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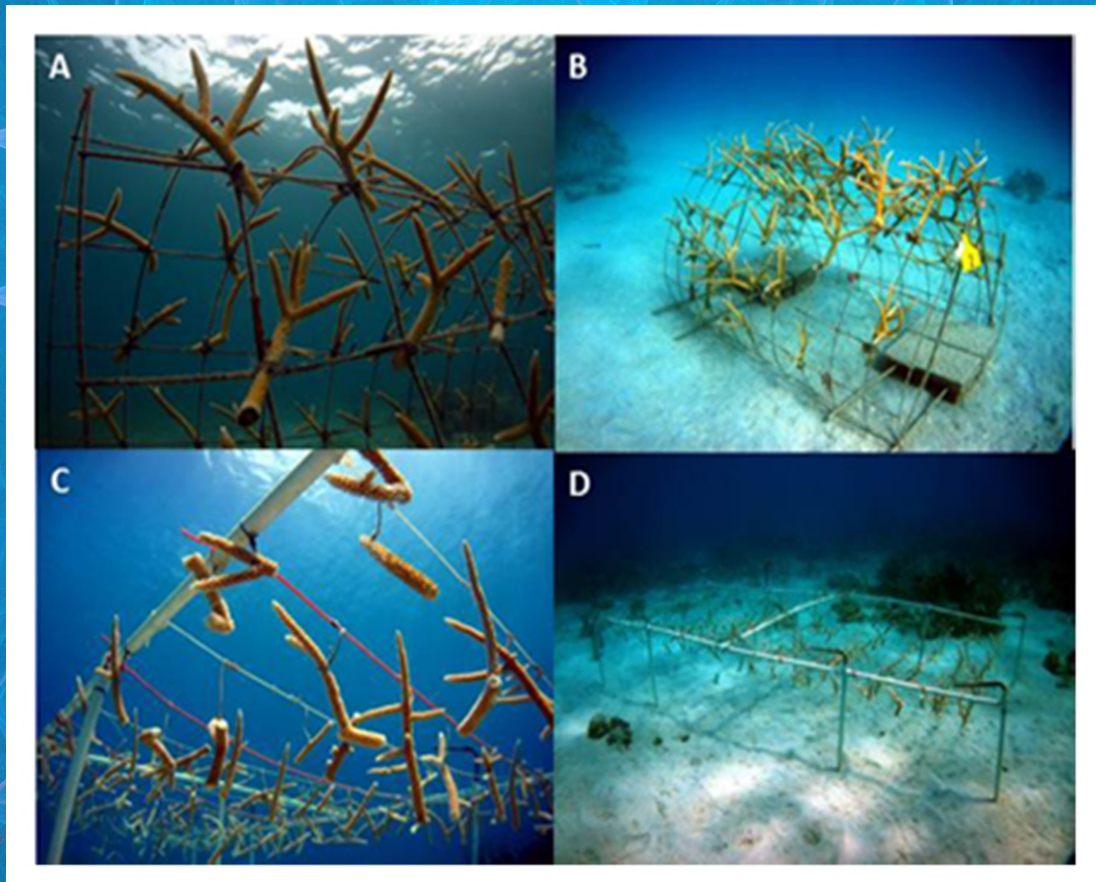
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Table of Contents

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Evaluation of Phenotypic Diversity by Use of Variable Analysis Multi of Various Populations of Oak Cork (*Quercus suber* L.) in Tunisia

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Abstract

Populations of oak cork (*Quercus suber* L.) originated from different areas in Tunisia were analysed based on morphological characters to assess the overall degree of phenotypic variability, to detect similarities between the genotypes and to evaluate significant forest features. One-way ANOVA analysis shows a significant difference between oak cork populations and demonstrates that parameters measured on leaves show an important degree of variability and permit to discriminate oak cork individuals. Vegetative variables are leaf length, leaf width, petioles length, number of leaf veins, petioles width, spines number, leaf area, length nipples, diameter nipples, weight nipples, and height trees. Diameter trees were scored and subjected to multivariate analysis. Results showed a considerable diversity among local germoplasme of oak cork. Principal component analysis (PCA) revealed that the characters related to the length and width of the sheets and the nipples accounted for a large proportion of the observed variability. Cluster analysis showed a typically continuous phenotypic diversity among oak cork accessions, and little associations between accessions from same geographic origin. Also, some heterogeneity within accessions that received the same denomination was evidenced. The variability observed between different populations is attached to phenotypic characters which depend primarily on two factors, gene and environment.

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Keywords

***Quercus suber* L., Populations, Phenotypic Study, Variability, Tunisia**

1. Introduction

The oak cork (*Quercus suber* L.) (2n = 24) is a Mediterranean species, which has a great ecological and socio-economic interest. Oak groves are seldom in a stable state everywhere in the world, but are subjected to many gradual changes. They sometimes undergo catastrophic dysfunctions and then present strong reductions of growth, and damage in the crown. Because of the significant value of the products of the oak groves (wood of high quality, cork) and their ecological importance and landscape, the oak groves dominate of vast wide Mediterranean zones and constitute a significant component of landscapes apart from the zones of mountains; such dysfunctions raise a great concern among ecologists and foresters.

In Tunisia this indigenous leafy species occupies considerable surfaces in Kroumirie and Mogods about 127,000 ha in 1950 [1]; since the last decades, a worrying regression which was estimated at 1.22% of the surface per year [2]. The oak cork is characterized of a great polymorphism particularly in leaves and fruits. It has a great genetic variability which results in morphological differences between individuals and ecotypes. Thus technological qualities of cork and its speedily increase appear to strongly depend on the genetic factors [3]. [4] reported that the oak cork constituted definitely distinct races according to areas: the Numidia oak cork of Algeria and Tunisia, the Atlantic oak cork of Morocco, and the oak cork of the Western South of France with bi-annual maturation and more resistant to the cold; this confirmed great genetic richness of the oak cork to which primarily contributed cross-fertilization character of the species.

It can hybrid with the holm oak and this confers to him a great ecological plasticity and a greater adaptability in the mediums [3].

The production of cork shows variability between trees of same oak forest in term of the annual growth and quality; the variability of settlements shows the dominating effect of the tree like origin variability [5].

This article describes the evaluation of morphological variations which is based on the observation and the measurement of the morphological criteria on the leaves and on the fruit of oak cork populations in Tunisia. The principal objectives of this study consist to detect genetic ratios among cultivars, to determine total degree of polymorphism of this character, and to evaluate significant forest features for the classification of oak cork. The understanding of genetic variability and suitable identification of oak cork cultivar constitutes essential stages with regard to develop multiples strategies. Results obtained from these markers are compared and discussed in order to start an improvement program of this species.

2. Materials and Methods

2.1. Plant Material and Measurements

2.1.1. Plant Material

A total of 180 wild cork oak trees (*Quercus suber* L.) were inventoried from different locations in Kroumirie, Mogods and Cap Bon in Tunisia (15 individuals from each populations of Hammam Bourguiba [HB], Dar Fatma [DF], Oued Zeen [OZ], Aîn Zana [AZ], Mejen Essef [ME], Béni Mtir [BM], Djebel Zouza [DZ] (Ain Draham), El Feidja [EF] (Gar Dimaou), Bellif [B] (Nefza), Djebel Khroufa [DK] (Tabarka), Keff El Rand [KR] (El Haouaria) and Hammam Jdidi [HJ] (Hammamet) (**Table 1** and **Table 2**). The choice of the sites was carried out according to the geographical distribution, the bioclimat and the relief. The plant material consisted in leaves and nipples randomly taken from adult cork oak trees (*Quercus suber* L.) and used to undergo a morphological study.

2.1.2. Morphological Characters

Morphological characterization of each individual was according to describe vegetative characters of oak cork to namely sheets and fruits as well as the growth of the trees (**Table 3**). These are reported as part of standard descriptors of oak cork [6].

Table 1. Characteristics geographical, bioclimatic and relief of different populations of oak cork (*Quercus suber* L.) in Tunisia.

Site	Abbreviation	Area	Geographical distribution	Bioclimat (bioclimatic stage)	Relief	Altitude (m)	Characteristics geographical
El Feidja	EF	Ghardimaou	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	780 m	32.43˚84'22"E 40.40˚26'7"N
Beni M'ttir	BM	Ain Drahem	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	800 m	32.47˚48'36"E 40.65˚89'8"N
Hammam Bourguiba	HB	Ain Drahem	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	570 m	32.46˚76'08"E 40.71˚96'0"N
Dar Fatma	DF	Ain Drahem	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	909 m	32.47˚84'09"E 40.73˚59'6"N
Oued Zena	OZ	Ain Drahem	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	675 m	32.48˚22'49"E 40.75˚63'0"N
Mejen Essef	ME	Ain Drahem	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	800 m	32.48˚17'17"E 40.69˚20'4"N
Aîn Zana	AZ	Bouselm	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	851 m	32.48˚77'03"E 40.64˚69'4"N
Djebel Zouza	DjZ	Nefza	Western north (Kroumirie)	Wet superior at moderate winter	Montagne	542 m	32.49˚96'49"E 40.77˚41'2"N
Keff El Rand	KR	Haouaria	Northeast (Cap Bon)	Sub-wet superior at hot winter	Montagne	642 m	32.65˚77'63"E 40.77˚15'8"N
Hammam Jdidi	HJ	Hammamet	Northeast (Cap Bon)	Semi-arid lower than hot winter	Montagne	174 m	32.62˚08'79"E 40.35˚84'9"N
Djebel Khroufa	DjK	Tabarka	Western north (Mogods)	Wet inferior at hot winter	Chaine tellienne	160 m	32.49˚41'25"E 40.90˚49'5"N
Bellif	B	Tabarka	Western north (Mogods)	Wet inferior at hot winter	Chaine tellienne	88 m	32.50˚79'87"E 40.98˚60'9"N

To study the morphological variability of *Quercus suber* 50 leaves were randomly taken at different part of the tree and thirteen vegetative variables (leaf length, leaf width, petioles length, numbers of leaf veins, petioles width, spines numbers, leaf area, length nipples, diameter nipples, weight nipples, height trees, diameter trees) were measured. All morphmetric measurements were taken using a Vernier caliper with digital display. The leaf areas were calculated using Microtech Scanmaker HP SCANJET G2710 scanner and were measured by image analysis logiciel (Optimas V6.0).

2.2. Analysis of Morphological Data

In order to elucidate genetic variability and to establish relationships between trees, several statistical procedures were conducted. Phenotypic data were used to approximate contribution of the measured leaves, nipples and trees traits in the genetic diversity among the considered individuals. Data were computed using the SPSS software (Statistical Package for the Social Sciences, version V.17.) to achieve one-way analysis of variance (ANOVA) and XLSTAT software to achieve principal components analysis (PCA) as a multivariate analysis. The PCA analysis was applied to assess differences between individuals and elucidate partitioning of phenotypic variability at inter-populations level. Parameters means were compared by DUNCAN'S multiple range test ($P <$ 0.05) [7] (**Table 4**).

Table 2. Measured vegetative characters of oak cork (*Quercus suber* L.) in Tunisia. Leaf, nipples and trees characters retained for the study of oak cork (*Quercus suber* L.) populations diversity in Tunisia.

Parameter	Abbreviation	Unit	Character
	LOFE	cm	Leaf length
	LAFE	cm	Leaf width
	LLOA	-	Length/width of leaf
	LOPE	mm	Petioles length
Leaf descriptors	NBNV	-	Numbers of leaf veins
	LAPE	mm	Petioles width
	NBEP	-	Spines numbers
	SRFO	cm^2	Leaf area
	LOGL	cm	Length nipples
Nipples descriptors	DIGL	cm	Diameter nipples
	POID	g	Weight nipples
	HAU	m	Height trees
Trees descriptors	DIA	cm	Diameter trees

Table 3. Mean values of measured traits in 12 cork oak accessions. (See **Table 2** and **Table 3** for cultivars and parameters labels respectively.)

	LOFE	LAFE	LLOA	LOPE	NBNV	LAPE	NBEP	SRFO	LOGL	DIGL	POID	HAU	DIA
HB	5.841	3.265	1.818	10.251	11.280	0.989	11.430	4.51	3.124	1.9341	5.384	8.500	33.233
DF	5.436	3.082	1.7818	11.200	11.120	8.462	10.130	5.402	3.284	1.586	5.696	7.420	35.600
OZ	5.257	2.709	1.9678	8.770	10.180	9.030	9.350	5.240	2.764	1.488	3.825	9.014	44.479
AZ	6.508	3.412	1.967	12.500	10.590	8.967	9.470	4.988	2.985	1.542	4.527	7.172	34.562
ME	6.008	3.362	1.802	12.170	11.790	9.680	9.190	4.858	3.043	1.556	4.951	10.013	51.539
BM	5.632	3.094	1.841	11.360	11.080	7.607	9.900	4.958	3.104	1.598	5.430	10.376	38.581
DjZ	6.100	3.092	2.014	11.700	12.640	9.475	11.480	4.222	3.715	1.656	7.031	6.777	38.037
EF	5.105	2.817	1.833	11.880	10.950	7.408	9.960	5.255	3.610	1.404	4.878	9.407	41.008
DjK	5.820	3.102	1.898	12.750	11.260	9.219	10.790	5.157	3.317	1.716	5.763	8.733	38.800
B	5.424	3.148	1.742	10.035	10.530	8.011	10.200	4.749	3.587	1.774	7.562	7.367	44.6181
KR	5.759	3.211	1.824	10.120	11.420	10.105	10.440	5.843	3.351	1.878	6.736	9.120	24.066
HJ	5.493	3.073	1.804	9.570	10.665	1.2084	10.024	4.997	3.058	1.698	5.662	6.817	21.600

The Euclidean distance were estimated between individuals and a hierarchical cluster was constructed according to Ward's method [8] by module in the statistical packages [9] to establish relationships among Tunisian oak trees.

3. Results

Morphological parameters corresponding to biological characteristics, growth of the tree leaves, and fruit were measured in order to describe the genetic diversity and its partitioning.

Table 4. Result of Duncan test (0.05) conducted on morphological characters.

B	DF	KR	DZ	BM	HJ	HB	DjK	OZ	EF	AZ	ME
					Leaf length (cm)						
5.493	5.840	5.424	5.820	5.257	5.632	6.100	5.436	5.105	6.000	6.508	5.759
					Leaf width (cm)						
3.072	3.265	3.148	3.102	2.709	3.094	3.092	3.082	2.817	3.362	3.412	3.211
					Length/width of leaf						
1.803	1.817	1.741	1.897	1.967	1.841	2.013	1.781	1.833	1.801	1.967	1.823
					Petioles length (mm)						
9.569	10.251	10.035	12.750	8.770	11.360	11.700	11.200	11.880	12.170	12.500	10.120
					Numbers of leaf veins (leaf veins)						
10.665	11.280	10.530	11.260	10.180	11.080	12.640	11.120	10.950	11.790	10.590	11.420
					Petioles width (mm)						
1.208	0.988	8.011	9.219	9.030	7.607	9.475	8.462	7.408	9.680	8.967	10.105
					Spines numbers (spines)						
10.023	11.430	10.200	10.790	9.350	9.900	11.480	10.130	9.960	9.190	9.470	10.440
					Leaf area (cm^2)						
4.996	4.511	4.749	5.157	5.239	4.958	4.221	5.401	5.254	4.858	4.988	5.843
					Length nipples (cm)						
3.058	3.124	3.585	3.317	2.763	3.103	3.714	3.284	3.610	3.042	2.982	3.351
					Diameter nipples (cm)						
1.698	1.934	1.774	1.715	1.487	1.598	1.656	1.586	1.404	1.551	1.541	1.877
					Weight nipples (g)						
5.661	5.384	7.558	5.763	3.824	5.430	7.030	5.696	4.878	4.950	4.513	6.736
					Height trees (m)						
7.363	7.333	6.776	10.376	8.500	9.406	8.733	9.014	9.120	6.816	7.172	10.013
					Diameter trees (cm)						
44.618	35.600	38.037	38.580	33.233	41.007	38.800	44.479	24.066	21.600	34.561	51.539

B: Bellif; DF: Dar Fatma; KR: Keff Rand; DZ: Djebel Zouza; BM: Béni Mtir; HJ: Hammam Jdidi; HB: Hammam Bourguiba; DjK: Djebel Khroufa; OZ: Oued Zen; EF: El Feidja; AZ: Ain Zana; ME: Mejen Essef.

3.1. One-Way Variance Analysis: ANOVA

The ANOVA analyses applied on the morphological parameters enabled the determination of the most discriminating variables between the studied populations. The characters used are very efficient to estimate phenotypic variability of these populations.

The one-way ANOVA analysis showed a signifying diversity between cultivars, except for four parameters (length/width of leaf, petioles length, leaf area, diameter nipples). The ANOVA shows significant differences among cultivars for 9 traits out of 13. **Table 5** illustrates that out of the 13 parameters 6 were highly significant (leaf length, numbers of leaf veins, petioles width, length nipples, weight nipples, height trees and 3 were significant: leaf width, spines numbers and diameter trees). We note that the most discriminator parameters are those related to leaves and to nipples especially the length and weight and trees especially on height (**Table 5**).

Table 5. One-way analyses of variance (ANOVA) applied on quantitative parameters referring to leaf. Nipples and trees descriptors.

Morphological traits	Mean square	F. observed	*P*
Leaf length	1535	4395[**]	0.000
Leaf width	0.400	3008[*]	0.002
Length/width of leaf	0.072	2125[NS]	0.024
Petioles length	15,811	2202[NS]	0.019
Numbers of leaf veins	4222	3830[**]	0.000
Petioles width	96,429	59,571[**]	0.000
Spines numbers	5469	2905[*]	0.002
Leaf area	1769	0.974[NS]	0.475
Length nipples	0.818	24,721[**]	0.000
Diameter nipples	0.245	2320[NS]	0.013
Weight nipples	11,418	19,961[**]	0.000
Height trees	4745	6517[**]	0.000
Diameter trees	211,198	3810[*]	0.003

[NS]Non significant; [*]Significant; [**]High significant.

3.2. Multivariate Analysis: Principal Component Analysis

This method—carried out on covariance's matrix—provided a correlation matrix between the characters, as well as an individual's graphic representation in space which defined by the principal components.

Being given that the measuring units used vary from one character to another, it is essential to standardize the rough phenotypic data [10]. The reduced center matrix thus obtained was subjected to multivariate analysis. **Table 3** summarizes the results of the principal component analyses. Furthermore, we have performed a PCA analysis taking into account the morphological parameters. Result of PCA analysis shows that 50.091% of the total variation was explained by the first two principal components (**Figure 1**).

The first two principal components accounted for 28.783%, and 21.307%, respectively, of the total variations among oak cork accessions of based on the fourteen vegetative traits. (Length of leaves) (LOFE) (width of leaves) (LAFE) (number of leaf veins) (NBNV) (number of spines) (NBEP) (lengths nipples) (LOGL) (diameter nipples) (DIGL) and (weight nipples) (POID) had strong positive charge, while (leaf area (SRFO) tree height (HAU), diameter of the trees (DIA)) had strong negative charges in the first principal component.

In the second component, the number of spines (NBEP), leaf area (SRFO), (diameter Nipples) (DIGL) and nipples' weight (POID) had negative charges, whereas leaves' length (LOFE), petiole's length (LOPE) petioles' widths (LAPE) and trees' diameter (DIA) showed strong positive charges.

The graphic representation of cultivars on the plan axes (1-2) presented in **Figure 1** showed a significant opposition accessions of group IIa (DJ Zouza [DZ] and DJ Khroufa [DK]) and accessions of same group IIa (Oued Zen [OZ]) according to the first principal component.

Accessions of group IIa (DJ Zouza [DZ] and DJ Khroufa [DK]) are characterized by a length leaves, width leaf, petiole length, number of leaf veins; spines numbers, length nipples, diameter nipples and weight nipples were high positive charge, while, Oued Zen [OZ] has a larger height and diameter trees.

Moreover, individuals from populations of Dar Fatma [DF], El Keff Rand [KR], Bellif [B]; Hammam Bourguiba [HB], Hammam Jdid [HJ], Ain Zena [AZ], Méjen Essef [ME], Béni Mtir [BM] and El Feidja [EF] are shared between the two groups I and II (**Figure 2**).

In contrast, the accessions of group of (Dar Fatma [DF], El Keff Rand [KR], Bellif [B], Hammam Bourguiba [HB], and Hammam Jdid [HJ]) showed a significant opposition of accessions of group of (Ain Zena [AZ], Méjen Essef [ME], Béni Mtir [BM], and El Feidja [EF]) above by the number of spines length, diameter and weight of nipples.
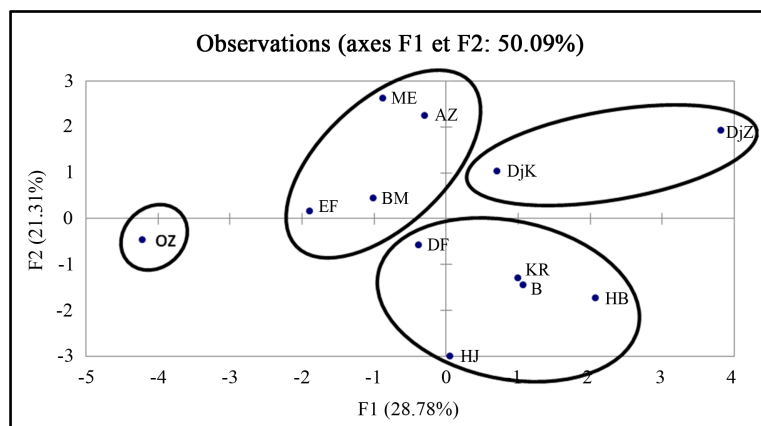
Figure 1. Representation of the projection of the populations in the plan 1-2 analysis in component principal.
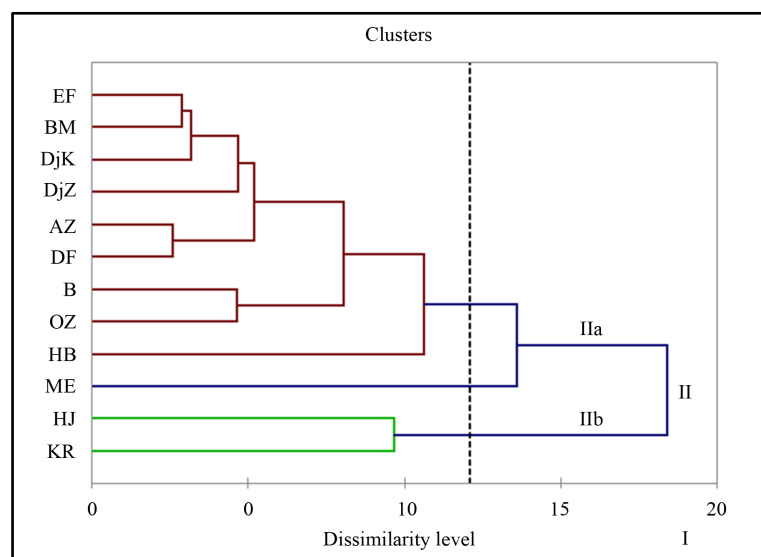
Figure 2. Hierarchical classification of the twelve populations of oak cork (*Quercus suber* L.) based on Euclidean distances applied on 13 morphological and dendrometric parameters.

The second axis opposes accessions of group IIa (DJ Zouza [DZ], and DJ Khroufa [DK]) with a great leaves length, leaves width, petiole length, petiole width, number of leaf veins, number of spines, lengths, diameter and weight of nipples and large accessions of group IIa (Oued Zen [OZ]) characterized by a large height and diameter trees (**Figure 2**).

3.3. Correlation Matrix

Table 5 shows the correlation matrix between the characters studied.

A high positive correlation (0.750 to 0.811) between the characters according to (**Table 6**):

- Leaf length was positively correlated to leaf dispatcher and petioles leaf length. Leaf dispatcher was positively correlated to petioles leaf length and nipples diameter. Petioles leaf length was positively correlated to numbers of veins. The number of veins was positively correlated to number of leaf spines and nipples length. The dispatcher of petioles of leaf was positively correlated to the diameter to the collets of young seedlings. The number of the spines was positively correlated to nipples diameter. The surface of leaf was positively correlated to the height of young seedlings. The nipples length was positively correlated to nipples weight. The nipples diameter was positively correlated to nipples weight.

Table 6. Pearson's correlation matrix between the different characters in cork oak accessions. With the threshold of risk of 5%.

Variables	LOFE	LAFE	LLOA	LOPE	NBNV	LAPE	NBEP	SRFO	LOGL	DIGL	POID	HAU	DIA
LOFE	1.000												
LAFE	0.811	1.000											
LLOA	0.441	−0.165	1.000										
LOPE	0.527	0.418	0.204	1.000									
NBNV	0.436	0.320	0.208	0.452	1.000								
LAPE	0.177	−0.024	0.333	0.395	0.216	1.000							
NBEP	0.146	0.084	0.109	0.033	0.556	−0.292	1.000						
SRFO	−0.362	−0.226	−0.251	−0.143	−0.397	0.306	−0.418	1.000					
LOGL	−0.119	−0.063	−0.118	0.282	0.498	0.194	0.563	−0.196	1.000				
DIGL	0.208	0.440	−0.308	−0.299	0.201	−0.381	0.658	−0.114	0.146	1.000			
POID	0.046	0.239	−0.295	−0.042	0.426	0.069	0.586	−0.198	0.750	0.604	1.000		
HAU	−0.219	−0.136	−0.173	0.120	0.005	0.231	−0.327	0.320	−0.250	−0.192	−0.380	1.000	
DIA	−0.069	−0.125	0.063	0.286	0.052	0.470	−0.340	−0.292	0.017	−0.488	−0.219	0.392	1.000

The values in fat are different from 0 on a level of significance alpha = 0.05.

A high negative correlation (−0.488 to −0.024) ia also to note between the characters according to (**Table 6**):

- The leaf length was negatively correlated to the surface of leaf, nipples length, the height and the diameter with the collets of young seedlings. Leaf dispatcher was negatively correlated to the width of the leaf, the surface of leaf, the nipples length, the height and the diameter with the collets of young seedlings. The number of veins was negatively correlated to the surface of the leaf. The width petioles leaf was negatively correlated to the number of the leaf spines and the diameter of nipples. The number of leaf spines was negatively correlated to the surface of the leaf, the height and the diameter with the collets of young seedlings. The surface of the leaf was negatively correlated to the length, the diameter and the weight of nipples, and to the diameter and to the collets of young seedlings. Nipples length was negatively correlated to height of young seedlings. The diameter of nipple was negatively correlated to height and diameter to the collets of young seedlings. The weight of nipple was negatively correlated to height and diameter to the collets of young seedlings.

3.4. Analyze Canonical Discriminating

Euclidean Distance

The Euclidean distances represent the extent of genetic separation between the groups of populations (**Table 7**) this information can be used to determine the crossings of the genotypes likely to give hybrids having more strength.

To identify the characters able to discriminate the 12 populations, averages and standard deviations of each character were calculated.

The obtained matrix exhibits genetic distances ranging from 2.593 to 31.432 and shows a high phenotypic diversity among the studied individuals. The lowest distance is observed between the individuals of [DF] and [AZ] populations. The distance that shows the maximum difference was noticed respectively between the individuals of [ME] and [HJ] populations.

The population of Méjen Essef [ME] is furthest away from the populations of Keff El Rand [KR] and Hammam Jdidi [HJ] and with distances respectively from 27,678 and 31,432. The most remarkable bringing together between populations exists between the population of Dar Fatma [DF] and the population of Ain Zena [AZ] with the weakest distance (about 2593 like between the population of Béni Mtir [BM] and the population of El Feidja

Table 7. The Euclidean distances for the 12 populations of oak cork.

Stamp proximity (Euclidean distance):											
	HB	**DF**	**OZ**	**AZ**	**ME**	**BM**	**DjZ**	**EF**	**DjK**	**B**	**KR**	**HJ**
HB	0											
DF	8153	0										
OZ	14,251	9650	0									
AZ	8842	**2593**	10,887	0								
ME	20,552	16,305	8244	17,290	0							
BM	8939	4333	7042	5631	13,209	0						
DjZ	10,265	3952	8888	5390	14,295	5051	0					
EF	10,462	5986	5230	7258	10,929	**2864**	5665	0				
DjK	10,301	3955	7486	4999	12,972	2890	3270	3424	0			
B	13,696	9343	4633	10,940	8512	7263	7469	5388	7007	0		
KR	13,123	11,893	20,791	11,358	**27,678**	14,936	14,463	17,395	15,051	20,803	0	
HJ	11,898	15,877	24,384	15,483	**31,432**	18,586	18,772	20,701	19,364	24,097	9678	0

[EF] with a distance about 2864. An ascending hierarchical classification of the populations was carried out thanks to the matrix of the Euclidean distances (**Table 7** and **Table 8**).

3.5. Cluster Analysis: Hierarchical Classification Method

The cluster obtained in the basis of Hierarchical classification analysis [11] was constructed. Dissimilarity level ranged from 2.70 to 12.10. **Figure 1** shows two major clusters (I) and (II). The first one (I) is composed by trees from "Hammam Jdidi" [HJ] population originating from Hammamet, and from "Keff Rand" [KR] population originating from El Haouaria.

The second group (II) contains the remaining population. It is composed by two minor clusters (IIa) composed by trees from Hammam Bourguiba [HB], Dar Fatma [DF], Oued Zeen [OZ], Aîn Zana [AZ], Béni Mtir [BM], Djebel Zouza [DZ] populations originating from Ain Drahem, El Feidja [EF] population originating from Bouselm; Bellif [B] and Djebel Khroufa [DK] populations originating from (Tabarka); and clusters (IIb) composed by a single population Mejen Essef [ME] originating from Ain Drahem.

Within this group Aîn Zana [AZ] and Dar fatma [DF] population were very closely related with the lowest registered dissimilarity level (d = 2.70). The same group of clusters IIa included all the 7 accessions; El Feidja [EF] originating from Bouselm and Béni Mtir [BM], Bellif [B], Hammam Bourguiba [HB] and Oued Zeen [OZ] originating from Ain Drahem; with accessions of Djebel Khroufa [DK] originating from (Tabarka) and accessions of Djebel Zouza [DZ] belonging to Nefza. Furthermore, Méjen Essef [ME] accession from Ain Drahem was very phenotypically different from the two other Keff El Rand [KR] originating from El Haouaria and Hammam Jdidi [HJ] originating from Hammamet. The topology cluster shows a considerable morphological variability no correlated to geographical origins of the prospected sites (**Figure 2**).

4. Discussion

The present study was carried out to evaluate genetic diversity and characterize the oak cork (*Quercus suber* L.) tree in 12 populations Tunisian using morphological parameters of leaves, nipples and trees.

Results exhibited considerable genetic variation among Tunisian oak cork populations. Similar data have been previously reported in oak cork accessions. Indeed, [12] bring back a high degree of phenotypical polymorphism in 26 sources of oak cork originating in Spain, Portugal, Italy, Morocco, Algeria and Tunisia by using seven morphological metric characters, considered to be discriminating related to characters of vegetative growth of oak cork. These authors also reported the utility to make the distinction between the sources of oak cork and to

Table 8. Eigenvalues, proportion of variation and eigenvectors associated with the first three axes of the PCA in 12 cork oak accessions (Table 5 for parameters' label.)

	PC1		PC2		PC3	
Eigenvalues variance	3742		2770		1884	
Variance proportion individual (%)	28,783		21,307		14,493	
Cumulative (%)	28,783		50,091		64,584	
	var	e.i	var	e.i	var	e.i
	NBNV	(0.350)	LOFE	(0.356)	LOFE	(−0.442)
	LAPE	(−0.082)	LOPE	(0.474)	LAFE	(−0.415)
	NBEP	(0.426)	LAPE	(0.399)	LLOA	(−0.121)
Eigenvectors[a]	SRFO	(−0.264)	NBEP	(−0.121)	LOGL	(0.535)
	DIGL	(0.351)	SRFO	(−0.144)	DIGL	(−0.154)
	POID	(0.397)	DIGL	(−0.298)	POID	(0.304)
	HAU	(−0.249)	POID	(−0.139)	DIA	(0.257)

var: variable; e.i: Eigenvalue. [a]Only variables showing high loading in different principal components were taken into account.

validate the morphological metric approach which is a tool for the early selection of sources for the deforestation.

The accessions indicated that the characters (lengths of leaves); (widths of leaves); (number of the veins' leaves); (spines' number); (lengths' nipples); (diameter's nipples) and (weight's nipples); (petioles' lengths); (petioles' widths) and (collet's diameter of seedlings) accounted for a large proportion of the observed variability.

In addition, the coefficient of correlation of Pearson revealed that certain characters were largely correlated the ones with the others. A strong correlation enters leaves length with dispatcher of leaves or between nipples length and weight of nipples length.

The typological analysis showed that a phenotypic diversity which generally continuous, characterizes the local oak cork germoplasm. The topology of the derived dendrogram strongly supported this assumption.

Moreover, the results of typological analysis UPGMA showed an association between the accessions which show similar characteristics, such as the accessions of Keff El Rand [KR] (El Haouaria) and Hammam Jdidi [HJ] (Hammamet) on the one hand and the accessions of El Feidja [EF] (Bouselm); Djebel Khroufa [DK] (Tabarka); Béni Mtir [BM]; Aîn Zana [AZ]; Dar Fatma [DF]; Bellif [B]; Oued Zeen [OZ]; Hammam Bourguiba [HB] (Ain Drahem) and Dj Zouza [DZ] (Nefza) on the other hand. The accession of Méjen Essef [ME] (Ain Drahem) presents a class independent of the other accessions.

This diversity of the populations in the morphological characters, like generally reported to many species of forest trees, can result from type to cross the reproductive system and intense gene flows between the species that were shown in particular between the holm oak and cork oak [13]. Indeed, the biotic effect was implied like factor responsible for the regeneration of cork oak loss [14] because the intensity of these various types of the predation can vary spatially and temporally between the populations.

This source of regression in the regeneration of the cork oak can act like a powerful source of diversification of selection among the populations of *Quercus suber* L.

The results also revealed a phenotypic heterogeneity within the same species.

In the cork oak, 64.584% of the total genetic variation measured on the growth characteristics is ascribable to differentiation between the populations. This value higher than that was found by [12] (value of about 17.8%) and by [15] (value of about 11%) and was relatively higher than the average: 7% obtained more than 25 species of oaks studied for the polymorphism enzyme [16], which suggests that the natural selection supported various phenotypes in different populations. Indeed, [17] show that the natural selection is a considerable source of differentiation between the populations. It has two possible explanations for this reason for variation: the populations of *Quercus suber* became differentiated because heterogeneity (monoclonal) of mode of selection, which

led the population to its local optima, and/or differentiation, is a consequence of the genetic drift produced by the genic absence of flow between the populations, and by the events founders. One could expect geographically adjacent populations with the environment which have the same conditions and, possibly, abiotic similar selective pressures [18].

The effect of genotype is responsible for the phenotypic variability in cork oak in eastern Spain since two chlorotypes (chlorotype No. 21 this Spanish Catalonia and chlorotype No. 23, which differs from previous two mutations present in the pyrenees, subject to new results from cork of Spanish Catalonia, one can assume that the cork oak chlorotype characteristic of this region [19]. Sums in accordance with [20] which considers the hybridization as a rare occurrence, accidental and very low percentage of occurrence with respect to time of cohabitation. [21] think it is unusual to find more than 5 percent. 100 hybrids in a mixed population (though he identifies himself three out of twenty-five individuals, in Bagley Wood).

[22], assume that imperfect understanding more variable than is commonly believed specific characters, explain why some studies have determined to a significant degree hybrid (loc. cit.) in natural populations. This variability could be sure of genetic or phenotypic origin. Note that [23] showed strong intraspecific variability agglutinins leaves and fruits by location.

RUSHTON using a multi variable analysis. There are however studies that leaf traits, assuming they correlate well to the characters of seed heads. Now it is often because of contradictions between leaf traits and characters infructescences that botanists cannot determine with certainty an oak!

Morphological variation and field observations suggest that there is gene flow among the four taxa of the subgenus Quercus in the population sampled in this study, Gene flow is an important factor in population genetics, shopping the diversity of species [24] and [25]. This study suggests that in the twelve populations sampled (Hammam Bourguiba [HB], Dar Fatma [DF], Oued Zeen [OZ], Aîn Zana [AZ]; Mejen Essef [ME]; Béni Mtir [BM]; Djebel Zouza [DZ] (Ain Draham) El Feidja [EF] (Gar Dimaou); Bellif [B] (Nefza); Djebel Khroufa [DK] (Tabarka); Keff El Rand [KR] (El Haouaria) and Hammam Jdidi [HJ] (Hammamet) interspecific gene flow occurs and is associated with great morphological variation.

5. Conclusions

As a result of the present study, we can conclude that traits related to vegetative organs of *Quercus suber* L. show considerable phenotypic diversity in this important crop. Those related to the sizes from leaves, nipples and growth present the most discriminating value. A case of homonymy was detected in cultivar originating from Ain Drahem.

This study reveals considerable phenotypic (and presumably genetic) diversity among oak cork accessions, and accurate oak cork cultivars identification; however, it needs a large number of morphological markers and the exploration of other eco physiological, biochemical and molecular markers such as isozymes, AFLP fingerprinting, microsatellites and sequencing to analyze genotypic diversity.

Acknowledgements

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Scientific Research

The Community of Parasites Infecting *Clarias gariepinus* in the Tanzanian Waters: A Case of Lake Victoria

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Abstract

Quantitative variations in parasites were investigated with respect to sex and size of *Clarias gariepinus*, season and localities sampled. Parasitic infection in male and female fish was not significantly different; prevalence (Man-Whitney paired sample test, U = 135.5, *p* = 0.7697), mean intensity (U = 136, *p* = 0.7829). Based on Kruskal-Wallis test analysis, the prevalence and mean intensity of five parasite species varied significantly among the three localities sampled. Adult parasites in *Clarias gariepinus* showed high abundance during the dry season while the larval forms showed high abundance in the wet season. In most of the parasite species analyzed the abundance varied significantly with host size. The abundance of *Dolops ranarum*, *Paracamallanus cyatopharynx* and *Eumasenia bangweulensis* for instance, increased with fish size, while that of *Diplostomum mashonense*, *Tylodelphys* species, *Astiotrema reniferum*, piscicolid leeches and *Monobothrioides woodlandi* increased initially but decreased as fish length reached 31 - 40 cm. Total parasite burden increased in fish of 20 - 40 cm standard length. Generally, most parasites were highly overdispersed and the negative binomial model described their distribution.

Keywords

Population Biology, Parasites, *Clarias gariepinus*, Lake Victoria, Tanzania

1. Introduction

Fish provides the necessary proteins for human health worldwide. In Tanzania specifically Lake Victoria, communities rely on catfishes as one of the major protein sources. The catfishes are members of the order Siluriformes and comprise about 2800 species worldwide [1]. There are three families in the African suborder Silu-

roidea that contain species suitable for aquaculture. These are the Claroteidae (formerly Bagridae), the Schilbeidae and the Clariidae. In Tanzania, the family Clariidae has six genera with 21 representative species that occupy varying habitats in streams, rivers and lakes [2]. Lake Victoria alone has three genera with six species namely: *Clarias gariepinus* (Burchell, 1822), *Clarias werneri* (Boulenger, 1906), *Clarias alluaudi* (Boulenger, 1906), *Clarias liocephalus* (Boulenger, 1898), *Clariallabes petricola* (Greenwood, 1956) and *Xenoclarias eupogon*. *Dinotoptera* and *Bathyclarias* are endemic to Lakes Tanganyika and Malawi, respectively [3].

Several comprehensive reviews of parasites of *Clarias gariepinus* from Lake Victoria basin are available [4]-[6]. Many data on the systematics of the parasites of *Clarias gariepinus* from the continental Africa and elsewhere are available in scientific papers [7]-[9]. However, studies dealing with quantitative data and ecology on *C. gariepinus* in Lake Victoria basin are still scarce. In addition, among the clariids, parasites of *C. gariepinus* are so far the only extensively studied while those of other species in the family are poorly known.

In the present paper the metazoan parasite fauna of *Clarias gariepinus* from the Mwanza Gulf, Lake Victoria (**Figure 1**) is discussed. It is anticipated that the study will show the abundance, distribution and seasonality of parasites in the localities surveyed.

2. Materials and Methods

2.1. Study Site

Figure 1 illustrates the area where this study took place. Sampling was carried out at three sites along the Mwanza Gulf namely, Butimba/Kirumba bays (BK), Nyegezi bay (N) and Lake Malimbe (LM) along Luanso bay. The two sites, Butimba/Kirumba and Nyegezi bays form a continuum along the shore of the gulf and therefore bear the features of the main lake. Nyegezi bay is located at 2°35'S, 32°55'E. The eastern part of Nyegezi bay is muddy and is fringed by abundant vegetation. It is relatively shallow with a maximum depth of nearly 9 m in some parts. A single seasonal stream empties into the eastern part of the bay. The western shore is mainly sandy and rocky.

Butimba/Kirumba bays are located at the entrance of the gulf, most part of which is sandy and rocky and along the shores are industries and human habitats. Two streams enter the lake via these bays; Mirongo empties into Kirumba bay and Malama into the eastern part of Butimba bay. The bottom at the mouth of these streams is mainly muddy and fringed by vegetation cover.
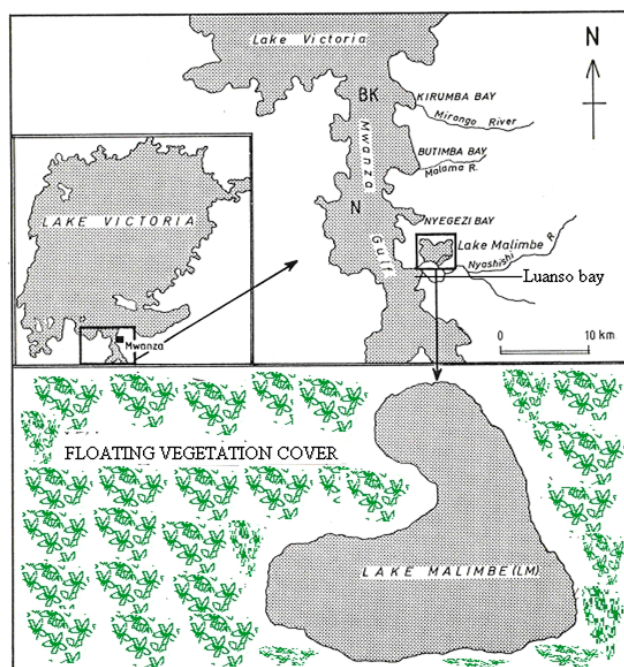

Figure 1. The Mwanza Gulf showing the three localities sampled [Butimba/Kirumba bays (BK), Nyegezi bay (N) and Lake Malimbe (LM)].

Lake Malimbe (2˚34.471'S, 32˚53.867'E) adjoins Lake Victoria, located about 10 km from Mwanza town, off the eastern part of the Mwanza Gulf. It is a satellite lake, with a surface area of about 10 km^2. Floating macrophytes such as *Papyrus* and *Phragmites* cover most of the lake. The remaining open water area is about 0.5 km^2 with a maximum depth of 2.5 m. Entry to the Lake is very difficult especially during the rainy season. There are no tributaries from the main lake and the main source of water is from seepage and rain run off. The bottom of the lake is essentially muddy. During much of the year (perhaps most years) Malimbe is cut off from the main lake. Connection is re-established in the rainy season from March-May particularly in years with exceptionally heavy rains like the El Ninõ of 1998.

2.2. Methods

Initially, 2004-2006, the study was intensive being sponsored by the Lake Victoria Environmental Management Project (LVEMP) and samples were collected twice per year *i.e.* wet (November to May) and dry (June to October) season. Later, 2007 to 2013, this was a monitoring period sponsored by Lake Victoria Research Initiative (VicRes); samples were collected once per year alternating between wet and dry seasons. About 18 - 50 fish depending on the availability were examined per every visit. Fish were caught by longlines and handlines with baited hooks. Dead fish were transported in an ice-cooled box and live fish in buckets filled with water from the lake to the laboratory. Examination of fish for parasites, handling and processing of parasites followed standard procedures as described by Moravec *et al*. [10] [11].

Comparison of abundance in relation to locality was analyzed by Kruskal-Wallis test. Comparison of abundance in relation to sex and size of the host were analyzed by Man-Whitney paired sample test and Kruskal-Wallis test, respectively. Seasonality of parasites distribution and abundance were analyzed by Friedman's test [12]. Friedman's test was done by Statistica Package Version 9 (2009) while Kruskal-Wallis and Man-Whitney tests (U) were done using Instat Version 3.1. Most of the parasite's count data did not fit in the normality test and hence nonparametric test were employed in this study. Ecological terms are as defined by Margolis *et al*. [13].

3. Results

3.1. Parasite Abundance in Relation to Sex

The abundance of parasites between male and female fish were not significantly different, prevalence (Man-Whitney tests, U = 135.5, p = 0.7697), mean intensity (U = 136, p = 0.7829). **Table 1** is a summary of the results for *C. gariepinus* sampled for the period stated. The results show that relatively more female than male fish were infected by most parasites though mean intensity was considerably higher in male than in female fish. The number of female fish examined from each locality did not differ from that of male fish (t = 1.85, p = 0.082). Due to the similarities observed, the data for both male and female fish were pooled in subsequent analysis.

3.2. The Abundance of Parasites with Respect to Localities

The abundance of five parasite species was significantly different among the three localities sampled. The digenean *D. mashonense* was more abundant at Nyegezi bay than at Butimba/Kirumba bays and Lake Malimbe. *Tylodelphys* species, *P. clarias*, *M. woodlandi* and *P. cyathopharynx* were more abundant at Lake Malimbe than at Butimba/Kirumba and Nyegezi bays. *D. ranarum* was only observed in Lake Malimbe while piscicolid leeches and *Eustrongyloides* species only from Nyegezi bay. *Proteocephalus* species was found only from Butimba/Kirumba bays (**Table 2**).

3.3. Seasonal Variation of Parasites Abundance

Although most parasites occurred in all the localities, marked differences in the pattern of occurrence were observed. *D. mashonense*, *Tylodelphys* species, *P. clarias* and *P. cyathopharynx* occurred consistently in the three localities throughout the study period, except some few months at Butimba/Kirumba bays. The prevalence of *D. mashonense* varied during the study period as was at Nyegezi and Butimba/Kirumba bays when the occurrence of the digenean fell below 76%. However, the variations were not statistically significant (Friedman's test χ^2 = 2.97, p = 0.227), as was *P. clarias* (χ^2 = 3.5, p = 0.174). Prevalence of *Tylodelphys* species (χ^2 = 104, p = 0.005) and *P. cyathopharynx* (χ^2 = 19.45, p = 0.001) varied significantly during the study period. In general, prevalence

Table 1. Mean intensity (in parentheses) and prevalence of parasites in male and female *Clarias gariepinus* in Mwanza Gulf, Lake Victoria (Pooled data).

Parasite	n_f	Female	n_m	Male
Diplostomum mashonense	515	87.88 (536.36)	426	87.84 (540.83)
Tylodelphys sp.	181	30.89 (93.30)	146	30.10 (104.62
Dolops ranarum	20	3.41 (3.10)	14	2.89 (3.64)
Spinitectus petterae	5	0.85 (4.40)	9	1.86 (3.33)
Procamallanus laevionchus	12	2.05 (2.08)	11	2.27 (2.45)
Polyonchobothrium clarias	178	30.38 (2.70)	146	30.10 (2.86)
Astiotrema reniferum	29	4.95 (5.62)	30	6.19 (5.67)
Allocreadium mazoensis	15	2.56 (5.20)	22	4.54 (8.32)
Piscicolid leeches	12	2.05 (3.50)	17	3.51 (3.29)
Monobothrioides woodlandi	37	6.31 (5.0)	29	5.98 (14.76)
Paracamallanus cyatopharynx	168	28.67 (3.11)	161	33.20 (4.06)
Contracaecum sp.	28	4.78 (14.57)	19	3.92 (25.84)
Euclinostomum sp.	5	0.85 (9.60)	5	1.03 (6.80)
Gyrodactylus sp.	31	5.29 (5.10)	24	4.95 (3.63)
Eumasenia bangweulensis	32	5.46 (5.81)	30	6.19 (5.77)
Eustrongyloides sp.	-	-	1	0.21 (1)
Proteocephulus sp.	-	-	1	1.44 (7)
Total No. of fish examined	586		485	

of most autogenic parasites was high during the dry season but declined in the rainy season. Prevalence of allogenic species such as *Tylodelphys* species and *Contracaecum* species were high in the rain season. Monthly variation in mean intensity showed no consistent pattern during the study period. However, for most autogenic parasites the highest mean intensity was recorded during the dry season and that of allogenic parasites increased during the rainy season.

3.4. Seasonal Variation of the Variance to Mean Ratio

All parasites recorded from *C. gariepinus* were overdispersed. The dispersion index (variance to mean ratio) among localities was not significant. The degree of overdispersion fluctuated in the same pattern as the prevalence and intensity of infection. The dispersion index also indicated that the number of heavily infected fish increased in the wet season for parasites using *C. gariepinus* as intermediate host, and the rise in infection was high in dry season for parasites using *C. gariepinus* as a definitive host. Some of the parasites however, did not show a consistent pattern probably due to low number of hosts infested.

3.5. Prevalence of Parasites with Respect to the Length of *C. gariepinus*

Prevalence of parasites in each size group of fish is shown in Table 3. In most of the parasite species analyzed prevalence varied significantly with host size. The prevalence of *D. ranarum*, *P. cyathopharynx* and *E. bangweulensis* increased with fish size. The prevalence of *D. mashonense*, *Tylodelphys* species, *A. reniferum*, piscicolid leeches and *M. woodlandi* increased initially but decreased as fish length reached 31 - 40 cm. The remaining parasite species did not show a recognizable pattern.

Table 2. Mean intensity (in parentheses) and prevalence of parasites on *Clarias gariepinus* from Mwanza Gulf, Lake Victoria.

Parasite	Butimba/Kirumba	Nyegezi	Malimbe	Rmk
Diplostomum mashonense	(420.7A) 85.2A	(716.1B) 86.4A	(461.7A) 91.2A	*
Tylodelphys sp.	(58.9A) 8.05A	(158.5B) 23.7B	(77.8A) 53.9C	*
Dolops ranarum	-	-	(3.5) 8.06	-
Spinitectus petterae	(1.5) 2.67	(1.9) 2.66	(3.0) 0.50	ns
Procamallanus laevionchus	(1.0) 2.67	(3.0) 1.06	(2.24) 4.28	ns
Polyonchobothrium clarias	(2.77A) 31.5A	(2.48A) 21.01B	(2.90A) 38.04A	*
Astiotrema reniferum	(7.0) 8.05	(1.67) 8.79	(5.0) 8.06	ns
Allocreadium mazoensis	(10) 3.69	(8.5) 3.72	(12.67) 3.02	ns
Piscicolid leeches	-	(1.56) 6.12	-	-
Monobothrioides woodlandi	(2.17A) 4.03A	(3.5A) 0.53B	(11.15B) 13.1C	***
Paracamallanus cyatopharynx	(2.31A) 15.77A	(1.93A) 20.47B	(4.46B) 15.6A	***
Contracaecum sp.	(9.9) 4.03	(9.94) 4.52	(13.9) 4.53	ns
Euclinostomum sp.	(3.0) 0.34	(2.0) 0.80	(3.17) 1.51	ns
Gyrodactylus sp.	(2.62) 4.36	(2.32) 5.9	(3.39) 4.53	ns
Eumasenia bangweulensis	(4.0) 5.34	(6.79) 6.38	(5.02) 9.52	ns
Eustrongyloides sp.	-	(1.0) 0.27	-	-
Proteocephulus sp.	(7.0) 0.34	-	-	-
Total No. of fish examined	298	376	397	

Kruskal-Wallis test, $p > 0.05$, considered not significant. [*]Significant; [**]Very significant; [***]Extremely significant and "ns": not significant. Figures with different letters are statistically different.

3.6. Mean Intensity of Parasites According to the Length *C. gariepinus*

Mean intensity was calculated for each parasite species within each fish size group. The data obtained were compared using Kruskal-Wallis test. For most parasite species mean intensity varied significantly among fish of different sizes. For *D. mashonense*, *Tylodelphys* species and *M. woodlandi* for instance, mean intensity increased as the fish size increased but decreased in fish over 31 - 40 cm long. In other parasites, *P. clarias*, *D. ranarum*, *P. cyathopharynx*, *E. bangweulensis* and *A. mazoensis*, mean intensity increased as fish size increased without a decline in fish over 31 - 40 cm. In some parasites, the rise in mean intensity observed was not consistent, as there was an initial rise followed by a decline and a rise again.

The mean number of parasites irrespective of species, excluding *D. mashonense* and *Tylodelphys* species increased with fish size up to 31 - 40 cm size class and declined thereafter (**Figure 2**). The mean number of parasite species per fish increased in the first group of fish size, remained constant in the subsequent three groups before declining to 6 species in fish over 40 cm standard length (**Figure 3**).

3.7. Variation of Frequency Distribution with the Length of *C. gariepinus*

The distribution of the intensity of infection of parasites in *C. gariepinus* showed that the zero class was the greatest in most parasites except in *D. mashonense* where the zero class was always smaller than the next class in the series. The number of hosts with low intensity of parasites was always higher than the number of heavily infected hosts. The distribution of the intensity of parasites was aggregated; hence the negative binomial fitted the distribution of all parasites analyzed.

Table 3. Prevalence of parasites according to the length of *C. gariepinus*.

Parasite	4 - 10 cm	11 - 20 cm	21 - 30 cm	31 - 40 cm	<40 cm	Rmk
Diplostomum mashonense	66.18	87.95	92.17	91.74	85.88	ns
Tylodelphys sp.	22.06A	29.75A	37.95B	33.03A	22.35A	*
Dolops ranarum	0	0.56A	0.60A	9.17B	11.76B	*
Spinitectus petterae	0	1.51	3.01	0.46	1.18	ns
Procamallanus laevionchus	1.47	1.13	3.61	3.21	3.53	ns
Polyonchobothrium clarias	26.47A	32.58B	36.14B	23.85A	24.71A	*
Astiotrema reniferum	0	2.26A	4.22A	14.22B	10.59B	*
Allocreadium mazoensis	0	1.13A	9.64B	4.13C	7.06B	*
Piscicolid leeches	0	3.77A	4.82A	0.46B	0	*
Monobothrioides woodlandi	1.47A	1.51A	4.82A	17.43B	12.94B	**
Paracamallanus cyatopharynx	2.94B	25.42A	34.94A	44.05A	43.53A	***
Contracaecum sp.	5.88	4.33	4.82	3.21	5.88	ns
Euclinostomum sp.	1.47A	0.38B	0.60B	2.75A	0	*
Gyrodactylus sp.	5.88A	4.14A	10.24B	5.50A	0	*
Eumasenia bangweulensis	2.94A	3.95A	3.01A	7.80B	18.82B	*
Eustrongyloides sp.	0	2.5	0	0	0	-
Proteocephulus sp.	0	0	0	0	3.0	-
Total No. of fish examined	68	531	166	218	85	

[*]Significant; [**]Very significant; [***]Extremely significant; "ns": Not significant, $p > 0.05$, considered not significant. Figures with different letters are statistically different.
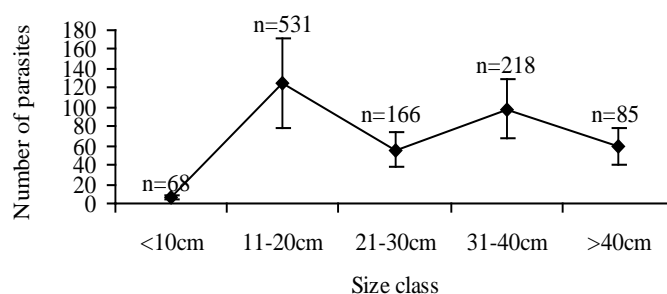

Figure 2. Mean number of parasites per fish, irrespective of species according to fish size (excluding *D. mashonense* and *Tylodelphys* sp.) (Error bars are ± SD).

The behaviour of the index of dispersion (Variance: mean ratio) with the length of *C. gariepinus* like mean intensity fell into two categories; in the first group there was an initial rise as fish size increased, followed by a decline in the fish over 40 cm standard length (*D. mashonense*, *Tylodelphys* sp. and *M. woodlandi*). The second category showed continuous increase in the dispersion index as the fish increased in size.

4. Discussion

4.1. Abundance of Parasites According to the Sex of *C. gariepinus*

The present study suggests that male and female *C. gariepinus* do occupy the same ecological guild and hence
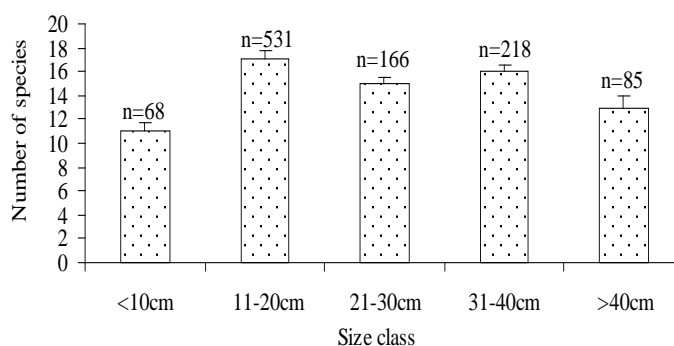
Figure 3. The number of parasite species per fish according to the length of *C. gariepinus* (Error bars are ± SD).

the prevalence and mean intensity of their parasite fauna were found to be similar. Similar studies found the same results [14]. Furthermore, it has been found that female vertebrates are less heavily infected with parasites than males [15]. Elevated levels of oestrogen are believed to protect female hosts from parasitic infections [16]. On the other hand Reimchen [17] suggested that competition for mates and subsequent elevation of testosterone levels could lead to immunosuppression in male hosts, hence an increase in susceptibility to parasitic infections. This could account for the relatively high mean intensity values observed in male as compared to female *C. gariepinus* in the present study.

4.2. Variation of Parasite Abundance among the Three Localities Surveyed

Previous studies have revealed variation in prevalence of parasites of fishes among lakes [18], rivers [19] and among sampling stations in large bodies of water [20]. The present study, like previous studies cited above, has demonstrated the existence of variation of prevalence and mean intensity of parasites in the same host species from different localities in the same lake. The variation observed indicates the presence of an uneven distribution in terms of species and density [21] of parasites intermediate hosts, some of which constitute food items for *C. gariepinus* among the localities surveyed [22]. This further affects the type and quantity of food materials taken by *C. gariepinus*, consequently differences in prevalence and mean intensity of parasites recruited among the three localities.

4.3. Abundance of Parasites According to the Length of *C. gariepinus*

Host size is suggested as the most important predictor of parasite load [21]. In the present study, two patterns were observed between parasites prevalence and mean intensity on the one hand and fish length on the other. The first pattern showed an initial rise in prevalence and mean intensity followed by a decrease in large fish. This is by far, the most commonly observed pattern in parasitological studies [23]. In the second pattern prevalence and mean intensity increased continuously with increasing length of *C. gariepinus*. The second pattern is similar to the first but differs due to low representation of large fish in samples.

In fish populations, the intensity of infection by metazoan parasites increases with the size of fish as observed in the present study. The increase sometimes stops beyond a certain host size, but the positive relationship generally holds when several classes are pooled. Large fish provide more internal space for parasite establishment and incur higher infection rates as they eat more parasitized prey [24]. Several biological parameters or ecological processes can determine which types of parasites are likely to correlate closely with host size. Density-dependent regulation of parasite numbers, for instance, may obscure the relationship between fish size and intensity of infection by preventing the accumulation of large numbers of parasites even in the largest fish [25]. Another example is the differences in the life span exhibited by different parasite taxa within a fish host; this could result in different rates of parasite accumulation as a function of fish size. Larval digeneans, for example, are long-lived in fish hosts and tend to accumulate over time and hence for these parasites intensity tends to correlate with fish size [24].

In the present study, it was observed that prevalence and mean intensity of the ectoparasites (*D. ranarum*, piscicolid leeches and *Gyrodactylus* species) and those of larval trematodes (*D. mashonense*, *Tylodelphys* species

and *Clinostomum* species) increased with the size of *C. gariepinus*. Pennycuick [16], and Amundsen *et al.* [21] stated that large fish represent greater targets for the ectoparasites and parasites dependent on skin penetration; and that larger fish also circulate more water over their gills and oral cavity than do smaller fish, thus bringing more ectoparasites and cercariae into contact with them. This argument may account for the high prevalence and mean intensity of ectoparasites and larval digeneans in larger fish observed in the present study.

Furthermore, it has been suggested that a prolonged life span of the parasite may contribute to maintain a basic infection level within the host population, and thus act as a stabilizing factor for the parasite population as it will contribute infective stages for the next generation of fish [21]. It is therefore to be expected that there will be a steady increase in the mean intensity of parasites with increase in size of fish. However this was not always the case in the present study as heavily infected fish were not always the largest ones [24]. Morphological and behavioural changes, and development of immunity in older fish that would render them less accessible to invading parasites have been cited as possible causes of the lower intensity often observed in large fish [21].

Irrespective of the variations described for the individual parasite species with fish size, the total parasite burden was greatest in the largest fish. The mean number of parasite species also increased with fish size initially then decreased in larger fish, probably due to development of immunity towards certain parasite species and/ or behavioural and diet changes in large and old fish. Similar observations and conclusions have been reached in other studies [24].

4.4. Frequency Distribution of the Parasites of *Clarias gariepinus*

Most of the parasites recovered from *C. gariepinus* at the three localities sampled showed an overdispersed distribution. The dispersion index like mean intensity presented two patterns with respect to host size. The first pattern was that of an initial increase in overdispersion followed by a decline in fish over 40 cm and the second was a continuous rise in dispersion index with host size. The decline of the dispersion index accompanied by a decreased mean abundance may be suggestive of parasite-induced host mortality [26].

When the distribution of parasites is overdispersed a large number of parasites are accommodated in a smaller number of hosts. It should also be noted that most parasites are harmful to their host and the more parasites there are in a host the larger are the adverse effects. With an overdispersed distribution there will be a smaller number of hosts with many parasites and these may die and reduce the parasite population by many more individuals than it does the host population and hence the infection is kept to a moderate level (density-dependent regulation) [24]. On the other hand heavily infected fish may be eaten by a predator; this is advantageous to the parasite if it requires its host to be eaten by another host for the parasite to develop to the next stage of the life cycle [27]. However, if there are few hosts with large numbers of parasites then the chances for the predator finding these will be slight even if susceptibility to predation is greatly increased. There is thus an optimum shape for the distribution in which a large number of the parasites is in a fairly small number of the hosts [28]. Thus with overdispersed distribution the effect of the parasites on the host population is minimal and the distribution is advantageous to both host and parasite in ensuring continuous development of the parasite with minimal losses to the host population.

Overdispersion has been attributed to the heterogeneous distribution of the infective stages of the parasites such that when a fish visits such lacunae with infective stages of parasites, it results in heavy infections. Also behavioural differences and/or genetic factors and random continuous re-exposure of fish to infection over time have been cited as attributing factors to overdispersion [29].

4.5. Seasonality of Abundance and Frequency Distribution of Parasites

Results showed that mean intensity and prevalence of all parasites (with the exception of *D. mashonense*, whose prevalence was almost constant throughout the study period), varied with the seasons in all localities sampled. The mean intensity and prevalence of parasites using *C. gariepinus* as an intermediate host (*D. mashonense*, *Tylodelpys* species, *Contracaecum* species and *Clinostomum* species) were high during the rain season and tended to decline in the dry season. However mean intensity and prevalence of *Tylodelphys* sp. at Malimbe were low during the rain season. Fluctuations in prevalence and mean intensity of parasites such as metacercariae of trematodes, which utilize snails as first intermediate hosts, could be attributed to the fluctuations in the population of their snail intermediate hosts in the shores of the Mwanza Gulf. These shores are characterized by seasonal flood plains fringed with marshes and vegetational cover. Concentrations of snails are high in the flood plain

pools during the dry season, but infectivity in *C. gariepinus* is low due to the water-land barrier separating fish from these intermediate hosts in the pools. At the onset of the rain season the snails are washed into the shores of the main lake where the parasite infective stages come into contact with *C. gariepinus*, hence resulting in high prevalence and mean intensity.

Also *C. gariepinus* is known to traverse long distances into the wetlands where they may be trapped in the pools of water at the onset of the dry season and predated by piscivorous birds, with subsequent removal of parasites from the pool. This could also account for the seasonal variations observed. Nkwengulila [29] reported increased prevalence and mean intensity of the parasites of *Clarias* species during the dry season contrary to the results of the present study. These seemingly contradictory observations might be attributed to the differences in the size and nature of the water bodies involved. Nkwengulila [29] studied *D. mashonense* in *C. gariepinus* from Mindu Dam in Tanzania. The volume of water in small water bodies tends to decline in the dry season; consequently contact between parasites and fish hosts is increased. This is probably not the case in large water bodies like Lake Victoria whose volume of water remains almost constant throughout the year. The intermediate hosts for some of the parasite's infective stages, such as snails, tend to accumulate in the shallow waters of lakes [30], mainly in the pools of the nearby wetlands. Contact between fish and the intermediate hosts of the parasite infective stages is thus blocked during the dry season and is only re-established at the onset of the rain season as discussed above.

Parasites using *C. gariepinus* as a definitive host showed high prevalence and mean intensity during the dry season. It should be noted that the intermediate hosts for most of these parasites are the planktonic crustaceans and aquatic insects, and that most are acquired through the food chain. In the dry season, concentration of planktonic crustaceans, aquatic insects and zoobenthic organisms is high along the shores of Lake Victoria. It has been demonstrated that zooplankton accumulates in the littoral shallow waters due to accelerated reproduction; in addition embryonic development and hatching of parasite eggs occur along the shallow waters [30]. The presence of the definitive hosts (fish) in synchrony with the increased density of parasite's intermediate hosts leads to increased frequency of contact between the fish hosts and the infective stages of the parasites, thereby increasing prevalence and mean intensity in the fish host.

Acknowledgements

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Scientific Research

Evaluation of Characteristics Floristico-Edaphic of the Steppes at Alfa (*Stipa tenacissima* L.) in the Saida Region (Western Algeria)

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Abstract

To establish an assessment of floristic and edaphic characteristics of steppe formations we conducted a phytoecological study on a local scale by comparing the current plant diversity and soil in three (3) stations representative of the vegetation to alfa (*Stipa tenacissima* L.) located south of Saida (western Algeria). The relations between the station, the vegetation and the characteristics of soils in the steppe are very imperfectly known. This study intends to analyze the relationship between floristic composition and edaphic parameters of the steppe at alfa. Anthropogenic pressures on plant structure are different imbalance resulting in one ecological. The floristic and edaphic data have been the subject of a factorial correspondence analysis (A.F.C). The floristic inventory denotes a regressive dynamics of the formations at alfa passing from one site to another. Thus 39 plant species have been inventoried in the site in good condition, against 26 species for the moderately degraded site and 16 species only for the damaged site. The coupled results between species and edaphic parameters show the relations between the plant diversity and the physico-chemical characteristics of the soils. The statistical analyses do appear a strong correlation between floristic composition, the state of vegetation and the edaphic parameters.

Keywords

Evaluation, Steppe, *Stipa tenacissima* L., Soil Analysis, Plant Diversity

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1. Introduction

Steppe plant formations of North Africa are the result of the combined action of climatic factors and old and moderate anthropogenic pressures. The agro-sylvo-pastoral balance observed during the latter centuries has been broken in recent years by growing demographic pressure and changes in production systems (extensive sheep rearing and mechanized agriculture).

Abdelguerfi and Laouer [1] found that, for several years, in the countries of the Maghreb an agro-sylvo-pastoral imbalance settled and manifested itself by a degradation of the pastoral and forest potential.

In Algeria the steppe regions represent 9% of the territory and are real areas of steppe rangelands. Their population is composed mainly of pastoralists [2]. In 1971, the works of Le Houérou [3] showed that the steppe of Algeria was satisfactory and their perennial aboveground phytomass was of the order of 800 to 1500 kg MS/ha (dry mass per hectare) between 1950 and 1970. The same author noted in 1985 that perennial biomass was less than 200 kg MS/ha and the islets of degradation had became jointifs and covered considerable expanses.

The phenomenon of degradation of the steppe formations, found that there was a 40-year, trained more and more alarming desertification and translated on the ground by a modification of the component of the ecosystem (flora, fauna, soil and its elements, habitats) [3]-[5].

The further restructuring of the Algerian steppe area in recent years was characterized by the development of agglomerations and the sedentarization of nomads in a fragile space. The highlands territories have been occupied by tens of thousands of people; there is barely more than a century and currently this is occupied by several million people. These changes have the most disturbing ecological consequences [6] [7]. The former pastoralists and occasional farmers have become one of the workings of the economy of the country [8] [9]. The degradation of the steppe spaces is mainly due to the increase of livestock and overgrazing. The increase of the population and extensive farming has harmful consequences on the future of natural resources already weakened by climate pejorations.

These socio-cultural changes are accompanied by important qualitative and quantitative changes aboveground phytomass following a decrease and/or disappearance of species with good pastoral values [10], leaving room for species not palatable (spiny or toxic species) [11] [12]. It appears that overall pastoral production in these rangelands is marked by a very significant decline. Rapid and intense changes have been remarked in these areas under the growing demand of the people.

Given the degradation of these natural ecosystems, phytomass distribution seems to be linked to certain soil parameters (particle size, organic matter, pH, electrical conductivity, depth). These parameters have been studied, particularly for soil-vegetation relationships in the steppe [13]-[17]. In the wilaya of Saïda the rangelands extend over an area of 163,063 ha and are representing a total area of 24.1% and are located in the municipalities of Moulay Larbi, Sidi Ahmed, Maamoura and Skhouna. There are four types of steppe rangelands: *Stipa*, *Artemisia*, *Atriplex* and salsolaceae [18].

To identify the dynamics of vegetation and edapho-resources we have inventoried the flora and evaluated the characteristics edaphic relating to perimeters of alfa (*Stipa tenacissima* L.) located in the municipality of Mâamoura (south of Saïda). This work fits in tracking the physiognomy change of natural ecosystems exposed to anthropogenic stress on one hand and the relationship between the degradation of the site to *Stipa* on the other hand.

2. Materials and Methods

2.1. Physical Layout and Choice of Stations

The study area is located in the southern part of the wilaya of Saïda (western Algeria). It is naturally limited by a series of Djebel oriented north to northeast with Djebel Sidi Youssef (1338 m) and Djebel Harchoune (1259 m). These mountains are arranged in a southwest and north and are bowing to the south in the direction of high steppe plains (**Figure 1**).

The human impact on ecosystems in the study area is quite contrasting. Thus the choice of stations was motivated by the persistence at alfa locally little or not damaged. This physiognomic descriptor has allowed us to identify tree stations (**Table 1**):

1) Perimeter at alfa in good condition (Site 1) = A.B.V;
2) Perimeter to moderately degraded at alfa (Site 2) = A.M.D;
3) Perimeter at alfa damaged (Site 3) = A.D.

Table 1. Characteristics of the study sites.

		A.B.V	A.M.D	A.D
Geographic coordinates		0˚35'28"W, 34˚41'11"N	0˚36'19"W, 34˚40'9"N	0˚36'15"W, 34˚59'1"N
Description of the stations	Average recovery rate	50% - 70%	30% - 50%	Less than 30%
	Average height of the tufts at alfa	60 cm	30 cm - 60 cm	Less than 30 cm
Average altitude		1100 metres slope: 2% to 5%		
Climate		• Mediterranean climate dry and hot summer season and winter rainy, cool and cold; • Rainfall accumulation of last two decades is between 81.5 mm and 356 mm/yr [12]; • Seasonal pattern: HAPE; • Frost days: 37 (from December to March); • Sirocco days: 11 (from May to August); • Bioclimate: arid than cold variant.		

Figure 1. Location of the study area.

These three stations are representative of steppe rangelands. In each of these formations ten floristic surveys related to ten soils were made.

2.2. Study Methods

2.2.1. Study of the Vegetation and Soil

The approach used to study vegetation has adapted to the characteristics of the steppe formations whose stands are discontinuous. We've made 10 floras by station surveys during the good phenological time. The surface of records must be sufficient to understand the maximum of plant and floristically homogeneous species [19] [20]. An area of 400 m^2 was chosen deliberately to our sampling. Inventory of vegetation was made based on the stigmatic abundance-dominance of Braun-Blanquet method [21].

The soil survey approach is based on 10 samples of soil by site. Each sample of soil was associated with a floristic survey and the vegetation (A.B.V; A.M.D and A.D). Soil samples have been made at the level of the rhizosphere of subjects to alfa.

The edaphic variables are measured on fine soil (less than 2 mm) and are as follows: granulometric analyses; pH, conductivity electrical (CE), total limestone (Ct, %), active limestone (Ca, %) and the organic matter (Mo) of a hand and the depth (Pr) of the profile to the limestone crust on the other.

2.2.2. Data Processing

To explain the links between vegetation and edaphic parameters we confronted the different results using at A.F.C. This approach allows highlighting the correlations between plant data and the parameters of the studied soil [22] [23]. Plant species have been treated in presence-absence.

Digital processing was performed using the Minitab Version 15 Software.

3. Results and Discussion

3.1. Analytical Results of Soil

3.1.1. Grading Results

The granulometric results obtain in laboratory are transferred on the diagram of textures, this allowed us to classify sites sampled. **Table 2** reflects the most significant results.

The results obtained show a fairly divided composition. On site A.D the amount of clay is reduced, it is between 5.2% and 10.3% and for the other sites it oscillates between 10.3% and 20.6%. As silt, measurements vary between 08.2% and 29.5% for A.M.D; 25.8 to 29.3% for the A.D and 28.1% to 42.8% for A.B.V. The percentage of the sands is important in three sites. The measures range from 60.3 and 71.3% for A.D and A.M.D sites however the A.B.V site presents a moderate percentage (values between 46.9 and 51.3%). Our results from the fraction of clay from the site 3 confirm the results of Trabut [24]. However we note that in degraded and/or degraded places the percentage of clay is low. The role of the latter is to establish links between the elementary particles of the soil or with many substances (ions, organic molecules, water).

3.1.2. Physico-Chemicaly Results of the Soils Studied

The results are grouped in **Table 3**.

The Analyses show a clear difference of Mo content from one site to another (A.B.V; A.M.D and A.D). Despite a grade of 0.91 per cent on average of Mo for A.B.V; it remains too low. According to estimates by Pouget [13] and Djebaili [15] the Mo average is 1% - 2% for steppe soils. As for the other sites (A.M.D; A.D) the values in Mo are respectively 0.5% and 0.3%.

Also we note a clear difference between the Pr of sites profiles. The global average of Pr of soil is 16 cm for A.B.V; 9.7 cm for A.M.D and 5.4 cm for the A.D. These values show a regression of Pr of soils and their truncation are net.

The rate of Ca is roughly equal in the three sites; it is 2.33% to 2.58% for A.B.V; 2.30% to 2. 83% for A.M.D and 2.05% to 2.73% for A.D.

The CE is close in the three sites, it oscillates respectively: 0.3 to 0.7 mmhos for A.B.V; 0.2 to 0.9 mmhos for A.M.D and 0.1 to 0.6 mmhos for A.D.

3.2. Analysis of Vegetation

The species inventoried using the keys Quézel and Santa [25] are grouped in **Table 4**. Species were coded and their calculated frequencies of occurrence. 39 species have been recorded in A.B.V, against 26 sp. for A.M.D and only 16 sp. for A.D.

Table 2. Results of granulometric analyses of the studied soils.

	Clays (%)	*Silt* (%)	*Sands* (%)	**Texture**
	15.5	35.1	49.4	Silty
A.B.V	20.6	28.1	51.3	Silty
	10.3	42.8	46.9	Silty
	10.3	29.5	60.3	Silty-sandy
A.M.D	15.5	20.6	63.9	Silty-sandy
	20.6	08.2	71.3	Silty-clayey-sandy
	10.3	29.3	60.4	Silty-sandy
A.D	10.3	27.3	62.4	Silty-sandy
	5.2	25.8	69.1	Silty-sandy

Table 3. Analytical results of the studied soils.

Site of vegetation	N survey	pH	pH KCl	CE mmhos	Ct%	Ca%	Mo%	Pr cm
A.B.V	R 1	8.2	8.4	0.6	4.9	/	1.1	20
	R 2	7.9	7.7	0.3	6.5	2.53	0.9	14
	R 3	8.4	8.1	0.3	2.4	/	1.3	14
	R 4	8.2	7.9	0.4	7.9	2.48	0.7	15
	R 5	8.7	7.9	0.5	6	2.45	0.8	15
	R 6	8.4	7.5	0.7	7.1	2.36	1	14
	R 7	8.7	7.9	0.4	8.1	2.58	0.6	15
	R 8	8.5	8.4	0.3	5.6	2.33	0.7	14
	R 9	8.4	7.8	0.4	5.8	2.45	0.5	15
	R 10	8.1	7.9	0.4	8.4	2.38	1.1	19
A.M.D	R 1	8.5	7.9	0.4	9.7	2.41	0.9	10
	R 2	8.2	7.8	0.8	7.9	2.30	0.4	11
	R 3	8.4	8.1	0.8	8.1	2.83	0.6	9
	R 4	8.4	8.2	0.3	7.6	2.64	0.8	10
	R 5	8.3	7.8	0.8	7.6	2.31	1.1	11
	R 6	7.9	7.9	0.3	8.1	2.61	0.3	9
	R 7	8.4	7.9	0.2	7.1	2.63	0.4	8
	R 8	8.3	7.8	0.9	8.4	2.65	0.4	11
	R 9	8.4	8.0	0.7	8.4	2.63	0.2	11
	R 10	8.6	8.3	0.4	9.2	2.38	0.5	9
A.D	R 1	8.5	8.3	0.3	8.3	2.58	0.4	5
	R 2	8.7	8.3	0.1	11.6	2.71	0.3	7
	R 3	8.6	7.9	0.3	8.4	2.65	0.5	5
	R 4	8.7	8.7	0.4	8.6	2.72	0.2	6
	R 5	8.6	8.3	0.6	10.1	2.05	0.5	7
	R 6	8.6	8.4	0.4	7.1	2.65	0.2	7
	R 7	8.7	8.0	0.5	9.9	2.73	0.4	6
	R 8	8.8	8.2	0.4	7.1	2.35	0.1	3
	R 9	8.7	8.2	0.5	9.1	2.58	0.3	6
	R 10	8.4	8.1	0.4	7.1	2.61	0.2	5

The comparative analysis of the frequencies and taxa shows the disappearance of many species along an anthropogenic gradient. Some species have adapted to the pressures and their frequencies have practically not changed (*Poa bulbosa*, *Noaea mucronata*, *Astragalus incanus*, *Bromus rubens*, *Hordeum murinum*); others have seen their increased frequency (*Avena sterilis*, *Muscari comosum*, *Paronychia argentea*, *Peganum harmala*, *Senecio flavus*, *Artemisia herba alba*) and others have disappeared (**Table 4**). We have noted the appearance of a species in A.D (*Helianthemum virgatum*).

Table 4. List of species and their frequencies in the study sites.

Plant species	Code	A.B.V frequency	A.M.D frequency	A.D frequency	Trend
Stipa tenacissima	stte	0.8	0.5	0.4	↘
Poa bulbosa	pobu	0.7	0.7	0.6	→
Stipa parviflora	stpa	0.7	-	-	Disappearance
Alkanna tinctoria	alti	0.6	0.5	-	Disappearance
Astragalus sesameus	asse	0.6	0.5	-	Disappearance
Bassia muricata	bamu	0.6	0.4	0.3	↘
Diplotaxis virgata	divi	0.6	0.6	-	→
Erucaria uncata	erun	0.6	-	-	Disappearance
Muricaria prostrata	mupr	0.6	-	-	Disappearance
Noaea mucronata	nomu	0.6	-	0.5	→
Schismus barbatus	scba	0.6	0.6	0.3	↘
Scorzonera undulata	scun	0.6	0.5	-	↘
Senecio vulgaris	sevu	0.6	-	-	Disappearance
Astragalus incanus	asin	0.5	0.5	0.6	→
Avena sterilis	aval	0.5	0.5	0.8	↗
Bromus rubens	brru	0.5	0.6	0.6	→
Centaurea pungens	cepu	0.5	0.4	-	Disappearance
Eruca vesicaria	erve	0.5	0.4	0.6	↗
Hordeum murimum	homu	0.5	0.6	0.5	→
Astragalus armatus	asar	0.4	0.7	-	Disappearance
Helianthemum lippii	heli	0.4	-	-	Disappearance
Helianthemum pilosum	hepi	0.4	0.4	-	Disappearance
Iris sisyrinchium	irsi	0.4	-	-	Disappearance
Ornithogalum pyramidale	orpy	0.4	-	-	Disappearance
Reseda lutea	relu	0.4	-	-	Disappearance
Salvia verbenaca	save	0.4	-	-	Disappearance
Saponaria glutinosa	sagl	0.4	0.4	-	Disappearance
Chrysanthemum coronarium	chco	0.3	-	-	Disappearance
Erucastrum leucanthum	erle	0.3	0.3	-	Disappearance
Marrubium vulgare	mavu	0.3	-	-	Disappearance
Matthiola livida	mali	0.3	0.3	-	Disappearance
Muscari comosum	muco	0.3	-	-	Disappearance
Paronychia argentea	para	0.3	0.6	0.5	↗
Peganum harmala	peha	0.3	0.5	0.7	↗
Senecio flavus	sefl	0.3	0.3	0.7	↗
Artemisia herba alba	arhe	0.2	0.3	0.3	↗
Ferula communis	feco	0.2	0.5	-	Disappearance
Herniaria hirsuta	hehi	0.2	0.4	-	Disappearance
Micropus bombicinus	mybo	0.2	0.5	0.5	↗
Helianthemum virgatum	hevi	-	-	0.4	Appearance

The arrow refers to the tendency of the plant (evolution or regression).

This analysis shows a decline of plant biodiversity and the physiognomy of the route to alfa is changing. Taxas them toxic and/or thorny replace taxas them appetables and aboveground biomass is in clear regression.

3.3. Relationships Factors Soil-Vegetation

The interdependence of the parameters studied (floristic surveys and their edaphic values) is represented in **Figure 2** and **Figure 3**. Floristic surveys, edaphic factors and plant species are represented by symbols accompanied by their coding (**Table 4**).

Analysis of factorial:

The study of factorial 1-2, 1-3 and 2-3 shows significant variations, including those of edaphic factors, are organized according to axis 1 that represents the highest contribution (0.344 for axis 1; 0.222 for axis 2 and 0.155 for axis 3).

1-2 and 1-3 plans that will be represented and discussed contain most of the statistical information.

Plan 1-2 (Figure 2):

Records are ordered according to axis 1, which corresponds to a gradient of degradation, from A.B.V in the positive part to A.D in contrast, with A.M.D intermediate position. The edaphic point of view, the point cloud is highly structured for the Ct, the Pr and the Mo. High Ct (Ct3) levels correspond to A.D site which is also characterized by a low Pr (Pr1) and the lowest rate in Mo (Mo1).
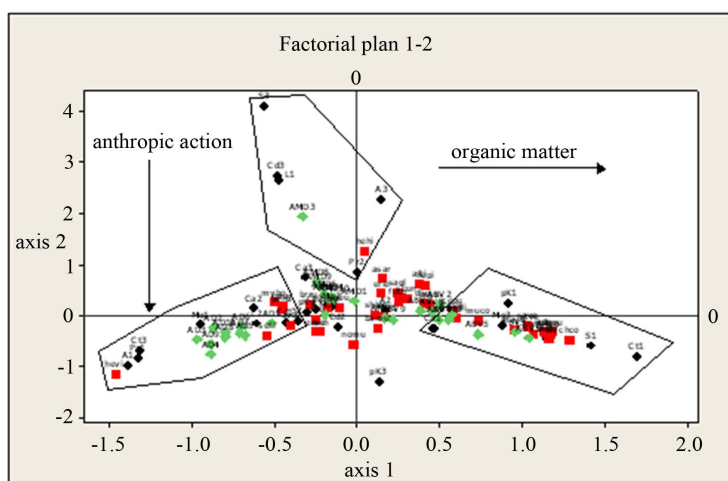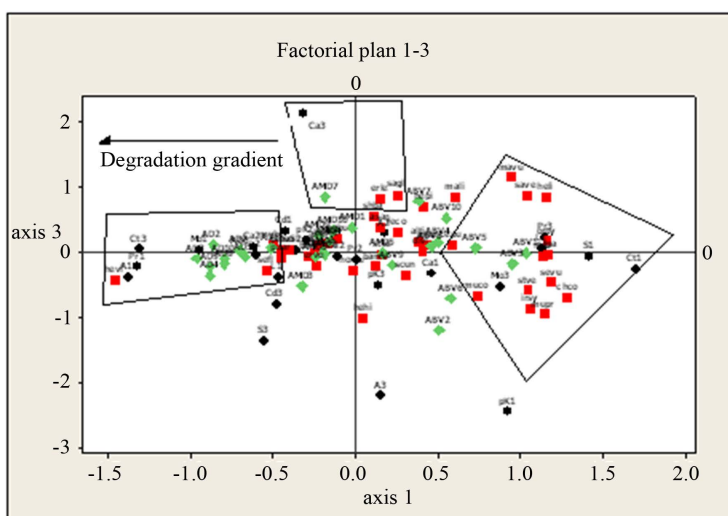

Figure 2. Factorial plan 1-2.

Figure 3. Factorial plan 1-3.

Interpretation of the axes:

Axis 1: Parameters edaphic Ct1, S1, Mo 3, Pk1, Pr 3 and L3 are well correlated and lie side axis 1 positive. The negative are: A1, Ct3, Pr1, Mo1, A2 and L2. Axis 1 is characterized by Mo and the negative Pr gradients toward the positive pole.

On the floristic map the species that are found side positive and are associated with edaphic (Ct1, S1, Mo 3, Pk1, Pr 3 and L3) parameters are as follows: *Helianthemum lippii*, *Chrysanthemum coronarium*, *Stipa parviflora*, *Muricaria prostrata*, *Senecio vulgaris*, *Marrubium vulgare*, *Salvia verbenaca*, *Ornithogalum pyramidale*, *Matthiola livida*, *Centaurea pungens*, *Muscari comosum*, *Iris sisyrinchium*, *Eruca vesicaria* et *Reseda lutea*. These taxa represent a facies at alfa in good condition (A.B.V).

The side negative of the axis is marked by the presence of the following species: *Helianthemum virgatum*, *Senecio flavus*, *Micropus bombicinus*, *Avena alba*, *Peganum harmala* and *Paronychia argentea*. These species are associated with the following soil parameters: A3, Ct3, Pr1, L2 and Mo1 (**Figure 2**). These indicators both floristic and edaphic indicate us a regression of vegetation and the physiognomy of the alfa facies is A.D.

Axis 2: On the positive side we find the following soil parameters: S3, L1, A3, Pr2 and Cd3. These parameters have a great affinity with A.M.D. A single species seems marked this facies is *Herniaria hirsuta*. The edaphic parameters: A1, Pr1 and Ct 3 strong and are in opposition with S3, Cd 3 and L1.

Plan 1-3 (Figure 3):

It completes one given by the 1-2 plan. It may be noted, in addition to information from plan 1-2 (axis 1), that *Erucaria uncata* and *Astragalus sesameus* A.B.V-related and occur side axis 1 positive. The negative axis 1 *Helianthemum virgatum*, *Senecio flavus* and *Paronychia argentea* have an affinity with A1, Pr1, and Ct3 characterize A.D.

Axis 3: *Saponaria glutinosa*, *Erucastrum leucanthum* and *Schismus barbatus* species show some affinity characterized by a spatial proximity on the factorial and A3 side positive and negative axis 3 *Herniaria hirsuta* with A3 side.

Analysis of plan 1-3 confirmed evolutionary gradient of the vegetation and the soil depth. The edapho-flora combinations show the existing affinities between different parameters considered in the studied facies types (**Figure 3**).

4. Conclusions and Recommendations

The strong climate-anthropogenic impact causes the disruption of ecosystems at alfa in the Algerian highlands. The residuals pockets at alfa observed in the region of Saida are threatened with extinction. One of these, used as control, allows studying the influence of damage anthropozoogenes on the floristic composition and some soil parameters of the two neighbouring sites, respectively moderately degraded and seriously affected.

The combination of floristic and edaphic data by the A.F.C highlights a degradation gradient associated with the floristic composition and three major soil variables: the depth of the soil, organic matter and the rate of total limestone.

The vegetation is mainly affected by a regression of the floristic richness that passes of 36 species for the control (A.B.V), 26 species for A.M.D and only 16 species for A.D (the more degraded stage). This regressive evolution translates into a decrease in the aboveground phytomass, a regression and an endangered species. We noticed the individualisation of a species (*Helianthemum virgatum*) in the more altered site. This dynamic causes a change in the appearance of the steppes with mainly the installation of a poor ecosystem herbal thorny and/or toxic (*Peganum harmala*, *Ferula communis*, *Paronychia argentea*, *Senecio flavus*, *Micropus bombicinus*).

Degraded stage is marked by a truncation of the soil, intense erosion which translates into a low depth, a small amount of organic matter and a high total limestone rate.

Edaphic and floristic data indicate unambiguously and convergently double erosion of natural resources (soil and biological). The trampling of livestock, the low recovery of the vegetation, the reduced structural stability of soils in an arid climate can causing a significant erosion by rain water or wind. Physico-chemical changes in soils and plant physiognomy induce a scarcity of biological potential. The dunes that are observed on the highlands are witnesses on these phenomena, and the situation calls for immediate measures of protection and restoration of the steppe ecosystems. This must involve the creation of a seed bank on one hand and a biological study of the species that structuring the steppes on the other hand.

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Scientific Research

The Impact of Water Level Decline on Water Quality in the Epilimnion of Lake Kinneret (Israel): Perennial Perspectives

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Abstract

Long term record (1933-2014) of Water Level (WL), nutrient concentrations, plankton densities, and temperatures in the epilimnion of Lake Kinneret was analyzed. The aim is to identify if water quality is deteriorated when the WL is low. It was found that water temperature increased and the composition and biomass of plankton communities were modified. Nitrogen and TDP decreased but TP slightly increased in the epilimnion during low WL conditions. The quality of epilimnetic water was not deteriorated and followed by a slight oligotrophism trend.

Keywords

Kinneret, Low Water Level, Plankton, Temperature, Epilimnion

1. Introduction

Lake Kinneret is the only natural freshwater lake in Israel located in the Syrian-African Rift Valley in northern Israel. The Israeli climate conditions are varied between desert in the south to subtropical in the north and mild Mediterranean in the center. Above 95% of the Israeli natural water resources are utilized. Rain distribution over Israel (total 7.9 bcm/y) varied between 1300 (north) < 100 (southern desert) mm/y: 70% evapo-transpiration, 5% runoffs, and 25% infiltration. Total national water supply is 2.11 bcm (10^9 m^3) of which 0.55 bcm from the Kinneret-Jordan system and 0.7 bcm from desalination sources. Thirty percent (0.750 bcm/y) of supplied water is given as drinking qualities for housing consumption. The only open options to enhance water resources are desalination and recycling. Averaged water budget for Lake Kinneret is given in **Table 1** [1]).

　　Lake Kinneret was designed as major source of drinking water supply. In 1933 the south end of the lake was

Table 1. Annual water budget (million cubic meters [mcm] per year) of Lake Kinneret (Gvirzman, 2002).

Input	
River Jordan	480
Golan Heights Rivers	145
Direct rain	75
Eastern Galilee	75
Diversion from Jarmuch	25
Total input	800
Output	
Evaporation	280
National Water Carrier	380
To South Jordan via Dam	80
Local consumers	60
Total output	800

blocked by a dam. From that time the outflow is controlled by lake management policy which is limited by hydrological, national regulations, management design and environmental constrains. Recently, desalinated water replaces most of the Kinneret resources. The National Water Carrier (NWC) was constructed during the 1950s (operated 10.6.1964). During the last 49 years the NWC system withdrew approximately 15 bcm of water from the lake (ca 3.8 times the lake volume) for drinking, agriculture, industry and aquifer recharging. Nevertheless, as a result of water level restriction management, about 4 bcm of water were released through the south dam to the Dead Sea. Lake water salinity is highly fluctuated: 300 - 330 ppm chloride before 1960, 400 ppm during early 1960s and 200 - 210 ppm during early 1980s and then gradually increased to 280 - 300 ppm in 2002. Lake kineret became a significant supplier of salts to the Israeli soils and aquifers. About 9 million tons of dissolved salty ions (Cl, CO_3, Na, Mg, K, Ca, SO_4) deteriorate soils and aquifer qualities in the southern part of Israel.

Regional Hydrology

Three major rivers (Hatzbani, Banyas and Dan) flow from the Hermon mountain region, located in the north part of the Kinneret drainage basin. These rivers joint into one river of Jordan which is crossing the Hula Valley through two major man-made canals: 80% and 20% of the Jordan water flow via the eastern and western canals respectively. In the south end of the valley the two canals joint into one water flow, the Jordan route flowing downstream into Lake Kinneret. Hula Valley altitude is between 170 - 61 m above sea level and Lake Kinneret WL is fluctuated between 208.80 - 214.87 m below sea level. The upper and lower WL legislation was highly discussed over the years. The upper limit (red line) was decided as −208.8 and was never changed. The background for that regulation was due to existed housing distance from shoreline and predicted compensations resulted by potential damage. The lower limit was mostly due to limnological trait and predicted impact on water quality. Because of the flexibility of this issue and water supply vs rain gauge (drought) constrains, the lower limit was changed several times. The constrains of water supply during drought forced to lower the bottom limit which was therefore changed several times: −212, −213, and −215. The cardinal dispute that is never clearly settled is therefore: How low can WL be? The Jordan river contributes about 63% of the Kinneret water budget and more than 50% of total external nutrient inputs of the total originate in the Hula Valley Region. The drainage basin area of Lake Kinneret is 2730 km^2, located mostly northern to the lake of which "Hula Valley" is about 200 km^2. From 1972 the hydrological management of the Kinneret-River Jordan system was controlled by both rain gauge and the "NWC management". NCW management included maximum close dam that was aimed at maximum storage and national supply by pumping through the "carrier". It is likely that close dam policy might be disadvantageous. Before Dam construction nutrient rich winter input floods crossed the lake in upper layers resulted by their higher temperature than that of the epilimnetic water and naturally left out through the open outlet.

Close dam management enhanced nutrient retaining in the hypolimnion and the sediments. Water withdrawal was done by daily pumping from upper layers. In comparison with "no dam" condition, "close dam" management might enhance nutrients accumulation. Moreover, winter demands for supply are lower than in summer, therefore additional nutrients retaining in the lake sediments is predicted because in winter nutrient concentrations are higher. Nevertheless, when lakes are comparatively analyzed it has to be considered that in Lake Konstanz for example only 1% of the input is pumped and no Dam construction whilst in lake Kinneret app. 60% of the total inputs were pumped prior to the desalination period. Moreover, water retention time is very significant parameter. In Lake Kinneret this value is 5.6 years when maximum permitted WL (208.8 m below sea level) is maintained.

The Kinneret ecosystem has undergone significant changes during the last 70 years. Some of the changes are natural like droughts and floods, and others are anthropogenic like land-use in the drainage basin or fishery and salts diversion in the lake. Increasing population up to above 200,000 inhabitants in the drainage basin, sewage removal and fishpond restriction in the catchment, operating new agricultural technologies (crop types, irrigation) and development of eco-tourism [2]-[4]. The Lake Kinneret water quality is of a national concern therefore the lake is depends on the nation and the nation is depends on the lake [2] [3]. The lake supplies 16% - 30% of the national water demands and >55% of drinking water requirements. Kinneret is a warm monomictic lake which is stratified from May through mid December (anoxic hypolimnion) and totally mixed during mid December through April. Among fluctuations of lake conditions are high (6.3 m) amplitude of water level (208.57 - 214.87 mbsl); high inflow discharges (>10^9 m^3 per annum) and droughts (<260×10^6 m^3 per annum); low (333 mm/y) and high (1060 mm/y) precipitations in the drainage area; plankton biomass and composition changes, fish stocks, epilimnetic temperatures and nutrient concentrations. The seasonal pattern of the distribution of hydrological, chemical and biological parameters consistently represents subtropical climate conditions: high levels in winter and low in summer months but high hypolimnetic inventories of dissolved phosphorus, ammonium, sulfides and CO_2 in summer-fall period as a result of the thermal and chemical stratification.

The lake is exploited for its fishing by ca 200 licensed fishermen which remove commercially an average of 1600 ton of fish (94 kg/ha) per annum. The zooplanktivorous *Lavnun* (bleak, *Acanthobrama spp.*) comprised 55% by weight of total catches and >50% of the stock biomass. Among 8 commercial species out of 24 recorded the native Tilapias (averaged 326 t/year) and the exotic Mugil (averaged 160 t/year) are the most important in the commercial landings [2] [3]. This paper is aimed at contributing answer and verifications to the question: How low can WL of Lake Kinneret be? The issue is limited between two extremely unchangeable constrains: the −208.8 upper WL limit and the lowest possible WL −215.00 as constrained by technical operational option of the intake of the NWC.

2. Methods

Data of temperatures, nutrient concentrations and plankton densities in the epilimnion, were taken from the Lake Kinneret Data Base ([5] of the Kinneret Limnological Laboratory which are reported annually (1970-2014). The data of the Jordan River nutrient loads, and concentrations and discharge were taken from the annual and temporal reports published by The National Water Company of MEKOROT. Eighty years of daily WL record (TAHAL-Water Planning of Israel, MEKOROT-Israel Water Supply Company, Kinneret Limnological Laboratory) were breaking down by two ways: 1) The entire period of post Dam construction (1933-2014) was divided into four groups of 20 years each: 1 = 1933-1953; 2 = 1954-1973; 3 = 1974-1993; 4 = 1994-2014; and 2) Post operation of the NWC (1965-2014) by two groups of 24 years each: 1 = 1965-1989; 2 = 1990-2014; The whole record contain monthly averages of daily measured WL. The WL of the four temporal groups (20 years each) was comparatively analyzed by ANOVA test ($p < 0.05$), and results are presented in Table 2. Further analysis of the nutrient and plankton concentrations in the epilimnion was referred comparatively to these 4 periods.

Statistical analyses used in this study were taken from STATA 9.1, Statistics-Data Analysis and Stat View 5.1, SAS Institute Inc. The analyses used were: ANOVA ($p < 0.05$), polynomial and linear regressions, fractional polynomial prediction, LOWESS (0.8). These statistical evaluations were used in two ways: 1) Regressions between each parameter and its monitored orderly decline respected WL values. Results are presented in figures; 2) Analysis between periodical (years) groups by comparative evaluation of the parameters (plankton, nutrients, etc.) in that time frame. The WL values were also included in the analysis to enable consideration of WL impact. Results are presented in tables.

Table 2. Comparative analysis (ANOVA test; $p < 0.05$) of WL between 4 twenty year groups. S = significant; NS = not significant.

Number	Period	Average WL (mbsl)	Relative scale (high > low)	Probability (p)
1	1933-1953	-210.55	1 < 2	<0.0001 S
2	1954-1973	-209.77	1 < 3	0.1069 NS
3	1974-1993	-210.39	1 > 4	<0.0001 S
4	1994-2013	-211.84	2 > 3	<0.0001 S
			2 > 4	<0.0001 S
			3 > 4	<0.0001 S

3. Results

The period after the construction of the south Dam, (1933-2014) or the period later than the operation of the NWC (1965-2014) were considered and divided differently: the first divided into 4 groups of 20 years each. The second period was divided into two groups, 24 years each. The two methods indicate clearly low WL later than 199O. The paper is focused on comparative analysis of the epilimnetic water quality and temperature aimed at an attempt to identify long term impact of low WL. The formal daily monitoring of WL in Lake Kinneret WL started in 1926. The precision of WL information prior to 1931 is doubtful. Therefore these data will not be considered. Measurements of the Jordan outlet threshold bottom indicated an altitude of −212.35 mbsl and WL data consequently indicate water depths at the Jordan outlet of 4 m and 2 m in winter and summer respectively (**Figure 1**). Reports and photos from late 1920s and early 1930s evidently show people, and horses pulling wagons crossing this river section by foot. Consequently the period of 1926-1933 is neglected in this paper. The WL of Lake Kinneret was prominently lower during 1994-2013 (**Table 2**). WL during period 3 (1974-1993) was lower than period no. 2 (**Table 2**). Lake conditions after 1994 are titled as "low level period". When post NWC operation period, 1965-2014, is considered, two distinct periods were indicated: High WL in 1965-1989 and Low WL in 1990-2014 which is similar to the previous classification. WL was averaged as 210.05 mbsl (SD 0.84 m) during 1965-1989, and 211.65 mbsl (SD = 1.55 m) during 1990-2014. Averaged WL during the first period was higher than that of the second period by 1.6 m with lower amplitude and fluctuations frequency than during 1990-2014 expressed as higher SD's. WL fluctuations during 1926-2014 (**Figure 2**). It is prominent that before the NWC operation (1964) Wl was consistently high with small amplitude of seasonal changes than after the NWC operation when demands for domestic supply intensified and droughts became more frequent. Results given in **Figure 3** obviously confirm the direct relation between Jordan River discharge and the Kinneret WL. Data shown in **Tables 3-7** and **Figures 4-6** identify a trend of oligotrophism in the epilimnion of Lake Kinneret later than early 1990s. It is expressed by the decline of Nitrogen compounds and dissolved Phosphorus. Nevertheless TP not only did not declined but slightly increased. Those trends of N and P changes initiated conditions which are in favor by N_2 fixers Cyano-bacteria but causing reduction of the N required alga, the Peridinium. **Figures 7-12** and **Table 5**, **Table 6** confirm decline of herbivorous and increase of predator zooplankton accompanied by decreasing biomass of Phytoplankton when WL was low. Independently with trophic conditions, epilimnetic temperature increased when WL decline (**Figure 12**). Lower WL is primarily the result of lower rain gauge and consequently lower river discharge (**Figure 3**) but also due to higher pumping regime (resulted by demands elevations). Lower discharge is obviously accompanied by lowering of external load inputs of nutrients which significantly enhance oligotrophism. Direct relations (r^2 range 0.596 - 0.886, probabilities < 0.0001) were documented between Jordan River discharge and monthly load inputs of nutrients (TN, TP, TIN, Sulfate, Chloride and Organic Nitrogen). Results in **Table 3** indicate significant direct relations between epilimnetic nutrient (except TP) concentrations and WL: the lower the WL is, the lower is also the nutrient concentration in the epilimnion. Moreover, the lower the River Jordan discharge is, the lower is also the nutrient concentration in its input water. Statistical analysis (linear regression) between annual landings of Tilapias and total native fish did not indicate significant relations (data is not shown here). Results presented in **Tables 3-6**, where nutrients and zooplankton conditions are comparatively analyzed, prominently indicates that the periods with low WL are characterized by low values. **Figure 3** clarify the impact of Low WL (started in early 1990s), when Jordan river
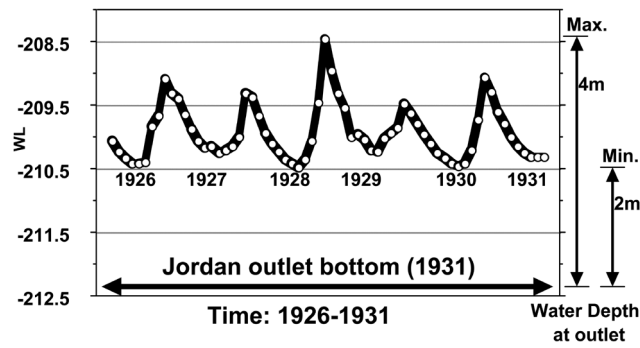
Figure 1. Kinneret WL fluctuations (monthly means) during 1926-1931 before Dam constructions. Respective water depth at the Jordan outlet point are given.
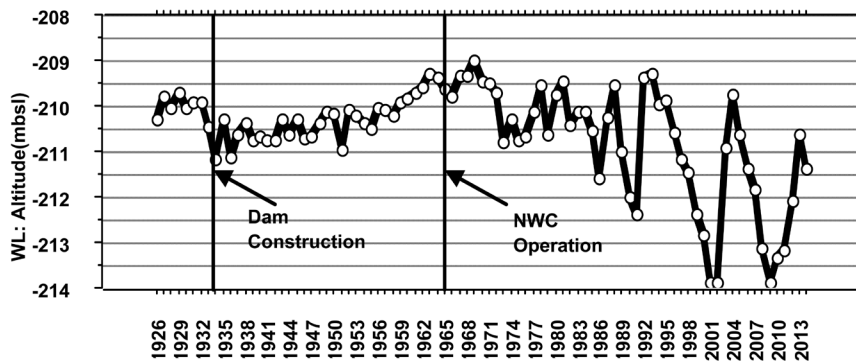

Figure 2. Kinneret WL fluctuations (monthly means) during 1926-2014. Timing of Dam constructions and NWC operation are indicated.
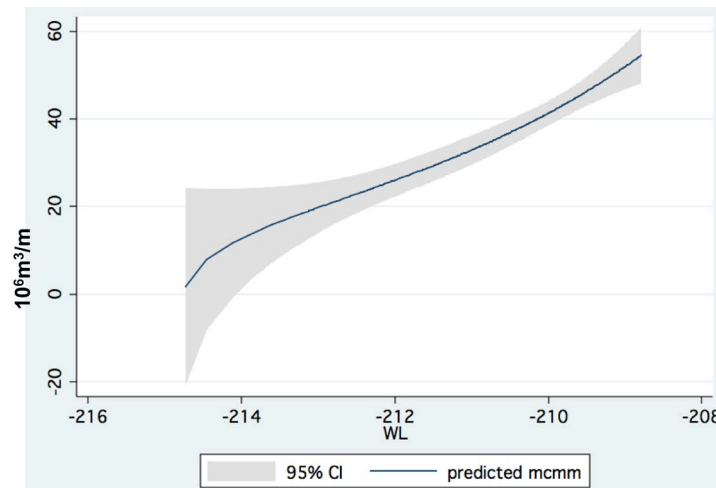

Figure 3. Fractional polynomial prediction (95% CI) of Jordan monthly discharge (10^6 m^3; mcmm) vs. Kinneret WL (monthly means).

discharge was reduced on the concentrations of TN, TIN, TDP, zooplankton (all groups, herbivorous and predator), pyrrhophyta biomass in the epilimnion of Lake Kinneret (**Figures 4-11**).

4. Discussion

Wl decline in Lake Kinneret is mostly resulted by rain reduction and partly by water supply (pumping) regime. The major outcomes of the Kinneret WL decline were primarily changes of nutrients dynamic and consequently

Table 3. Polynomial regressions between nutrient concentrations (ppm) and pyrrhophyta biomass (g/m^2) averaged for the epilimnion vs. WL (upper part) and Jordan discharge (mcm/m) (lower part). All regressions except TP indicate direct positive relations with WL: decline with lowering WL. Parameters of r^2, p = significance level, S = significant, are given.

| Nutrient	r^2	p	Significance
Lake WL			
PON	0.126	<0.0001	S
TIN	0.086	<0.0001	S
Kieldhal total	0.085	<0.0001	S
TDN	0.130	<0.0001	S
TDP	0.090	<0.0001	S
TP	0.010	0.1359	NS
Pyrrhophyta	0.127	<0.0001	S
TN	0.187	<0.0001	S
Jordan disch. (mcm/m)			
PON	0.098	<0.0001	S
TIN	0.558	<0.0001	S
Kieldhal total	0.099	<0.0001	S
TDN	0.368	<0.0001	S
TDP	0.079	<0.0001	S
TP	0.208	<0.0001	S
Pyrrhophyta	0.135	<0.0001	S
TN	0.339	<0.0001	S
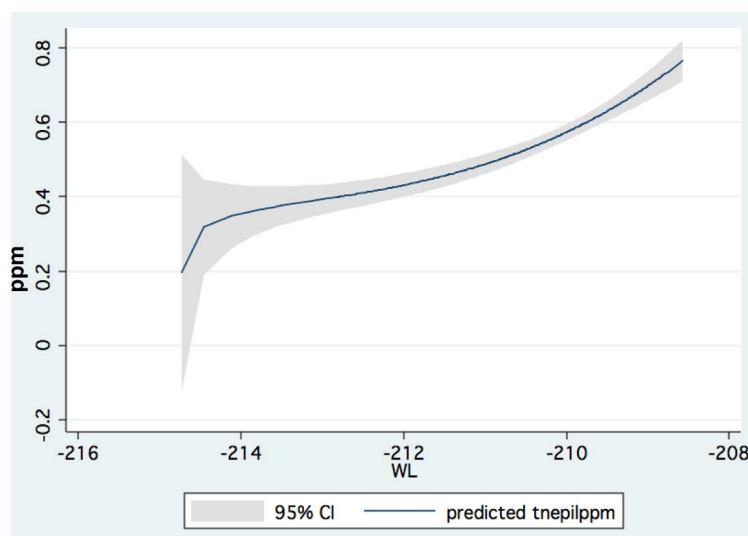
Figure 4. Fractional polynomial prediction (95% CI) of epilimnetic TN concentration (ppm; TN-epilimnion-ppm) vs. Kinneret WL (monthly means).

Table 4. Results of ANOVA Test ($p < 0.05$) comparative analysis between monthly means of zooplankton biomass (g/m^2) in three periods (2 = 1954-1973; 3 = 1974-1993; 4 = 1994-2013). Significance levels (p), indications (S = significant; NS = not significant) and scaled levels (higher > lower) are given.

Total zooplankton			
Periodical mean WL (mbsl)	Periodical average (g/m^2)	Relative scale	Significance (p)
2 (−209.77)	41.4	2 > 3	<0.0001 S
3 (−210.39)	25.7	2 > 4	<0.0001 S
4 (−211.84)	29.9	3 < 4	0.0079 S
Cladocera			
2 (−209.77)	23.4	2 > 3	<0.0001 S
3 (−210.39)	15.2	2 > 4	0.0032 S
4 (−211.84)	18.3	3 < 4	0.0102 S
Copepoda			
2 (−209.77)	14.9	2 > 3	<0.0001 S
3 (−210.39)	8.7	2 > 4	<0.0001 S
4 (−211.84)	9.5	3 < 4	0.2255 NS
Rotifera			
2 (−209.77)	3.1	2 > 3	0.0037 S
3 (−210.39)	1.9	2 > 4	0.0365 S
4 (−211.84)	2.1	4 > 3	0.4445 NS

Table 5. Results of ANOVA test ($p < 0.05$) comparative analysis between monthly means of the biomass (g/m^2) of zooplankton feeding behavior groups in three periods (2 = 1954-1973; 3 = 1974-1993; 4 = 1994-2013). Significance levels (p), indications (S = significant; NS = not significant) and scaled levels (higher > lower) are given.

Herbivorous zooplankton			
Periodical mean WL (mbsl)	Periodical average (g/m^2)	Relative scale	Significance (p)
2 (−209.77)	32.0	2 > 3	<0.0001 S
3 (−210.39)	20.3	2 > 4	<0.0001 S
4 (−211.84)	23.9	3 < 4	$p = 0.0084$ S
Herbivorous copepoda			
2 (−209.77)	5.5	2 > 3	<0.0001 S
3 (−210.39)	3.2	2 > 4	<0.0001 S
4 (−211.84)	3.5	3 < 4	$p = 0.2230$ NS
Predator copepoda			
2 (−209.77)	9.4	2 > 3	<0.0001 S
3 (−210.39)	5.5	2 > 4	<0.0001 S
4 (−211.84)	6.0	3 < 4	$p = 0.2230$ NS

Table 6. Results of ANOVA test ($p < 0.05$) comparative analysis between monthly means of the epilimnetic mean nutrient concentrations (ppm) in three periods (2 = 1954-1973; 3 = 1974-1993; 4 = 1994-2013). Significance levels (p), indications (S = significant; NS = not significant) and scaled levels (higher > lower) are given.

TN (ppm)			
Periodical mean WL (mbsl)	**Mean concentration (ppm)**	**Relative scale**	**Significance (p)**
2 (−209.77)	0.568		
3 (−210.39)	0.582	4 < 3	<0.0001 S
4 (−211.84)	0.439	4 < 2	$p = 0.0005$ S
TP (ppm)			
2 (−209.77)	0.013		
3 (−210.39)	0.017	2 < 3	<0.0001 S
4 (−211.84)	0.017	2 < 4	<0.0001 S
TIN (ppm)			
2 (−209.77)	0.160		
3 (−210.39)	0.150	4 < 2	$p = 0.0294$ S
4 (−211.84)	0/115	4 < 3	$p = 0.0220$ S
TDN (ppm)			
2 (−209.77)	0.368		
3 (−210.39)	0.431		
4 (−211.84)	0.321	4 < 3	<0.0001 S
TDP			
2 (−209.77)	No data		
3 (−210.39)	0.008		
4 (−211.84)	0.006	4 < 6	<0.0001 S
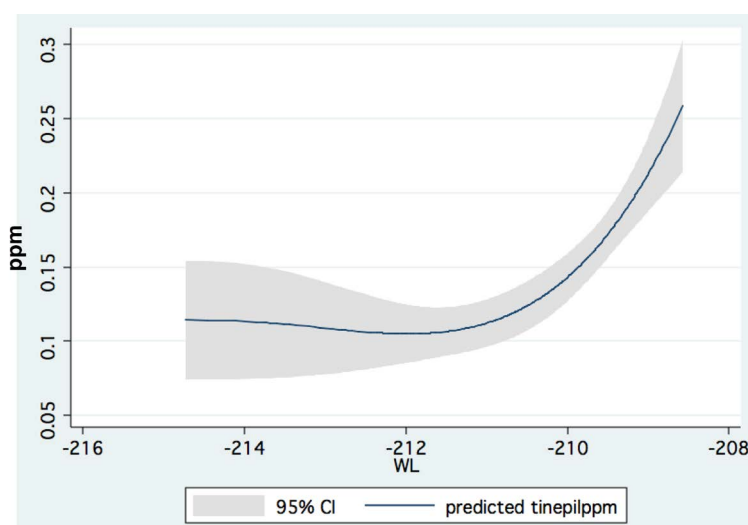
Figure 5. Fractional polynomial prediction (95% CI) of epilimnetic TIN concentration (ppm; TIN-epilimnion-ppm) vs. Kinneret WL (monthly means).

Table 7. Metabolic parameters of zooplankton in Lake Kinneret. Herbivorous copepods are young life stages and predator stages are adults and copepodites 4 and 5. Values are given in mgC/body mgC/day [9].

Zooplankters	Temper. (°C)	Production (mgC/mgC/day)	Respiration (mgC/mgC/day)	Consumption (mgC/mgC/day)	Efficiency (%)
Hebivorous copepods	15	0.050	0.288	0.750	45
Hebivorous copepods	20	0.113	0.550	2.750	24
Hebivorous copepods	27	0.167	1.125	3.250	39
Predator copepods	15	0.048	0.163	0.465	45
Predator copepods	20	0.103	0.287	2.142	18
Predator copepods	27	0.160	0.499	2.517	26
Cladocera	15	0.140	0.153	1.200	24
Cladocera	20	0.160	0.190	2.750	13
Cladocera	27	0.210	0.413	4.650	13
Rotifera	15	0.047	0.153	0.750	27
Rotifera	20	0.080	0.190	2.375	11
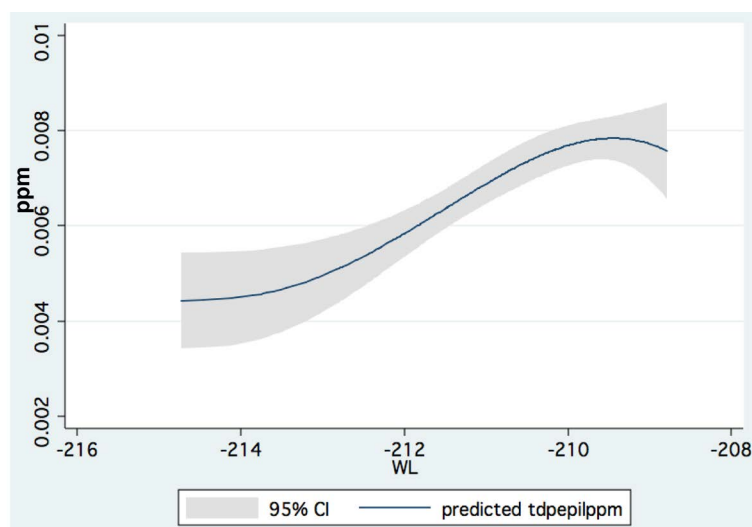
Figure 6. Fractional polynomial prediction (95% CI) of epilimnetic TDP concentration (ppm; TDP-epilimnion-ppm) vs. Kinneret WL (monthly means).

modification of phytoplankton community structure and biomass. Other effects were modifications of zooplankton population composition and biomass densities. Fish stocks changes as reflected by annual landings were insignificantly correlated with WL Studies of the impact of WL decline on the limnological conditions of lakes commonly include examples of extreme cases like Lake Chad, Aral Sea and Lake Sivan. These kind of comparative consideration as well as those with man-made reservoirs should be carefully evaluated. Moreover, even if WL fluctuations are moderate in lakes under different anthropogenic operational management the system response might be different. WL decline in Lake Kinneret varied mostly within the maximum amplitude of 6 meters and the Kinneret is a deep lake with Max. and mean depths of 45 m and 26 m respectively. The lowest WL permitted in Lake Kinneret is limited to the depth of the intake of the NWC (215 m mbsl) and the upper limit is legislated to 208.8 mbsl. About 55% of water inputs in Lake Kinneret are pumped for human consumption. The comparison of WL decline conditions between two lake ecosystems which have different type of Hypsometric curve are misleading. The shoreline length of Lake Kinneret is 53 km and the Value of the Development of
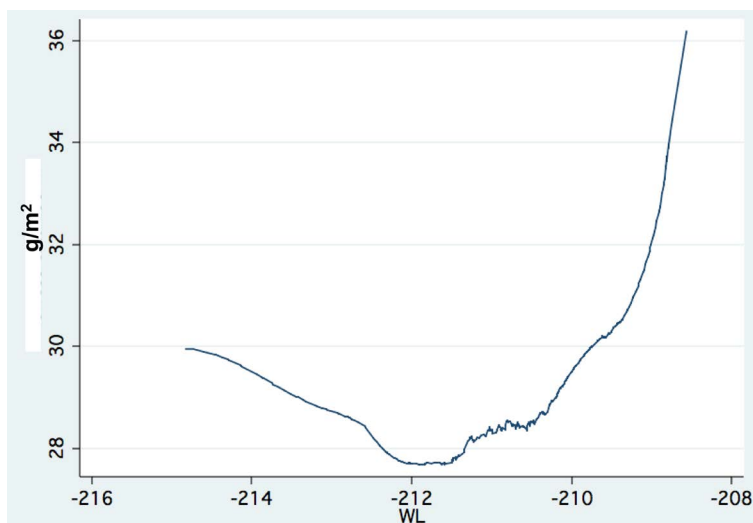
Figure 7. Trend of changes (LOWESS) of total zooplankton biomass (g/m²) vs. Kinneret WL (monthly means).
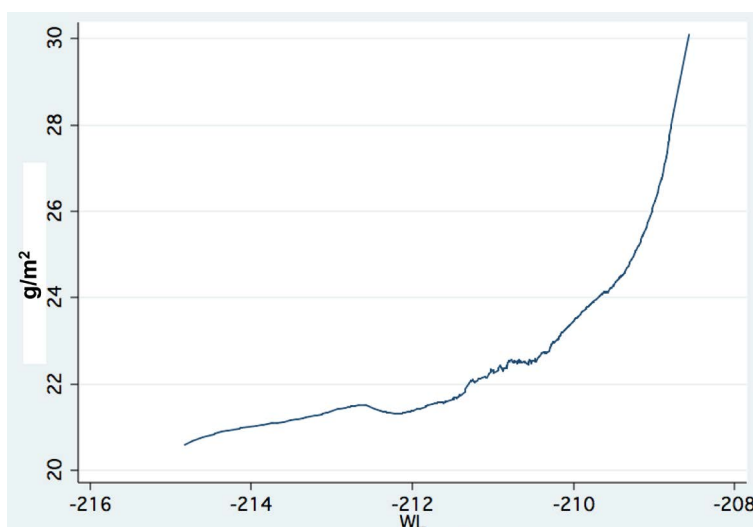

Figure 8. Trend of changes (LOWESS) of herbivorous zooplankton biomass (g·m²) vs. Kinneret WL (monthly means).

Shoreline is 1.16 and residence time is 5.6 years. Therefore comparative study of WL decline in Lake Kinneret with shallow lakes or very deep lakes, with different residence time, thermal structure (amictic, monomictic or polymictic), the amplitude range of the WL change, and others, are mostly irrelevant due to significant differ of limnological conditions between those ecosystems. Human population (and sewage production) and pollutant inputs are significant parameters involved. In Lake Konstanz (Germany-Austria-Swiss) population density in the vicinity of the lake is app. 4170 whilst in Lake Kinneret 950 residents per 1 km of shoreline, respectively, or, 19,500 residents and 12,500 capita per 1 km³ of lake water in Konstanz and Kinneret respectively. In Lake Sivan in Armenia, WL was lowered by 19 meters, whilst in Lake Kinneret only maximum of 6 m but usually about 1.5 - 2.0 m. The shallow lakes of Aral Sea and Lake Chad were dried as a result of extreme WL decline whilst in Lake Kinneret only 10.3 km² are exposed when WL decline from maximum to minimum permitted (208.8 - 214 mbsl). WL decline in lake Chad caused water surface reduction of 12,000 km² (48%) due to climate change and increased water consumption. In the Aral Sea 70% of the water inflows were diverted for agriculture and WL declined by 15 meters. Neither Chad, and Aral nor Sivan lake are dissimilar ecosystems to Lake Kinneret and comparison is misleading.
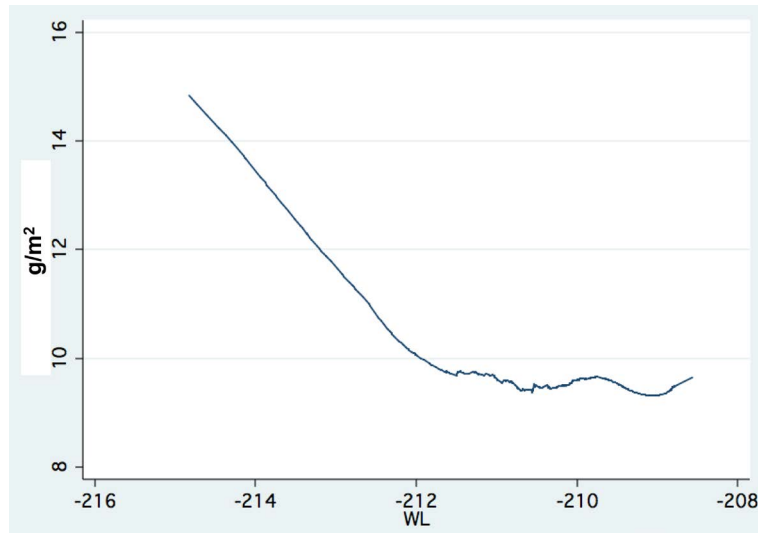
Figure 9. Trend of changes (LOWESS) of copepoda biomass (g·m^2) vs. Kinneret WL (monthly means).

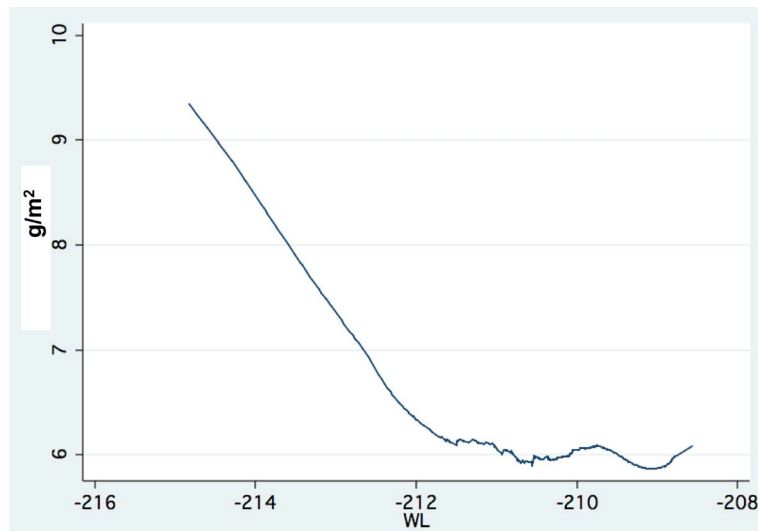**Figure 10.** Trend of changes (LOWESS) of predator copepods biomass (g·m^2) vs. Kinneret WL (monthly means).

4.1. Epilimnetic Temperatures

Epilimnetic temperatures data indicates cooling trend of Kinneret water during 1970-mid 1980s when WL was more or less consistently high. Epilimnetic lake water warming (by 1.8˚C) documented afterwards during WL decline. During WL decline the temperature of the thermocline increased and its depth was reduced (shallower) with consequent reduction of the volume of the epilimnion. Warmer epilimnion, shallower and warmer thermocline indicates elevation of epilimnetic specific Heat capacity (cal/m^3). It is in agreement with intensification of light absorbance expressed as shallower Secchi depth, due to enhancement of the density of small particles (nano-phytoplankton). The shift of phytoplankton composition from large cells *Peridinium* to small sized algae with higher particle density probably enhanced heat capacity in the epilimnion. The implication of the warming process might have an impact on the lake metabolism: enhancement of biological, microbiological, chemical and obviously physical rate of processes. A parameter which contributed to the warming process of the epilimnion under regime of WL decline is the Albedo factor. Temporal changes of the epilimnetic and 3 m above water surface (**Figure 12**) temperatures in Lake Kinneret were documented. These fluctuations are similar to the
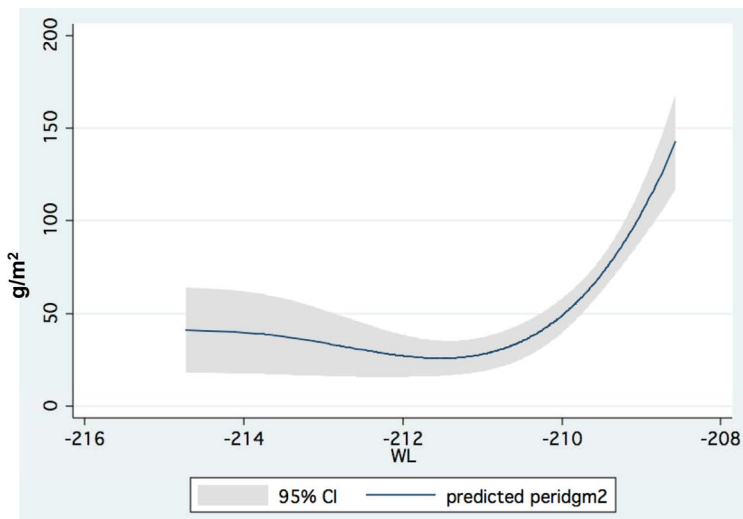
Figure 11. Fractional polynomial prediction (95% CI) of pyrrhophyta (g·m²) vs. Kinneret WL (monthly means).
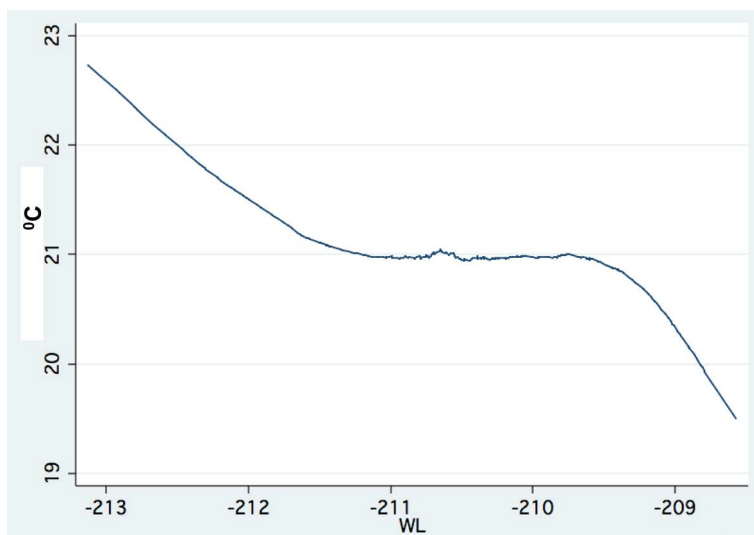

Figure 12. Trend of changes (LOWESS) of averaged epilimnion temperature vs. Kinneret WL (monthly means).

pattern of air temperature changes in Hula Valley, located northern to the Kinneret. It was suggested that in the Hula Valley changes are due to fluctuations of Albedo values resulted in by Land Use modifications in the Hula Valley. Increasing of epilimnetic heat load in Lake Kinneret occur because of consecutive events: WL decline, reduction of the surface water area, and reduction of the epilimnion volume. The ratio between radiation and reflectance of solar energy (Albedo) from water surface (app. 5%) is the same in both high and low WL. Nevertheless, change of heat capacities emerged from dimensions (surface area and epilimnetic volume) reduction. The decline of Kinneret water level was followed by diminishing of water surface area which lowered total capacity of evaporation induced cooling impact, even so, heat capacity was enhanced. WL decline accompanied by surface reduction has created two factors of heat enhancement: 1) reduction of cooling effect by smaller capacity of evaporation; and 2) lower Albedo total heat addition. Those two factors probably caused an elevation of epilimnetic temperatures. If low WL is a continual case, elevated heat capacity is accumulated. To understand how WL decline contribute to the increase of epilimnetic temperature the following computation is presented:

Berman (1976) Stanhill and Neuman (1978), and Serruya (1978), documented annual averaged incident radiation (solar plus sky) for the Kinneret region as 15.1×10^{6} J·m⁻²d⁻¹.

Albedo value for water covered surface is consider as 5%.

Lake water surface at altitude of 209 mbsl and at 214 mbsl is 169.5 km^2 and 159.2 km^2 respectively (10.3 km^2 = 6.1% reduction).

Epilimnion volume at 209 mbsl is 3184 × 10^6 m^3 (thermocline depth 22 m) and at 214 mbsl −2258 × 10^6 m^3 (thermocline depth 16 m) [5].

Annual total solar and sky radiation on the entire surface of lake water is 949 × 10^{15} J and 892 × 10^{15} J for 209 and 214 mbsl respectively.

If reflectance of 5% (Albedo) is subtracted from incident radiation, annual absorbance is 9020 × 10^{14} J and 8470 × 10^{14} J for 209 and 214 WL altitude respectively.

Each m^3 of the epilimnion absorb annually an additional 2.833 and 3.751 × 10^{14} J per m^3 at 209 and 214 WL altitude respectively.

The increase of heat absorbance from 2.833 to 3.751 × 10^{14} J per m^3 represent additional heat capacity of 32.4% per year. Consequently, WL decline by itself enhance additional heat load to the epilimnion of Lake Kinneret.

4.2. Nutrient Dynamics

Undoubtedly the volume of the hypolimnion is significantly ($r^2 = 0.462$) reduced but volume of the epilimnion is just slightly become smaller when WL is decline from 209 to 214 mbsl. Even if the rate of biological activities in the epilimnion does not modified, their wastes (plankton mortality, fish and zooplankton excretion, etc.) are settled into a smaller volume of the hypolimnion. Consequently, the concentration of nutrients (H$_2$S, NH$_4$, CO$_2$, etc.) together with un-aerobic microbial products significantly increased (H$_2$S: $r^2 = 0.582$, NH$_4$: $r^2 = 0.401$; TP: $r^2 = 0.409$; data is not given here). Moreover, biological activities are probably enhanced as a result of the epilimnetic temperature increase resulted by WL decline. Recent report of The Kinneret Limnological Laboratory [5] published information about the negligible upward transfer of Phosphorus. Data presented in this paper confirm that the period of low WL, later than 1993, is characterized by improvement of epilimnetic water quality in terms of lower nutrient concentrations.

4.3. Phytoplankton Changes

Serruya & Pollingher [6] documented that the replacement of non-grazed *Peridinium* having high C:P ratio by the actively grazed diatoms and green algae having low C:P ratio led to a substantial decrease of algal standing crop in Lake Kinneret. Dominance of diatoms, chlorophytes and cyanophytes was recorded. Nevertheless, report about change of algal assemblages as a result of 17 meters WL decline in Lake Sivan (Armenia) indicated doubling values of algal biomass. The dominance of diatoms and *Anabaena sp*. in Lake Sivan was documented. The overall response to WL decline in Lake Kinneret was the opposite to Lake Sivan: Increase of algal biomass and eutrophication in Lake Sivan and decrease of algal biomass and oligotrophism in Lake Kinneret. Low WL conditions in Lake Kinneret enhanced the increase of the biomass of *Cyanobacteria*, Diatoms, and *Chlorophyta* and obviously their portion (%) in the assemblages (Table 8). The biomass of the *Peridinium* which was dominant under high WL was almost eliminated from the Kinneret Phytoplankton community when WL declined and rarely flourished after heavy rainy seasons. On the other hand, the chlorophyll concentration was declined as a result of total algal biomass decline which accompanied the reduction of nutrient concentrations in the epilimnion. The prominent change of N/P ratio in the epilimnion was probably the major reason for the proliferation of *Cyanobacteria* and the decline of *Peridinium*. *Peridinium* is a well known N limited algae and N$_2$ fixers *Cyanobacteria* are P limited. The low WL conditions in Lake Kinneret were therefore highly favored by *Cyanobacteria*. The biomass enhancement of Diatoms and *Chlorophyta* is probably due to the high level of epilimnetic bioavailable phosphorus. Because of low content of intracellular N in nano-planktonic algae (especially diatoms and chlorophytes), the preferred food for grazing zooplankton, higher biomass was grazed in order to cover demands for Nitrogen supply [7]. The result of this change was enhanced supply of recycled P in the system.

4.4. Zooplankton

The zooplankton biomass density (g/m^2) data presented in Table 4, Table 5 and Figure 7, Figure 8 indicates similarity of Total zooplankton, *Copepoda*, *Cladocera*, *Rotifera*, Herbivorous Zooplankton (including herbivorous copepods, cladocerans and rotifers) predator copepods and herbivorous copepods. The top-down predation

Table 8. Changes of Epilimnetic characteristics affected by WL decline.

Parameter	Low WL characterization
Temperature	Increase
Nitrogen	Low
Phosphorus	High
%Peridinium	Low
%*Cyanobacteria*	High
%*Chlorophyta*	High
%Diatoms	High
Total phytoplankton	Low
Zooplankton	Low
Water quality: W toxic *Cyanobacteria*	Low
Water quality: WO toxic *Cyanobacteria*	High

(W = with; WO = without).

impact of zooplnktivorous fish, (bleaks) on zooplankton was previously discussed [2]. Due to its very low market value fishery of bleaks was abandon and consequently its stock in the lake was flourished. Bleak stock size is related to fishery and much less to WL fluctuations with two exceptional winters occur in 1992 and 2002 when WL increase rate was high and Bleak population flourished [2]-[4]. Nevertheless, metabolic parameters of zooplankton (**Table 7**) indicates decline of feeding efficiencies of all zooplankters at high temperatures. The biomass density of zooplankton, and especially herbivorous animals which create >80% of the total biomass, is highly affected by the availability of food resources, mostly, nano-planktonic *Chlorophyta* and Diatoms. WL decline that was accompanied by increase of temperature caused reduction of zooplankton biomass [8] which was not confounded by elevation of chlorophytes and diatoms biomass. The increase of chlorophytes and diatoms is due to increase of bioavailable Phosphorus (TDP) (**Table 3**). It is suggested that warmer epilimnion is an advantage for the fishery and biology of the tropical originated fish, *Sarotherodon galilaeus*. This fish has a high commercial and ecological values but temperatures <12°C are un-tolerable and >21°C are favored by this fish.

5. Conclusions

The impact of WL decline on the epilimnion of Lake Kinneret is summarized in **Table 8**.

Conclusive Remark

This paper should not be considered as a recommendation to decline WL in Lake Kinneret and high WL is also not ultimately preferred. The paper is aimed at an attempt to consider provisional WL decline resulted by external constrains as an acceptable temporary option.

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Scientific Research

Impact of Selected Environmental Pollutants on the Ultrastructure of the Gills in *Pinctada radiata* from Coastal Zones, Egypt

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Abstract

There has been an increasing interest in marine oysters (order: Petridae) in recent years because their numbers are declining in many parts of the world and also because they are used as monitors of pollution. The present study describes the microscopic structure of gills as viewed by light and electron microscopy in two locations selected in Alexandria coast, Eastern Harbor (E.H.) and El Asafra. The specimens in the E.H. represent the presence of extracellular mineralized granules.

Keywords

Gill, Bivalve, Transmission Electron Microscope, *Pinctada radiata*

1. Introduction

There are over 6500 species of marine bivalves in the phylum Mollusca [1], including oysters. Marine bivalves are known to be natural unique accumulators of contaminants [2]. The environment had become increasingly aware of the importance environmental risk management in the economic development, health and quality of life [3]. [4] and [5] reported that *Pinctada radiata* could be used as indicator species for heavy metals accumulation studies. The sensitive aquatic environment is suffering of pollution that affects both quantity and quality of

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benthic invertebrate biodiversity [6].

Bivalves are used in monitoring programmes in the marine environment due to their ability to concentrate pollutants to several orders of magnitude above ambient levels in seawater [7]-[9]. [10] and [11] reported that bivalves had been used as successful biomonitors of aquatic metallic pollutant levels internationally prominent examples including the United States National Oceanic and Atmospheric Administration Mussel Watch Programmes and the Australian Oyster Watch Programmes. Cadmium levels in hepatopancreas were lower than those in gills [12]. Shellfish plays an important role in the ecology of aquatic pathogens [13], and [14]. Histopathology is a tool for monitoring anthropogenic contamination [15] [16].

2. Materials and Methods

2.1. Sampling

2.1.1. Water Samples

Coastal water samples were collected from the three selected locations on the coast of the Alexandria at 3 - 5 m depth for the determination of copper, and cadmium. Seawater samples were filtered through 0.45 µm millipore filters to remove any debris particles then stored at −20˚C until analysis. All concentrations are reported as µg/l for seawater. All the precautions recommended by [17] to minimize risks of sample contamination were followed during collection and treatment of samples.

2.1.2. Mollusk Samples

The Bivalve were collected in sterile plastic bag (no. 250/location/season, replicant, 5 time) and were cleaned from attached organisms and then rinsed with seawater from their sampling locations and transported to the laboratory within 4 - 6 hrs.

2.1.3. Analytical Methods

1) Heavy metals in seawater

The concentration of heavy metals were determined in the collected seawater samples using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer model 2380) under the recommended conditions and the detection limits in the manual for each metal [18].

2) Physicochemical analysis of seawater

Surface water samples were collected five times; bi-week from three sites representing the coastal area in front of Alexandria city at each location, water samples were collected using polyethylene bottles (2-liters capacity). The polyethylene bottles were previously cleaned with detergent rinsed several times with distilled water, soaked in 1 N HCL for several days and finally rinsed with re-distilled water. At each site a 150 ml dissolved oxygen bottle was firstly filled and immediately fixed, using manganous sulphate and alkaline potassium iodide solution [19]. Some parameters were totally or partially measured in the field *i.e.* as soon as the sample was collected. These steps of the methods would be explained by the term "*in situ*" in the text. Temperature measurements: *In situ* at each station, air and water temperatures were measured at the time of water sampling using an ordinary thermometer. Salinity (S‰): Salinity was determined by measuring the electrical conductivity using an inductive Salinometer (Beckman; model RS. 10). Hydrogen-ion concentration [pH]: The pH-value of water sample was measured in the laboratory immediately after collection using Bench type (JENWAY, 3410 Electrochemistry Analyzer pH-meter). Dissolved oxygen (BOD: It was determined by a modified Winkler's method [20].

3) Heavy metals in tissue

The preparation of samples to determine concentration of heavy metals was carried out animals were separated from the shells; weighed and digested using conc. HNO_3 in Taflon digestion vessels. Wet digested samples were diluted with deionized distilled water and analyzed by Ion-selective electrod AVL. The obtained data were expressed as µg/g wet weight [21]. The analytical method was checked by (5 replicate) measurements for the studied metals in a sample of marine.

4) Histological studies on gills of (Bivalve, Mollusca)

a) Light microscopical technique: In order to establish the histological state, twenty individuals from each sampling site/month, were processed for light microscopical study [22]. The shells were removed gently then the soft specimen was quickly dissected.

b) Electron microscopy: Specimens were fixed in 2.5% glutaraldehyde solution (pH 7.2, buffered 0.1 M phosphate buffer) for 2 - 4 hrs at 4˚C and rinsed in 0.1 M phosphate buffer and then post-fixed in 1% osmium tetroxide (OsO_4) solution for 2 hrs at 4˚C. After fixation, the specimens were washed with 0.1 M phosphate buffer 4 times for 2 hrs and dehydrated with ascending grades of ethanol. Specimens for transmission electron microscope (TEM) were embedded in Epon 812, cut at ultrathin sections (70 nm in thickness) and placed on copper grids (200 mesh) in order to double-stain with uranyl acetate and lead citrate. Specimens were examined using a TEM (JEM-1200EXII, JEOL, Japan).

Semithin sections (0.5 - 1.0 µm) were cut using LK Bill ultra-microtome. In order to stain the resin embedded sections, they were rinsed for 1 - 2 minutes in about 1% toluidine blue solution in 1% borax. They were then washed in tap water, dried on hot plate (60C) and mounted in Canada balsam. Toluidin blue-stained sections were examined and photographed using Diallux 20EB Leitz research microscope provided with Canon camera. Ultrathin sections were cut from the resin blocks at a thickness of 10 nm using glass or diamond knives. Sections were mounted on coated grids (1% partodion in amyl acetate) and stained in solution of aqueus or alcoholic uranyl acetate for about 15 - 20 minutes. After drying, they were examined.

2.2. Statistical Analysis

Statistical analysis was performed using two-way ANOVA using SPSS computer program (version 14.0) to check for significant difference between metal concentrations in different localities.

3. Results

The ecological investigations of water were restricted to two locations; El Asafra and the Eastern Harbor (E.H.). All parameters were measured monthly and they are shown in **Figure 1(a)**, **Figure 1(b)**.

The gills consists of two plates at each side of animal, the gill plate is comprised of parallel filaments, connected by cillialry discs. Each gill filament is divided into abfrontal, intermediates and frontal zone (**Figure 2(a)**, **Figure 2(b)**). In the center of the filaments, haemocytes circulate through the haemolymph vessel. The frontal surface of a gill filament bears frontal cilia, latero frontal and lateral cilia (**Figure 3(a)**, **Figure 3(b)**). The wall of the gill filament is lined with ciliated columnar epithelial cells with ovoid nuclei, between them there are a number of mucous secretory cells with circular nuclei.

Three types of cells had been reported by transmission electron micrographs. Cells containing several large mitochondria, flattened epithelial cells covered the first type and with elongated microvilli, mucous cells enclosed between the first one. There were no obvious surface morphological abnormalities of gill filament in animals collected from E.H. or El Asafra. Little increase in the mucous secretion and lateral cilia increased in length and number in oyster gill collected from the E.H. (**Figure 4(a)**, **Figure 4(b)**).

The histo pathological changes directed to the arrangement of regularity of gill lamellae and occasional areas avoid of microvilli appeared on some frontal and lateral surfaces. Black granules were detected throughout the cells and in some cases the mitochondrial membrane started to decay. There were some areas where abfrontal surface were lacking of cilia, in some cases frontal and abfrontal cells appear necrotic, their internal organelles getting out into the extracellular space.

There were some alteration in filaments morphology with increase in vacuoles and decrease in mitochondria number which sometimes completely disappeared and several gill filaments were dilated. In the present study, histology showed haemocyte infiltration (**Figure 6**).

In Electron microscopic sections, the central zone of the gills, consists of a sheet of tissue with outer and inner epithelia separated by loose interstitial tissue containing haemolymph spaces, haemocytes, muscles and variable numbers of large vesicular cells (**Figures 2-4**). In the central zone of the gills of *Pinctada radiata*, there were generally loosely packed vesicular cells and large extracellular spaces, often traversed by thin muscle fibers. In some specie men the interstitial tissue was with numerous clusters of granules among the vesicular cells. In some specimens, the tissue was much denser, as shown in (**Figure 5(a)**, **Figure 5(b)** and **Figure 6(b)**). Larger granules were also often found scattered through the tissue.

The vesicular cells (**Figure 5(a)** and **Figure 6(a)**) were large, with a central region filled with fine granular storage material and a thin peripheral layer of cytoplasm containing the nucleus. Muscle cells contained thin filaments and dense bodies, with no cross striations. Mitochondria lay peripherally, often in large lateral cytoplasmic projections. Lateral projections often contained large amounts of granular material. Muscle cells varied in
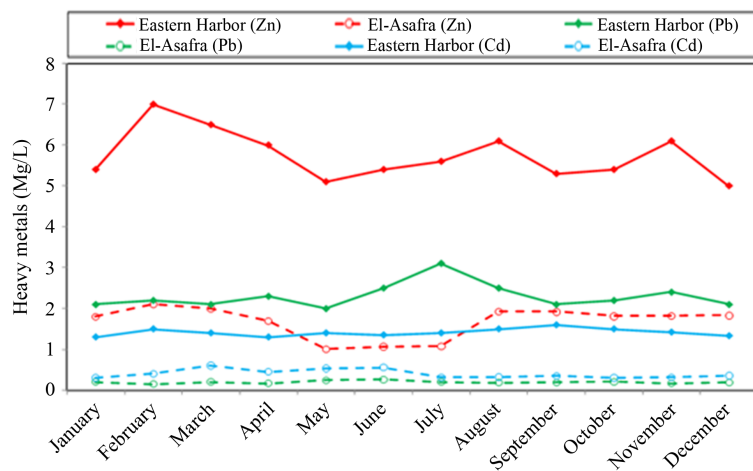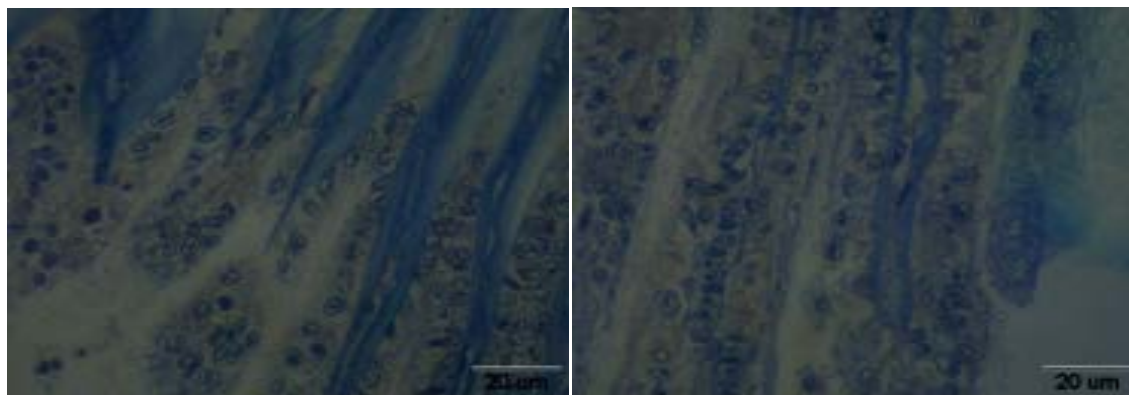
(a)
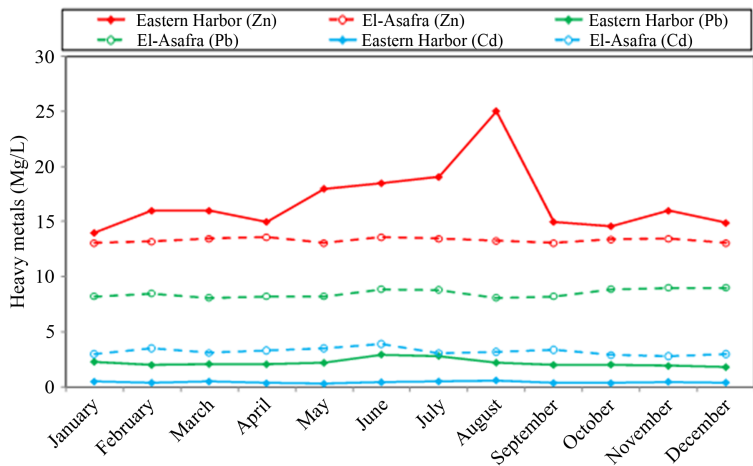
(b)

Figure 1. (a) Comparison between E.H. and El Asafra in the sea sample according to heavy metals. (b) Comparison between Eastern Harbor and El Asafra in gills of *Pinctada radiata* (µg/g) according to heavy metals.

(a)

(b)

Figure 2. Photomicrograph, semithin, of gill of *Pinctada radiata*, collected from El Asafra showing gill filaments (G.F.), inter filamentary junction (IFJ), frontal (FS) and lateral (LS) and inter lateral surfaces (ILs) of gill filaments.
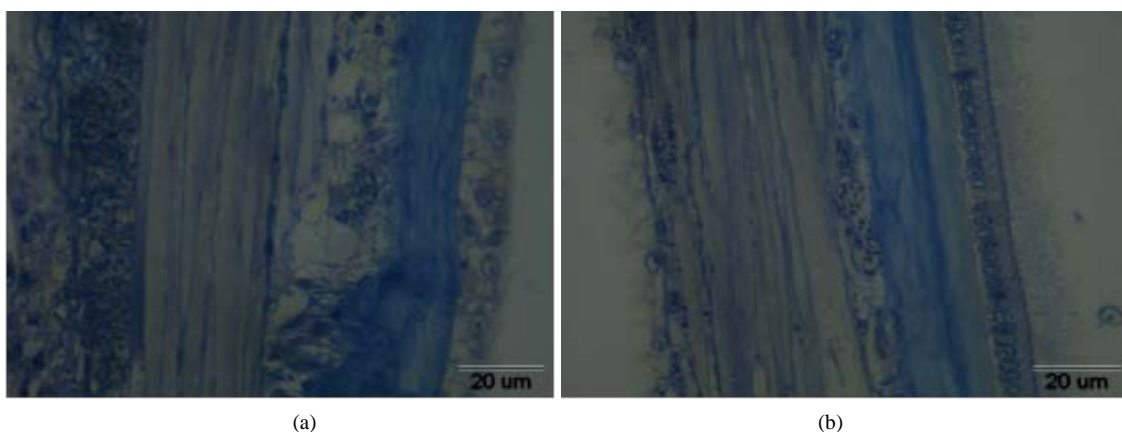
(a)   (b)

Figure 3. Photomicrograph, semithin, of gill of *Pinctada radiata* collected from El Asafra showing; mucocytes (Mu), frontalsurface (FS), haemocoel (H), lateral cilia (LFC), lateral cilia (LC), mucocyte (Mu) and nucleus (N).
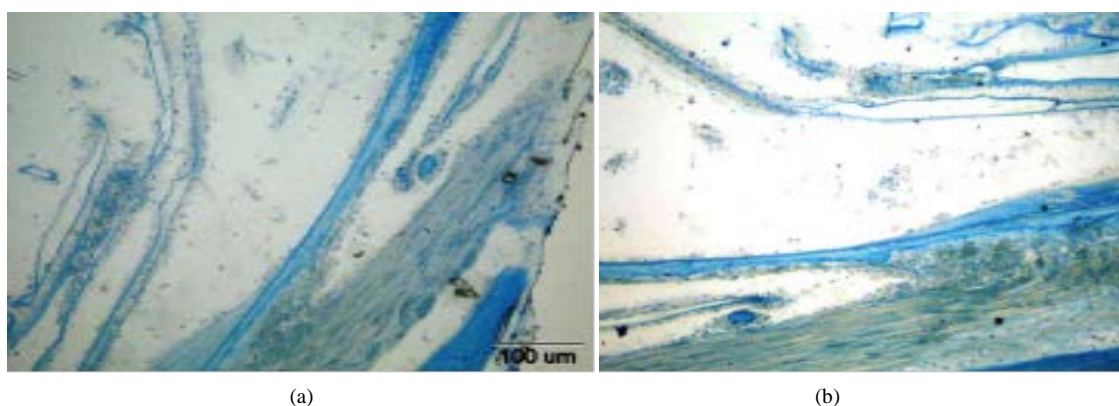

(a)   (b)

Figure 4. Photomicrograph, semithin, of *Pinctada radiata* collected from E.H. showing abnormal gill showing abnormal shape and irregular arrangement of gill filaments (G.F.), haemocoel (H.) and decay of frontal surface.
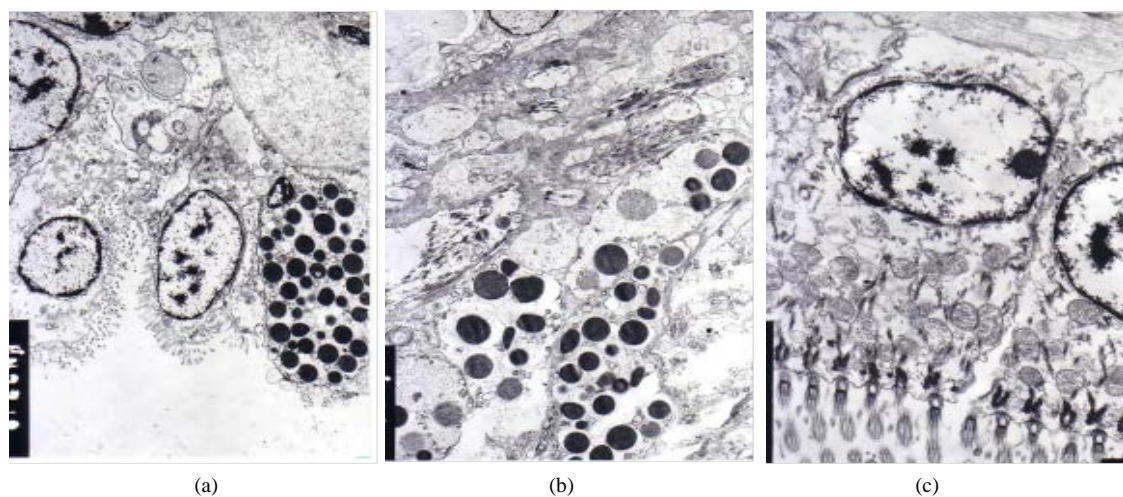

(a)   (b)   (c)

Figure 5. (a) (b) TEM micrograph of normal gill filament in the frontal zone of gill filament of oyster collected from El Asafra, showing the thin cells with microvilli (MV) cover the apical surface of cells. (c) TEM micrograph of normal gill filament from El Asafra showing the mucous cell (Mu), cells with many mitochondria (M) and nucleus (N).

size, from large thick cells in the muscle bands traversing the distal margin of the gills, to thin fibers laying under the epithelia (**Figures 7(a)-(c)**).
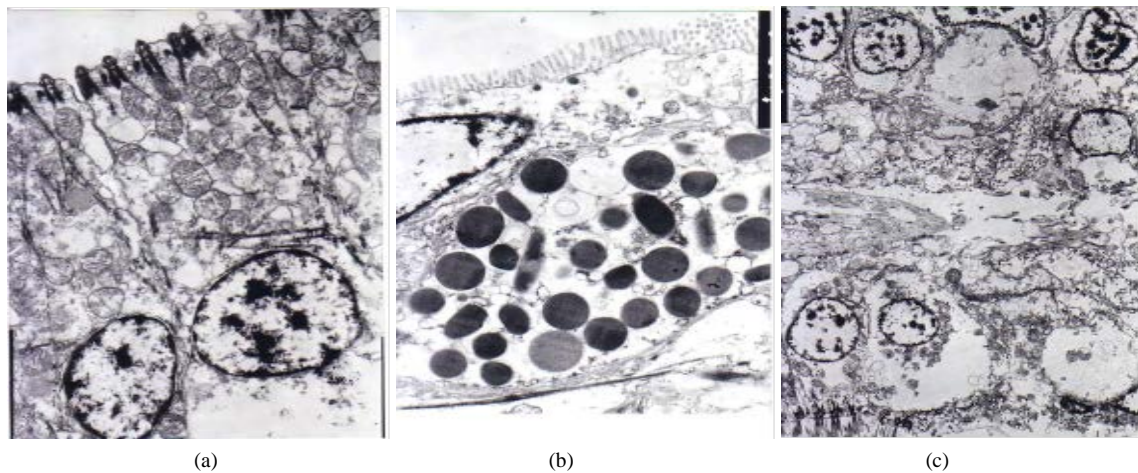
Figure 6. (a) (b) TEM micrograph of normal gill filament in the frontal zone of gill filament of oyster collected from El Asafra, showing the thin cells with microvilli (MV) cover the apical surface of cells. (c) TEM micrograph of normal gill filament from El Asafra showing the mucous cell (Mu), cells with many mitochondria (M) and nucleus (N).
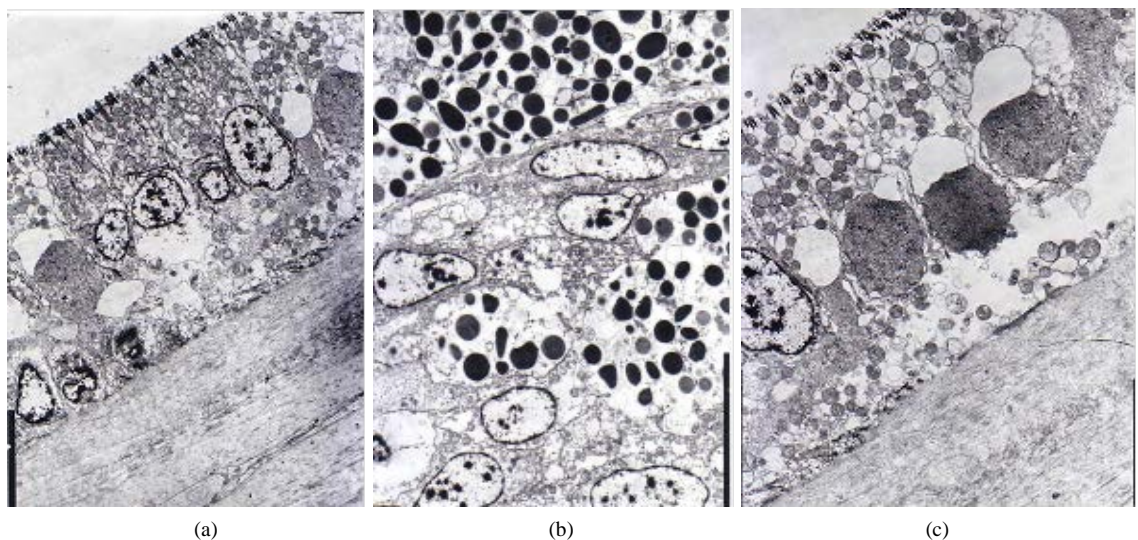

Figure 7. (a) (b) (c) TEM micrograph of gill illustrating the decayed mitochondria (M) and mitochondrial memberane; gill oyster collected from E.H.

The most common type of haemocyte observed in the oysters was a large granulocyte with vesicles containing amorphous material (granules), in descriptions of haemocytes because of their granular appearance by light microscopy. There were also smaller numbers of haemocytes without large vesicles. These had variable nucleus, cytoplasm ratios and variable numbers. Gills contained varying quantities of granules. Most granules were extracellular, Granules were also scattered in the interstitial tissue. In some specimens, granules were generally very numerous, the majority of granules occurred as large clusters of small granules.

4. Discussion

Sea food is considered as an important source of protein for human [23]. In the present study the concentration of heavy metals is considered less than that reported by [24]. It is concluded that the coastal area in Mediterranean sea of Egypt might be considered relatively unpolluted with heavy metals. [25] reported that the coastal area in Mediterranean sea of Egypt might be considered relatively unpolluted with heavy metals. In all cases the average concentrations of trace elements in Egyptian Mediterranean coast are far from the hazardous concentrations [26]. They revealed that the origin of trace elements in the sampled water of the Egyptian Mediterranean

was mainly the waste water discharge. [27] reported that the uptake of metals may take place at the gills of bivalve. Only gills of bivalves can be considered as an adequate target tissue for heavy metals [28]. [29] suggested that the gill in Mussel *Mytilus edulis* appeared to be more suitable organ for biomonitoring heavy metals. Bivalves are frequently used in marine ecotoxicology for the purpose of assessing seawater quality because they are very sensitive to pollutants [30]. Gills are frequent targets of environmental pollutants because they are the main interface between the organisms and their environment [31]. Gills are the target organ in oysters exposed to high concentrations of heavy metals [32].

Histopathological alterations of gills of bivalve tissues have been shown to be responsive and sensitive to wide range of contaminants because they play an important role in respiration and food collection [33]-[35]. The histopathological changes of gills of bivalve in the present study are in agreement with [2] and [36] as they reported irregularity of gill lamellae of the cells, swelling of gill filaments and haemocytes infiltration of bivalve. Epithelial cells of the gills play a crucial role [37]; the damage in the epithelium results in serious dysfunction of tissues consequently leading to deleterious effect on the organization levels [2]. Bivalves possess different measures of defense against environmental hazards e.g. particle rejection and formation of pseudo faces, reduced filtration rate and valve closure [38]. Haemocytes in bivalves possess a variety of functions, including regeneration, digestion and phagocytosis of foreign particles and pathogens [39]. The major environmental problem in the coastal area is directly related to the impact of domestic effluents [40].

[41] reported that metal concentrations recorded in the soft tissues of mussels *Mytilus galloprovincialis* increased without a source of extra metals in water. [26] revealed that the origin of trace elements in the sampled water of the Egyptian Mediterranean was mainly the waste water discharge. They added that the surface east water current and south west winds blowing on the Mediterranean coast of Egypt contributed mostly in spreading the trace elements to wide areas of the coast. [28] found that the degree of environmental contamination was only one among several factors that influenced metal concentrations in animals. Bioavailability or specific sources may be responsible for higher concentrations in apparently less impacted environments. [29] suggested that gills of bivalves appeared to be a more suitable organ for biomonitoring than that of the hepatopancreas. Bivalves are frequently used in marine ecotoxicology for the purpose of assessing seawater quality because they are very sensitive to pollutants [30]. Gills are frequent targets of environmental pollutants because they are the main interface between the organisms and their environment [31] and [32].

Mediterranean is surrounded by 18 countries from three continents (Europe, Africa and Asia) [42]; intense human activities from these countries produce a strong environmental impact in form of marine degradation [43], and cause heavy metal stress on the Mediterranean waters mainly through discharging different sources of pollutants through discharging different sources of pollutants through coastal waters [30]. The Egyptian Mediterranean coast has been influenced by untreated urban and industrial effluents that caused coastline degradation [44] and [45], particularly in Alexandria coast due to the high population growth and rapid development [45]. [46] reported Zn in gills of Mussels in France as 17.8 + 2.1 µg/g. In the present study data the gills of *P. radiata* is considered less than that, as it is reported to be 13.33 ± 0.2 in El Asafra and 16.84 ± 3.04 µg/g in the E.H.

In British Columbia, Canada along an apparent pollution gradient of acid mine drainage, tissue Zn concentrations were likely not high enough to have a direct impact on mussels (*Mytilus edulis*) health [47]. Metal binding induction differs markedly among the gills of the bivalve: mollusks *Mytilus galloprovincialis* and *Ruditapes decussatus*. [48] concluded that gills could preferentially be used in biomonitoring studies in the blue mussels. The gill tissue of *Mytilus galloprovincialis* is responsible for the uptake of metal ions from water [49]. Cd is not an essential element for animals [50]. He added that the occurrence of Cd in the marine environment was rare, therefore the impact of Cd on the environment was considerably small. [51] stated that cadmium was not needed for clams' growth and may be deleterious. Exposure to high levels of Cd does not stimulate Reactive Oxygen Species [52]. Gills are suggested as a possible route for accumulation of Cd as a possible route for Cd excretion [53]. [54] reported that Cd was present in the effluent and had accumulated significantly in mussels' gills. [55] reported that, the concentration of dissolved cadmium in gills of *Pinctada radiata*, collected from coastal zones was fluctuated between 3.03 µg/g wet weight and 0.57 µg/g wet weight. [46] reported; the level of Cd level in the gills of oysters collected from (Loire Atlantique, Bourgneuf, France) as 0.27 + 0.01 µg/g wet weight. They added; the level of Cd level in the gills of mussels collected from (Loire Atlantique, Bourgneuf, France), as 0.1 + 0.01 µg/g wet weight.

An assessment of potential risks to human health due to consumption of the mussels (*Mytilus edulis*) and (*Perna viridis*) was undertaken for the metals. Metals could pose a health risk to heavy seafood consumers [56].

[54] reported; Cd accumulated significantly in mussels' gills. Uptake of metal in bivalves may take place at the gills and their relative importance is a function of the speciation of the metals in the environment [27]. The surveys of contaminants in shellfish conducted by Agency for Toxic Substances and Disease Registry [17] which reported the mean of Cd level for shellfish as 360 µg/g dry weight. [29] suggested that gill appeared to be more suitable organ for metal biomonitoring more than the hepatopancrease. [49] reported that the gill represented the quick answer of mussels to water concentrations of metals. [41] concluded; in nature metals in *Pygananodon grandis* are bound in the gills.

Very high Cd concentration may result from food chain bioaccumulation of elevated Cd levels brought into the productive surface water by upwelling into the region [40]. Lead is leader member of the toxic metals in the marine environment [50]. He added that metal variations were result of both natural and human activity. Moreover, some mollusk species represent a valuable seafood source. Therefore, high concentration of heavy metals in mollusk species gives dangerous indicator to deteriorate the marine life and pose a health risk to human. Exposure to Pb generally resulted in reduced oyster growth [11]. Reduction in growth has been reported to oyster *Pinctada imbricata* exposed to 270 µg/l Pb [57]. Lead uptake at the gill surface may occur via a number of possible pathways including passive diffusion, active transport [11]. [51] stated that in an environment affected by contaminant oyster tissues have an opportunity to adsorb heavy metals. Surveys of contaminants in shellfish conducted by [17] found that the mean Pb concentration in health tissues of the shellfish should not exceed 250 µg/g, when Pb concentration exceeded 250 µg/g, shellfish became harmful to consumers.

The bioavailability of metals such as lead concentrations in soft tissues of oysters *Pinctada imbricata* is highly dependent on the speciation or physicochemical forms of the metals in seawater [11]. *Ruditapes philippinarium* was exposed to different concentrations: Pb (350 - 700 µg/l) for seven days. The highest concentrations were found in the gills for Pb [28]. They reported that gills of clam *Ruditapes philippinarum* could be considered as an adequate target tissue for heavy metals. Some studies showed that both Mediterranean and red sea seawater are relatively unpolluted with heavy metals as compared to other regions in the world [25]. The selection of histology as indicator of disease and contamination was based on previous studies that showed strong relation between pollution and the histology of gills [12]. Histological examinations showed clearly different pathological changes in the structure of the gills of *Pinctada radiata* (Bivalve) exposed to pollution and collected from Alexandria coast, Egypt [55]. Some of these areas are polluted with different kinds of contaminants caused by the discharges of industrial and municipal effluents containing chemical and biological contaminants such as heavy metals [3]. Untreated sewage and waste waters were discharged annually from large numbers of outlets into Alexandria coastal area through local sewage system and endangers human health [6]. Marine bivalves have been used to monitor environmental health conditions and potential pollution by using the whole animal or specific organs to determine contamination levels and facilitate comparisons over space and time [5]. Several indicators of exposure to stress were reported in bivalves including, histopathological changes mostly confined to organs directly involved in the metabolism and detoxification of pollutants and elevated expression of stress proteins [58]. To protect public health, we have to harvest shellfish from approved waters where water quality standards have been met.

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Scientific
Research

Community-Based Coral Reef Rehabilitation in a Changing Climate: Lessons Learned from Hurricanes, Extreme Rainfall, and Changing Land Use Impacts

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Abstract

Coral reefs have largely declined across multiple spatial scales due to a combination of local-scale anthropogenic impacts, and due to regional-global climate change. This has resulted in a significant loss of entire coral functional groups, including western Atlantic Staghorn coral (*Acropora cervicornis*) biotopes, and in a net decline of coral reef ecosystem resilience, ecological functions, services and benefits. Low-tech coral farming has become one of the most important tools to help restore depleted coral reefs across the Wider Caribbean Region. We tested a community-based, low-tech coral farming approach in Culebra Island, Puerto Rico, aimed at adapting to climate change-related impacts through a two-year project to propagate *A. cervicornis* under two contrasting fishing management conditions, in coastal areas experimenting significant land use changes. Extreme rainfall events and recurrent tropical storms and hurricanes had major site- and method-specific impacts on project outcome, particularly in areas adjacent to deforested lands and subjected to recurrent impacts from land-based source pollution (LBSP) and runoff. Overall, coral survival rate in "A frame" units improved from 73% during 2011-2012 to 81% during 2012-2013. Coral survival rate improved to 97% in horizontal line nurseries (HLN) incorporated

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during 2012-2013. Percent tissue cover ranged from 86% to 91% in "A frames", but reached 98% in HLN. Mean coral skeletal extension was 27 cm/y in "A frames" and 40 cm/y in HLN. These growth rates were up to 545% to 857% faster than previous reports from coral farms from other parts of the Caribbean, and up to 438% faster than wild colonies. Branch production and branchiness index (no. harvestable branches > 6 cm) increased by several orders of magnitude in comparison to the original colonies at the beginning of the project. Coral mortality was associated to hurricane physical impacts and sediment-laden runoff impacts associated to extreme rainfall and deforestation of adjacent lands. This raises a challenging question regarding the impact of chronic high sea surface temperature (SST), in combination with recurrent high nutrient pulses, in fostering increased coral growth at the expense of coral physiological conditions which may compromise corals resistance to disturbance. Achieving successful local management of reefs and adjacent lands is vital to maintain the sustained net production in coral farms and of reef structure, and the provision of the important ecosystem services that they provide. These measures are vital for buying time for reefs while global action on climate change is implemented. Adaptive community-based strategies are critical to strengthen institutional management efforts. But government agencies need to transparently build local trust, empower local stakeholders, and foster co-management to be fully successful. Failing to achieve that could make community-based coral reef rehabilitation more challenging, and could potentially drive rapidly declining, transient coral reefs into the slippery slope to slime.

Keywords

1. Introduction

1.1. Unprecedented Coral Reef Decline

Coral reefs have suffered a widespread decline through the Atlantic during the last three to four decades [1]-[3]. This has resulted in losing entire coral functional groups and in the net loss of coral reef ecosystem resilience, ecological functions, net services and benefits across multiple spatial scales. Staghorn coral populations, *Acropora cervicornis* (Lamarck, 1816), have collapsed across their geographic range in the western Atlantic over the last three to four decades [4]-[7]. Acroporid corals have also disappeared from many coral reefs in Puerto Rico where they are previously common [8] [9]. Staghorn coral used to dominate vast extensions of shallow to moderately deep (5 - 20 m) fore-reef terraces across the Caribbean [10]-[12] for over the last 600,000 years through the Pleistocene [13]-[17]. It was also a dominant reef species even as far as the mid Miocene (3.5 M.A.) and Pliocene (15 M.A.) [18]. The long persistence of *A. cervicornis* across the geological record goes back to Neogene deposits (40 - 50 M.A.) across the northeastern Caribbean [19]. Nevertheless, current declining trends are unprecedented in the recent geological record [20] [21], suggesting non-natural causes for its rapid decline. This has prompted its listing as a critically endangered species on the International Union for the Conservation of Nature Red List, and its designation as a threatened species under the US Endangered Species Act across the US Caribbean since 2006.

Acroporid corals in general are highly vulnerable to impacts from different natural factors, such as disease epidemics [5] [22] [23], emergent microbial infections [24] [25], and hurricanes [26]-[29]. They are also susceptible to multiple human factors, including land-based source pollution (LBSP), mostly in the form of chronic sewage pollution, sedimentation and turbidity [1] [30]-[33], and climate change [34]. *Acropora cervicornis* can reproduce both sexually and asexually [35]-[38]. Nonetheless, natural recovery, with few exceptions, has been very limited and virtually inexistent in most localities across the region largely as a result of their extremely low densities, significant mean colony size reduction and geographical isolation of surviving colonies [39]. These factors can significantly reduce their reproductive potential, and impair successful sexual reproduction and larval recruitment. Sexual recruitment in *A. cervicornis* has been very limited or non-existent across the Caribbean Region [40]-[42], mostly as a result of recurrent climate-related disturbances such as increased sea surface tem-

perature (SST) and massive coral bleaching [43]. Lack of recovery of *A. cervicornis* could result in a permanent decline in coral reef spatial heterogeneity and in losing a significant part of its essential fish habitat functions. Therefore, the use of low-tech coral farming and reef rehabilitation methods had become a paramount tool to enhance natural recovery ability of depleted reefs, foster the re-establishment of self-sustainable, sexually-reproducing populations on local reef scales, and contribute to sustainably maintaining and enhancing reef ecosystem functions and resilience.

1.2. The Role of Coral Aquaculture and Coral Reef Restoration

Coral aquaculture and coral reef restoration have become important tools to help replenish depleted coral reefs [44]-[52]. There has been a significant increase in coral farming activities at a global scale over the last decade [53], including the Wider Caribbean, which has involved a variety of low-tech approaches to propagate *A. cervicornis*, including the use of wire frames, wire coated frames, and a sort of horizontal line structures [54]. Early attempts to propagate *A. cervicornis* in Puerto Rico date back to 1980 [55]. There have been important recent efforts to replenish coral reefs in Puerto Rico impacted by significant vessel groundings through low-tech coral farming activities [56] [57]. Also, there have been successful participatory and collaborative efforts between the academia and community-based non-governmental organizations (NGOs) with an aim to mitigate or restore climate change-related impacts and depleted reefs historically impacted by military training activities [58] [59]. This effort has resulted in the successful implementation since 2003 of the *Community-Based Coral Aquaculture and Reef Rehabilitation Program* led by Sociedad Ambiente Marino (SAM) and the Coral Reef Research Group (CRRG) of the University of Puerto Rico's Center for Applied Tropical Ecology and Conservation (CATEC), with the direct collaboration of NGO Coralations and the Culebra Island Fishers Association. The program has successfully propagated and reintroduced over 15,000 *A. cervicornis* colonies around Culebra since 2003. The general goals of the program include the aim to restore *A. cervicornis* depleted populations and to rehabilitate coral reef ecosystem functions by fostering increased fish and coral recruitment, and increased herbivory levels. These processes are critical for the sustainability of coral reef ecosystem functions and resilience under forecasted climate change scenarios [60].

1.3. Climate Change and Extreme Weather Threats to Coral Reefs

Climate change has become one of the most significant threats to coral reef ecosystems [61]. Forecasted trends of change based on climate modeling suggest major threats due to increasing SST and increased risk of massive coral bleaching events [62]. These could have potentially devastating consequences for selected reef-building species [63] [64] and for marine ecosystems [65]-[68]. Global-scale climate change impacts may also threaten the success of coral aquaculture and coral reef rehabilitation activities due to extreme weather events leaving base-communities nearly defenseless against factors such as declining reef accretion in face of increasing sea level rise (SLR), ocean acidification, net loss of ecosystem resilience and productivity, and declining socio-economic value, services and benefits (*i.e.*, losing fisheries sustainability potential, tourism revenues). Such impacts can be more critical for small island-nations with limited geographic, socio-economic, and human resources, particularly under non-sustainable economic models [69]. Nonetheless, studies addressing the impacts of climate-related factors such as increasing SST, increased tropical storm or hurricane frequency, or extreme rainfall events are missing. We postulate that a chronic increase in SST may increase the frequency and/or severity of extreme rainfall events and hurricanes. In turn, this should result in an increased frequency and/or severity of impacts from sediment-laden runoff pulses and LBSP to coral reefs and to community-based coral farming efforts. This suggests the need to test low-tech adaptive strategies to minimize such impacts and maximize coral growth and survival. Further, the role of no-take marine protected areas (MPAs) as potential buffers of multiple anthropogenic disturbance impacts to coral reefs, including climate change, still remains controversial [70] [71]. We suggest that even a no-take MPA designation is not enough to ameliorate impacts from climate change and extreme weather events. Their potential benefit could be further diminished by LBSP. There are still no published accounts comparing outputs of coral farming within and outside no-take MPAs.

This study was aimed at addressing the impacts of high SST, hurricanes, and extreme rainfall events on community-based low-tech *A. cervicornis* farming in Culebra Island, Puerto Rico. We tested two different methods to propagate corals as an adaptive strategy to mitigate impacts by hurricanes, extreme rainfall and sediment-laden runoff pulses (**Figure 1**). Finally, results were compared between coral farming sites located within a no-take MPA and control sites outside open to fishing.
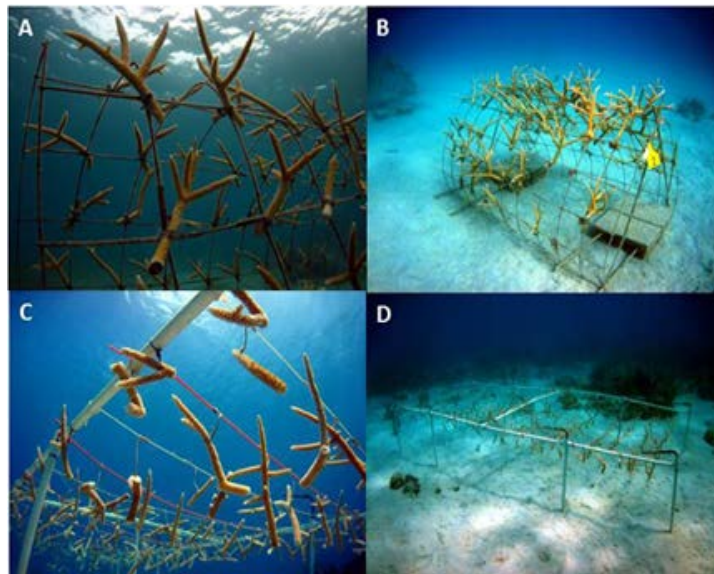
Figure 1. Example of community-based, low-tech *Acropora cervicornis* farming units. (A) and (B) Wire "A frames"; (B) and (C) Horizontal line nurseries (HLN) or "table" units.

2. Methods

2.1. Study Sites

Coral farming activities were carried out at three different sites in Culebra Island, a 72 km^2 volcanic island, with 2000 residents, and located at 27 km off eastern Puerto Rico, in the Caribbean Sea (**Figure 2**). Bahía Tamarindo (BTA) and Punta Melones (PME) are located within the Canal Luis Peña no-take Natural Reserve (CLNR). Punta Soldado (PSO) is located at a control site open to fishing. Propagules from wild coral populations were obtained from Culebrita Island (CBT). All coral farming was conducted at depths ranging from 4 to 6 m, over sandy and rubble bottoms adjacent to fringing reefs. Each "A frame" unit consisted of a 180 × 150 cm wire panel (15 × 15 cm sq. holes) partially bent to form an "A" shape structure that was anchored to the bottom using two concrete blocks (50 kg each), and a combination of 2.1 m rebars driven to the bottom, sand screws, and polypropylene lines. "A frames" supported up to 80 colonies each attached using plastic ties. Each horizontal line nursery (HLN) unit consisted of a 3 × 3 m square-shaped "table" with six legs built using 1.88 cm-wide PVC pipes. Corals were suspended at approximately 0.9 m off the bottom from fishing line or trimmer cable using plastic covered telephone copper wire. HLN units were anchored as above. Each unit also supported 80 colonies. Materials used in this study were commonly available in hardware stores, were not expensive and units were easy to construct and implement by local community-based volunteers. This project constituted part of the *Community-Based Coral Aquaculture and Reef Rehabilitation Program* which has been in operation since 2003.

2.2. Sea Surface Temperature and Rainfall Patterns

SST was permanently measured at each study site in Culebra Island using Hobo Temp V2 data loggers (Onset Computer Co.) placed a 5 m depth. Measurements were continuously obtained at 12-min intervals during the entire two years of the project (N = 87,600 SST lectures per site). Daily rainfall records were provided by William Kunke, resident of Culebra. The island has not had official rainfall records since 1975. Data on extreme rainfall events was also obtained from the Puerto Rico TJUA Doppler radar imagery using GRlevel 3 v.1.79 software (Gibson Ridge Software).

2.3. Genetic Characterization of *Acopora cervicornis* Source Populations

Samples from original *A. cervicornis* wild population sources selected for coral propagation were subjected to genetic analysis to determine their genetic identity. Genomic DNA was extracted from ~5 - 7 polyps with the
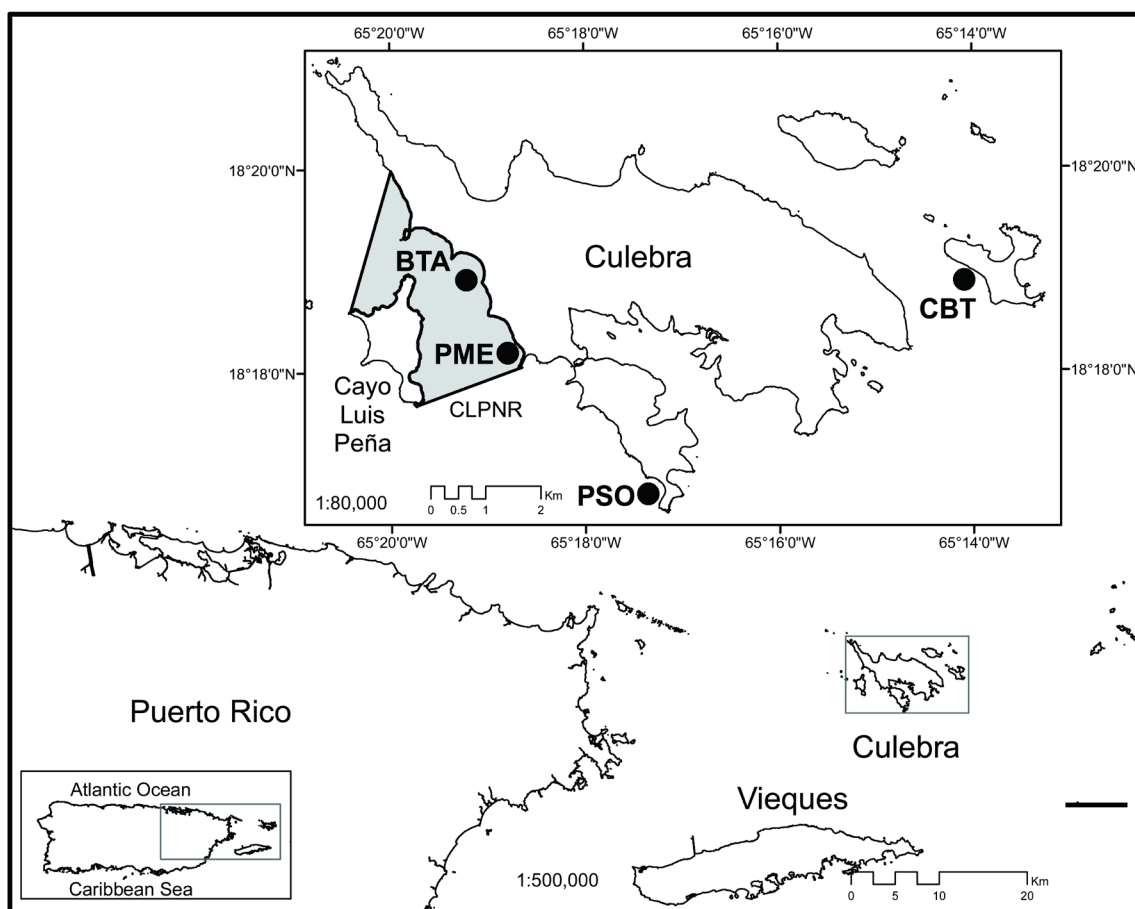
Figure 2. Study sites at Culebra Island, Puerto Rico. BTA = Bahía Tamarindo; PME = Punta Melones; PSO = Punta Soldado. CLPNR = Canal Luis Peña no-take Natural Reserve (gray shaded area). CBT = Culebrita Island (source of wild coral colonies).

DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's animal tissue protocol from each sample. Samples were then screened for 4 polymorphic microsatellite markers [72], namely #166, #181, #182 and #207, following a modified protocol from [73]-[75]. PCR amplifications were performed in 10 µl reactions, containing 1 µl genomic DNA (5 - 15 ng·µl^{-1}), 0.8 mM dNTPs, 0.1 µM of forward primer with M13 tail, 0.1 µM of fluorescently labeled M13, 0.2 µM of reverse primer, MgCl$_2$ (2 mM), 0.3 µl of 1 U·µl^{-1} Taq DNA polymerase (Fermentas), and 1× of the PCR buffer. Temperature cycling was performed by denaturing 1 min at 94°C, followed by 20 cycles of 20 s at 94°C, 35 s at 56°C, and 30 s at 72°C. Then, followed by 15 cycles of 20 s at 94°C, 35 s at 50°C, and 30 s at 72°C, and a 10 min extension step at 72°C. Amplicons were diluted up to 50× to approach 10 - 20 ng·µl^{-1}, and were run on an ABI3130xl Genetic Analyzer with ROX labeled size standards. Microsatellite alleles were scored using Gene Marker v2.2. The probability of identity (PI) is the probability of two samples with different genotypes to have identical haplotypes given a set of genetic markers. Identical haplotypes are then considered ramets of the same genet (clones of a same genotype) with a confidence probability PI. The Computation of PI was performed in Genalex v6.4 [76].

2.4. Coral Farming Sampling Design

Coral farming was conducted in 2011-2012 and in 2012-2013 using wire "A frames" (**Figure 1(A)**, **Figure 1(B)**). HLN or "table" units were also used in 2012-2013 at BTA and PSO (**Figure 1(C)**, **Figure 1(D)**). Each farm was subdivided in 6 replicate units per site, one per each genetic clone, at approximately 5 m depth. A total of 80 replicate 22 cm-long fragments per clone, per site, were grown during a year in 2011-2012 in "A frames", when fragments were harvested and used either for out planting or for coral farm expansion. Fragments ranging from

32 to 37 cm-long were used in "A frames" during 2012-2013, while 15 cm fragments were used for HLN units. Fragment survival, growth (total linear extension), total branch abundance (>0.5 cm length), branchiness index (BI) (# harvestable branches > 6 cm), percent live tissue cover, percent recent mortality, percent old mortality, causes of mortality (when possible to identify), disease prevalence, percent bleaching frequency, and bleaching severity index (BSI) were monitored in 20 permanently marked, haphazard replicate fragments per clone at each site, at fixed 0, 1, 3, 6, 9 and 12 month-intervals during each cycle. Data were also collected during month 5 (September 2011) of the first year to address impacts by Hurricane Irene, and tropical storms Emily and María. Colony growth measurements were performed using a cm-calibrated metric line at 0.2 cm resolution. BSI was visually determined on all monitored colonies using a semi-quantitative classification system using the following scoring points: 1) unbleached coral; 2) pale bleaching; 3) patchy bleaching; 4) mostly bleached; 5) fully bleached. Scores per clone were averaged for each 20 coral cohort. Predator density was also documented, including corallivore snails (*Coralliophila caribaea*, *C. abbreviata*), fireworm (*Hermodice carunculata*), territorial damelfishes (Pomacentridae), and butterflyfish (Chaetodontidae). Data during 2012-2013 were simultaneously collected from "A frame" and HLN units at similar fixed time intervals.

Selected coral farming data from the 2011-2012 and the 2012-2013 cycles were individually tested using multivariate approaches with PRIMER-E v6.1.16 & PERMANOVA+ v1.0.6 statistical package [77] [78]. The 2011-2012 data were tested using a three-way permutational analysis of variance (PERMANOVA) for site (BTA, PME, PSO), time (0, 1, 3, 5, 6, 9, 12 months), clone (n = 6 clones), and management effects (no-take MPA, control site outside). PERMANOVA was used to test the null hypothesis of no significant difference in mean values of any of the coral parameters measured, as well as their interaction effects. Data from the 2012-2013 cycle were tested using a four-way PERMANOVA for site (BTA, PME, PSO), time (0, 1, 3, 6, 9, 12 months), clone (n = 6 clones), method (A frames, HLN), and management effects (no-take MPA, control site outside). Data were square root-transformed prior to analysis. All tests were based in 10,000 permutations.

3. Results

3.1. Sea Surface Temperature

SST during the study period of time always exceeded every monthly historical mean value (**Figure 3(A)**). This resulted in positive SST anomalies during the entire 28-month long project extent. According to historical SST records across the northeastern Caribbean, the mean monthly maximum (MMM) SST (28.5˚C) is often typical of late-August to mid-October. However, the MMM was reached and exceeded as early as mid June during 2011 and 2012, extending down to November. Hot spot SST (28.5˚C + 1˚C) was reached or exceeded for about 5 continuous weeks (Degree Heating Weeks; DHW) during 2011, but only during two weeks in 2012 and 2013. The highest SST anomaly of 2011 was observed during late August (+1.69˚C), and the lowest at early March (+0.16˚C). The highest SST anomaly of 2012 was documented during early November (+1.87˚C), and the lowest during late April (+0.23˚C). The highest SST anomaly of 2013 occurred also during early November (+1.65˚C), and the lowest during late April (+0.20˚C). Winter SST anomalies were also a concern during this study with +1.49˚C during late January 2011, +1.78˚C during early January 2012, and +1.65˚C during early January 2013. Mean seasonal SST anomalies ranged during 2011 from +0.50˚C in Spring to +1.16 in the Summer, from +0.65˚C in the Spring to +1.36˚C in Fall during 2012, and from +0.65˚C in Spring to +1.42˚C in Fall (**Figure 3(B)**). The presence of year-round positive SST anomalies suggests continuing physiological stress for coral colonies and potentially increased vulnerability to other local sources of stress (*i.e.*, extreme rainfall, sediment-laden runoff, LBSP).

3.2. Extreme Rainfall Events: A Proxy for Recurrent Runoff and LBSP Pulse Impacts

Culebra Island has a subtropical dry climate, with a historical annual rainfall mean of (84.5 cm) based on 1907-1975 records (http://weather-warehouse.com). Annual rainfall total for the periods of 1907, 1909, 1919, and 1955-1972 averaged 85.6 cm, and ranged from 36.2 cm in 1967 to 142.8 cm in 1970 [79]. But mean annual rainfall trends have increased to 114.4 cm during the period of 1987-2013, or 34% above the historical mean (William Kunke, unpub. data). Mean rainfall in Culebra Island during 2010 reached a record 189.8 cm. During 2011, rainfall reached 111 cm, only 74.8 cm in 2012, and 107.3 cm in 2013. Recent rainfall trends between 2010 and 2013 have been +125%, +31%, −11.8%, and +27% in relation to historical annual mean. There were also
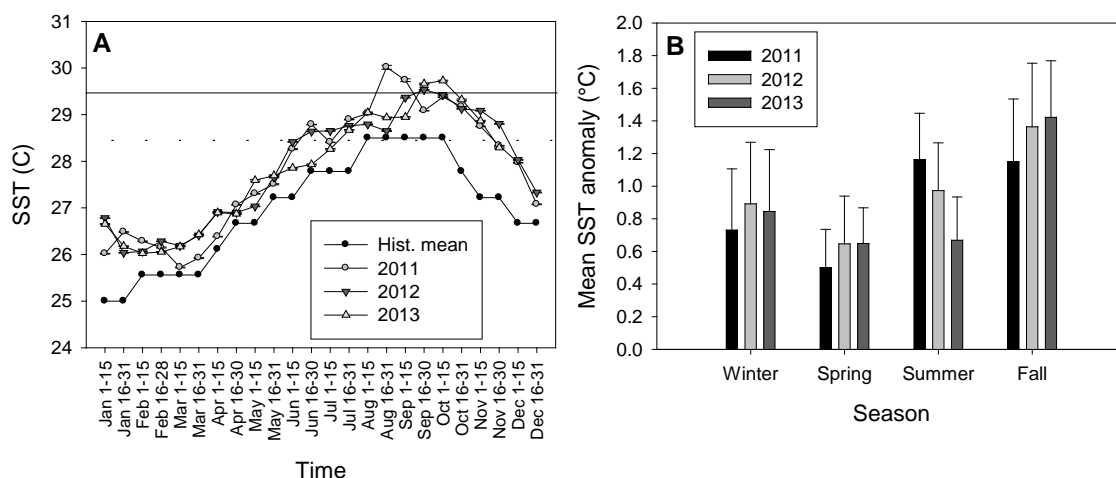
Figure 3. Sea surface temperature (SST) records at Culebra Island between January 2011 and November 2013: (A) Mean annual values. Dashed horizontal line = mean monthly maximum (MMM = 28.5˚C). Continuous horizontal line = hot spot (28.5˚C + 1˚C). (B) Mean seasonal SST anomaly (±95% confidence intervals).

significant impacts by extreme rainfall events in Culebra during recent years that were documented from Puerto Rico TJUA Doppler radar imagery using GRlevel 3 software, which were not recorded from rain gauge data. There is record of at least 20 different extreme rainfall events in Culebra Island only for the period of May to September 2011, many of which had direct impacts on watersheds adjacent to coral farming sites and resulted in major runoff episodes that affected coral survival.

For our purpose, extreme rainfall was defined as heavy rainfall in a short period of time (*i.e*., >2.5 cm/hr). There were six events recorded in a local rain gauge during May 2011 (4 - 15 cm/event), five during June (4 - 15 cm/event), four during July (5 - 13 cm/event), and two during August (13 cm and 18 cm/event) associated to tropical storm Emily and Hurricane Irene (August, 2011). These also produced 2 m, and 4 - 5 m swells, respectively.

Based on rain gauge data, summer extreme events in 2011 represented rainfall anomalies of 127%, 140%, and 152% in relation to mean monthly values in May, June, and August, respectively (**Figure 4**). However, Doppler data suggested extreme rainfall events that resulted in rainfall records 319% above mean May value, 521% in June, 246% in July, 168% in August, and 165% in September. All of these events resulted in coral mortality episodes in both wild and cultured *A. cervicornis* populations as a result of rapid shut-down reaction (SDR) and tissue loss following heavy rainfall and sediment-laden runoff.

The northeastern Caribbean Region was also impacted during 2012 and 2013 by several significant rainfall events, as well as by recurrent long-period bottom swells. These included important events such as tropical storm Isaac (August 2012), which also produced waves of approximately 4 m across coral farms. During late October 2012 long-period swells from Hurricane Sandy produced 5 m NW breaking waves, in combination with bottom swells across the region, and produced Doppler estimates of 13 cm. Another important event in December 2012 was tropical storm Rafael, which produced not much rain, but generated 3 - 4 m swells. Bottom swells resulted in significant sediment resuspension. During the first 9 months of 2013 Culebra Island was initially impacted by three consecutive months of very dry conditions (<2.5 cm), followed by a few significant isolated strong rainfall episodes associated to the mid-Atlantic through positioning over Culebra. These included extreme events during late March (13 cm over a few hours) and a strong tropical wave during mid June (20 cm over a few hours). Then, tropical storm Chantal (July) produced some significant rain bands over eastern Puerto Rico and Culebra, with 2.5 m-high SW swells and a monthly rainfall anomaly of 215%. Tropical storm Gabrielle (September) produced about 18 cm of rain over Culebra in about 48 hours. Runoff impacts by recurrent storm events, particularly those associated to passing tropical storms, were magnified by the recurrent practice of the Culebra Island municipal government of clearing all vegetation across several small creeks and natural drainage channels adjacent to urban areas as a preventive measure to manage potential flooding. The end product of such practices is extreme runoff pulse events and major erosion of ephemeral stream banks with significant concomitant turbidity impacts on costal ecosystems.
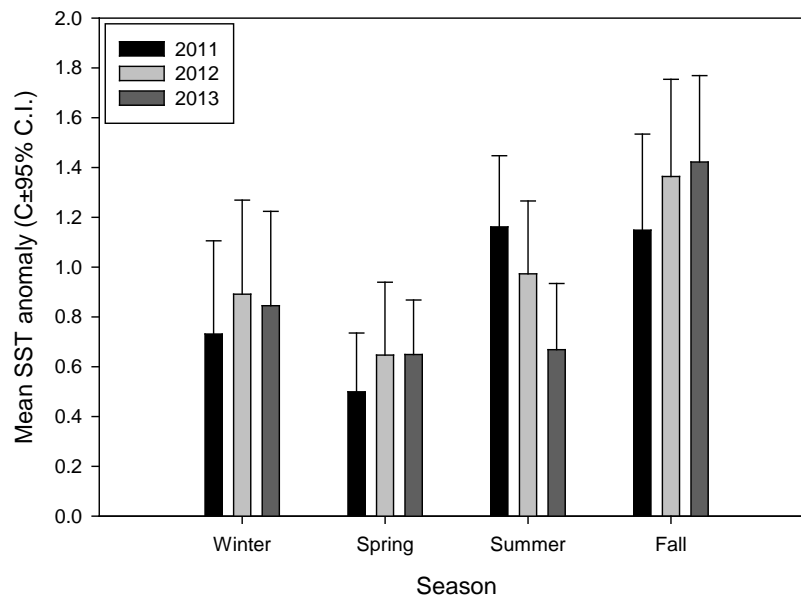
Figure 4. Mean monthly rainfall anomaly from Culebra Island during the project (2011-2013) in comparison to 1907-1975 mean (http://weather-warehouse.com). Data source: William Kunke (unpub. data).

3.3. Genetic Characterization of *Acropora cervicornis* Source Populations

At least six different haplotypes within the eight samples from *A. cervicornis* wild source populations were found in this study. One of the haplotypes (H6) included several ramets (clones, PI = 1.3×10^{-3}), represented by samples #09 and #10. Missing values for alleles for 3 out of 4 markers in sample #7 prevented ruling out clonality for this sample, as its profile for marker #207 made it a possible clone of haplotypes H1, H2, H3 or H6. Based on our eight samples, the probability for two different genotypes to have identical haplotypes by chance (PI) using these four microsatellite markers was ~1.3×10^{-3}. But it is likely to be well underestimated due to the low number of samples at our disposition. By comparison, the PI calculated on the combination of the same four markers based on *A. palmata* 306 haplotypes [80] was estimated to be ~7.9×10^{-6}. Therefore, it was a reasonable assumption that identical haplotypes represented different biological clones.

3.4. Coral Farming (2011-2012)

In spite of hurricane and extreme rainfall impacts during 2011, *A. cervicornis* farming during the first year was highly successful. Corals in "A frame" units showed 84% and 78% survival rate at BTA and PSO, respectively, after the first year (**Figure 5(A)**). Survival rate at PME reached only 57% due to significant mechanical impacts by Hurricane Irene during August 2011, which caused localized destruction of some of the farming units and significant immediate and delayed coral mortality due to SDR and a White Band Disease-like (WBDL) condition. Also, frequent extreme rainfall events caused recurrent sediment-laden, nutrient-loaded turbid runoff impacts. PME, as well as BTA, received substantial recurrent runoff impacts. PME farms were eventually relocated after September 2011 to an alternative site adjacent to BTA, where surviving fragments showed an outstanding recovery. Coral fragment survival rates were also highly site-specific (**Table 1**). Temporal and management effects significantly influenced coral colony survival rates in "A frame" units, but clone variation did not, which means that extreme rainfall events had widespread adverse impacts among all clones in the population. Most interaction effects were also highly significant. Mean percent live tissue cover on coral farms after one year was 89% at BTA, 93% at PME (based on data from colonies that survived physical destruction by Hurricane Irene), and 91% at PSO (**Figure 5(B)**). Coral colonies showed rapid and remarkable tissue regeneration after hurricane sand blasting impacts. Temporal patterns, site effects and management effects were highly significant, producing a clear spatio-temporal gradient. Site × time, time × clone, management × site, management × time, and management × site × time interactions were also significant.

Table 1. Three-way PERMANOVA of coral fragments from "A frame" units (2011-2012).

Variable	d.f.[*]	Survival rate pseudo-F (*p*)	% live tissue pseudo-F (*p*)	Fragment size pseudo-F (*p*)	Branch abundance pseudo-F (*p*)	Branchiness index pseudo-F (*p*)
Time	6, 119	12.59 (<0.0001)	8.88 (<0.0001)	11.81 (<0.0001)	7.63 (<0.0001)	10.32 (<0.0001)
Site	2, 123	10.57 (<0.0001)	4.21 (0.0120)	4.98 (0.0096)	6.11 (0.0019)	4.45 (0.0094)
Clone	5, 120	1.33 (NS)[**]	0.60 (NS)	2.14 (NS)	5.03 (0.0003)	2.04 (0.0094)
Management	1, 124	4.84 (0.0311)	3.99 (0.0429	8.90 (0.0029)	11.80 (0.0008)	8.67 (0.0017)
Site × time	20, 105	10.92 (<0.0001)	6.70 (<0.0001)	5.51 (<0.0001)	3.79 (<0.0001)	4.33 (<0.0001)
Site × clone	17, 108	3.40 (0.0002)	1.21 (NS)	2.48 (0.0020)	2.91 (<0.0001)	2.90 (0.0005)
Time × clone	41, 84	1.72 (0.0231)	1.56 (0.0467)	2.60 (<0.0001)	2.43 (0.0004)	2.10 (0.0015)
Mgmt. × site	2, 123	12.59 (<0.0001)	4.21 (0.0126)	4.98 (0.0082)	6.11 (0.0029)	4.45 (0.0114)
Mgmt. × time	13, 112	6.47 (<0.0001)	5.78 (0.0002)	8.11 (<0.0001)	5.68 (<0.0001)	6.46 (<0.0001)
Mgmt. × clone	11, 114	2.16 (0.0237)	1.11 (NS)	2.60 (0.0051)	5.13 (<0.0001)	2.31 (0.0096)
Mgmt. × site × time	20, 105	10.92 (<0.0001)	6.70 (<0.0001)	5.51 (0.0051)	3.79 (<0.0001)	4.33 (<0.0001)
Mgmt. × time × clone	83, 42	0.98 (NS)	0.89 (NS)	2.17 (0.0047)	2.15 (0.0036)	1.26 (NS)
Mgmt. × site × clone	17, 108	3.40 (0.0002)	1.21 (NS)	2.48 (0.0019)	3.91 (<0.0001)	2.90 (<0.0001)

[*]Degrees of freedom (within, between); [**]NS = not significant (*p* > 0.05).
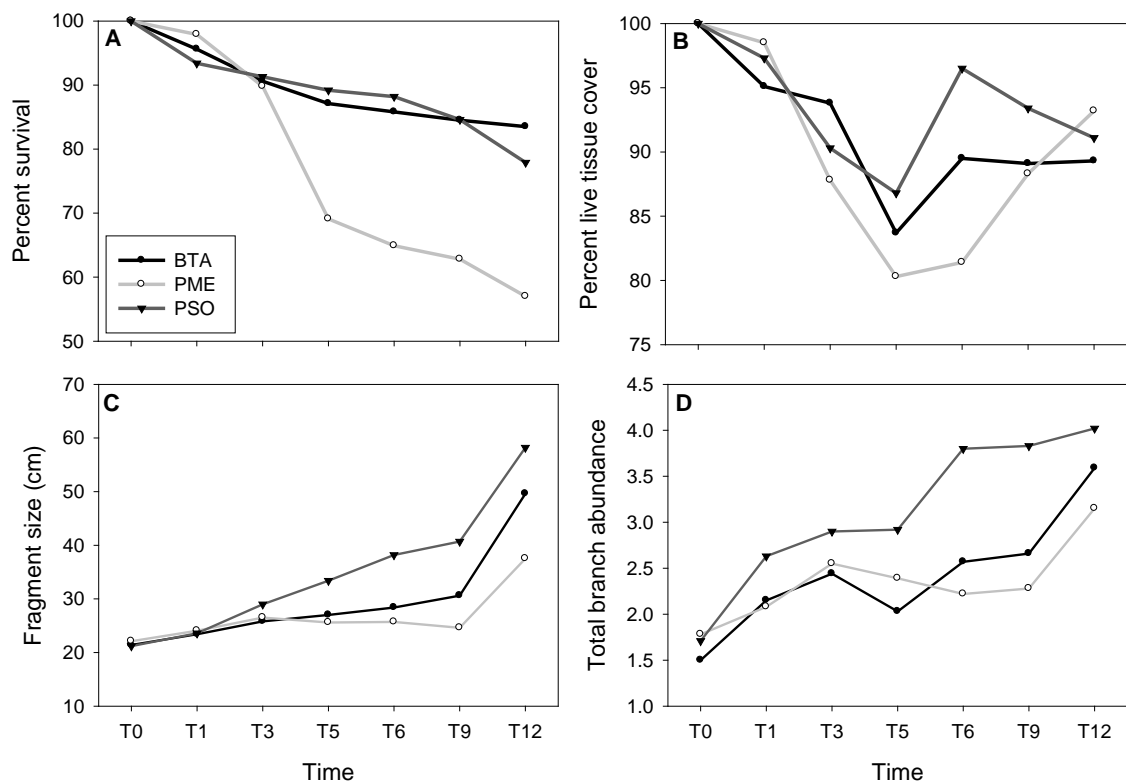

Figure 5. Coral fragment parameters mean values in "A frames" during 2011-2012: (A) Percent survival rates; (B) Percent live tissue cover; (C) Fragment size (cm); and (D) Total branch abundance.

Mean total coral colony length after one year at BTA increased from 21.4 to 49.6 cm (28.2 cm/y), from 22.1 to 37.5 cm (15.4 cm/y) at PME (based on data from colonies that survived physical destruction by Hurricane Irene), and from 21.2 to 58.2 cm (37 cm/y) at PSO (**Figure 5(C)**). The magnitude of coral colony growth was 132% at BTA, 70% at PME, and 175% at PSO. Overall mean monthly skeletal extension at BTA was 2.35 cm, 1.28 cm at PME, and 3.08 cm at PSO. PSO was the location with the lowest runoff impacts. Temporal patterns, site effects and management effects were highly significant (**Table 1**). Interaction effects were also significant.

Mean coral branch abundance after one year at BTA increased from 1.50 to 3.59 branches/colony, from 1.78 to 3.15 branches/colony at PME (based on data from colonies that survived physical destruction by Hurricane Irene), and from 1.71 to 4.02 branches/colony at PSO (**Figure 5(D)**). The magnitude of increase in coral colony branch abundance was 139% at BTA, 77% at PME, and 135% at PSO, which also reflects the limitation imposed by hurricane impacts. Temporal patterns, site effects, clone and management effects were highly significant (**Table 1**). Interaction effects were also significant. Mean coral branchiness index at BTA increased from 0.26 to 1.84 branches/colony, from 0.17 to 1.19 branches/colony at PME (based on data from colonies that survived physical destruction by Hurricane Irene), and from 0.24 to 2.34 branches/colony at PSO. This index represents the number of harvestable branches > 6 cm long. The magnitude of coral branchiness index increase was 608% at BTA, 600% at PME, and 875% at PSO (**Figure 6**), which represents a 6 to nearly 9-fold increase in the abundance of harvestable branches per colony, in spite of hurricane and extreme rainfall effects. Temporal patterns, site effects, clone and management effects were highly significant (**Table 1**). Almost all interaction effects were also significant.

Variability in coral colony conditions, including SDR and WBDL showed no significant spatio-temporal difference, or differences among clones (data not shown). But incidence of both conditions was always associated to major runoff episodes. Bleaching incidence was also highly variable in space and time, with no significant difference. Nonetheless, minor bleaching sporadically occurred in some colonies, with no mortality, just after initial transplanting as an acclimation response to altered irradiation at the outplanting site. Minor bleaching also occurred in some colonies during the late summer when SST was highest. Mean BSI reached 1.24 at BTA, 2.80 at PME, and 1.06 at PSO (out of a maximum scale value of 5.0 during full bleaching) after Hurricane Irene impact, suggesting only minor colony paling. BSI never exceeded 1.31 during the rest of the study. Bleaching was never a critical factor affecting project outcome. Corallivore gastropod (*Coralliophila abbreviata*, *C. caribaea*) recruitment pulses appear to be more active during Fall and late Spring, but no significant differences among sites were observed. Fireworm (*Hermodice carunculata*) predation was not a factor on coral farms, although predation damage was largely common on colonies outplanted to natural reef bottom at PSO, outside the no-take reserve.
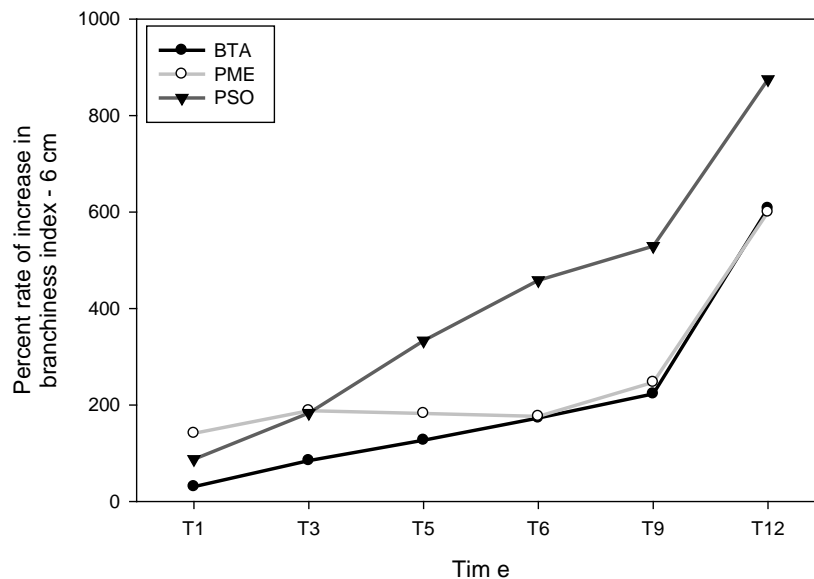

Figure 6. Percent rate of increase in branchiness index.

3.5. Coral Farming (2012-2013)

During the period of 2012-2013 coral farming was expanded to simultaneously include "A frame" and HLN units. HLN or "table" units were designed and implemented to minimize sand blasting impacts and predation from corallivore gastropods and fireworms. Farms were impacted by tropical storms Ernesto (August), Isaac (August), and Rafael (December). Also, 3 - 5 m-high long-period swells generated from Hurricane Sandy across the northwestern Atlantic (October 2012) also swept coral farms causing localized breakage of large-sized colonies from older coral farms. However, a quick response from the community-based volunteers prevented any direct mortality. In spite of such impacts, *A. cervicornis* gowth during this cycle was highly successful, particularly for HLN units. Fragments in HLN units showed a significantly higher mean percent survival rate at PSO-T (99.6%) and BTA-T (93.3%), when compared to "A frame" units at PSO-A (83.7%), PME-A (80.1%), and at BTA-A (79.1%) (**Figure 7(A)**). There were highly significant temporal, site, methods and management effects (**Table 2**). Survival rates were overall higher at PSO, and higher on HLN units. All interaction effects were also significant. Coral survival rates were significantly enhanced when colonies remained suspended in the water column in HLN units, in comparison to those that grew attached to the wire mesh structure of "A frames" under recurrent runoff pulse impacts and LBSP. Competing taxa, such as macroalgae, cyanobacteria, sponges, tunicates, bryozoans, fire coral (*Millepora alcicornis*, *M. complanata*), hydroids and reef oysters, can rapidly outcompete and overgrow *A. cervicornis*. Also, proliferating algal mats on the wire mesh structure after runoff impacts

Table 2. Four-way PERMANOVA of coral fragments from "A frame" and HLN units (2012-2013).

Variable	d.f.[*]	Survival rate pseudo-F (p)	% live tissue pseudo-F (p)	Fragment size pseudo-F (p)	Branch abundance pseudo-F (p)	Branchiness index pseudo-F (p)
Time	5, 180	16.04 (<0.0001)	6.96 (<0.0001)	18.68 (<0.0001)	17.11 (<0.0001)	11.50 (<0.0001)
Site	4, 181	11.55 (<0.0001)	34.01 (<0.0001)	15.35 (<0.0001)	14.68 (<0.0001)	22.23 (<0.0001)
Clone	6, 179	1.74 (NS)[**]	1.45 (NS)	3.74 (0.0015)	2.17 (0.0435)	2.27 (0.0242)
Method	1, 184	42.12 (<0.0001)	132.31 (<0.0001)	53.63 (<0.0001)	47.76 (<0.0001)	87.99 (<0.0001)
Management	1, 184	8.63 (0.0044)	9.27 (0.0030)	0.45 (NS)	1.23 (NS)	1.71 (NS)
Time × site	29, 156	10.64 (<0.0001)	13.77 (<0.0001)	12.07 (<0.0001)	11.12 (<0.0001)	11.02 (<0.0001)
Time × clone	41, 144	2.43 (0.0003)	1.18 (NS)	3.26 (<0.0001)	3.22 (<0.0001)	1.94 (0.0009)
Time × method	11, 174	26.83 (<0.0001)	32.76 (<0.0001)	28.95 (<0.0001)	24.93 (<0.0001)	29.62 (<0.0001)
Time × mgmt.	11, 174	9.79 (<0.0001)	4.53 (<0.0001)	9.08 (<0.0001)	8.79 (<0.0001)	5.94 (<0.0001)
Site × clone	30, 155	2.85 (<0.0001)	6.71 (<0.0001)	5.18 (<0.0001)	3.43 (<0.0001)	5.71 (<0.0001)
Site × method	4, 181	58.31 (<0.0001)	34.01 (<0.0001)	15.35 (<0.0001)	14.68 (<0.0001)	22.23 (<0.0001)
Site × mgmt.	4, 181	11.55 (<0.0001)	34.01 (<0.0001)	15.35 (<0.0001)	14.68 (<0.0001)	22.23 (<0.0001)
Clone × method	12, 173	4.81 (<0.0001)	12.94 (<0.0001)	9.18 (<0.0001)	6.92 (<0.0001)	11.94 (<0.0001)
Clone × mgmt.	12, 173	2.18 (0.0146)	1.80 (0.0486)	2.72 (0.0009)	1.41 (0.0009)	1.60 (NS)
Method × mgmt.	3, 182	15.47 (<0.0001)	45.41 (<0.0001)	20.46 (<0.0001)	19.24 (<0.0001)	29.63 (<0.0001)
Time × site × method	29, 156	10.64 (<0.0001)	13.77 (<0.0001)	12.07 (<0.0001)	11.12 (<0.0001)	11.02 (<0.0001)
Time × site × mgmt.	29, 156	10.64 (<0.0001)	13.77 (<0.0001)	12.07 (<0.0001)	11.12 (<0.0001)	11.02 (<0.0001)
Site × clone × method	30, 155	2.85 (0.0002)	6.71 (<0.0001)	5.18 (<0.0001)	3.43 (<0.0001)	5.71 (<0.0001)
Site × clone × mgmt.	30, 155	2.85 (0.0002)	6.71 (<0.0001)	5.18 (<0.0001)	3.43 (<0.0001)	5.71 (<0.0001)
Clone × method × mgmt.	24, 161	16.04 (<0.0001)	7.55 (<0.0001)	5.91 (<0.0001)	4.04 (<0.0001)	6.80 (<0.0001)
Site × clone × method × mgmt.	30, 155	11.55 (<0.0001)	6.71 (<0.0001)	5.18 (<0.0001)	3.43 (<0.0001)	5.71 (<0.0001)

[*]Degrees of freedom (within, between); [**]NS = not significant ($p > 0.05$).

partially smothered coral tissue and functioned as a sporadic refuge for potential coral predator invertebrates, such as fireworms and corallivore gastropods. HLN units resulted in higher coral colony survival rates, better overall coral colony health condition, and never showed problems of predation by corallivore invertebrates.

Mean percent live tissue cover remained outstandingly high on HLN units, with mean values of 99.1% at PSO-T and of 96.5% in BTA-T, in comparison to BTA-A (84.8%), PME-A (86.6%), and PSO-A (86.5%) (**Figure 7(B)**). There were highly significant temporal, site, methods and management effects (**Table 2**). Percent live tissue cover was overall higher at non-reserve PSO and on HLN units. All interaction effects were significant, with the exception of time × clone interaction. Mean total colony length at the end of the second year was significantly higher at HLN units than at "A frames". Mean colony size at BTA-A increased from 30.6 to 63.8 cm (33.2 cm), from 36.0 to 57.3 cm (21.3 cm) at at PME-A, and from 36.0 cm to 62.4 cm (26.4 cm) at PSO-A (**Figure 7(C)**). But the range of colony growth on HLN units had no precedent in comparison to a decade of coral farming data in Culebra, with a range from 13.8 to 43.1 cm (29.3 cm) at BTA-T, and from 15.7 to 65.4 cm (49.7 cm) at PSO-T. Mean percent increase in total colony length was 58.1% at PME-A, 92.7% at PSO-A, and 107.3% at BTA-A. But mean percent increase in total colony length was 215% at BTA-T and 314% at PSO-T. The magnitude of skeletal extension of cultured corals on HLN units was significantly higher than those grown at "A frames". Monthly coral colony skeletal extension through the second year averaged 1.8 cm at PME-A, 2.2 cm at PSO-A, 2.8 cm at BTA-A, and 2.4 cm at BTA-T, and 4.1 cm at PSO-T. There were highly significant temporal, site, clone, and method effects (**Table 2**). Mean total colony length was overall higher at PSO, and on HLN units. All interaction effects were also significant. These results reflect the negative impacts produced by recurrent runoff pulses at BTA due to increased deforestation along the existing road to BTA.

Total branch abundance per colony during 2012-2013 shifted from 2.1 to 4.6 per colony at BTA-A, from 2.9 to 4.3 per colony at PME-A, and from 2.6 to 6.6 per colony at PSO-A, with a shift from 0.9 to 3.4 per colony at BTA-T, and from 1.1 to 5.4 at PSO-T (**Figure 7(D)**). The ratio of increase in total number of branches per colony was 149% at BTA-A, 59% at PME-A, 218% at PSO-A, 324% at BTA-T, and 438% at PSO-T. There were also highly significant temporal, site, clone, and method effects (**Table 2**). All interaction effects were significant, with the exception of methods × management interaction. Branchiness index (>6 cm) increased from
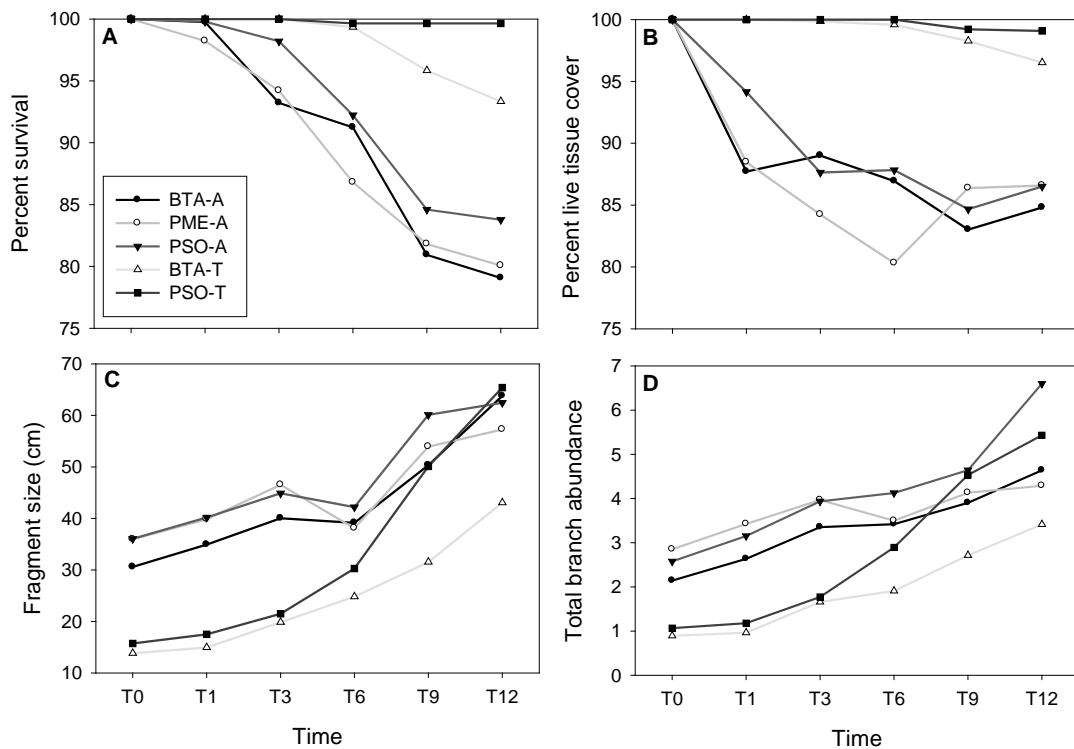

Figure 7. Coral fragment parameters mean values in "A frames" during 2012-2013: (A) Percent survival rates; (B) Percent live tissue cover; (C) Fragment size (cm); and (D) Total branch abundance.

0.73 to 2.31 branches per colony at BTA-A, from 1.03 to 2.00 branches per colony at PME-A, and from 0.92 to 2.40 branches per colony at PSO-A. There was also an increase from 0.13 to 1.14 branches per colony at BTA-T, and from 0.10 to 1.60 branches per colony at PSO-T. Ratios of branchiness index reached by the end of the second year were 129% at PME-A, 237% at BTA-A, and 854% at PSO-A, or a 1.3 to 8.5-fold increase (**Figure 8**). There was a 1875% increase at BTA-T and a 1925% increase at PSO-T, or a 19-fold increase in branchiness index at each site. There were highly significant temporal, site, clone, and method effects (**Table 2**). All interaction effects were also significant. Coral skeletal extension and proportional colony growth and branch production were also significantly higher at PSO and at HLN units.

Coral bleaching was not a critical factor either through 2012-2013. Mean bleaching index values rarely exceeded 1.0 to 1.2, which meant that only sporadic fragments showed minor paling either rapidly after transplanting or during the late Summer. Spatio-temporal patterns were not significant. Similarly, bleaching frequency remained fairly low at the end of the second year, and with very limited impacts that were largely clone-specific and limited to the warmest period of late Summer-Fall of 2012. Bleaching was not considered a cause of coral mortality through the entire project.

4. Discussion

This project produced important lessons for adapting future community-based coral farming and coral reef rehabilitation efforts to forecasted climate changing conditions. Lessons include aspects regarding wild population source propagule selection to maintain high genetic diversity, coral farming site selection, impacts from LBSP and the crititical significance of managing adjacent land uses, and adaptive modifications to coral farming methods to improve success under changing environmental conditions. There were also important lessons learned regarding community-based participation in coral reef management activities.

4.1. State-of-the-Art Genetic Tools vs. Low-Tech Traditional Field Identification of Clones

Maintaining high genetic clone diversity should be a critical component of every coral aquaculture and coral reef rehabilitation project to buffer against any future impacts by disease outbreaks, other epizootics, massive bleaching or environmental variability associated to local human factors or climate change. State-of-the-art genetic characterization confirmed that the six coral clones of *A. cervicornis* used in coral farms in this study were different genetic individuals. Genetic diversity and structure in scleractinian corals vary significantly, reflecting the evolutionary differences between species, but also the type of genetic markers employed. Microsatellite markers were more successful at detecting weak genetic structure than mitochondrial markers, ITS or allozymes
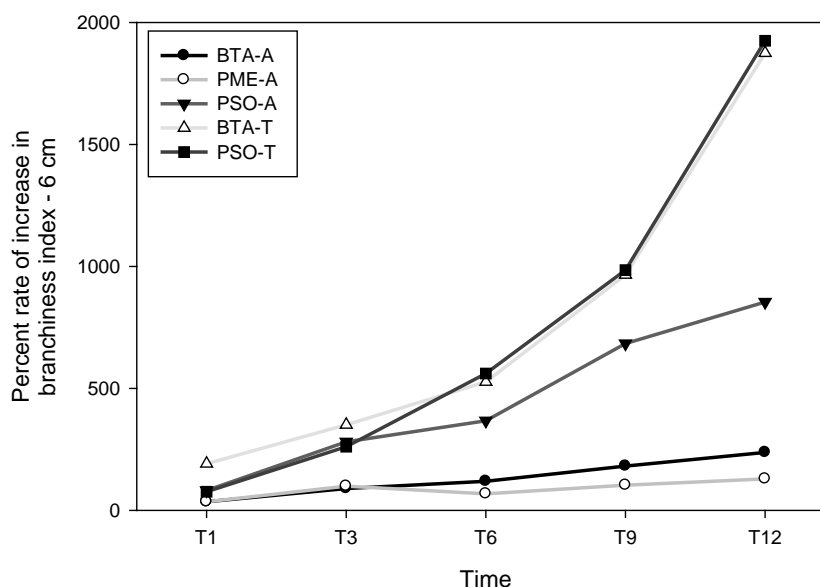

Figure 8. Percent rate of increase in branchiness index.

[80]-[83]. Microsatellite markers used in this study were particularly successful in determining genetic differentiation in *A. cervicornis* colonies located within reef patches in scales smaller than 2 km^2. Genetic differentiation in corals between reefs separated by a few kilometers is generally not significant, except when introgression of alleles is observed [39] [84] [85]. Such small-scale structure was recently evidenced in *A. cervicornis* using spatial autocorrelation of nuclear and mtDNA data [86]. Here, we also evidenced a similar small-scale structure suggesting that remnant *A. cervicornis* wild populations around Culebrita Island and Los Corchos reef system have a high genetic diversity per unit of area and can provide genetically diverse propagules for coral farming and reef rehabilitation efforts. Further, this suggests that high genetic diversity could be rapidly achieved collecting source fragments from relatively small spatial scales.

This study also confirmed that the original phenotypic pre-selection of wild coral samples for coral farming using low-tech traditional field identification of coral genetic clones by simple observation and comparison of source fragments showed a 90% coincidence with different genetic identity as confirmed through complex high-tech laboratory testing. Therefore, experienced coral farmers can very certainly have the ability to select in the field different genetic clones within relative small spatial scales without having to conduct expensive state-of-the-art genetic studies. This finding is very important as it validates traditional, low-tech, community-based methods for selecting coral clones in the field which will continue to be used in remote islands and in traditionally-underserved communities which lack resources and high-tech tools.

4.2. Extreme Rainfall and Poor Land Use Patterns Are a Major Threat for Coral Farms

Extreme rainfall events and storm-associated rainfall, in combination with poor land uses, represented the most significant environmental threat to coral farming success in this study. It is paramount to consider the long-term environmental history of sites selected for coral farming and that of adjacent lands and watersheds during the planning stage of any proposed coral farming project. In our particular case, selected sites showed since 2003 outstanding environmental conditions in support of successful coral farming. But since 2011, there has been a major burst of tourism activities at BTA that has propelled increased traffic through a small local road parallel to the beach, resulting in an increased number of visitors, multiple recreational activities (*i.e.*, charter vessels, kayaking, snorkeling, SCUBA diving), and from 100 to 250 visitors per day. This has also resulted in increased deforestation of land adjacent to the existing road for unpaved parking and areas for turning around public buses. There has been also a recent increase in deforestation of steep slopes at PME for the construction of access roads to private properties adjacent to the shoreline, which has resulted in increased turbid runoff pulses. Areas that have undergone significant alterations in land use patterns or that have undergone significant construction activities may not be suitable sites for coral farming projects as local watersheds can be largely vulnerable to major runoff events and erosion during extreme rainfall episodes. Extreme localized rainfall events have become a common phenomenon across regional to global scales over the last century [87] [88], and particularly across the Caribbean [89], as a typical manifestation of extreme weather events associated to increasing climate change impacts. Major variation in large-scale rainfall patterns [90] and sea surface warming trends across the region [91] have largely been associated to El Niño Southern Oscillation (ENSO) dynamics. Increasing hurricane frequency and severity across the Atlantic has been associated to sea surface warming trends [92]-[94]. There has also been a trend of increasing rainfall extremes associated to tropical storm and hurricane impacts, which may result in localized monthly rainfall anomalies of +150% or higher [95], similar to extremes documented in this study. But the lack of long-term weather monitoring data and the lack of functional monitoring stations networks across multiple locations such as Culebra Island often result in weak spatial and temporal resolution of weather patterns on local scales, and in common failure to document impacts by extreme events. The observed pattern of extreme rainfall impacts through this study has largely coincided with recent trends of increasing SST across the northeastern Caribbean and mid-Atlantic regions [89] [90] [96]. When such events occur, high amounts of rainfall can impact relatively small, localized areas (*i.e.*, individual watersheds) within a relatively short time span (*i.e.*, from a few hours to about 24 - 48 hr). Under such conditions, runoff impacts on coastal waters of small tropical islands can be significant.

Extreme rainfall across waters adjacent to Puerto Rico (<20 km from Culebra Island) based on Doppler radar imagery through this study were significantly stronger than values recorded at a single rain gauge station over the island. Examples of these included a 31 cm rainfall event during a 24 hr period (July 2-3, 2011) and a 78 cm maximum rainfall event associated to Hurricane Irene within approximately 60 hr (August 21-24, 2011). There

was also a 104 cm rainfall event over open sea adjacent to Culebra associated to tropical storm María within only 23 hr (September 13, 2011). Overall, extreme rainfall measured with a rain gauge in Culebra Island during this study resulted in up to 127% to 215% monthly rainfall anomalies. But simultaneous Doppler estimates suggested up to 165% to 521% monthly anomalies for some locations across Culebra Island adjacent to coral farming sites, pointing out at the importance of significant small-scale spatial variability of extreme events.

Extreme weather events have also been shown to influence multiple ecosystems across global scales [97]. Rainfall rates > 2.5 cm/hr, in combination with impacts from soil erosion and sediment-laden, nutrient-loaded runoff, and with significant shifts in wind direction and velocity, and in ocean surface circulation can cause major localized shifts in water salinity, and in sediment, nutrients and microbes delivery to adjacent coral reefs. There is solid evidence that extreme rainfall and runoff pulse events can deliver significant concentrations of dissolved organic carbon (DOC) [98] and dissolved inorganic carbon (DIC) to recipient water bodies [99] [100], capable of locally altering water pH due to increased CO_2 delivery [101]. We did not measure pH during this study, but mean pH across the study sites was determined in a previous study to be 8.24 ± 0.02 (95% confidence interval) across a one year period of time [102]. There is also isotopic evidence of fluctuating impacts of rainfall events in Puerto Rico that show the marine origin of most of the rainfall across the northeastern Caribbean Region [103]. Isotopic analysis can also help elucidate long-term variability in regional rainfall patterns at regional scales [104], and could be a significant tool to quantify the magnitude of runoff impacts and LBSP on coral farming and reef rehabilitation efforts in the future.

Extreme events can have major rapid impacts on adjacent coral reefs communities, particularly on highly susceptible taxa such as Acroporid corals, often resulting in chronic alterations of coastal water quality [30] [33] [105], in localized coral mortality events [33] [106] [107], and in irreversible alterations of coral reef benthic community structure [31]. Therefore, besides major turbid runoff impacts on corals, extreme offshore rainfall events can also produce significant localized alterations of salinity close to the surface that, depending on oceanic surface currents and wind patterns, can further impact adjacent shallow reefs. Such events are predicted to increase with global warming trends [107] and the potential use of temporal variability in DOC/DIC pulses and in rainfall isotopic signals should be incorporated to address future impacts of extreme rainfall events on marine ecosystem dynamics and in coral outplanting success.

4.3. Community-Based Coral Farming: A Race against Adversity

Community-based coral farming efforts in Culebra have been highly successful since 2003 by reintroducing over 15,000 threatened Staghorn coral colonies back to local coral reefs, by maintaining multiple different storm-swell resistant structures, by restoring sexual reproduction potential on local reef scales, by fostering increased fish migration, recruitment and the creation of new essential fish habitats, by fostering fish spillover effects, and by demonstrating that under proper training and education, community-based volunteers can be highly successful in co-managing and recovering coral reef resources with minimal government intervention. This has happened in spite of the increase in illegal deforestation activities adjacent to coral farming sites over recent years, of uncontrolled recreational uses, of the limited support provided by government institutions to the project, and in spite of the weak institutional governance and political will to implement and enforce existing environmental regulations and the existing management plan of CLPNR.

The community-based adaptive shift from "A frames" to HLN units resulted in a major improvement of coral farming outcomes during the second year of the project, in spite of recurrent impacts by extreme rainfall events and storms. For instance, overall coral fragment survival during 2011-2012 in "A frames" improved from a global mean of 73% to 81% in 2012-2013. HLN units incorporated in 2012-2013 had a mean 97% survival rate. These results are substantially higher than those previously documented in several studies elsewhere across the Caribbean, where very high method-specific colony mortality occurred across several locations (**Table 3**). Percent tissue cover averaged 91% in "A frames" during 2011-2012, and 86% during 2012-2013, but improved to 98% in HLN units in 2012-2013. Total fragment length in "A frames" during 2011-2012 averaged 26.9 cm/y and 27.0 cm/y in 2012-2013, but improved to 40.0 cm/y in HLN units in 2012-2013. These results compared favorably with colony growth rates reported from similar coral farming efforts in the literature (**Table 3**) and from colony growth rates in the wild (**Table 4**). Fragment growth rates in "A frame" units in this study was 50% to 545% faster than that reported from coral farming efforts in Florida and 17% more than that documented in Jamaica. But fragment growth rates in HLN units in this study was 122% to 857% faster than that reported from coral

Table 3. Summary of *A. cervicornis* skeletal extension and survival rates measured on coral farms.

Location	Time (m)	Skeletal extension (cm/y)	Method	Coral mortality	Reference
Jamaica	13	23.0	A frame	31% - 100%	[108]
Florida, USA	4	4.18 - 18.0	Lattice	17% (2 months)	[109]
Florida, USA	20 days	5.4 - 7.6	Cinder blocks	87% (2.5 cm size) 13% (3.5 cm size)	[110]
La Parguera, PR	3	-	A frame	2.5%	[46]
Guayanilla, PR	12	-	Nails, epoxy	12%	[57]
Guayanilla, PR	12	52.5	FUCA[*]	2.6% - 12.5%	[56]
Culebra, PR	12 (Y1) 12 (Y2)	15.4 - 37.0 (Y1) 21.3 - 49.7 (Y2)	A frame HLN	16% - 43% (Y1, hurricane) 0.4% - 20.9% (Y2, storms)	This study

[*]FUCA = floating underwater coral array.

Table 4. Summary of *A. cervicornis* skeletal extension rates measured on wild colonies.

Location	Mean skeletal extension (cm/y)	Reference
Buck Island, USVI	7.1	[111]
Buck Island, USVI	10.0	[112]
Florida, USA	4.5	[113]
Florida, USA	11.0	[114]
Florida, USA	4.5	[115]
Florida, USA	12	[36]
Barbados	14.6	[116]
Jamaica	26.6	[116]
Jamaica	12.0	[37]
Jamaica	11.06 (backreef) 12.02 (forereef) 4.35 (pinacle)	[117]
La Parguera, PR	7.8	[118]
La Parguera, PR	10.6 (backreef) 14.7 (forereef)	[119]
Culebra, PR	21.0 - 45.0[*] 41.0 - 77.0[**]	Hernández-Delgado (unpub. data)

[*]Data from 2-year-old outplants; [**]Data from 3-year-old outplants.

farming efforts in Florida and 74% more than that documented in Jamaica. *Acropora cervicornis* mean growth rate in the wild is about 10.66 cm/y, but has been observed to reach values of up to 41 to 55 cm/yr on 2-year-old outplanted colonies, and of up to 41 to 77 cm/yr on 3-year-old outplanted colonies in Culebra Island following previous coral farming efforts. These observations are consistent with previous studies from Guayanilla, Puerto Rico, where growth rates of 52 cm/y were documented [56].

Colony growth on "A frames" was 153% faster than the Caribbean wide mean in the wild. Growth rate on HLN units was 275% faster than that in the wild. The rate of increase in branch abundance per colony was also outstanding, although there is no data in the literature to establish comparisons. These results suggest that community-based low-tech adaptation of coral farming methods used in this study resulted in improved coral colony

survival rates, percent tissue cover, linear skeletal extension, increased branch production, and in increased production of harvestable branches, in spite of continued adverse impacts from extreme rainfall and runoff events, and from storm-related wave action. But recent disturbing evidence suggests that fast skeletal extension rates may not necessarily reflect healthy coral reef conditions. Elevated symbiotic respiration rates as a result of chronic enlarged *Symbiodinium* populations and year-round elevated SST can result in significantly enhanced skeletal extension rates in corals [120]. In turn, such elevated respiration rates can reduce the photosynthesis: respiration ratio of symbionts, restricting the capacity of the coral host to maintain sufficient and sustainable levels of energy reserves (*i.e*., lipids) needed to sustain their essential functions (*i.e*., homeostasis, tissue repair ability, mucous production, immune functions, sexual reproduction, biophysical stress resistance). Therefore, increasing coral skeletal extension rates should be interpreted with caution as it could be implying unequivocal combined impacts from increased LBSP and climate change, and may significantly limit the ability of corals to withstand and recover from disturbance. This aspect should be carefully studied.

4.4. Community-Based Participation: A Key for Successful Coral Reef Rehabilitation

Community-based participation in this project, in direct collaboration with academic resources, continued being fundamental for the success of the *Community-Based Coral Aquaculture and Reef Rehabilitation Program* since 2003 [55]. Community-based participation and project engagement was critical in five ways: 1) It was important to strengthen community-based participants theoretical, technical, and hands-on training and education in coral farming, and reef conservation and restoration methods in support to the management of the local MPA; 2) Strengthen the existing community-based emergency rapid response team capable of rapidly providing technical assistance to local government institutions on emergency reef restoration efforts (*i.e*., after hurricane or tropical storm impacts; or after vessel grounding incidents); 3) Improved the preventive management and maintenance of coral farming units by trained community-based volunteers to reduce impacts from storm swell generated damage to near zero in both, coral farming units and outplanted colonies. Regular maintenance was also important to maintain the structural integrity of farming units and to reduce impacts from potential competing taxa (*i.e*., macroalgae, sponges, hydroids, fire corals) and to remove coral predators (*i.e*., gastropods, fireworms); 4) This project further confirmed our decadal-long experience that community-based participation and engagement is a critically successful behavior-transforming outreach and educational tool; and 5) Strengthening and empowering base communities to improve their problem analysis and decision-making tools regarding managing their adjacent shallow coral reef ecosystems that constitute their first line of defense against storm swells and sea level rise.

Nonetheless, reciprocal feedback from government institutions was lacking most of the time, providing very limited support, in particular, a very weak enforcement of existing no-fishing regulations within the no-take MPA. There was also no enforcement at all regarding LBSP, deforestation of adjacent lands, and the implementation of mandatory erosion and sedimentation control measures. Weak governance and natural resource management failures have been previously identified as key roadblocks to the successful conservation of coastal resources in Puerto Rico [33] [121]. Weak governance can be a major roadblock to MPA, coral farming and reef rehabilitation success, and deserves major attention. A particular concern has also been the recent burst in dubious political decisions favoring zoning changes and private development in highly sensitive areas on very steep slopes prone to erosion and in lands adjacent to the shoreline through fast-tracked permits, which halt public scrutiny. Fast-tracked permitting procedures often lack full evaluation of environmental impacts and have resulted in non-sustainable development practices with strong adverse impacts in small tropical islands [69].

4.5. Timing of Coral Farming Activities Is Critical for Project's Success

Project timing and phasing on funding agencies is often never coupled with the appropriate environmental conditions needed for successful coral propagation. It is critical that coral farming projects start during cooler months (Winter to Spring) and this must be stressed out to funding and regulatory agencies when planning funding and reporting cycles for such projects. Coral transplanting during summer or fall should be avoided to reduce or prevent coral mortality and the probability of failure due to high SST stress, runoff impacts, or potential disease outbreaks. Most coral species are also completing their gametogenesis cycle during the warmer months, which require strong energy expenditure and may further compromise their survival if fragmenting and transplanting is conducted before mass spawning occurs.

4.6. The Role of No-Take MPAs: A Matter of Political Will

No-take MPAs are often established to protect and help recover reef fish stocks, as well as to restore herbivorous fish guilds, from fishing impacts [122]. Increased herbivory can reduce algal cover inside MPAs [123] and has been shown to be important for recovering coral reef ecosystem resilience by fostering increased coral growth [124] [125]. But it has been suggested that the Wider Caribbean Region has a higher vulnerability to climate change impacts due to its lower resilience, faster rates of macroalgal growth, higher rates of algal recruitment, basin-wide iron-enrichment of algal growth from aeolian dust, lack of Acroporid corals, lower herbivore biomass and missing groups of herbivores, in comparison to Indo-Pacific coral reefs [126]. Lack of coral and fish functional redundancy of Caribbean reefs can also increase its long-term vulnerability to climate change and ocean acidification impacts [127]. This may suggest that even the establishment of fully functional MPAs could have only a limited impact on coral reef recovery unless policy makers confront the accelerating negative effects of the global-scale sources of coral mortality [123]. There is mounting evidence that even coral reefs located within no-take MPAs are rapidly declining [63] [64] [71] [128]. Unequivocally, these trends point out at the need of vigorously implementing integrated management strategies that support reef resilience, complemented by strong policy decisions [129], to reduce the rate of impacts associated to multiple local human-driven factors and climate change. But such integration requires strong, management-oriented, novel scientific supporting research with a strong community-based integrative and participatory approach. This is where community-based coral farming and reef rehabilitation efforts become critical to improve no-take MPA success.

In this study, the no-take CLPNR resulted in no significant improvement of coral farming success due to rapidly changing land use and recreational use patterns adjacent to BTA site. Coral survival rates, percent live tissue cover and colony growth always resulted higher at non-MPA control site PSO. There has been a dramatic increase since 2011 in site visits at BTA by tourists either from beach access, kayaking, jet skiing or from charter vessels. Coral outplanting activities to natural reef bottoms within the reserve have resulted in a significant aesthetic improvement of the local sheltered coral reefs and in a significant increase in fish diversity, abundance and biomass, and in the abundance of endangered green turtles (*Chelonia mydas*), an impact that has not been reached yet at the non-reserve control site. This suggests that no-take MPAs, in combination with coral farming and outplanting, can be highly successful to improve overall reef resilience. But concomitantly, successful reef rehabilitation has also fostered an interest to visit the reef, resulting in a net increase in the number of visitors, and in increased traffic and road widening near BTA. This has exposed multiple areas to soil erosion, which has in turn resulted in dramatic increases in runoff impacts. Sediment delivery has been identified as a major threat for nearshore coral reef ecosystems in Culebra [130]. There was already a successful implementation of runoff controls through community-based efforts, with the collaboration of government agencies [131]. But these measures have not been enough as governance and enforcement still remain weak. An example of such weakness is that by the end of the study period, primary cacti forests across steep lands adjacent to the non-MPA control coral farming site at PSO were also being fully deforested by local residents with the support of the local government to allow illegal invasions of public lands to establish permanent camping grounds for weekend enjoyment of local residents. This now represents a major unprecedented threat of potential sediment-laden runoff impacts similar to that of PME and BTA. Coral farms originally established at PME (within the CLPNR) during the first year of the project had to be relocated due to excessive pulse runoff impacts. Runoff impacts dramatically increased during the project at BTA as well, and now runoff is becoming a major threat at PSO, but no preventive or remedial action from government institutions have been implemented yet. Therefore, ignoring the reality of chronic environmental degradation and the total lack of political will to enforce existing zoning and environmental regulations constitute also a major roadblock to success.

There is still a need to incorporate stronger management and enforcement measures to the existing management plan of the CLPNR to further reduce such adverse impacts and to regulate impacts from recreational activities. But also there is still a need to review, update and fully implement a science-based land use management plan to reduce impacts of LBSP and inappropriate land uses on adjacent coastal ecosystems. Coral farming and reef rehabilitation success will largely depend upon strong governance, and successful management and enforcement of adjacent land uses and recreational activities. There is also a major need to link ecological resilience to governance structures, economics and society in order to successfully and sustainably manage and rehabilitate marine ecosystems [132]. Failing to recognize that reality could also represent a major roadblock for successful conservation and management through a sustainable participatory process.

5. Conclusions

Community-based, low-tech coral aquaculture approaches used in this study proved to be successful, reliable and highly cost-effective tools to conserve and restore threatened Staghorn coral populations with minimum intervention and maintenance, and generated multiple management-oriented lessons learned. The *Community-Based Coral Aquaculture and Reef Rehabilitation Program* has continued to be a successful model to empower wider Caribbean community stakeholders to implement basic coral reef conservation and coral transplanting methods. This could have major implications in helping base communities engage into conservation-oriented coral reef management activities, and to help communities adapt and manage climate change impacts on their "backyard" coral reef ecosystems. Hands-on, behavior-modifying, transformative education continued to be a crucial product of community integration and active participation, improving local stewardship, and fostering their successful integration into planning, decision-making processes, and in the implementation of local-based coral reef and fisheries conservation-oriented and restoration-oriented management. However, rapid adaptive responses in low-tech coral farming and reef rehabilitation will become critical to keep up with climate change impacts in the near future. Community-based efforts will continue to be fundamental to successfully foster the rehabilitation of reef ecosystem's resilience, biodiversity, ecological functions, and its socio-economic, ecological and environmental benefits and services. Further, the integration of the academia, NGOs, fisher communities, base communities, private stakeholders and government institutions has become a successful collaborative model that can be applied through the wider Caribbean Region and will be important in a time of economic constraints across developing island nations.

Nonetheless, there is a particular concern with the still prevailing lack of adaptation capacity of multiple coastal base communities to climate change impacts, including sea level rise and loss of coral reefs, across many small island nations through the wider Caribbean, which could affect the sustainability of coastal community livelihoods [69]. Further, weak governance and lack of political will to enforce existing regulations can be a major deterrant of community compliance and a roadblock to project success. This points out at the importance of coral reef rehabilitation to foster increased coral reef ecosystem resilience, functions, and services, further improving the adaptability of coastal communities and coral reef ecosystems to climate change. A concerning call for precaution is also brought up by documented coral skeletal extension rates in this study, as well as in some recent studies of *A. cervicornis* farming [56], as it could be the result of successful methodologies being used, but may also imply significant combined impacts from increased LBSP and climate change which may significantly compromise coral colonies ability to withstand disturbance and may explain significant recurrent mortality episodes from multiple disturbance events.

It is not clear whether we can foster acclimatization responses in coral reefs to futures characterized by recurrent ecological surprises, non-linear change and unexpected long-term consequences of climate change and ocean acidification. The speed at which climate change is impacting reef ecosystems leaves little opportunity for evolutionary processes to come to the aid of corals and other reef inhabitants, thus survival will be highly dependent upon any natural resistance already existing in the gene pools today [66]. It will also rely upon successful governance, management of reef resources and land use patterns, and upon consistent enforcement of existing environmental regulations. Therefore, the identification of high-temperature resistant genetic clones has become a critical tool for successful coral propagation and reef restoration [133]. On that line, we were successfully able to identify, propagate and restock local depleted populations using six different shallow-water genetic clones of *A. cervicornis* highly resistant to existing warm surface water conditions. But efforts should also be implemented to propagate deepwater genetic clones across deepwater habitats to improve ecological scales of connectivity across multiple spatial scales.

Rehabilitated coral reefs are part of a human-coupled ecosystem. The most important challenge or potential roadblock to future progress of coral farming and reef rehabilitation overall is largely related to climate change-associated conditions, in combination with poor land use management and weak governance. Therefore, human activities must be integrated into the equation for coral farming and reef rehabilitation success. Environmental history, ecological trajectory and ecosystem conditions are critical factors for any coral propagation success. This suggests that positioning of coral farms is fundamental. Special considerations should be given to factors such as: 1) environmental history of each proposed coral farming site; 2) environmental conditions of adjacent reef communities; 3) sediment movement (*i.e.*, bedload, sedimentation rates); 4) distance of sources of runoff; 5) exposure to wave action, winter storm and hurricane swells; 6) distance to large sand deposits (risk from

sediment bedload and sandblasting during bottom swells and storms); and 7) magnitude and extent of recreational activities impacting the system, even with so called "low-impact" activities. Therefore, carrying capacity and the limit of acceptable change need to be determined for such sites.

Successful coral farming and reef rehabilitation will also require functional synchronized and integrated management efforts to address land use patterns, water quality issues and fishing activities (to improve herbivore fish guilds) in order to improve ecosystem conditions for enhancing coral and fish recruitment, and overall biodiversity recovery [127]. It will be critical to fully implement LBSP controls, as well as appropriate watershed-scale management plans to control runoff impacts. Runoff-associated bleaching events and colony mortality can be highly clone-specific, as well as site-specific and event-specific. No generalizations can be made because not all coral clones respond the same, or because impacts from any given extreme rainfall event can produce different impacts on different locations, and on different times of the year. The combination of high SST and factors such as meso-scale water quality (*i.e*., from gyre currents) can also have significant impacts on project outcomes and should be closely monitored. Achieving successful local management of reefs is vital to maintain the sustained net production of coral farms, and of reef structure, and therefore the provision of the important ecosystem services that they provide. These measures are also vital for buying time for reefs while global action on climate change is implemented [61]. Any problem or imbalance in any of these elements will have in the long run a negative impact on rehabilitation success. If such impacts occur in combination with increasing forecasted climate change-related negative impacts in the near future and increased reef degradation rates, they could make community-based coral reef rehabilitation more challenging. In combination with declining reef condition and increased inability of coral and fish larvae to identify suitable natural reef bottoms for settlement [134], it could potentially drive rapidly declining, transient coral reefs into the slippery slope to slime [135].

Acknowledgements

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