

**RESEARCH ARTICLE** 

# Recent changes in macroalgae distribution patterns in the Orbetello lagoon (Italy)

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## Abstract

- 1 This study related recent distribution changes in seven macroalgae taxa (Acetabularia acetabulum, Chaetomorpha linum, Cladophora sp., Gracilariopsis longissima, Spyridia sp., Ulva laetevirens, Valonia aegagrophyla) to spatial (basin) and temporal (time) trophic differences in a meso-eutrophic Mediterranean coastal lagoon (Orbetello, Italy).
- 2 In July 2003 and July 2009, the coverage percentage (CP) of each considered taxon was measured in 38 stations equally distributed in the Western and Eastern lagoon basins. All data were analysed using SURFER v8.0 software along with ANOVA, ANOSIM and multivariate analyses to produce geostatistical spatial distribution maps and to estimate statistical probabilities for "basin" and "time" factors.
- 3 Data from this study were integrated with data on phanerogams reported by Giovani et al. (2010) to evaluate changes occurring at a functional group level from 2003-2009 using the Ecological Evaluation Index (EEI).
- 4 Observed macroalgae changes may have been due to lagoon management activities performed during the study period. The removal of direct nitrate inputs seems to have forced specie-specific shifts that were highly significant only in the Western basin.
- 5 Statistically significant differences at the taxon level have been indicated for Spyridia sp. (basin and time) and Cladophora sp. (time) taxa. Other non-significant changes included a rarefaction of Chaetomorpha linumin the Western basin and Spyridia sp. in the Eastern basin, and an increase of Gracilariopsis longissima, C. linum and Valonia aegagrophyla in the Eastern basin.
- 6 EEI application indicated a general improvement in water quality due to management measures applied in Western basin, whereas the Eastern basin evidences stability/slight degradation from 2003-2009.

Keywords: macroalgae, transitional water, trophic level, Chlorophyta, Rhodophyta

#### Introduction

During the past 150-300 years, a general tendency towards a progressive decline in

biodiversity has been observed in estuaries and coastal lagoons, leading to a loss of submerged aquatic vegetation estimated at nearly 65% of the total for phanerogams and nearly 48% for other taxa (Lotze et al., 2006). This phenomenon has been associated with various human activities linked to physical modifications and chemical pollution in the marine environment, particularly in areas close to urban settlements (Duarte, 2002; Orth et al., 2006; Short et al., 2006). Nutrients affect macrophyte distribution; in fact, phanerogams dominate in oligo- and meso-trophic systems, while macroalgae dominance increases when eutrophication occurs (Goodman et al., 1995; Plus et al., 2003). The primary causes of shifts and succession in the macrophyte community are nutrient loadings, mainly nitrogen, as well as changes in coastal hydrology or interactions between them (Viaroli et al., 2008). In meso-eutrophic conditions, both macroalgae and phanerogams are present, but if eutrophication occurs, macroalgae tend to replace rooted species (Zaldívar et al., 2008). Macroalgae species in coastal eutrophic lagoons are often freely-moving, thus hydrodynamic and meteorological features such as water currents and winds could strongly affect their distribution within the lagoon basin (Giusti et al., 2010).

The implementation of the Water Framework Directive CE 2000/60 (EC, 2000) in Europe initiated numerous research programmes focusing on Mediterranean transitional ecosystems by applying, among other things, relevant indices to asses eutrophication based on macrophytes (Orfanidis et al., 2003; 2011; Sfriso et al., 2009). However, further investigation is needed, as fluctuations within intermediate levels of pollution as well as early shifts from meso-trophy towards eu-trophy cannot be easily predicted (Arévalo et al., 2007; Orfanidis et al., 2008a) especially using tools based on community biological levels (Orfanidis et al., 2001; 2011). Therefore, knowledge of factors affecting macroalgae distributions and shifts in transitional waters becomes important.

In a meso-eutrophic ecosystem dominated by opportunistic macroalgae development, frequent shifts among opportunistic macroalgae species are observed (fluctuations described on the basis of technical reports in Lenzi et al., 2003), but dynamics and factors affecting dominances are not yet clear.

The Orbetello lagoon (Tuscany, Italy) is a well-studied (Lenzi, 1992; Lenzi et al., 2003; Innamorati and Melillo, 2004; Specchiulli et al., 2008) meso-eutrophic ecosystem which, over time, has evidenced frequent shifts in macrophyte dominance due to natural or human-induced factors, and is thus an interesting case-study for understanding macrophyte patterns.

This study aims to evaluate the spatialtemporal changes in the distributions of seven macroalgae during the period 2003 to 2009. Considered taxa are Acetabularia acetabulum (Linnaeus) P.C. Silva, Chaetomorpha linum (O.F. Müller) Kützing, Cladophora sp., Ulva laetevirens Areschoug, Valonia aegagrophyla Kützing (Chlorophyta), and Gracilariopsis longissima (S.G. Gmelin) M. Steentoft, L.M. Irvine et W.Farnham, and Spyridia sp. (Rhodophyta). Results presented in this paper will be looked at along with previously published research performed contextually with the present study and focusing on phanerogams (Giovani et al., 2010). In order to assess water quality changes, data from both studies were used to calculate the Ecological Evaluation Index (Orfanidis et al., 2001; Orfanidis et al., 2007).

## Materials and methods

## The Orbetello lagoon

The Orbetello lagoon is a meso-eutrophic (Specchiulli et al., 2008) transitional ecosystem located along the Southern Tuscan coast (42°25'-42°29' Lat. North; 11°10'-11°17' Long. East) of the Central Tyrrhenian Sea. It covers 25.25 km<sup>2</sup> (1.20 m average depth ranging from 0.30-1.70 m); an artificial

dam divides the lagoon into two connected basins referred to as Western and Eastern, covering 15.25 and 10.00 km<sup>2</sup>, respectively. The presence of the dam reduces water circulation and water exchanges between the two basins, while non-significant fluxes of floating macroalgae throughout the dam are reported in the literature (Innamorati and Melillo, 2004). A large amount of data concerning hydrodynamics, winds, and the geomorphologic structure of this ecosystem have been acquired in previous research (Lenzi et al., 2003; Giusti and Marsili-Libelli, 2006; Specchiulli et al., 2008). As described in detail in Giovani et al. (2010), the Orbetello lagoon is characterized by a high level of human impact due to both historical and recent human activities responsible for the bioavailable nutrient and chemical distributions within the system. Among others, the major human activities producing environmental impacts are: i) summer tourism (Renzi et al., 2009; 2012), ii) chemical runoff from urban areas (Perra et al., 2010; Specchiulli et al., 2011), iii) a fertilizer production plant (no longer in operation, Focardi et al., 2009; iv) effluents from municipal wastewater treatment plants (Renzi et al., 2009), and v) discharges from fish farming activities (Porrello et al., 2005; Renzi et al., 2007). Although localized hot-spot sources of human origin are a significant concern, widespread natural ones (i.e. inflows from the Albegna river) are also relevant (Giovani et al., 2010). Frequent macrophyte fluctuations have been documented in this ecosystem over time (Lenzi et al., 2003). Due to human-driven water circulation, artificially maintained from April to October by the pumping of seawater into the Nassa and Fibbia channels (Western basin), the two basins present dissimilar ecological characteristics. In particular, with the exclusion of an area located in the Western basin closed off to the effluents from municipal wastewater

treatment plants, higher salinity and nutrient levels are recorded in the Eastern basin (Specchiulli et al., 2008). This phenomenon is, however, related to water evaporation and sediment release phenomena, which enrich water during its flow throughout the lagoon. Nutrient and salinity differences produce significant effects on species assemblage and distribution of both macro-zoobenthos (Focardi, 2004) and phanerogams (Giovani et al., 2010).

## Samplings

Sampling for this study was carried out at the same time as for the study by Giovani et al. (2010). Therefore, details on criteria, strategies, and procedures selected for samplings can be found in that paper. Cover percentage (CP) of seven taxa of macroalgae (Acetabularia acetabulum, Chaetomorpha Cladophora sp., Gracilariopsis linum, longissima, Spyridia sp., Ulva laetevirens, Valonia aegagrophyla) was studied in July 2003 and July 2009 at thirty-eight georeferenced sampling stations equally divided between the Western and Eastern basins (Fig. 1). Criteria adopted for the a *priori* selection of the geographical locations of sampling replicates, for the in situ localization of the selected coordinates and for the production of the georeferenced map, were designed to minimize sampling error (Cochran, 1977). A logical model developed to reduce type I errors (Underwood, 1992; 1993; Benedetti-Cecchi, 2004) and based on two factors - basin (two levels, fixed) and time (two levels, fixed) - was adopted, producing a total of 76 observations for each taxa considered. This sampling strategy aimed to optimize the number of sampling replicates, sizing them in terms of extent of sampling surface (9 m<sup>2</sup>), as shown in Giovani and colleagues (2010). Cover in percentage (CP) was visually estimated applying a modified version of the Boudouresque field method (1970). With the exception of zero



Figure 1. Sampling stations (1-19) of the Orbetello Lagoon basins. W=Western, E=Eastern.

values (assigned only when a total absence of macroalgae cover was observed), the lowest detection limit assumed in this study was 30%. Field estimations of CP could be subject to error due to the presence of floating biomasses which are packed together in different densities, thus the estimation of lower CP values could be misrepresentative and reflect significant errors. A value of 30% is the limit above which a very good reproducibility (± 5% SD) was recorded during inter-calibration procedures. Inter-calibrations performed include comparison among CP data collected separately by researchers on the same replicate following the same methodology. Only well-represented macroalgae taxa have been considered in this paper, with

the rarest species neither quantified listed. Furthermore, distinctions nor among difficult-to-identify species of the genera Cladophora sp. and Spyridia sp. (Innamorati and Melillo, 2004) were avoided by recording CP only at the taxonomic level of the genus. Data on temperature, salinity and nutrient levels in water were collected by the local regional monitoring agency and/or from published literature.

## Ecological Evaluation Index

The Ecological Evaluation Index (EEI) was calculated using data from both the present study and Giovani et al. (2010), following the methodology reported by Orfanidis and colleagues (2001). For each sampling station, CP values of considered species were summed together into two different ecological status groups: the late-successional (phanerogams and macroalgae with a thick or calcareous thallus; low growth rates; perennials) and the opportunistic (annual sheet-like and filamentous species with high growth rates). EEI values ranged between 2 (bad Ecological Status Class) and 10 (HighESC).

## Statistical analyses

Spatial distribution maps were calculated by the software package SURFER version 8.0 using the kriging, a geostatistical gridding method which produces visually appealing contour and surface plots from spatial data (Matheron and Armstrong, 1987). The one/ two-way ANOVA analysis of variance was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad. com). These analyses were performed considering two factors: basin (Eastern versus Western) and time (2003 versus 2009). The specific hypotheses examined  $(H_{o})$  was that the quantitative differences in abundances observed between basins and between times would not be significant. Data were also analysed separately for each basin considering the taxa as a factor, with the aim of investigating the significance of differences between the two considered lagoon basins with regard to taxa assessment. The multivariate analysis was developed using Primer E Software package version 6.0 (Plymouth Marine Laboratory, UK). This analysis was applied to evaluate the statistical significance of observed distributions in a multivariate dimension (Clarke and Warwick, 2001; Benedetti-Cecchi, 2004). The Bray-Curtis distances resemblance matrix was calculated on abundances, considering taxa as variables and sampling replicates as samples, after the application of the log(x+1) and successively the square root transformations of collected CP data. Data transformations

were performed to reduce dataset variability and, in particular, to estimate the statistical effects of the rarest species (A. acetabulum, U. laetevirens), as suggested by Clarke and Green (1988). Logarithmic transformation, carried out using the log(x+1) algorithm, was also necessary to eliminate zeros, which are frequent in abundance acquisitions.

Nm-MDS was performed using the Kruskal stress formula 1, imposing a minimum stress of 0.01 and restarting the process 50 times, and cluster analysis was performed applying the single-linkage cluster mode and the Simprof test (1000 permutations per mean profile, 999 simulation permutations, 5% significance level). Due to the complete absence of CP data available for the taxa U. *laetevirens* in 2003, and for A. acetabulum, Cladophora sp., U. laetevirens in 2009, these variables were deleted a priori from the reported plot to avoid excessive aggregation of the other variables, thereby improving the readability of obtained results.

Two-way crossed ANOSIM tests based on the Bray-Curtis similarity matrix derived from CP data were performed to test for significant differences concerning species distribution depending on the factors basin and time. Probabilities associated with observed segregations were calculated by means of the one/two-way test statistic R (analysis of similarity), imposing a run of 9,999 permutations. This similarity was expressed through the R index and its related percentage of significance (p): when this index was close to 1, the samples within a given variable were closer together than those of another variable; whereas an R index close to zero indicated a greater similarity between variables.

## Results

Spatial distribution maps of considered taxa, obtained for both the sampling campaigns (2003, 2009), are reported in figures 2-8.



Figure 2. Spatial distribution maps of cover (%) of *Acetabularia acetabulum* for 2003 and 2009.



Figure 3. Spatial distribution maps of cover (%) Ulva laetevirens.



Figure 4. Spatial distribution maps of cover (%) of *Cladophora* sp.

The species A. acetabulum (Fig. 2) showed a localized and sporadic distribution in the Orbetello lagoon. In fact, this species is observed only in sampling replicate 9W from summer 2003, albeit at high CP values (50%). A similar behaviour is observed for the species U. laetevirens (Fig. 3) for a different sampling replicate (3W). The genus Cladophora sp. (Fig. 4) was present in 2003 in both basins at the Nassa and Fibbia channels (Western) and along the Feniglia sand bar (Eastern), and in 2009 completely disappeared from the entire lagoon system. Similarly, the genus Spyridia sp. (Fig. 5) went from a quite extensive presence in the Eastern basin and only one replicate in the Western one (12W) in 2003 to complete disappearance from both basins (only one recorded presence at station 11E) observed in 2009. The opposite trend was observed for the species V. aegagrophyla (Fig. 6), which



Figure 5. Spatial distribution maps of cover (%) of *Spyridia* sp.



Figure 6. Spatial distribution maps of cover (%) of *Valonia aegagrophyla*.



Figure 7. Spatial distribution maps of cover (%) of *Gracilariopsis longissima*.



Figure 8. Spatial distribution maps of cover (%) of *Chaetomorpha linum*.



Figure 9. Mean values (± standard deviations, n=10) of temperature and salinity in Orbetello lagoon from May 2003 to February 2009.

in 2003 was found in one sampling replicate (8W), but in 2009 showed a notable expansion in both basins. This species is actually widespread throughout the whole Orbetello lagoon, achieving absolute dominance in wide lagoon areas (unpublished data). A notable increase in the presence of G. longissima (Fig. 7) was observed from 2003 to 2009, with broad high-density areas located in the Northern part of the Eastern basin; wider distribution in the central area of the Western basin was also observed in 2009. On the contrary, C. linum (Fig. 8) showed quite stable behaviour in the Western basin,but increasing distribution in the Eastern one, with higher densities observed for sampling replicates 1-3E. Temperature mean values (± standard deviations) ranged between 6.2°C (January) and 35.3°C (July), while salinity mean values ranged between 23.8 (April) and 47.8 (August) (Fig. 9).

ANOVA test indicated significant differences for both factors ("basin" and "time") for *Spyridia* sp. and for the "time" factor for *Cladophora* sp. (Table 1).

Table 1 - A summary of one-way ANOVA test performed for abundance data of each studied taxon for the factors basin (Eastern *versus* Western) and time (2003 *versus* 2009). Type I error probability p<0.01 was considered significant (\*\*) while 0.01 was considered slight butnot significant (\*). ns: not significant.

Taxa	basin	time
Acetabularia acetabulum	ns	ns
Chaetomorpha linum	ns	ns
Cladophora sp.	ns	F=6.790; P=0.013
Gracilariopsis longissima	ns	ns
Spyridia sp.	F=12.860; P=0.001	F=9.202; P=0.004
Ulva laetevirens	ns	ns
Valonia aegagrophyla	ns	ns

 $V_{\cdot}$ aegagrophyla evidenced significant differences for the time factor only in the Eastern basin, with a complete absence recorded in 2003 followed by an increase (ca. 20%) in 2009. In contrast, in the Western basin, the average CP of V. aegagrophyla was near 10% for both times. As evidenced in figure 10, C. linum shows an inverse temporal trend in the two considered basins: a notable average CP increase in the Eastern basin associated with a non-significant CP decrease in the Western basin. No significant difference between basins is observed for this species due to the elevated standard deviations (higher than 10%) associated with CP means.

The nm-MDS run on variables (macroalgae taxa) and overlaid on the cluster analyses are reported with varying levels of similarity in figure 11.

Concerning C. linum, the similarity among data acquired in both times is greater than 40%. This result evidences this species' similar behaviour in both basins in the same sampling replicates. On the other hand, a similarity below 20% observed for V. aegagrophyla supports the idea that this species behaved differently in terms of distribution and related CP in 2003 than in 2009. A similarity within 35% for the species V. aegagrophyla (2009) and C. linum (2003, 2009) indicates a similar trend for both in 2009. G. longissima

Table 2 - A summary of one-way crossed ANOSIM Global tests for the factors basin (Eastern versus Western) and time (2003 *versus* 2009). The test performed is based on a similarity matrix derived from abundance data (cover in percentage). R: ANOSIM statistic; *p*: associated probability level (%); NPS: number of permutated statistics greater than total R on a total of 9,999 permutations.

Factor	R	Р	NPS
basin	0.091	0.30	24
time	0.060	1.60	161

and *Spyridia* sp. distributions are also characterized by a similarity within 35%. ANOSIM tests indicated significance



Figure 10. Mean values ( $\pm$  standard deviation) of the cover (%) for the taxa grouped into basins (Eastern, Western) and times (2003, 2009).

Table 3 - A summary of one-way ANOVA test performed for each basin separately, where taxa (*A. acetabulum*, *C. linum*, *Cladophora* sp., *G. longissima*, *Spyridia* sp., *U. laetevirens*, *V. aegagrophyla*) and times (2003, 2009) were considered as variables. ns: not significant.

Basin	Taxa	time
Eastern	F=7.227; P<0.0001	ns
Western	F=11.630; P<0.0001	F=5.670; P=0.0188



Figure 11. Non-metric multi-dimensional scaling (nm-MDS) performed on variables (taxa) superimposed to the cluster analysis. Similarity levels of 20 and 40% are evidenced using dotted-line circles, whereas asterisks and solid-line circles indicate the time 2003 and 2009, respectively.

concerning both of the tested factors, particularly for the basin factor (Table 2). Results obtained with the ANOVA test performed using taxa and time as factors (Table 3) support the hypothesis that considered taxa would evidence different general assessments in the two considered basins. In particular, taxa assessment differed significantly according to the *time* factor in the Western basin, whereas no differences were recorded in the Eastern basin.

Spatial distribution maps of EEI in both 2003 and 2009 are reported in figure 12.

Taking into consideration the entire lagoon, the mean EEI value increased from 2003 (EEI=5.7) to 2009 (EEI=6.4). While 45% of sampling stations showed a positive increase in EEI value, 21% showed a decrease and 44% no change from 2003 to 2009. While the mean EEI value for the Western basin increased, the mean EEI value for the Eastern basin decreased from 2003 to 2009.



Figure 12. Spatial distribution maps of Ecological Evaluation Index (EEI) calculated for the times 2003 and 2009. EEI values ranged between 2 (bad Ecological Status Class) and 10 (high ESC).

## Discussion

The absence of significant differences observed for the species A. acetabulum and U. laetevirens is principally due to a lack of representativeness resulting from the low frequencies recorded. An extreme fluctuation of data produces the absence of significant differences concerning either the time or basin factors for G. longissima. Fluctuations observed for V. aegagrophyla are the principal cause of the result (lack of significance) obtained in a comparison between basins.

A similar mathematical artefact due to the elevated standard deviations (higher than 10%) associated with CP means underlies the statistical results (absence of significance)

of analyses performed on *C. linum* data concerning both the tested factors.

Macroalgae distributions observed in Orbetello lagoon basins during the period 2003-2009 are influenced by existing conditions. The ecological dominance of opportunistic (r-selected) macroalgae species such as C. linum and Spyridia sp., with a simple anatomical structure and a wide range of physiological limits, indicates eutrophic conditions (Zaldívar et al., 2008; Orfanidis et al., 2008a; Viaroli et al., 2008). These species take up nutrients and grow much faster than slow-growing, longlived phanerogams (Wallentinus, 1984; Pedersen and Borum, 1997) which eventually disappear. Eutrophication is also confirmed by the limited distribution of A. acetabulum, a sessile and perennial macroalga growing on a hard substratum associated with oligotrophic conditions (Zaldívar et al., 2008; Orfanidis et al., 2011). Also limited was the distribution of Ulva laetevirens, a species associated with hyper-trophic conditions. Between 2003 and 2009, management strategies adopted in the Orbetello lagoons led to a significant reduction in nutrient loads in the Western basin, but the direct discharge of aquaculture effluents without any abatement treatment determined a general increase in nutrients in the Eastern basin (11-12E and 17-19E). Fish-farm effluents produce significant ammonium inputs (Innamorati and Melillo, 2004). These changes in nutrient inputs could explain the observed macroalgae shifts. Although Acetabularia acetabulum completely disappeared in 2009 on hard substrates of the lagoon, similar behaviour in the species Ulva laetevirens, Spyridia sp., and Cladophora sp. may be indicative of a general trend of increasing water quality. The increase of C. linum (nitrophilous species) in the Eastern basin near the Ansedonia area was obviously induced by direct discharges from fish-farms effluents. On the other hand, the notable reduction in C. linum and the contextual increase in G. longissima were associated with the reduction of effluents from municipal wastewater treatment plants (Western basin). In 2009, high CP values of C. linum, and G. longissima highlighted a critical area in the Eastern basin (1-3E). This area is characterized by very low redoxpotential associated with high phosphorous releases (Renzi et al., 2007). With regard to Gracilaria sp., phosphorus enrichment could increase its photosynthesis both in winter and summer (Garcia-Sanchez et al., 1996), whereas Cladophora sp. suffers from both nitrogen and phosphorous limitation, but is primarily limited by the latter (Lapointe and O'Connell, 1989). Researches performed in transitional ecosystems (Stal et al., 1996; Viaroli et al., 2008 and citations therein) suggested that a key role in controlling primary productivity and algal growth may be played by levels of ferric iron and carbonates in sediments, which could buffer phosphorous dynamics, influencing N/P ratios and consequently specie-specific macroalgae proliferations. The occurrence of non-homogenous ferric iron and carbonate levels in Orbetello lagoon sediments could be a significant factor in determining differential phosphorous availability. Further

research must be carried out to evaluate significant relationships between macrophyte distribution and sedimentary levels of ferric iron, carbonates, sulphides, and phosphorous. Numerous non-human-controlled natural factors (geomorphologic dynamics, wind intensity and climate changes) may determine significant changes in a lagoon ecosystem, producing numerous temporary physical and ecological boundaries and gradients within it. In spite of this natural variability, many factors influencing lagoon dynamics are controlled and controllable by humans and are the key aspects on which appropriate management strategies should be focused. An appropriate strategy for the management of lagoon hydrodynamics, sea-lagoon water exchanges, human-generated pressures and resource exploitation could mark the boundary between a highly productive ecosystem and a severely stressed one. In such variable and temporary ecosystems, the best strategy is to maintain a dynamic equilibrium characterized by an optimal balance of productivity and environmental quality. Achieving this goal requires energy in terms of technological, economical, and social resources, and effective efforts will call for the optimization of management strategies, which cannot be obtained without a detailed knowledge of the dynamics affecting the system as well as factors influencing macrophyte population dominance shifts.

The effects produced by management measures to improve the environmental quality of the Orbetello lagoon have been monitored at a functional group level taking into account both macroalgae and angiosperms through the application of the EEI. Several studies implementing this index in transitional waters (Orfanidis et al., 2007; Orfanidis et al., 2008b; Mascaró et al., 2013) indicated that it could be usefully applied to evaluate the occurrence of human-generated pressures and trophic changes within a lagoon system associated with changes in nutrient loads over time. In accordance with the management measures adopted in the Orbetello lagoon, the EEI indicated an improvement in water quality only in the Western basin.

#### Conclusions

Management activities undertaken to improve the water quality of the Orbetello lagoon seem to have brought about significant changes in specie-specific dynamics of macroalgae, such as the disappearance of Cladophora sp., the notable reduction in Spyridia sp., and the stabilization of G. longissima. The implementation of the EEI indicated an improvement in water quality due to management measures taken in the Western basin, whereas in the Eastern basin water quality appears to have remained stable or slightly degraded during the study period. Therefore, more efficient strategies must be planned to improve water quality in this ecosystem in the future.

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