{-2} in June before *H. poli* juveniles were deployed under the fish farm. Several studies have reported excess sedimentation of organic matter below the fish cage that decreased with distance away from the cage (Pérez, et al. 2002; Sarà et al., 2004; Corner et al., 2006; Holmer et al., 2007). Findings suggest that the benthic community structure is affected when carbon flux (> 1 - 2 gC m^{-2} day^{-1}) exceed carbon loading tolerances (Holmer et al., 2005; Chamberlain and Stucchi, 2007). Based on these deductions, the modelled deposition of organic carbon in May and June may suggest changes to benthic conditions, at least during this period. These conditions would be expected to have effects on benthic sediment quality, among which elevated sediment oxygen demand (Wu, 1995; Holmer et al., 2005). The predicted load and accumulation of organic matter over a short period may be cause for the mass mortalities recorded below the fish cage. This suggests that integrating sea cucumber experimental sites within commercial monoculture requires consideration for suitable culture methods, appropriate setup design and siting considerations for IMTA systems.

The predicted levels of sedimentation decreased rapidly within a short distance from the fish cage. This gradient suggests that benthic enrichment may be localised at the studied farm site. The accumulation of organic particulates in surface sediments near the fish cages can be associated with the settlement of fish waste products and uneaten feed pellets (Sarà et al., 2004; Cromey et al., 2012). Waste from fish farms can be an additional organic-rich source that releases particulate nitrogen and carbon in varying proportions. The low C/N values near fish cages, particularly in October, follow trends of lower C/N ratios under sea bream and sea bass net cages in other Mediterranean countries (Holmer et al., 2007). In this study, nitrogen enrichment in sediments near fish cages and TN values that declined away from the farm also corroborates findings from isotopic studies in similar environments (Sarà et al., 2004; Holmer et al, 2007). The lack of consistent spatial patterns in sedimentary organic carbon content and lower than predicted values may be attributed to farm and site-specific variables like sedimentary metabolic processes that would require further research.

The growth performance of *H. poli* at E10 and E25, relative to that at E0 and the reference sites, indicates that the quantities and quality of organic content in seafloor sediment at these sites were suitable for sea cucumbers. Nevertheless, the reported escapees and low survival rates across all sites when compared to other field studies (Yokoyama, 2013; Yu et al., 2014a, Neofitou et al., 2019) suggest other critical factors may need to be considered for open-water culture of juvenile *H. poli*. In addition to the impact of severe weather events, the survival and growth of *H. poli* may have been influenced by the initial stocking density. The stocking density for juvenile *H. poli* in this experiment was higher than that (10 individuals m^{-2}) recommended by Nefitou et al. (2019) for *H. tubulosa*. The total stocking biomass used in this experiment (313 g m^{-2}) of *H. poli* was higher than that used in the laboratory culture of *H. poli* (270 g m^{-2}) by Tolon (2017) and that proposed for long-term culture of *H. tubulosa* (6 individuals m^{-2}, 253 g m^{-2}) (Tolon et al., 2017a) where no mortalities were recorded. Studies have reported survival rates up to 100% and higher growth rates for *A. japonicus* when cultured at low densities in similar studies (Yokoyama, 2013; Yu et al., 2014a). Conversely, survival rates in *H. tubulosa* showed a significant drop during a 30-day field trial at higher culture density (ca. 3300 g m^{-2}) (Neofitou et al., 2019) than that used in the present study. Competition is expected to have contributed to the missing *H. poli* individuals and consequentially, the low survival rates. This may be affirmed by the absence of individuals and escape attempts from experimental cages, in response to competition for space and food. This further demonstrates the need to use appropriate setup design for the bottom-culture of this deposit-feeding sea cucumber. While no published information is available on the local natural population densities of *H. poli*, the survival and growth performance of *H. poli* would be expected to improve at lower stocking densities.

Quantitative data on the growth performance of *H. poli* in the wild environment is limited and extensive trends of growth are unknown. The RWG of *H. poli* juveniles at E10 and E25 suggests a suitable food source for sea cucumbers, corroborating findings that deposit-feeders grow better in organic-rich sediments (Nelson et al., 2012; Yu et al., 2012; Hannah et al., 2013; MacDonald et al., 2013; Yokoyama, 2013; Yu et al., 2014a, b). These findings are consistent with other open-water studies that have demonstrated the ability of sea cucumbers to grow better in proximity to fish cages rather than at reference sites (Yokoyama, 2013; Yu et al., 2014a, b, Tolon et al., 2017b; Grosso et al., 2021). Over 8 months, Yokoyama (2013) reported up to a 421-fold weight increase in *A. japonicus* of initial mean weight (± SD), 0.12 ±0.10 g, when deposition was between 0.11 gC m^{-2} day^{-1} and 2.17 gC m^{-2} day^{-1}. The RWG of *H. poli* near the fish cages in the present study is comparable to that of *A. japonicus* juveniles in Yu et al. (2014a).

During a 3-month study by Tolon et al. (2017b), *H. tubulosa* grew an average of 0.32 % day^{-1} below commercial cages in sediments enriched in organic carbon content (4.68 - 4.84 %) and without record of mass mortalities. The average growth rate of *H. tubulosa* decreased (0.22 % day^{-1}) at 70 m from the farm in sediments that were less influenced by organic carbon flux (3.64 - 4.2 %) (Tolon et al., 2017b). Over 12 months, the growth rates of *H. poli* close to the fish cages (0.18 – 0.2 % day^{-1}) were lower than the rates achieved by *H. tubulosa*. Nonetheless, peak growth rates of *H. poli* observed in this trial in November and July were higher than that reported by Tolon et al. (2017b) and comparable to those of *A. japonicus* in similar studies (Yu et al., 2014a). The SGR of *H. poli* at E10 and E25 increased until January but then decreased by March. This compares well with the growth trends of *A. japonicus* in open-water IMTA (Yokoyama, 2013; Yu et al., 2014a).

In a land-based setup, the average SGR of *H. poli* was 0.03 % day^{-1} at 15^{o}C and 0.87 % day^{-1} at 25 ^{o}C with juveniles able to survive and maintain weight through the winter (Tolon, 2017). In the present study, growth performance in *H. poli* generally decreased and subsequently stagnated to maintain weight as water temperatures approached 15 ^{o}C. While optimal water temperatures are species-specific, the seasonal fluctuations in growth performance of *H. poli* agree with conclusions of higher growth in the warmer months, and marginal or lack of growth for *H. tubulosa* and *A. japonicus* as water temperatures dropped to sub-optimal temperatures (Yokoyama, 2013; Günay et al., 2015; Tolon, 2017).

Conclusions

This 12-month field study showed that *H. poli* was able to grow better in locations near a commercial fish farm rather than at the reference sites, suggesting that sea cucumbers may have utilised nutrients from the waste released from the fish farm. Further research is needed to establish connectivity and to show a trophic link between the fish farm and the sea cucumbers using biochemical tracers such as stable isotopes, and then assess the potential to reduce organic waste from the fish cages.

In this study, the sea cucumbers directly below the commercial fish cage were smothered by nutrient input in an event of mass mortality. This revealed that the rates of sedimentation and the organic load in sediment at this site were not suited for the bottom culture of *H. poli* at these densities. This study suggests that site layout in IMTA must consider how waste is dispersed from the fish cage.

Despite the biomass increase, high and comparable mortality rates across IMTA and reference sites reveal the need for improved culture conditions. The feasibility of using *H. poli* in open-water IMTA would primarily depend on better survival rates, possibly addressed through optimal stocking densities and with setup design consideration. This would also depend on a better understanding of the physiological and metabolic requirements of *H. poli* at different growth stages and environmental conditions.

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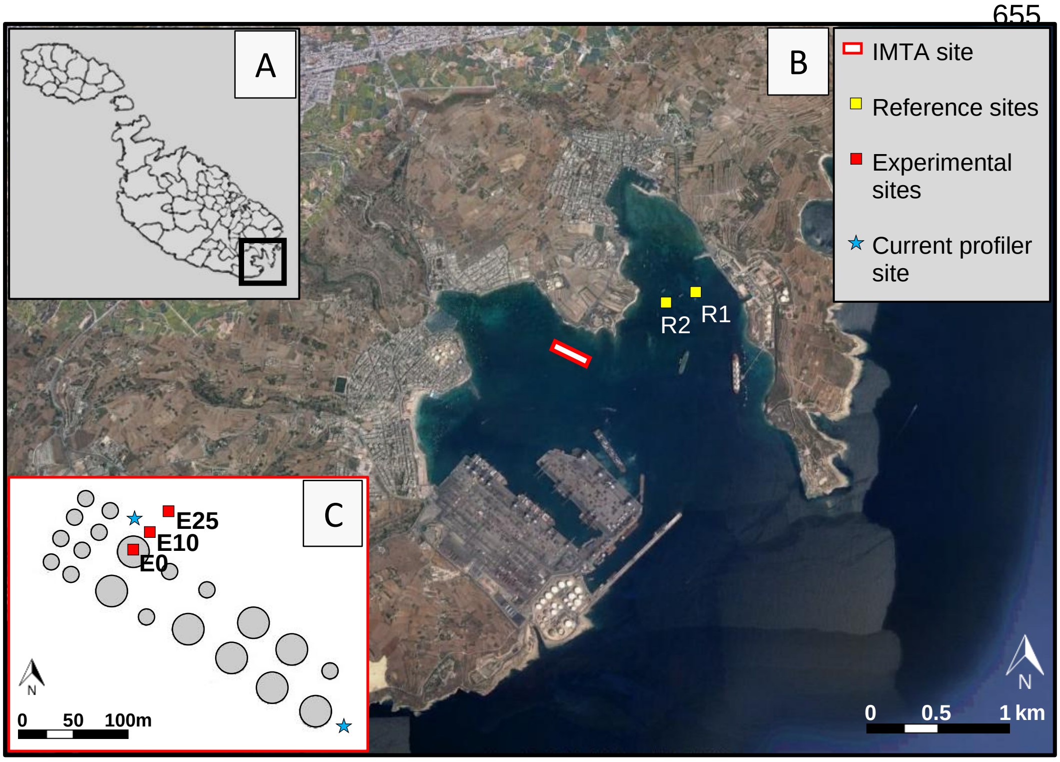


Figure 1A. Location of the test site within Marsaxlokk Port, southeast Malta. B. Zoomed Google Earth image of location showing the IMTA and reference sites, R1 and R2. C. IMTA site showing the sea cucumber cage positions at each experimental site and the deployment locations of the current profiler.

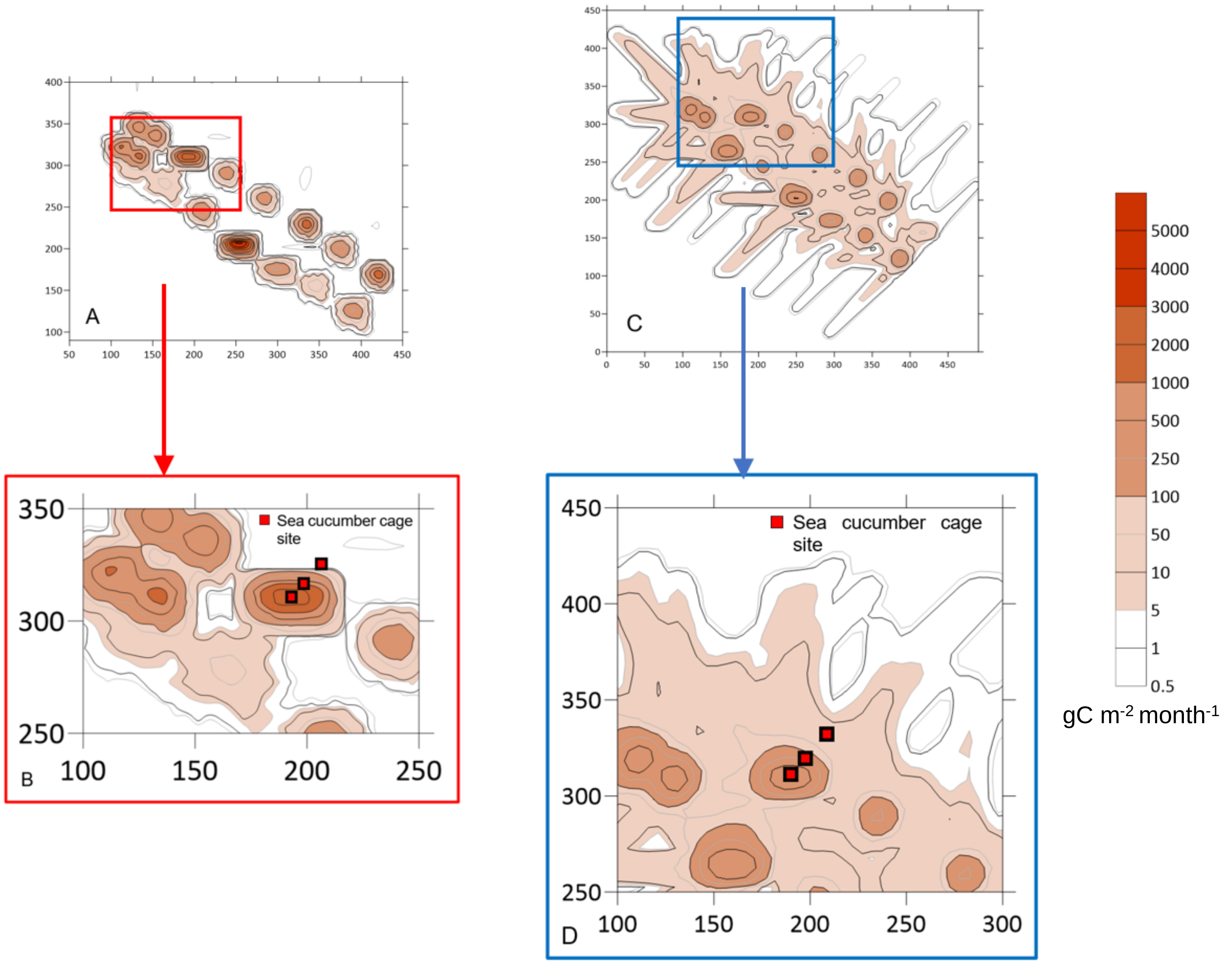


Figure 2. Modelled contour plot in 5m x 5 m grid resolution for carbon waste deposition (g m^{-2} $month^{-1}$) at the study site. Plot for deposition from A. all cages on the fish farm and B. fish cage used for co-culture of *Holothuria poli* in May 2018. C. Plot for deposition from all cages on the fish farm and D. fish cage used for co-culture of *Holothuria poli* in June 2018.

Table 1. Methods of analyses used to assess near-bottom water quality parameters

Parameters	Abbr.	Unit	Principal analytical method	Standard methodology	Limit of detection (LOD)
Ammonium Nitrogen	$N\text{-}NH_4^+$	µmol L^{-1}	Spectrophotometry	APAT CNR IRSA 4030 A1 Man 29 2003	0.03
Nitrate	$N\text{-}NO_3^-$	µmol L^{-1}	Spectrophotometry	APAT CNR IRSA 4040 A2 Man 29 2003	0.03
Nitrite	$N\text{-}NO_2^-$	µmol L^{-1}	Spectrophotometry	APAT CNR IRSA 4050 Man 29 2003	0.03
Total Phosphorus	TP	µmol L^{-1}	Spectrophotometry	APAT CNR IRSA 4110 A2 Man 29 2003	0.03
Total Suspended Solids	TSS	mg L^{-1}	Gravimetry	APAT CNR IRSA 2090 B Man 29 2003	0.01

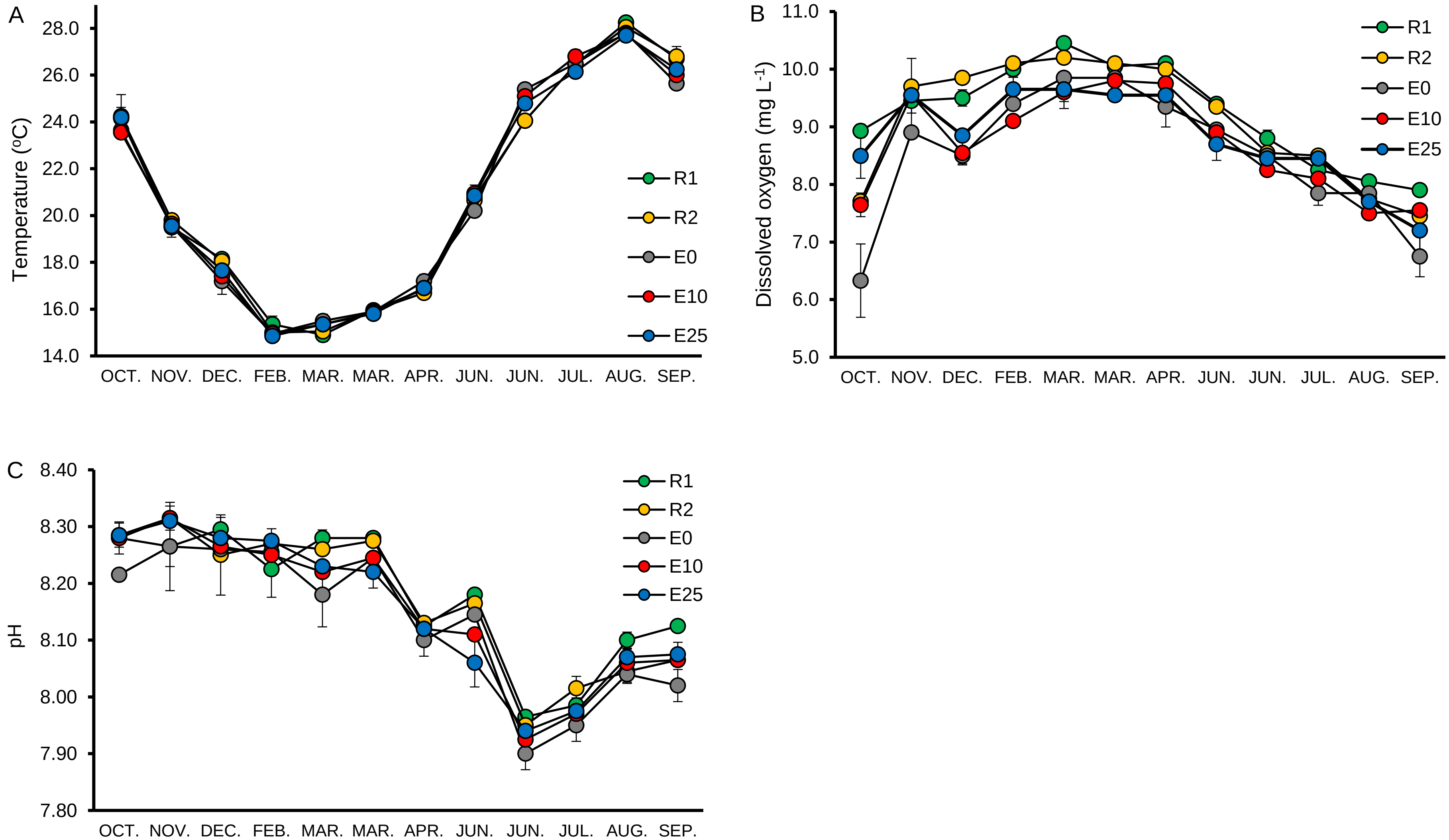
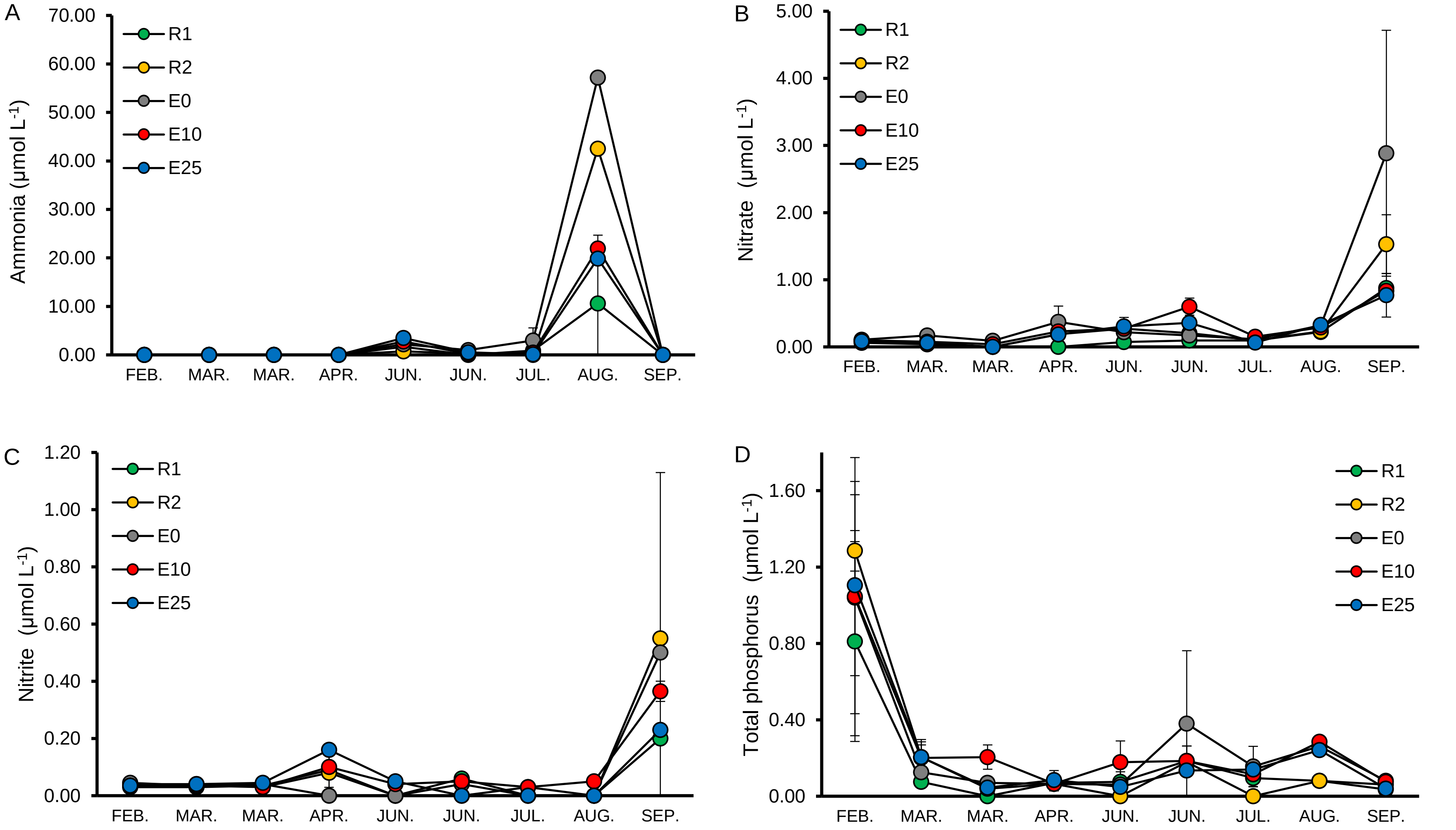

A
Temperature (ºC)
28.0
26.0
24.0
22.0
20.0
18.0
16.0
14.0
OCT. NOV. DEC. FEB. MAR. MAR. APR. JUN. JUN. JUL. AUG. SEP.
R1
R2
E0
E10
E25
B
Dissolved oxygen (mg L-1)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
C
pH
8.40
8.30
8.20
8.10
8.00
7.90
7.80

Figure 3. Temporal variation in near-bottom A. temperature, B. dissolved oxygen levels and C. pH, close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (*n* = 2). Error bars plotted for standard deviation.


A
Ammonia (µmol L-1)
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
R1
R2
E0
E10
E25
FEB.
MAR.
MAR.
APR.
JUN.
JUN.
JUL.
AUG.
SEP.
B
Nitrate (µmol L-1)
5.00
4.00
3.00
2.00
1.00
0.00
R1
R2
E0
E10
E25
FEB.
MAR.
MAR.
APR.
JUN.
JUN.
JUL.
AUG.
SEP.
C
Nitrite (µmol L-1)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
R1
R2
E0
E10
E25
FEB.
MAR.
MAR.
APR.
JUN.
JUN.
JUL.
AUG.
SEP.
D
Total phosphorus (µmol L-1)
1.60
1.20
0.80
0.40
0.00
R1
R2
E0
E10
E25
FEB.
MAR.
MAR.
APR.
JUN.
JUN.
JUL.
AUG.
SEP.

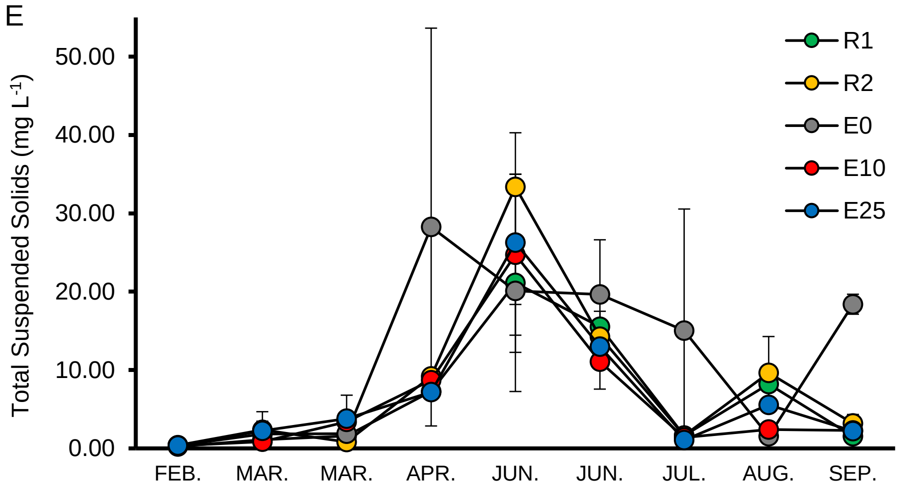


Figure 4. Temporal variation in near-bottom levels of A. ammonia, B. nitrate, C. nitrite, D. total phosphorus, and E. total suspended solids, close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (*n* = 2). Error bars plotted for standard deviation.

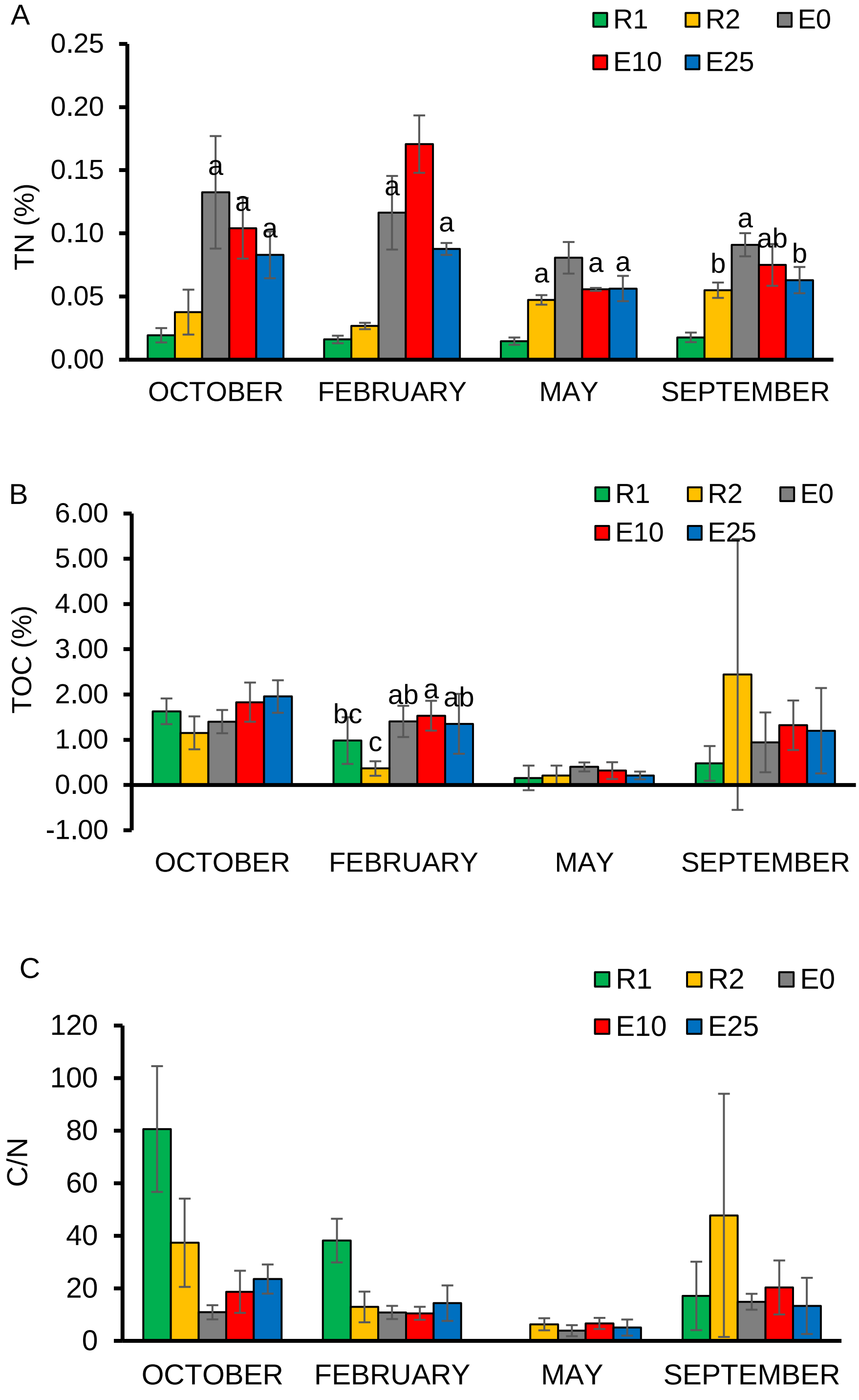


Figure 5. Temporal variation in the contents of A. total nitrogen (TN), B. total organic carbon (TOC) and C. weight ratio of carbon to nitrogen (C/N) in the surficial sediments (0-3 cm) close to fish cages at E0, E10 and E25, and at reference sites R1 and R2. Values are given as mean ± SD (n = 3). Error bars plotted for standard deviation. Different superscript labels indicate a significant difference (p< 0.05) between data for sites at the same sampling time.

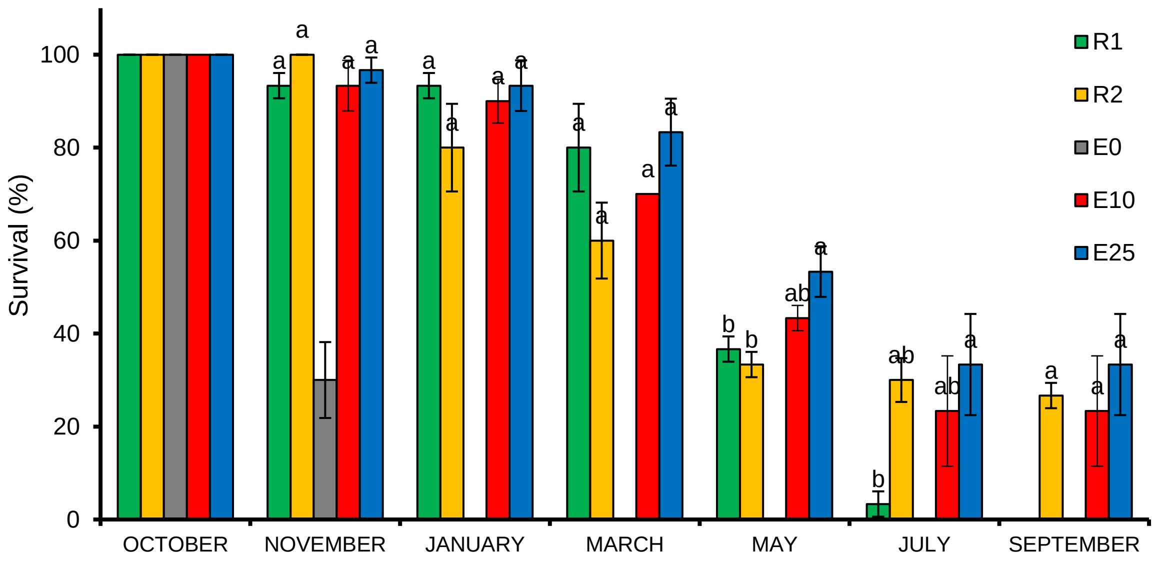


Figure 6. Percentage survival of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference (R1 and R2) sites over 12 months, between October 2018 (on deployment) and September 2019. Average survival for each site based on cage mean values. Standard deviation (n = 3) is represented by error bars. Different superscript labels indicate a significant difference ($p < 0.05$) between data for sites at the same sampling time.

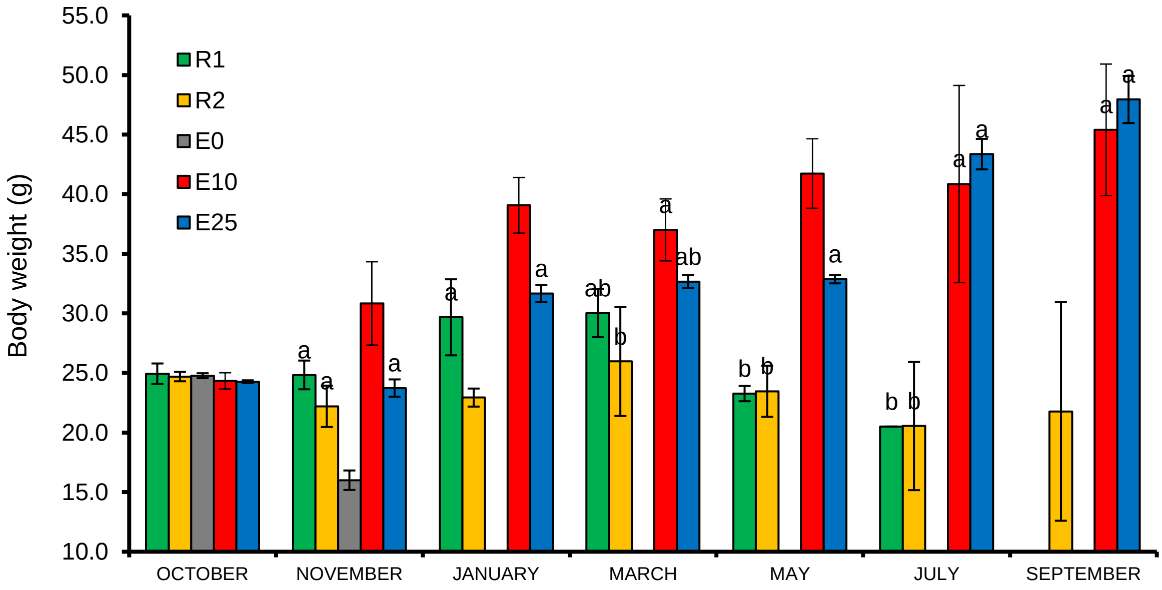


Figure 7. Mean wet body weight of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference (R1 and R2) sites over 12 months, between October 2018 (on deployment)

and September 2019. Average weight for each site based on cage mean values. Standard deviation (n = 3) is represented by error bars. Zero values are not included. Different superscript labels indicate a significant difference (p< 0.05) between data for sites at the same sampling time.

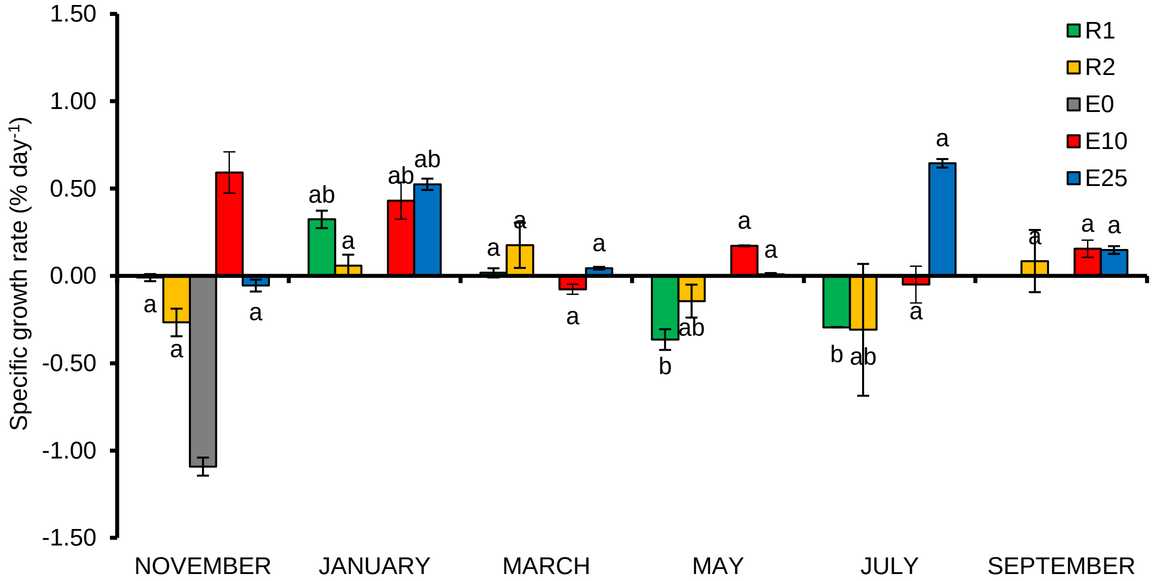


Figure 8. Specific growth rate of *Holothuria poli* deployed at different IMTA (E0, E10 and E25) and reference sites (R1 and R2) between successive sampling periods from October 2018 (on deployment) to September 2019. Average growth rates per site based on cage mean values. Standard error is represented (n = 3) by error bars. Zero values are not included. Different superscript labels indicate a significant difference (p< 0.05) between data for sites at the same sampling time.