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Water Quality Assessment in The Occurrence of *Acanthaster* spp. (Crown-of-Thorns Starfish, CoTS) on Coral Reefs in Menjangan Island, Bali, Indonesia

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Abstract

Aquatic stressors are known to cause biological impairment that can result in biodiversity loss in several Marine Protected Areas (MPA), including in Menjangan Island, West Bali National Park. The objectives of this study were to correlate the changes in water quality parameters with the biological effect of Crown-of-Thorns starfish (CoTS) population dynamics and to assess the most applicable parameters for continuous environmental monitoring. Field observations and surveys were conducted three times in 2017, during the wet season, dry season and season transition, in order to assess the effect of seasonal variability. Redundancy Analysis (RDA) was performed to determine the relationship between water quality parameters (temperature, dissolved oxygen, pH, salinity, turbidity, total suspended solids, dissolved inorganic nitrogen and phosphate, and chlorophyll-a) and phytoplankton as the indicator of CoTS larvae food supply. The results demonstrate that salinity, turbidity, and dissolved inorganic nitrogen (DIN) are responsible for the 47.7% of phytoplankton variation, which supports the hypothesis of nutrient enrichment as the trigger of CoTS population increase. CoTS outbreak is predicted to occur during the wet season, in areas with high salinity, moderate turbidity, and high DIN, so the outbreak may be related to a high rainfall rate that deliver a high nutrient loading on this region and thus management actions can be initiated during this specific period.

Keywords: Acanthaster spp., environmental monitoring, phytoplankton, redundancy analysis, water quality

Introduction

In the last decades, environmental degradation in the coastal area of Indonesia, an archipelagic state, has become more severe. Anthropogenic impacts and climate change are increasingly threatening coral reef ecosystems and therefore adaptive management strategies are needed to adequately address these threats [1]. At the same time, focus is currently directed mainly towards climate change research, which has distracted the attention from other ongoing and much more destructive threats to reef ecosystems (e.g., water quality decline) [2]. Removal of stress at any level will benefit coral conservation as it helps improve the resilience of coral reefs [3]. To identify the underlying factors of reef decline, changes of coastal aquatic ecosystems must be monitored in an accurate and actionable environmental way over an extended period of time [4,5].

The Marine Protected Area (MPA) of Menjangan Island was established in 1984 and it is now a part of the Bali MPA Network, managed by West Bali National Park. The coral reefs of this MPA are a part of Coral Triangle [6], which is known for its high biodiversity [7]. The destruction of coral reefs on Menjangan Island due to human factors, namely low environmental awareness of the local residents and visitors, marine litter, development activities, and destructive fishing by bomb and poison, is still monitored, as the island constitutes part of the MPA [8,9]. Coral reef degradation in Menjangan Island is caused also by climate change, specifically by the increasing sea surface temperature and ocean acidification that caused mass coral bleaching in 2009, 2010, and 2016 [7]. These anthropogenic and climatic disturbances have affected the majority of broken hard coral within the MPA [8].

In early 2017, we observed high density of *Crown-of-Thorns Starfish*/CoTS (*Acanthaster* spp.) population on the reefs of Menjangan Island waters. High-density population of CoTS has been observed in the waters of Menjangan Island several times during the last 30 years, with the highest recorded population being recorded in 1997 and 2009 [7]. CoTS is a marine invertebrate that feeds on coral (corallivore), with its outbreaks remaining one of the most significant disturbance factors and major causes of coral loss across the Indo-Pacific [2,10–14]. Environmental changes that are caused by the increase in the flow of nutrients originating from anthropogenic sources are considered to trigger the severity of CoTS outbreaks in the recent decades, since they increase the food supply for CoTS larvae, i.e., phytoplankton [15, 16]. However, the main reason of CoTS outbreaks in the Indo-Pacific is still uncertain [17], with CoTS outbreaks in Indonesia being generally underreported and understudied.

Usually, a healthy coastal area has low CoTS density (1 starfish per hectare), but the increase in the CoTS population can occur rapidly due to its high fecundity [18]. In a favorable environment, adult female CoTS can produce more than 10 million eggs per individual [19]. During the outbreak, CoTS can destroy up to 80% of coral reefs, causing fatal reef damage and threatening in this way the stability of reef ecosystems [20]. The outbreak mechanism of *Acanthaster* spp. is complex, but it is more amenable to direct intervention than other major disturbances that cause widespread damage to coral reef ecosystems (e.g., climate-induced coral bleaching) [21]. However, the method of culling CoTS, through direct intervention, in order to minimize the incidence of outbreaks (e.g., by chemicals injection) is still considered expensive and time consuming [22–24]. Therefore, the studies that improve the understanding and management of CoTS outbreaks, aiming to make this intervention more effective [25], are of great importance.

The description of the quality of the aquatic environment can be achieved through quantitative measurements of water quality parameters. A comprehensive water quality assessment is required in the overall process of evaluation of the physical, chemical, and biological nature of water in relation to natural quality, human effects, and intended uses [26]. In situ monitoring of water quality parameters, especially the ones related to nutrients and phytoplankton assemblages, is proposed as one of the most effective mechanisms for limiting future CoTS outbreaks while it provides information on whether CoTS larvae can develop in the absence of high nutrient level and phytoplankton abundance [25]. A proper monitoring program can contribute to the management actions required to protect the reef environments, such as the indirect intervention of improving water quality by reducing nutrient inputs from land-use practices [15].

Based on the findings and arguments above, this study attempted to correlate the changes of water quality parameters with the biological effect and to assess the most applicable parameters related to continuous monitoring in Menjangan Island MPA. The observed biological effect in this study was the dynamics of CoTS population over different seasons. In order to carry out an appropriate and cost-effective environmental monitoring process and since water quality parameters vary greatly, we tried, through this study, to select the parameters that best explain the health of the aquatic system within the specified area and intended use. We hope that a well-planned monitoring program will lead to better decisions and faster actions by the respective stakeholders, from the management of the National Park to the local fisherfolks and non-governmental organizations (NGOs), and consequently to the prevention of irreversible damage of coral reef due to the effects of environmental disturbances.

Materials and Methods

Study location and observation. This study was conducted on the waters of Menjangan Island that is located in the Regency of Buleleng, Bali, Indonesia (Figure 1). The size of this island is 175 hectares [9] and it contains seagrass, mangrove, and coral reef ecosystems. This island is an ecotourism hotspot for snorkeling and diving and is also routinely visited for religious activities taking place at several temples that are built on this island [8]. The observation of CoTS was performed during each sampling campaign, by insitu visual census, using an underwater camera. A detailed study on Menjangan Island benthic habitat mapping, which includes 82 observation points, is provided in another publication by Ampou *et al*. [27].

Water quality assessment surveys were performed three times in 2017: on February 15-16, August 14-15, and October 18-19, which represented the wet and dry seasons and the season transition, respectively. Ten survey sites were selected (Figure 1). Water quality parameters can be categorized as (1) basic physicochemical parameters: temperature, pH, dissolved oxygen (DO), salinity, turbidity, and total suspended solids (TSS); (2) nutrient parameters: nitrate, nitrite, ammonia, orthophosphate/dissolved inorganic phosphate (DIP), and silicate; (3) biological parameters: chlorophyll-a and phytoplankton. Due to the extremely low nitrite and ammonia concentrations across almost all observation sites and time periods, the data displayed represent the dissolved inorganic nitrogen (DIN), which is the sum of nitrate, nitrite, and ammonia.

Temperature, pH, DO, salinity, and turbidity were directly measured in triplicate on the respective sites, using a pre-calibrated Water Quality Meter (DKK-TOA WQC-24). Two liters of surface water were sampled and stored in dark polyethylene bottles. Phytoplankton was sampled using a plankton net with a mesh size of 25 µm, preserved by 10% formalin solution, and stored in polyethylene bottles. All samples were stored in cooling boxes until they reached the Institute for Marine Research and Observation (IMRO) on the same day of sampling.

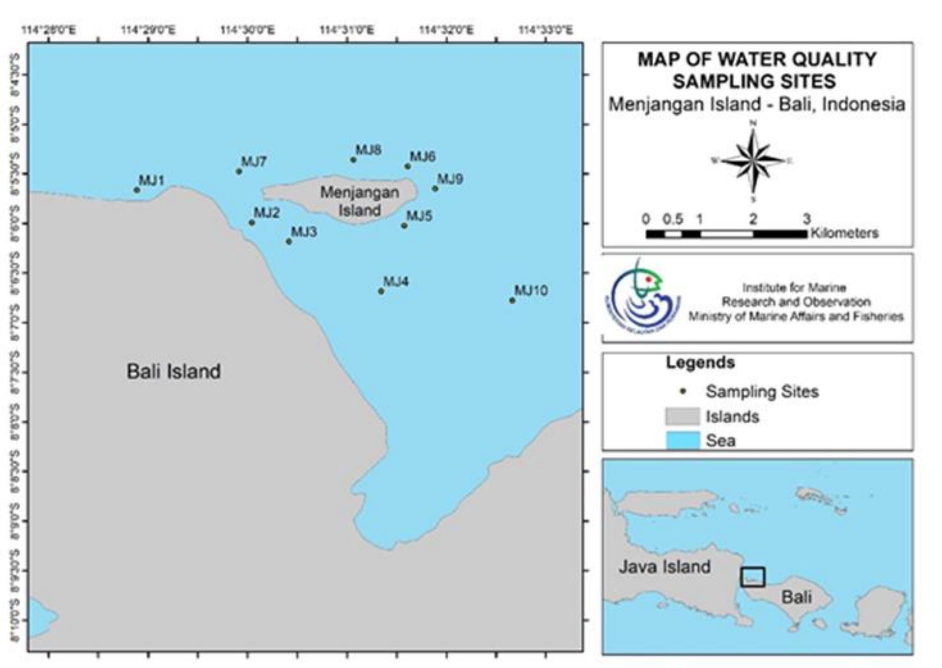

Figure 1. Location Map of Water Quality Sampling Sites

Nutrient and chlorophyll-a samples were analyzed spectrophotometrically in the Water Quality Laboratory–Marine Research Laboratory of IMRO. Prior to analysis, the samples were filtered using Whatman cellulose nitrate membrane filters with a pore size of 0.45 µm, except for TSS that was prepared using Whatman glass microfilters (934-AH) with a pore size 1.5 µm. Then, the samples were measured using the gravimetric method. Chlorophyll-a concentration was measured using the acetone extract of the membrane filters, while nutrients were measured using the filtrated water. Both measurements were performed using the spectrophotometric method (SCO-Tech SPUV-26). Nitrate and nitrite parameters were measured using the cadmium reduction column of the sulfanilamide method, ammonia using the indophenol method, orthophosphate using the ascorbic acid method, and silicate using the molibdosilicate method [28, 29]. Identification and quantification of phytoplanktons were performed using a trinocular microscope (Carl Zeiss Primo Star) according to [30] and [31].

Data analysis and visualization. Box plots with jitter were used to present the water quality data, visualizing the maximum, upper quartile, median, lower quartile, minimum, and overall distribution of the parameters. The significance of the parameters over different sampling times was assessed by the Mann-Whitney-Wilcoxon test. The abundance of Phytoplankton was displayed in percentage. Redundancy Analysis (RDA) is the direct expansion of multiple linear regression for multivariate data response modelling [32]. This type of analysis was selected to interpret the ecological structure in an area, taking into consideration the relationship of species with the environmental conditions. RDA is an asymmetric canonical analysis, which arranges all variances of the response variable Y (i.e., species biomass or abundance) that can be explained by the explanatory variable X (e.g., water quality parameter).

All data analyses were performed using R ver. 3.4.4 and R Studio ver. 1.2.5019. Prior to RDA, data scaling of explanatory and response variables was performed using the square-root transformation. Multicollinearity of explanatory variables was evaluated using the Variance Inflation Factor (VIF) and the correlation coefficient matrix, because multicollinearity reduces the statistical significance of the independent (explanatory) variables, thereby triggering high standard errors [33]. A VIF value of 5 is considered as highly correlated and the maximum allowed value for VIF is 10 [34]. Phytoplankton species were filtered using the presence-absence transformation to eliminate the effect of rare species against the analytical results. Variables that showed a high variance in RDA1 and RDA2 scores were visualized spatially by their medians, using the interpolation of Inverse Distance Weight (IDW) and ArcGIS ver. 10.6.

Results and Discussion

Field observation. In the first two surveys that were performed in February and August 2017, the population of CoTS was exceedingly high. However, during the following survey that took place in October 2017, the density was significantly lower. The density of marine litter that comprised of both plastic and biodegradable litter was similarly high in all sampling periods (Figure 2). Field observation showed that the dominant coral reefs that are located on the northern side of Menjangan Island were dominated by Scleractinian corals from *Acropora* sp. which in turn faced a severe CoTS invasion (Figure 3). Using the geomorphological habitat mapping of reef ecosystems in Menjangan Island by Ampou *et al*., [27], it is shown that the southern part of this island was dominated by massive and submassive corals from the species of *Porites cylindrical* and other species of *Porites* sp. All corals in those regions were in healthy condition and unimpaired by CoTS. It is suggested that adult CoTS prefers feeding on Acroporidae corals than non-Acroporidae and especially on *Porites* spp. Female CoTS that fed on *Acropora* showed an increase in their weight, produced heavier gonads and larger oocytes, compared to *Porites*-fed and starved females [35–38]. It is suggested that maternal provisioning can have important consequences on the quality and quantity of the offsprings. Moreover, it is suggested that local variation in maternal nutrition is likely to moderate reproductive success and may explain the temporal and spatial fluctuations in CoTS abundance.

Seasonal variation of water quality parameters. The variation of water quality parameters upon seasonal differences in February, August and October 2017 (Figure 4) showed that temperature, pH, DO, salinity, and TSS constituted significant parameters (p-value $\leq$ 0.05) across all sampling periods. The significance of turbidity, DIN, and DIP was confirmed in two out of the three seasonal differences. Some parameters, such as temperature and DIP, showed increasing trends from February to October. Conversely, TSS, DIN, and chlorophyll-a showed decreasing trends. More specifically, chlorophyll-a showed a significant difference between February and October. The median of pH was higher in August compared to the other sampling times, while on the contrary, the medians of DO, turbidity, and silicate reached their lowest in August but increased in October. This observation may be influenced by the low rainfall rate during the dry season in August. The variation of silicate concentration was insignificant across all sampling times, which suggests that silicate is minimally affected by seasonal variation and is readily available to be used by diatoms. This phytoplankton class had the highest phytoplankton abundance percentage across all sampling times (Figure 5).

Turbidity was influenced by the seasonal rainfall rate that reached its lowest value during the dry season of August. The suspended matter (TSS) was significantly higher in the wet season of February, which indicates an increased freshwater input in the wet season that delivers particulate material from land to sea. This condition becomes more severe with a lack of water and land use management in the mainland Bali, causing annual flooding during the wet season. High turbidity

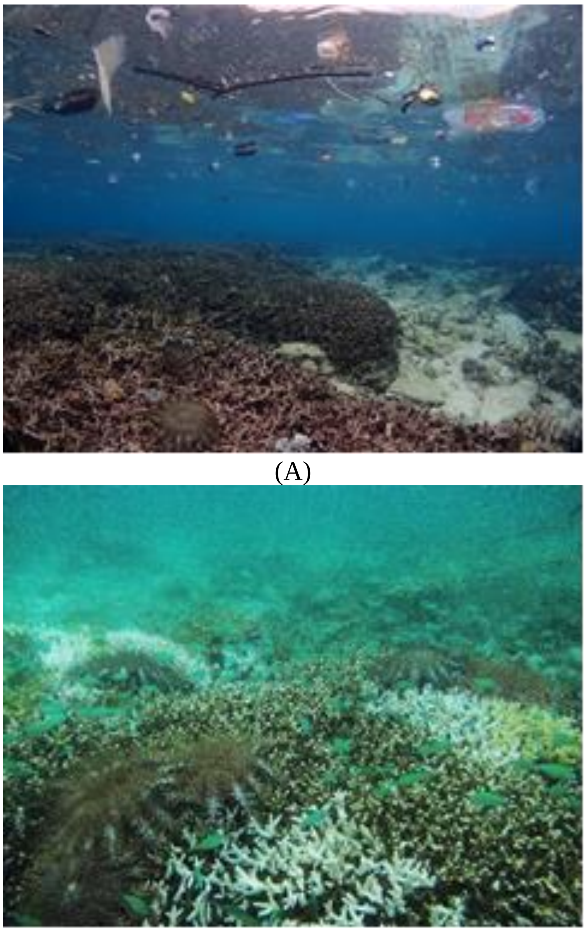

Figure 2. Environmental Condition of Menjangan Island MPA waters: (A) High Density Marine Litter (courtesy S. Andréfouet) and (B) Crown-of-Thorns Starfish (CoTS) Attacks on the Coral Reefs

Figure 3. CoTS Distribution on the Northern Part of Menjangan Island Showed in Yellow Shade. Detailed Benthic Mapping Shown in Green, Red, and Yellow Dots is Available in Ampou *et al.* (2018)

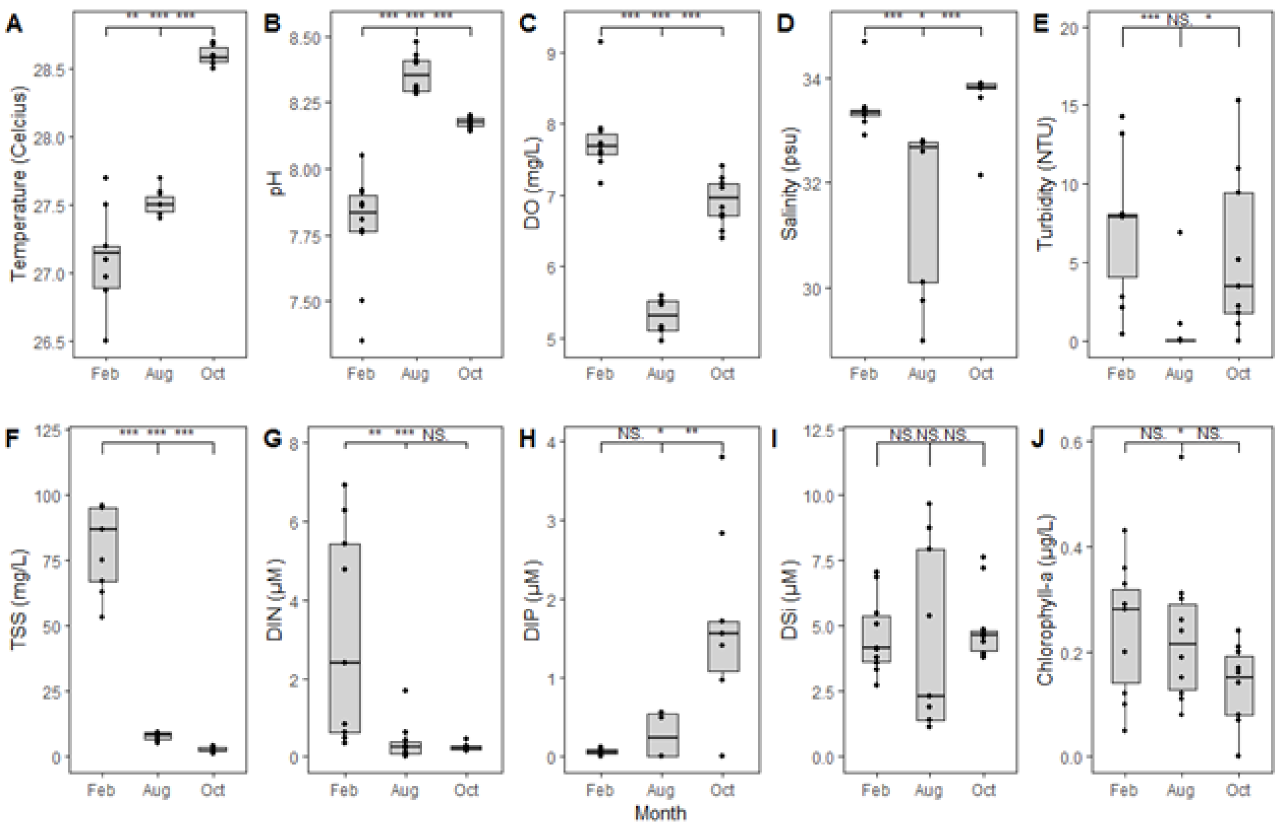

Figure 4. Box and Whisker Plot of Water Quality Parameters of 10 Sampling Stations in February, August, and October 2017. Note: Significance Test with p-value = 0.001 (***), 0.01 (**), 0.05 (*), and not Significant (NS). DO = Dissolved Oxygen, TSS = Total Dissolved Solids, DIN = Dissolved Inorganic Nitrogen, DIP = Dissolved Inorganic Phosphate, DSi = Dissolved Silicate

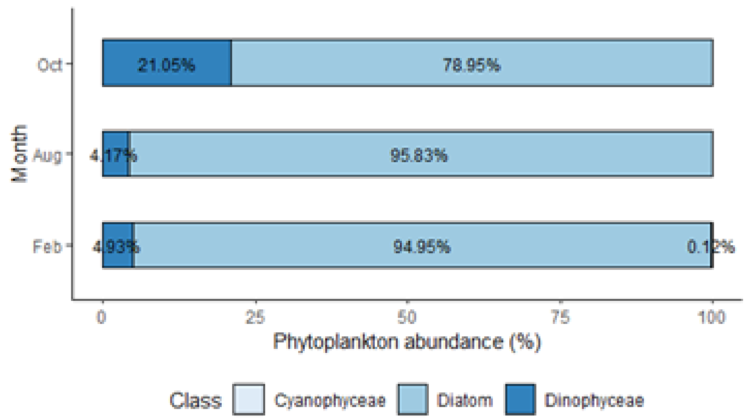

Figure 5. Phytoplankton Abundance Percentage from February, August, and October 2017

and suspended matter can decrease light penetration and therefore affect the growth of autotrophs, including phytoplankton, coral, and seagrass [39, 40]. For this reason, turbidity and TSS should be maintained below the standard criteria. The presence of aquatic colloids (e.g., humic and fulvic acid from terrestrial input) in seawater can increase hydrophobicity in the water column. This may result in stronger interactions between organic molecules, including toxic organic molecules (e.g., n-alkenes, isoprenoids, and polychlorinated biphenyl (PCB)) and bioinorganic molecules that contain heavy metals [41], which these molecules are detrimental for aquatic organisms.

Nutrient limitation was found to be affected by seasonal differences, with the P limited condition being observed during the wet season but gradually shifted to the N limited condition during the dry season and its transition. Terrestrial runoff characterized by high nutrient leads to a higher phytoplankton abundance, hence increases the survival rate of CoTS larvae that feed on them [42]. Chlorophyll-a concentration was relatively low and was categorized as oligotrophic. Chlorophyll-a can aid in the monitoring process of primary productivity. This parameter can be monitored using a water quality probe and remote sensing (e.g., Aqua sensor of MODIS satellite) [43], therefore is has been measured repeatedly in several studies [16, 44]. However, the chlorophyll-a levels that are considered to be within the optimal range for CoTS larval survival and development are quite moderate, between 0.4–1.0 µg/L [25]. As this study shows, elevated chlorophyll-a may not be urgently needed for the growth of CoTS larvae since the recorded chlorophyll-a concentration did not exceed 0.6 µg/L despite high CoTS density.

In February, the total number of identified phytoplankton species was 32, while in August and October that number dropped to 25 and 12, respectively. The sum of phytoplankton abundance recorded in those sampling periods subsequently decreased, from 243.600 cells/m^3, to 79.200 cells/m^3 and 5.700 cells/m^3, respectively. Diatom (Bacillariophyceae), showing the highest abundance percentage during the wet season in February (99.74%), constitutes the main phytoplankton class (Figure 6). Silicate is a nutrient that plays an important role in the building of the frustules of diatoms and the high availability of this nutrient over different seasons may contribute to a high diatom abundance. Phytoplankton from genus *Chaetoceros* spp. as well as some of their species that can be distinguished (i.e., *C. concavicornis, C. indicus, C. coarctacus*) had high abundance in February (75.300 cells/m^3) and August (39.600 cells/m^3), but no phytoplankton from this genus was recorded in October, which may be related to the N limitation. This observation matched the observation regarding the adult CoTS, which they showed a major presence in February and August, but their population decreased significantly in October. The high abundance percentage of Dinophyceae in October may suggest that the nitrogen fixation ability and better adaptation mechanisms of this phytoplankton class can overcome the N starvation stress during this period [45].

The abundance of specific phytoplankton species is considered much more important than the overall abundance of phytoplankton, regarding the rapid development and survival of CoTS larvae [25]. These larvae prefer phytoplankton with larger cell sizes (e.g., dinoflagellates and pennate diatoms > 4 µm) over those with smaller cell sizes (1–2 µm), even when the population of smaller cell size phytoplankton is excessive [46,47]. CoTS larvae also prefer algal species with the highest energy content (e.g., *Chaetoceros* sp., *Dunaliella* sp.) than the ones with a low energetic content (e.g., *Phaeodactylum tricornutum*, *Pavlova lutheri*) [44]. Therefore, the abundance of diatoms, especially from *Chaetoceros* spp., can be a suitable indicator for CoTS population dynamics in Menjangan Island waters.

Water quality assessment by redundancy analysis (RDA). The multicollinearity test of water quality parameters showed that temperature had a high Variance Inflation Factor (VIF) of 5.78, while this parameter showed also a significant correlation with pH, TSS, DIN, DIP, and chlorophyll-a, based on the correlation coefficient matrix (Figure 6). Therefore, temperature was excluded from the Redundancy Analysis (RDA). The variance of RDA suggested that the constrained partition was 47.7%, which explains the causal relationship between phytoplankton abundance and water quality parameters. The eigenvalues showed that the first and the second RDA dimensions (RDA1 & 2) explained 52.1% and 15.6% of all constrained partitions. Furthermore, the permutational ANOVA test showed that the proposed model and the RDA1 play a significant role (p=0.043). The redundancy analysis axes RDA1 and RDA2 regarding the relationship of water quality parameters, phytoplankton species, and sampling sites according to Euclidean distances were depicted in a triplot representation (Figure 7). RDA1 axis was strongly correlated with turbidity, while in RDA2 axis, salinity was shown to constitute a significant parameter. RDA3 that explained 11% of the total variations (not shown)

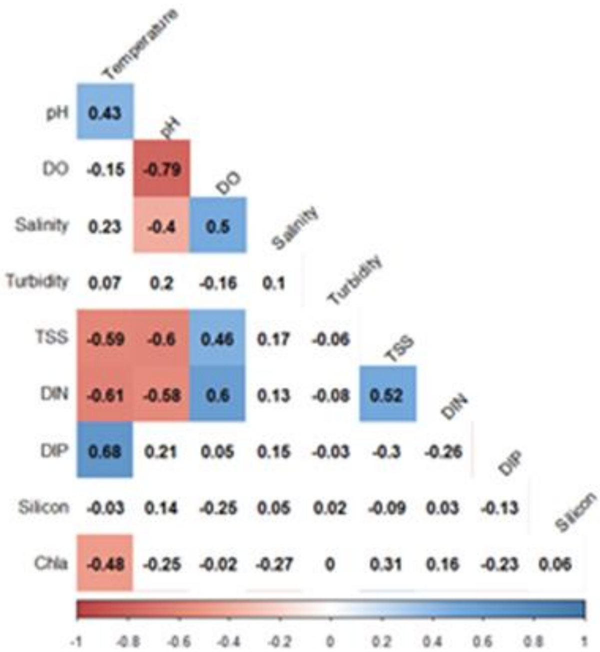

Figure 6. Correlation Matrix of Water Quality Parameters as Explanatory Variables with Significance Level (α) 0.05

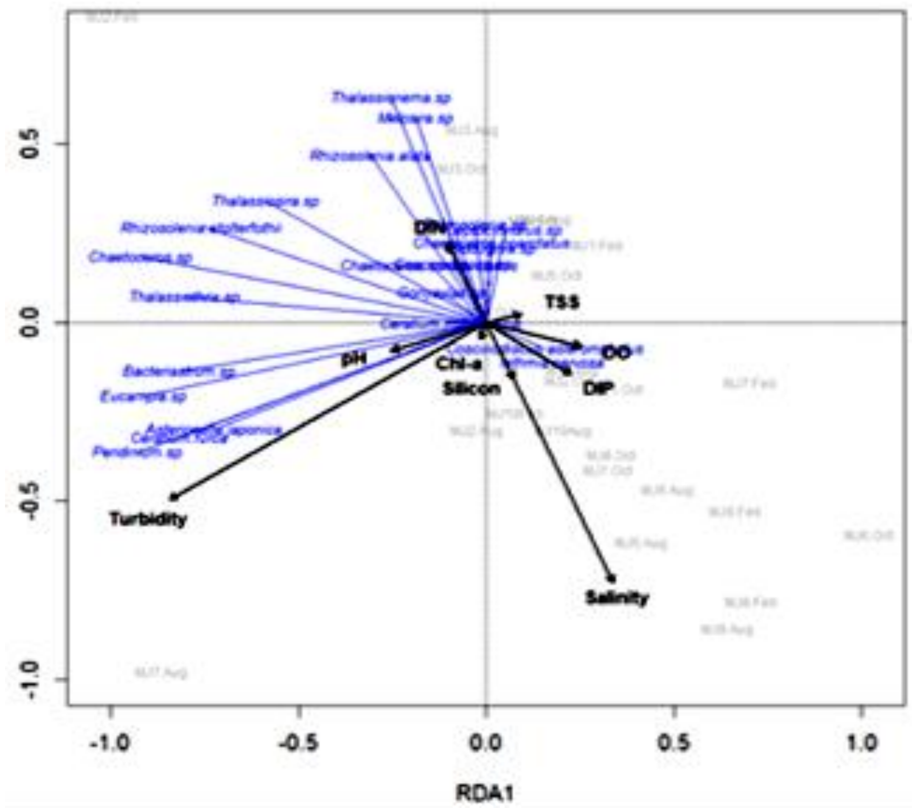

Figure 7. Result of RDA Shown as Canonical Ordination Triplot of RDA1 and RDA2 Axes, with the Elements of PHYTOPLANKTON Species, Samples and Water Quality Parameters Respectively Represented as Blue Lines, Grey Texts, and Black Arrows. Length of Arrows and Lines Represent the Relative Importance of the Variable or Species. The Distance between Samples Correlates the Dissimilarity in Species Composition, i.e., Close Samples in the Plot are more Similar than Distant Samples

was strongly correlated with DIN. Based on the variable ordination method, it is suggested that *Peridinium* sp., *Ceratium furca,* and *Asterionella japonica* prefer low turbidity conditions, *Isthmia nervosa* and *Coscinodiscus asteromphalus* prefer high salinity conditions, while *Rhizosolenia alata, Thalassionema* sp., and *Melosira* sp. prefer high DIN concentrations. In February, these phytoplanktons showed a higher abundance compared to the other sampling periods.

By correlating the result of RDA with CoTS population, it is suggested that the dynamics of salinity, turbidity, and DIN values upon different seasons affected significantly the composition of phytoplankton species, which is the main food source of CoTS larvae. Phytoplankton is known to have a life-cycle timescale of days, with a turnover that is highly affected by seasonal variation and related to the changes of physical properties and nutrient regimes [48]. The spatial visualization of these water quality parameters, as they were recorded in February (Figure 8), showed that CoTS prefer to live in the northern part of Menjangan Island that is characterized by high salinity and DIN with moderate turbidity. This area is facing the open sea, therefore it is affected by the currents of Java Sea Surface Water and Indonesian Throughflow (ITF), which are characterized by a recurring climatic pattern, namely El Niño Southern Oscillation (ENSO) [49]. *Acanthaster* spp. outbreak can occur primarily due to self-recruitment, but secondary outbreaks can occur as results of passive dispersal and subsequent mass settlements of planktonic larvae by ocean currents, originating from primary outbreak populations in other locations, as it has been observed in the Great Barrier Reef, Australia [42]. The latest CoTS outbreaks in Indonesia were reported in Spermonde Archipelago, South Sulawesi [50], thus the CoTS larvae in Menjangan Island may originate from another area. Nevertheless, CoTS populations before 1990 were observed only in Labuhan Lalang Harbor and Teluk Terima on the mainland Bali and not around Menjangan Island [9]. In February, these areas showed high turbidity levels caused from terrestrial organic sediment and therefore the possibility that this corallivore had migrated gradually from the mainland Bali to the northern part of Menjangan Island to avoid excessive turbidity levels is also taken into consideration.

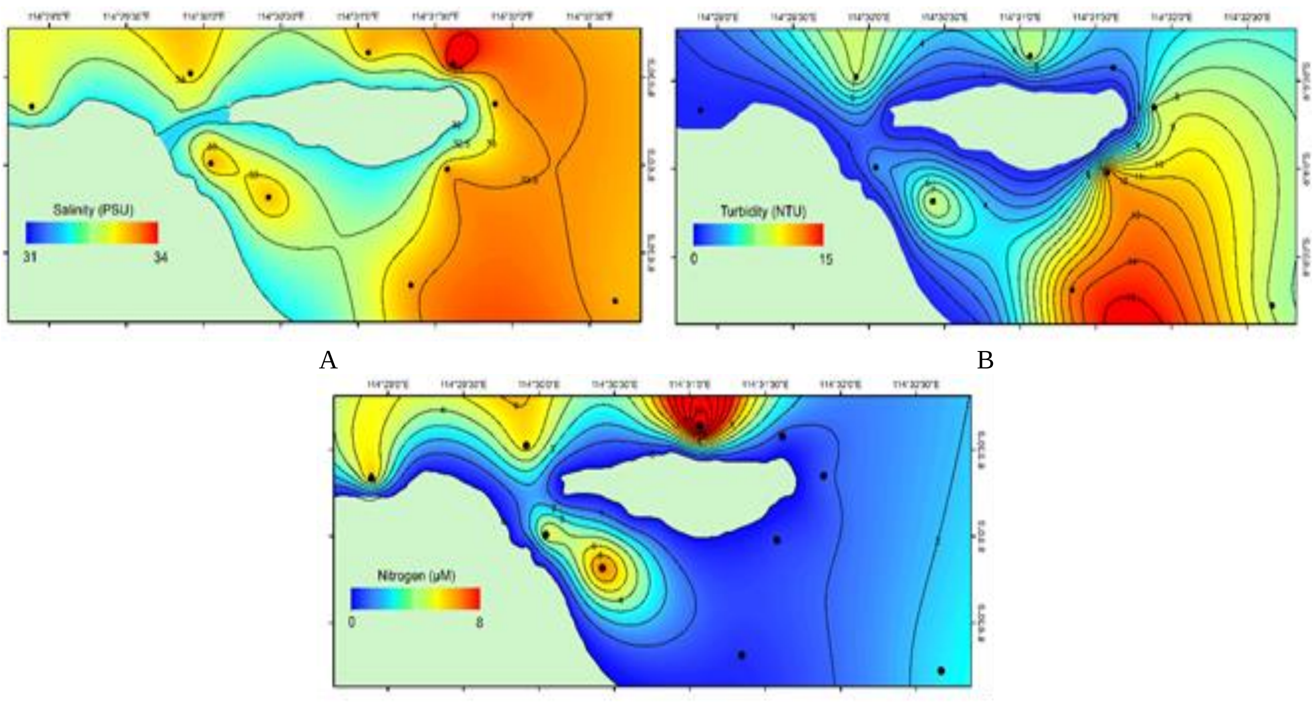

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Figure 8. Spatial Visualization during CoTS Outbreak of Water Quality Variables with the Highest RDA score: (A) Salinity, (B) Turbidity, and (C) DIN

Conclusion

In this study, ten water quality parameters were assessed for their suitability in coastal environmental monitoring. From the redundancy analysis (RDA), it is concluded that salinity, turbidity, and dissolved inorganic nitrogen (DIN) are satisfactory parameters in order to detect the changes in environmental conditions that are related to *Acanthaster* spp. outbreaks. Moreover, phytoplankton abundance is suitable as an indicator of Crown-of-Thorns starfish (CoTS) population dynamics in Menjangan Island Marine Protection Area (MPA). It is suggested that the increase of phytoplankton and *Acanthaster spp.* populations in this area is caused by high salinity, moderate turbidity, and high DIN levels during the wet season. Therefore, management of the possibility of CoTS outbreaks that may occur in the beginning of this season is recommended. The result of this study can be explored further by designing a time-series environmental monitoring program in Menjangan Island, e.g., by placing coastal buoys or establishing monitoring stations on the northern part of the island and using remote sensing approach through satellite images. It is hoped that this study will motivate the respective stakeholders in order to manage the water quality of Menjangan Island and the mainland Bali, thus preserving the health of aquatic ecosystems and promoting sustainable ecotourism. The analysis performed in this study is not limited to the issue of CoTS outbreak. It can also be implemented to determine the most important water quality parameter(s) for regular environmental monitoring on MPAs or coastal areas, especially in relation to seasonal variability.

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