Report of a phytoplankton and water quality survey made in the 'Sukkursu canal, Salina, in connection with a 'red water' episode in June 2017.

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1 Introduction

The present report was commissioned from the Department of Biology (DoB), Faculty of Science, University of Malta, by the Environment and Resources Authority (ERA) following the occurrence of a 'red water' episode in the Sukkursu Canal, Salina, Malta on the days immediately preceding 19 June 2017. This 'red water' event may have been caused by an 'algal' bloom. This therefore necessitated the collection of water samples from the canal for surveys of phytoplankton, in an attempt to identify the species causing the algal bloom, and for determination of water quality to assess the potential cause of the episode. The DoB commissioned the present authors to undertake the necessary studies.

2 Materials and Methods

Collection of water samples was carried out on Monday, 19 June 2017 at around noon. The weather on the day was sunny, with a light northwesterly wind and a maximum air temperature of around 33^{o}C. A gentle surface current moving towards the head of the inlet could be observed along the Sukkursu canal.

The intensity of the red colour of the water increased on going towards the head, with the pinkish-red colouration becoming most evident midway along the canal (Figure 1). *In-situ* measurements of water quality parameters were made at three sampling stations along the canal, the locations of which are shown in Figure 2.

Figure 1: Pinkish-red coloration of the water in the eastern part of the Sukkursu canal on 19 June 2017, at midday.

Replicate *in-situ* measurements of temperature, electrical conductivity, salinity, water turbidity, and dissolved oxygen concentration were made at the water surface at each of the three stations, using a YSI 650 MDS meter connected to a 6920 V2 multi-parameter probe. The unit was calibrated according to the manufacturer's instructions prior to the field session.

At each station, replicate water samples were collected at an approximate depth of 20cm below the water surface. Prior to the actual collection of samples, the sample bottles were pre-cleaned in the laboratory, and rinsed on site with water from the same station being sampled. Water samples used

for phytoplankton surveys were collected in glass containers, whilst those used for analyses of water quality were collected in plastic containers. Samples intended for phytoplankton surveys were fixed with Lugol's Iodine upon collection.

All samples were transported to the laboratory in a cooler box, with the temperature maintained at approximately 4°C, in order to maintain the integrity of the samples.

Analysis of phytoplankton was undertaken using the Utermöhl technique, whereby phytoplankton was first concentrated using settling chambers, followed by taxonomic identification (Hallegraeff, Anderson, Cembella, & Enevoldsen, 2003; Honer, 2002; Tomas, 1997) and quantification using an inverted microscope (Maranon & Hasle, 1978). After settling, each replicate sample was first scanned to ensure that an even distribution of microalgae was present on the chamber bottom, and to establish which target species were to be counted for abundance estimates. Taxonomic identification and enumeration of the sedimented microalgal cells was then made. This was restricted to the four most abundant species; hence identification of all microalgal species encountered in a sample was not attempted. Results for the four most abundant species are given as extrapolated number of cells per litre of sample.

Samples intended for chemical analysis were sent to an ISO 17025 accredited laboratory, CADA s.n.c. based in Italy, and accredited by ACCREDIA (the Italian national accreditation body). Samples were analysed for the parameters listed in Table 1 according to the indicated standard methodology; the laboratory is accredited to undertake analysis of marine waters for all the parameters listed in the Tables below.

Table 1: Standard methodology used for analysis of water samples by CADA s.n.c.

Parameter	Analytical methodology	Units
pH	APAT CNR IRSA 2060 Man 29 2003	pH units
Ammonia Nitrogen	APAT CNR IRSA 4030 C Man 29 2003	mg/l
Nitrates	APAT CNR IRSA 4040 A2 Man 29 2003	mg/l
Nitrites	APAT CNR IRSA 4050 Man 29 2003	µg/l
Phosphates	APAT CNR IRSA 4110 Man 29 2003	mg/l
Total Phosphorus	APAT CNR IRSA 2060 Man 29 2003	mg/l
Total Nitrogen	APAT CNR IRSA 5030 Man 29 2003 + APAT CNR IRSA 4050 Man 29 2003	mg/l

Figure 2: Aerial images showing the locations of Stations A - C in the eastern part of the Sukkursu Canal where sampling was carried out. The bottom image shows an enlarged view of the area enclosed within the red box in the top image. Image source: Google Earth

3 Results

3.1 Water Quality

Mean values and standard deviations of the water parameters measured *in situ* at each of the three stations are given in Table 2, whilst mean values and standard deviations of the water quality parameters analysed in the laboratory are given in Table 3. Certified analysis reports issued by CADA s.n.c. are appended to this report.

From the *in-situ* data, it can be noted that values of salinity increase along the length of the canal towards the head, while values of turbidity also increase along the same direction. Dissolved oxygen levels were very low, which is to be expected given the very low circulation of the canal water; this probably also linked with the clear smell of hydrogen sulphide especially at the inner parts of the canal, indicating that anaerobic processes were operating. Readings taken at Station C showed the highest variability for all parameters except turbidity.

The results of chemical analyses for nutrients (Table 3) did not indicate the presence of ammonia, nitrites and phosphates at detectable levels in any of the three stations. Nitrates were recorded at detectable levels only at Station A. Total phosphorus and total nitrogen had comparable levels at all three stations.

Table 2: Mean values (+/- 1 SD) of in situ measured parameters for the three stations along the eastern part of the Sukkursu Canal, Salini.

Parameter	Units	Station A		Station B		Station C	
		Mean	±SD	Mean	±SD	Mean	±SD
Temperature	˚C	27.07	0.00	28.11	0.01	28.20	0.59
Conductivity	µS/cm	48238	16.97	49599	46.67	54799	3486.04
Salinity	ppt	31.40	0.01	32.37	0.04	36.21	2.62
Turbidity	NTU	26.80	5.66	182.50	22.91	331.30	3.96
Dissolved Oxygen	%	22.65	7.00	28.45	6.58	30.20	12.87

Table 3: Mean values (+/- 1 SD) of water quality parameters analysed in the laboratory on replicate samples collected from each of the three stations along the eastern part of at the Sukkursu Canal, Salini.

Parameter	Units	Station A		Station B		Station C	
		Mean	±SD	Mean	±SD	Mean	±SD
pH	pH units	7.25	0.1	7.3	0.14	7.7	0.00
Ammonia Nitrogen	mg/l	ND < 0.01	0	ND < 0.01	0	ND < 0.01	0
Nitrates	mg/l	0.28	0.02	ND < 0.01	0	ND < 0.01	0
Nitrites	µg/l	ND < 10	0	ND < 10	0	ND < 10	0
Phosphates	mg/l	ND < 0.01	0	ND < 0.01	0	ND < 0.01	0
Total Phosphorus	mg/l	0.1	0.00	0.6	0	0.7	0
Total Nitrogen	mg/l	0.7	0.00	0.3	0	0.3	0

3.2 Phytoplankton

The results of the phytoplankton analyses are presented in Table 4, Figure 3, and Figure 4. The most abundant taxa recorded from the survey area (*Cryptomonas* spp. and *Rhodomonas* sp.) were naked marine flagellates (Kingdom Chromista; Phylum Cyrptista; Class Cryptophyceae). A thecate flagellate, *Alexandrium* sp. (Kingdom Chromista; Phylum Miozoa; Class Dinophyceae), was also recorded frequently although at lower abundance compared to the cryptophytes. As can be seen from the results, two of the four most dominant species (*Cryptomonas* sp.2 and *Rhdomonas* sp.) were collected from all three stations. *Alexandrium* sp. was collected from two stations whilst *Cryptomonas* sp.1 was only noted from Station A.

Microalgal abundance was relatively much higher in Station C, compared to the other two stations (Figure 3). This was a consequence of the relatively very high abundance of *Cryptomonas* sp.2 in Station C, which was approximately 30 times higher than the next most abundant value of the same taxon (Figure 4).

Table 4: Concentration of cells for the most abundant species recorded from individual samples collected at each station

Station A		
	Sample 1	**Sample 2**
	Conc x 10^5 cells / L	**Conc x 10^5 cells / L**
Cryptomonas sp. 1	2.16	1.92
Cryptomonas sp. 2	1.85	1.81
Rhodomonas sp.	8.96	5.73
Alexandrium sp.	0.31	0.47
Station B		
	Sample 1	**Sample 2**
	Conc x 10^5 cells / L	**Conc x 10^5 cells / L**
Cryptomonas sp. 2	2.55	2.95
Rhodomonas sp.	0.12	0.19
Station C		
	Sample 1	**Sample 2**
	Conc x 10^5 cells / L	**Conc x 10^5 cells / L**
Cryptomonas sp. 2	88.30	71.08
Rhodomonas sp.	5.64	6.89
Alexandrium sp.	1.57	1.88

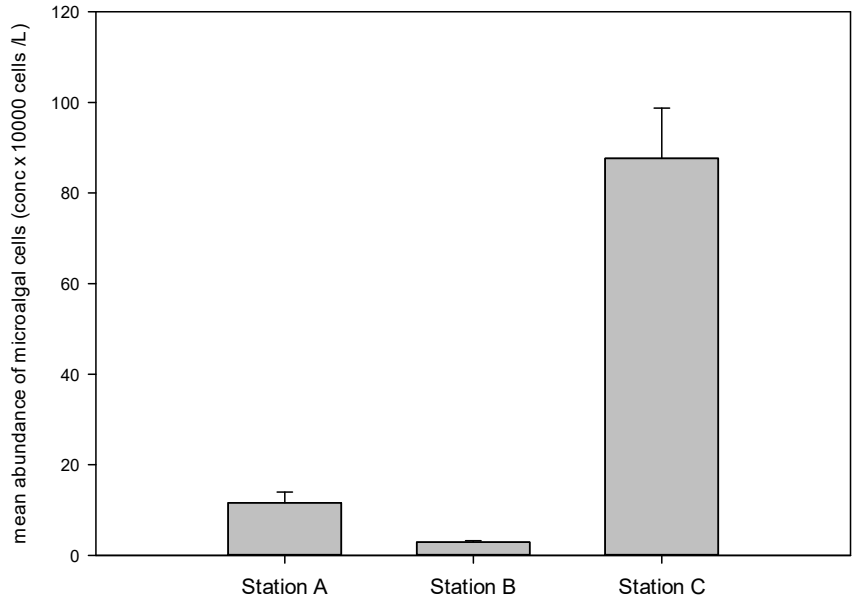

Figure 3: Mean abundance of microalgal cells from all four dominant taxa in the three sampling stations. Error bars represent one standard deviation from the mean value.

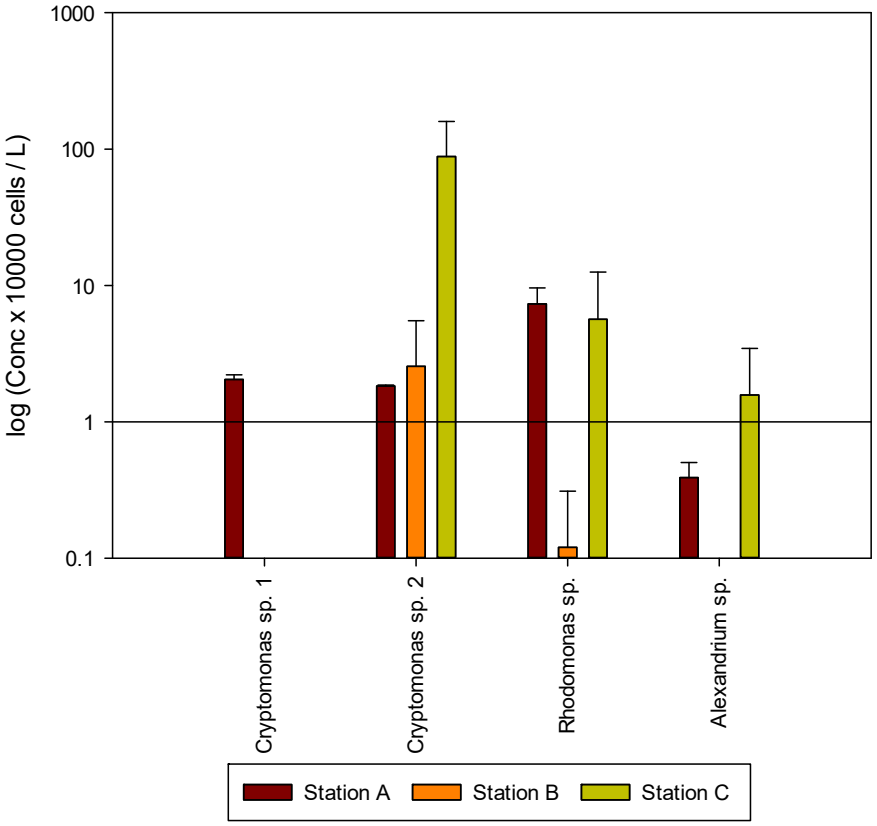

Figure 4: Mean concentration of microalgal cells from each of the four dominant taxa at each sampling station. Error bars represent one standard deviation from the mean value. The scale on the Y-axis is logarithmic.

It should be noted that the samples collected from Station B had a large amount of plant fibres and other debris that hindered the enumeration and identification process. This could be due to the shallow depth of the sampling station (approximately 1 m – 1.5 m) and the presence of a large amount of organic material, mainly debris from *Posidonia oceanica*, that was present on the bed and banks of the canal. Furthermore, the canal had been dredged in the days preceding the sampling session (Site Warden, pers. comm.). Therefore, the results from Station B should be treated with caution and only considered as indicative.

4 Discussion

Although no background data on the abundance of phytoplankton was available for comparison, the results suggest that the 'red water' event was the direct result of a short-lived and anomalous peak (a 'bloom') in the abundance of microalgae in the Sukkursu canal. This was most pronounced in Station C, where very high abundances of *Cryptomonas* sp.2, *Rhodomonas*, and *Alexandrium* sp were recorded. It cannot be assumed that the high abundance of algal cells this station was an autochthonous development, as cells originating in other parts of the canal may have been transported towards Station C by the movements of surface water observed at the time of survey. During the present study, the water in Station C was warmer, more saline, and more oxygenated than that in other stations at the time of survey. Moreover, levels of total Phosphorous were higher and levels of total nitrogen were lower than in other stations.

4.1 General conditions required for blooms to occur

The published literature gives no consensus value regarding the algal abundances that would be considered to constitute a 'bloom' event. Establishment of such criteria would be dependent on the species concerned, on background levels of algal abundance monitored over a period of several years, and on cell size. As such, the use of the term 'bloom' is being used descriptively in this context. The development of an algal bloom event is dependent on the interaction of multiple environmental factors including availability of nutrients, insolation, water circulation, hydrographic properties, and water chemistry. The combination of factors that generate and maintain an algal bloom is not well understood, and is likely to vary across locations, seasons, and species. Nonetheless, it should be assumed that high levels of nutrients would be one of the principal requirements for such an event to occur. This requirement has been observed to be a key trigger for algal blooms in water bodies in different parts of the world (Abdenadher et al., 2012; Deng et al., 2014; Mallin, 1994; Tian, Huang, Yu, Chen, & Hong, 2014).

4.2 Species composition

The four dominant taxa recorded from Is-Sukkursu canal have been documented as components of Mediterranean algal blooms in several studies (Abdenadher et al., 2012; Basterretxea, Garcés, Jordi, Masó, & Tintoré, 2005; Giacobbe et al., 2007; Gilabert, 2001; Vila et al., 2005).

More specifically, the species composition of the algal bloom in the Sukkursu canal (comprising *Rhodomonas* sp. and *Cryptomonas* spp) was similar to the 'winter period' successional stage of the annual succession in the Mar Menor lagoon on the Mediterranean coast of Spain (Gilabert, 2001), a succession which was correlated with nutrient dynamics. There is no long-term baseline data from Is-Sukkursu canal that may be used to place the species composition of the bloom in a successional context, and it is therefore not known whether phytoplankton succession in this habitat follows patterns that are similar to those observed in other sites.

The other genus recorded from Is-Sukkursu canal, *Alexandrium*, has also been noted to bloom in various parts of the Mediterranean, with the general initial stimulus being nutrient enrichment. This is a necessary, but often not sufficient condition for the development of algal blooms, as other factors may be required for blooms to occur.

Studies in the Mediterranean have shown that proliferation of *Alexandrium taylorii* was favoured by high levels of nitrogen and phosphorous, as well as by warm temperatures and calm conditions (Giacobbe et al., 2007). Similarly, blooms of *Alexandrium minutum* were promoted by nutrient enrichment as well as through confinement of the water by the physical structure of the water body, reducing cell dispersion and concentrating nutrients (Vila et al., 2005). Similar conditions for *A.catenella* and *A.minutum* blooms were recorded by Bravo, Vila, Masó, Figueroa, and Ramilo (2008) who also noted that accumulation of cysts in the bottom sediment was a potentially important factor for the recurrence of such blooms. Blooms of *Alexandrium taylori* in the Balearic Islands were associated with high nutrient loads but also required mild breeze conditions during summer (Basterretxea et al., 2005). The limiting nutrients also vary across species. In the case of *Alexandrium minutum* phosphate concentration appears to be more important than nitrogen or temperature in the regulation of population size of this species (Abdenadher et al., 2012).

4.3 Triggers for a bloom event in the Sukkursu Canal

The specific triggers stimulating proliferation of phytoplankton in the Sukkursu Canal are not known. However, several conditions that are generally associated with the formation of algal blooms are also present in this site.

(a) **Hydrographic confinement**: From the hydrographic point of view, the canal itself is confined, with only a relatively narrow opening to the sea, and this would be expected to concentrate both nutrients as well as algal cells, favouring the formation of blooms. The flow of water along the canal may be further restricted by the accumulation of organic debris, as was the case at Station B.

(b) **Nutrient enrichment:** The Sukkursu Canal is situated at the mouth of the Wied il-Ghasel valley system which drains a considerable land area, and which is presumed to represent a regular source of nutrients into these waters. The nutrient levels at the time of survey should be interpreted with caution, as their range and frequency of fluctuation is not known. However, the highest phosphate concentrations (Station C) were associated with higher abundances of *Alexandrium* sp., following the pattern reported by Abdenadher et al. (2012) for *A.minutum*. Conversely, the highest nitrogen concentrations (Station A) were associated with relatively higher abundances of *Cryptomonas* as observed by Deng et al. (2014) in Lake Taihu, China

(c) **Occurrence of light winds:** Light winds which, according to Basterretxea et al. (2005) promote bloom formation in *Alexandrium taylori*, are frequent in this area, although stronger winds, which tend to disrupt the formation of blooms, also occur regularly.

5 Recommendations

If prevention of future blooms is required, then removal of at least one of the three key conditions for bloom formation would be necessary.

(a) Ongoing monitoring of water quality and phytoplankton to establish background water quality and community characteristics and to provide early detection of blooms.

(b) Regular monitoring of the bottom of the canal, particularly at known ‘choke points’, and at its mouth, to determine whether any inordinate accumulations of sediment are present. If these sediment accumulations lead to significant reductions in the rate of water circulation, the water flow could be increased by judicious and ‘surgical’ dredging of the bottom of the canal. The frequency of monitoring should be higher at the transition of the wet season into the dry season, when blooms start to occur.

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