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LONGINES L'elegance du temps depuis 1832

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Cover Picture: Full moon alignment at Mnajdra lower temple. (Photo: Mr. Maurice Mieallef).

Malta



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Editorial

Is There a Future for Xjenza?

This issue of Xjenza is a single issue for the year 2000. It is not for the lack of sponsorship or funds, or an overworked editorial board. There simply were insufficient contributions to justify more than one issue. In the main, the papers featured in this issue were the only ones submitted to Xjenza during the year. One might well ask: "Has no more research of local interest been carried out or written up?" or on a more optimistic note: "Is all the research that is carried out being published in international journals?" If the answer to the latter question is yes, then there is no point in prolonging the soulsearching. If the answer is yes in either case, the conclusion must be that there obviously is not the market for Xjenza. In which case, do we call it a day, and fold?

Realistically speaking, though, do we honestly believe that this is the case? Considering the number of dissertations that are presented by students reading for a degree in the Sciences, at the end of each academic year, are we to believe that all this research receives international readership, or are the results left bound in a thesis somewhere in the supervisor's office or laboratory gathering dust? Are we giving our students the necessary training in scientific communication? The Biology Department hold an annual symposium on the current research effected in the area, and students do present their work and write abstracts and short papers that are collated in the form of conference proceedings. Xjenza publishes these abstracts in the December issue of that year. The Department of Pharmacy do likewise. However, these are exceptions to the rule.

It would be an excellent idea to have a formal course in science communication in every undergraduate science degree, where students are made to publish research results as part of their degree course work. In this way, scientists going into different walks of life would learn to communicate with colleagues and also with nonscientists, be the latter, the general public or the media. The ability to communicate one's ideas and thoughts effectively is becoming increasingly important as society and economics grow more entwined with science and technology.

Undergraduate dissertations are by no means the only potential source of worthy Xjenza papers. In fact we have only had a few articles based on students' undergraduate work in the nine issues of Xjenza over the past four years.

While acknowledging the contribution made by a number of colleagues, and mindful of the fact that the journal should not be a forum for these few, where are all the other fully-fledged scientists? Where are the results of their research on crucial local issues - on atmospheric pollution, on marine pollution, on waste management? Why is it that we only read snippets about such local research in newspapers – headlines to cater for the politicians' whims be they of one colour or of another – it really does not matter. So where are all our local experts? Is it truly mainly the case that it is foreign experts who are engaged to give an assessment of a particular situation requiring scientific expertise at an exorbitant fee while the local experts are denied the opportunity to make their valid contribution in a matter clearly within their field of specialisation? Or is it possible that the results of much work is precluded from being published for reasons of 'political sensitivity'?

Or is all this perhaps indicative of a more serious underlying malaise that is spreading throughout the academic corridors of this country? Working conditions for academics are so poor that scientists perhaps are asking themselves the question "What am I doing this research for? There is no remuneration attached to it, so why should I waste my time when I could be doing lucrative work?" Is the same perhaps being asked when it comes to writing up a paper with a view to publishing in Xjenza?

We embarked on the project of this journal in 1996, and we did so, knowing the difficulties and frustrations, with energy and enthusiasm because we felt there was a need for such a journal – a journal to provide a local outlet for the work of scientists in Malta. One aim was to enhance undergraduate science literacy and communication skills. To date we have had only one undergraduate contribution. We urge many more students to come forward, and we ask lecturers to encourage students to do so.

This is a plea to all of you out there to keep Xjenza going in such ways for the good of all, otherwise we shall be facing extinction like so many journals have before us. Believe me, it is worth saving if only because the effort of re-starting is devoutly to be un-wished upon anyone. On a personal note, if you will permit it, here is also hoping that this plea to you as Editor will fall on fertile ground. I leave it with you readers to mull this over, consider how you may contribute through submission of papers or help others to contribute a paper to our Xjenza, and keep it (and me) going for the benefit of Maltese science.

Angela Xuereb Editor.

Research Article

Alignments Along the Main Axes at Mnajdra Temples

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Summary: The Mnajdra temple has been the subject of controversy over the years with regards to the sun alignments with the lower (solar) temple. The aim of this paper is to investigate whether there were in fact any alignments with celestial bodies along the main axes at the Mnajdra complex. An analysis of the use of a megalithic unit for the erection of the Mnajdra temples was also carried out.

Keywords: equinox, solstice, precession, declinations, azimuth, ellipse – eccentricity.

I. Introduction

Did megalithic man possess the fundamentals of field astronomy, or archaeoastronomy as otherwise known today? The conclusions about the Mnajdra complex derived so far certainly show that prehistoric man was indeed a good observer of the movement of the sun. Without any kind of speculation, a prehistoric builder must have noted the position of the rising sun and was intrigued or alarmed by the stationary position of the sun's disc at the summer and winter solstices. Between these two extreme sightings, the sun appears to perform a pendulum movement through one whole year. Even more intriguing is the effect of the movements of other celestial bodies that could have been observed in the lower, high and small trefoil temples at the Mnajdra complex. Distant markers or foresights might have been natural alignment points (cleft or notch), but holes with stakes or stone pillars might have been other possibilities to establish a fixed foresight. The shifting of a distant mark or foresight is not very practical, so the observer must have marked the final position of a required back-sight by a rod or pointed stone. A pointed stone increases the accuracy when sighting a foresight in line with the rising sun. It is important to note that not all pointed stones are phallic symbols as usually accepted in archaeology. Pointed markers may also serve alignment purposes. At the entrance of the south temple at Mnajdra, one finds a small pointed stone on the left-hand side of the main entrance. A similar pointed stone seems to be missing at the right-hand side but there is a space allotted to it. These indicators agree with the procedure of builders who usually fixed markers in the ground to establish the direction of the proposed temple or building. Was the Mnajdra complex used as an astronomical observatory to observe the movement of celestial bodies? Who were the builders of the Mnajdra complex? Were they craftsmen of Maltese origin or did they arrive in Malta to design and build the temple for solar and lunar predictions which were associated with the annual agricultural phases? The objective of this paper is to correlate further and extend

the discussion regarding the orientation of the main axes of the Mnajdra temples, to investigate the possibility of any astronomical alignments, and to consider the geometrical properties of their ground plans.

The Mnajdra temple of Qrendi Parish has been surveyed and analysed for many decades since its excavation in 1836. Many archaeologists, excavators and technical missions have written about this temple from the archaeological and historical point of view but nobody had ever attempted to consider the temple's orientation up to the year 1979. Alfred Xuereb and Paul I. Micallef (1990), as well as Frank Ventura and George Agius (1980), initiated a new approach by investigating the orientation of this unique temple in the Mediterranean. The lower Mnajdra temple provided a challenge in fieldwork where linear and angular measurements are concerned. The preliminary task of the investigators was to survey in detail the lower temple and to establish the true geographical north by solar observations.

As all the results depended upon the accuracy of the true geographical north, angles were scrupulously measured. The first result that was obtained by Micallef, was that the Mnajdra temple is a device, the use of which allows one to predict the first day of the four seasons of the year, 21st March (Vernal equinox), 21st June (Summer solstice), 23rd September (Autumnal equinox) and 22nd December, (Winter solstice) (Micallef, 1990). Figure 1 shows how the seasons may be predicted at Mnajdra solar temple.

A person standing in the middle of the main passage of the lower temple observes the sun's disc exactly along the bisector of the passage, only at the equinoxes. The appearance of the sun's disc during sunrise along the centre-line of the main passage coincides with two particular dates, namely the vernal and autumnal equinoxes $(21^{st}$ March and 23^{rd} September). At the summer solstice $(21^{st}$ June), the sun's rays are projected upon the left edge of the vertical slab at the left-hand

A REFERENCE

Source: Micallef (1992).

Figure 1: Prediction of seasons at Mnajdra solar temple.

side of the oval space. At the winter solstice (22nd December), the sun's rays are projected upon the right edge of a similar slab, which is situated in the same oval space, to the right of the centre line.

The cross-quarter days, that is the days midway between the solstices and the equinoxes, as in the old Celtic calendar system, fall on the 6th May, 7th August, 6th November and 4th February. Alignments with these days are currently being studied by Maelee Thomson Foster (1999), of the University of Florida. These were the principal feast days in the old Celtic calendar, which according to Sir Norman Lockyer were believed to have been inherited from the megalithic builders (Micell, 1977).

II. The observational data and method of data analysis.

Azimuth and declination determination

The determination of the azimuth of a building, is a means of measuring its orientation from true north or south. The azimuths quoted below were measured positively from north through east. Since the objective is to correlate the orientation of Mnajdra complex along its main axes with celestial objects, the azimuths and altitudes must also be converted to declinations, that is, to positions on the celestial sphere. Figure 2 shows observation point O, on a plane where ON represents the direction of the true north.

OX represents an axis, whose azimuth and declination are to be determined. The azimuth is defined by angle NOX. Thus the declination can be obtained by considering the celestial sphere, around point O having poles P and P', and an equator Eq, as shown. The declination is zero on any point on the equator ($\delta = 0^\circ$). The poles have declinations of $\pm 90^\circ$, ($\delta = 90^\circ$ at P and



Source: Adapted from Agius, and Ventura, 1980.

Figure 2: Observer's horizon surrounded by the celestial sphere.

 $\delta = -90^{\circ}$ at P'). Thus any points in between have declinations between 0° and $\pm 90^{\circ}$ such that points above the equator have positive declinations and points below the equator have negative declinations.

If one considers axis OY, the azimuth angle NOY through east is greater than that of axis OX. The declination in this case has remained the same since XQYR represents the path of the same point as it rotates about the axis PP' of the celestial sphere, the so called "diurnal circle" of the point. OY is directed towards the setting position whereas OX is directed towards the rising position. The conversion of azimuths and declinations involves spherical geometry, which can be found in a number of standard works (Roy and Clarke, 1977).

The azimuth of Mnajdra complex was obtained from published work (Micallef, 1992 a), where a theodolite measuring to an accuracy of one minute of an arc was used, and from other work (Agius and Ventura, 1979) where it was measured with a theodolite. Agius and Ventura (1980) state that several sources of error, excluding instrumental errors, precluded the determination of very precise azimuths. These include the weathering and erosion that has taken place for the main portal entrances of the Mnajdra complex, that is, lower temple, high temple and small trefoil temple, as well as the inexact reconstruction of the temple itself. Other aspects that were taken into consideration prior to the conversion of azimuths to declinations were:

- the latitude of the temple.
- · the aspect of the horizon, and
- the refraction at the moment of sunrise.

The latitude of the Mnajdra temple was also taken from

Micallef's work, (1992 b). The latitude makes an appreciable difference on the declination corresponding to a particular azimuth (Roy and Clarke, 1977). Looking from inside the temple along its main axis the horizon opposite the main entrance can be below, at, or above eye level. This plays an important role on the declination and azimuth at which the sun would appear to rise from inside the lower temple.

The motion of the sun.

The sun rises in the east and sets in the west. The sun rises almost exactly in the east and sets in the west around 20^{th} , 21^{st} and 22^{nd} March. This particular date is referred to as Vernal Equinox in the Northern hemisphere since the sun is above the horizon during the day and below the horizon during the night for an equal period of time. After the Vernal Equinox the sun rises and sets from a progressively northern position. The sun rises and sets in its northerly position with this deviation to the north increasing until mid-summer. Around 21^{st} June, known as the day of the June solstice, the sun seems to stop, since during that day, the sun's declination stops increasing and hardly changes.

After the June solstice, the sun progressively rises and sets in a southward position. Around the 23rd September referred to as the autumnal equinox in the Northern hemisphere, the sun rises due east and sets due west. The sun's southerly position continues after 23rd September until around 21st December (winter solstice day in the Northern hemisphere), when the sun rises and sets in its southernmost position. The day is much shorter than the night at our latitude. After 21st December the sun's path turns northward again and the cycle is repeated. The following deductions can be made:

- The declination of the sun is zero at equinox since it follows a path along the celestial equator.
- The declination of the sun is maximum positive at the summer solstice.
- The declination of the sun is minimum negative at the winter solstice.

The declination of the sun at the solstices is actually \pm i°, where i is the inclination of the earth to the ecliptic, also called obliquity. Although the value of i is nearly constant, its value has been found to oscillate very slowly between 22°55' and 28°18' with a period of 40,000 years (Weigart and Zimmermann, 1976). This small change in the sun's declination has been taken in consideration using the astronomical computer program Redshift IITM. Table 1 shows the sun's declinations for different millennia at the solstices.

Motion of the Moon

The motion of the moon is quite complex. The main features of the moon's path around the Earth can be listed as follows:

Years before present	Declination at solstices (degrees)
4000	± 23.9°
5000	± 24.9°
6000	± 24.1°

Source: Roy and Clarke (1977).

Table 1: Values of sun's declination for different millennia.

- The moon orbits the Earth at an inclination of $5^{\circ}09'$ to the ecliptic. The two points where the moon's path intersects the ecliptic are referred to as the "nodes".
- The plane of the moon's orbit precesses slowly such that the position of the nodes appear to slide around the ecliptic in the retrograde direction. The complete rotation of the nodes takes about 18.6 years, when a new cycle starts. This causes different declinations of the moon, caused by the inclination of the lunar to the Earth's orbit, and the same orbital geometry is not repeated except once every 18.6 years.

The most significant declinations occur when either a new or full moon is at the maximum or minimum declination. Table 2 shows declinations the sun and moon respectively, for different millennia.

Summer solstice	Sun's declination	Mo decli	on's nation
		Max.	Min.
4000	23.9	29.1	18.8
5000	24.0	29.2	18.9
6000	24.1	29.3	19.0
Winter solstice		Min	Max
4000	-23.9	-29.1	-18.8
5000	-24.0	-29.2	-18.9
6000	-24.1	-29.3	-19.0
Equinox	0	5.2	-5.2

Motion of the stars.

Source: Roy and Clarke (1977).

Table 2: Sun and moon declinations across the millennia.

The unique engravings found on the broken fan shaped stone excavated at Tal-Qadi, on whose flat surface are carved radiating lines with crescent moon and groups of stars (Ridley, 1971), shows that the megalithic builders might have shown interest in the stars (figure 3). Although the interpretation of the symbols seems acceptable, one can never be sure of its correctness (Ventura and Agius, 1980).

Sir Temi Zammit referred to a pattern of five holes in



Figure 3: The "tal-Qadi" stone.

the forecourt of Tarxien temple, as an image of a constellation, that of the Southern Cross. This constellation was easily seen in the Maltese hemisphere during the time of the building of the Maltese temples (Zammit, 1929).

The apparent interest in the stars by megalithic man may not mean much. However this gives us an objective to investigate whether in fact any alignments did take place along the main axes of the lower, high and small trefoil temples at Mnajdra. One should not overlook the fact that during the rising and setting of stars, they appear much fainter due to atmospheric extinction. Another point worth mentioning is that certain stars can easily be missed due to hazy conditions as well as moonlight, although one must mention that the hill profile in front of Mnajdra lower temple helps to minimize such effects (Hawkins, 1974). Although the stars seem to be fixed year after year, on closer observation, one will notice that some are rising to higher declinations whilst others are going to lower declinations in the sky. Over thousands of years such movements become evident. The term that describes this movement is referred to as precession. Every 25,800 years the Earth's axis precesses at a slow rate. The Earth's poles revolve with reference to the celestial sphere of fixed stars. Thus stars that are presently visible from a particular latitude may be invisible in a few hundred years and vice-versa (Figure 4).

In Figure 4b (i), stars between A_1 and B_1 are invisible from the latitude L_1 . In Figure 4b (ii), stars between A_2 and B_2 are now invisible from L_2 . Thus stars that were invisible between B_1 and B_2 are now visible and viceversa between A_1 and A_2 .

The rate of precession is well known and considering the motion of the stars proper motion, the exact position could be calculated relative to the direction in space of the Earth's polar axis and to the equinox positions for



Source: (a) Adapted from Roy and Clarke (1977) (b) Agius and Ventura (1980)



any age. The relative longitude and latitude of the Mnajdra temples were fed to the computer astronomical program, Redshift II TM , together with the respective azimuth and declination of the main axes of the lower, high and small trefoil temples of the site. The base years that were investigated were 2876-2875 BC for the lower temple, 2751-2750 BC for the high temple and 3451-3450 BC for the small trefoil temple, pertaining to archaeological dating accepted by archaeologists.

The elliptical forms at Mnajdra complex.

Let us consider some of the most essential features necessary in understanding the geometric principles of megalithic mathematics before looking at the geometric forms at the Mnajdra complex. The Pythagorean 3, 4, 5 triangle which is the simplest right-angled triangle was certainly familiar to Early Bronze Age men, (Thom 1971), as this triangle or other similar ratios pertaining to the 3, 4, 5 ratio occurs over and over again in stone constructions of this period. John Edwin Wood claims that at some sites, megalithic man used triangles that are not quite Pythagorean (Wood, 1978 d). However on



 Type I egg-shape structure
 Type II egg shape structure
 Flat circles approx. by cllipses

 Source: Wood (1978)
 Flat circles approx. by cllipses

Figure 5: Identified Megalithic structures.

calculation, the angle between the two shorter sides is 89.6° instead of 90°. This shows that early megalithic man was not aware of this mathematical relationship in order to arrive at drawing a right angle on the ground. Therefore it seems that the concept of certain triangles giving right angles was discovered by accident. These triangles were used in the construction of stone circles, megalithic structures in the shape of an ellipse, type A and type B flattened megalithic circles, type I and type II egg-shape megalithic structures, and flattened circles approximated by ellipses megalithic structures (Wood, 1978 a), (Figure 5). Thom suggests that these megalithic builders had devised their own unit of length. Their aim was to make their structures with perimeters equal to 2¹/₂ times a whole number of these units (Wood, 1978 b). Thom calls the unit the Megalithic Yard, abbreviated MY, and claims that 1 MY = 0.829 m. Therefore 2 $\frac{1}{2}$ MY, the Megalithic Rod = 2.073 m.

One of the properties of an ellipse is that the distance between the focus to the top of the ellipse on the minor axis, (extreme point A) is equal to half the length of the major axis, (Figure 6). The proof comes directly in how the ellipse is drawn. When the stake is at C, the loop goes from F_2 to C and back again; its length is therefore 2a+2c. When the stake is at A, the loop of rope goes round F_1 , F_2 , and B; its length is $2AF_1 + 2c$. By subtraction, $AF_1 = a$. The triangle AOF_1 is right-angled, and it would not come as a surprise to find Pythagorean numbers incorporated in megalithic ellipses.



Figure 6: Geometry of an ellipse.

III. Results

Correlation with planets, stars, sun and moon along lower temple axis.

At this stage it was important to correlate the temple's declinations with those of significant celestial bodies alignments. I have chosen to date the lower temple according to standard archaeological dating (Ventura and Agius, 1980), that is, early Tarxien 3000-2500 BC. Thus, I have taken my investigative base year as 2875 BC, pertaining to the early Tarxien phase. The azimuth of the lower temple was fixed on 92°30'11" with a declination of 00°00'00" together with latitude of 35°49'40" and longitude of 14°26'15". Redshift II[™] was used to correct the declinations for the sun and moon for parallax and for the positions of sun and moon approximately 5000 years ago. Table 3 shows the alignments that occurred at 2876-2875 BC (and are still occurring) at Mnajdra lower temple. One should note

Date	Time	Celestial Body	Declination (Degrees, minutes, seconds)	Azimuth (Degrees, minutes, seconds)
5.12.2876 BC	01:34	Mars	-00.05.37	92.32.30
14.12.2876 BC	13:26	First quarter moon – Age 9 days	-00.37.34	92.32.29
28.12.2876 BC	00:37	Last quarter moon – Age 22 days	-00.21.19	92.32.45
29.12.2876 BC	11:52	Jupiter	-00.01.35	92.25.39
4.01.2875 BC	23:40	Saturn	00.53.17	92.29.18
31.01.2875 BC	07:45	Venus	00.00.07	92.34.26
7.02.2875 BC	08:06	Crescent Moon – Age 2 days	-00.09.15	92.33.40
21.03.2875 BC	06:20	Sun	-00.04.45	92.32.14
13.05.2875 BC	15:33	Gibbous moon – Age 12 days	00.15.15	92.29.37
21.07.2875 BC	23:00	Gibbous moon – Age 20 days	-00.32.03	92.26.21
29.07.2875 BC	09:11	Venus	00.07.31	92.30.45
3.08.2875 BC	10:09	Crescent moon – Age 4 days	00.11.43	92.34.54
26.08.2875 BC	19:29	Mercury	00.06.19	92.35.08
19.09.2875 BC	06:20	Sun	00.57.11	92.29.48
19.09.2875 BC	06:45	Saturn	-00,01.24	92.31.06
27.09.2875 BC	06:30	New moon – Age 0 days	00.06.30	92.29.40
7.10.2875 BC	04:58	Venus	00.01.53	92.30.16
11.10.2875 BC	17:39	Full moon – Age 14 days	-00.15.09	92.33.13
16.10.2875 BC	04:55	Mercury	00.12.58	92.30.29
12.11.2875 BC	03:27	Venus	-00.03.21	92.34.46
21.11.2875 BC	02:57	Crescent moon-Age 26 days	-00.54.36	92.30.31
5.12.2875 BC	14:10	Gibbous moon – Age 9 days	00.18.50	92.26.28

Table 3: Alignments for Mnajdra lower temple.

that the celestial bodies shown in bold could not be seen or are very difficult to observe due to the brighter sky background.

Another investigation was then conducted to check whether there were any star alignments that took place at Mnajdra lower temple at 2875 BC. All stars which were difficult to see were excluded, i.e. those with magnitude 4.00 or higher. To analyse all possible star alignments that could have taken place for the lower temple, two dates separated by 6 months were chosen, that is 8th December 2876 BC and 8th May 2875 BC. In fact possible rising star alignments on an azimuth of 92°30'11" and declination of 00°00'00" together with the temple's longitude and latitude were found for constellations of Aquila, Pegasus, Taurus and Hydra (table 4).

The same investigation was also held for any star alignments that took place at Mnajdra middle temple. Since the middle temple is dated as Tarxien phase (3000-2500 BC), then the year of analysis of 2750 BC was used. To analyse all possible star alignments that could have taken place for the middle temple, two dates separated by 6 months were chosen, that is 8^{th} December 2751 BC and 8^{th} May 2750 BC. In fact possible rising star alignments on an azimuth of 138°30'00" and declination of -38°06'00" were found

for constellations of Puppis, Canis Major, Vela, Ara and Aquarius (Table 4). No alignments with planets, sun or moon were found for Mnajdra middle temple and small trefoil temple.

Setting star alignments that took place at Mnajdra small trefoil temple (Ggantija phase) at 3450 BC were also investigated. To analyse all possible star alignments that could have taken place for the small trefoil temple, two dates separated by 6 months were chosen, that is 8^{th} December 3451 BC and 8^{th} May 3450 BC. In fact possible star alignments on an azimuth of 204°00'00" and declination of -46°06'00" were found for Columba, and Pavo (Table 4).

Significance and determination of ellipse equations for Mnajdra complex.

Neolithic and early bronze age man did not possess any of our modern ideas about mathematics. However there is no doubt whatsoever that megalithic man was fascinated with geometry. At Mnajdra the identified geometric shapes resemble closely the ellipse for the small trefoil temple and the solar temple. The high temple ellipses are rather flattened at the top and bottom ends. In order to study the ellipse forms at Mnajdra, primary data was retrieved on site. A measuring tape was used to determine the lengths of the major and minor axes of all the temples at Mnajdra. The ellipse

SAQ	Bayer Name Flamsteed Name	Magnitude	Declination (Deg, min, sec)	Right Ascension (Deg, min, sec)
Date: 8 th December 2876 BC				
76155	Maia - 20 Tauri	4.00	00.28.38	23.25.33
76140	Taygeta – 19 Tauri	4.40	00.32.53	23.24.56
76172 PLEIADES	Merope – 23 Tauri	4.30	00.05.04	23.26.18
76199	Alcoyne – 25 Tauri	3.00	00.17.28	23.27.15
76228	Atlas – 27 Tauri	3.80	00.18.42	23.28.48
182244	Hydrae – 49 Hydrae	3.50	(-)00.24.55	09.55.21
Date: 8th May 2875 BC				
76199	Alcoyne - η Tauri	3.00	00.17.20	23.27.13
127029	Enif - ε Pegasi	2.50	(-)00.21.49	20.44.24
76131	Electra – 17 Tauri	3.80	00.11.03	23.24.53
76155	Maia – 20 Tauri	4.00	00.28.30	23.25.32
76228	Atlas – 27 Tauri	3.80	00.18.34	23.28.46
135896		4.00	(-)01.19.55	04.20.04
144150	θ Aquilae	3.40	(-)01.15.41	15.58.25

Lower Temple star alignments.

High Temple star alignments.

SAO	Bayer Name Flamsteed Name	Magnitude	Declination (Deg, min, sec)	Right Ascension (Deg, min, sec)
Date: 8 th December 2876 BC				
196698	Furud – ξ Canis Majoris	3.10	(-)37.51.36	03.22.54
Date: 8 th May 2875 BC				
196698	Furud - ξ Canis Majoris	3.10	(-)37.52.01	03.22.53
197258	κ Canis Majoris	3.80	(-)36.48.46	03.56.37
197795	π Puppis	2.70	(-)37.48.16	04.32.41
219082		3.80	(-)37.04.08	05.11.08
237522	φ Velorum	3.70	(-)38.06.04	07.17.32
238813		4.00	(-)37.49.53	08.11.58
244168	η Arae	3.70	(-)37.28.11	11.40.59
191683	δ 8 Aquarii	3.80	(-)38.07.50	18.20.19

Small Trefoil Temple star alignments.

SAO	Bayer Name Flamsteed Name	Magnitude	Declination (Deg, min, sec)	Right Ascension (Deg, min, sec)
Date: 8 th December 2876 BC				
246574	Pavonis	2.1	(-)47.22.15	17.59.35
Date: 8 th May 2875 BC				
246574	α Pavonis	2.10	(-)47.22.18	13.18.10
196059	Phaet - α Columbae	2.80	(-)48.36.14	02.29.23
250374		4.00	(-)46.52.29	06.40.24

Table 4: Star alignments at Mnajdra solar, high and small trefoil temples.

Mnajdra complex	Small Trefoil temple	High temple (Larger ellipse)	High temple (Smaller ellipse)	Lower temple
Measurements	Units:	Units:	Units:	Units:
	metres/	metres/	metres/	metres/
	megalithic yards	megalithic yards	megalithic yards	megalithic yards
Length of major axis	9.2	16.5	13.8	13.8
	11.1	19.9	16.6	16.6
Length of minor axis	4.7	7.3	6.0	7.0
	5.7	8.8	7.2	8.4
Extreme point A	(0,2.35)	(0,3.65)	(0, 3)	(0, 3.5)
	(0,2.83)	(0,4.4)	(0, 3.6)	(0, 4.2)
Extreme point B	(0,-2.35)	(0,-3.65)	(0, -3)	(0, -3.5)
	(0,-2.83)	(0,-4.4)	(0, -3.6)	(0, -4.2)
Extreme point C	(-4.6,0)	(-8.25, 0)	(-6.9, 0)	(-6.9, 0)
	(-5.55,0)	(-9.95, 0)	(-8.3, 0)	(-8.3, 0)
Extreme point D	(4.6, 0)	(8.25, 0)	(6.9, 0)	(6.9, 0)
	(5.5, 0)	(9.95, 0)	(8.3, 0)	(8.3, 0)
Eccentricity (e) ($e^2 = 1 - b^2/a^2$)	0.86	0.89	0.9	0.86
	0.86	0.89	0.9	0.86
Determination of focus 1 (ae, 0)	(3.95, 0)	(7.4, 0)	(6.2, 0)	(5.946, 0)
	(4.77, 0)	(8.9, 0)	(7.5, 0)	(7.2, 0)
Determination of focus 2 (-ae, 0)	(-3.95, 0)	(-7.4, 0)	(-6.2, 0)	(-5.9, 0)
	(-4.77,0)	(-8.9, 0)	(-7.5, 0)	(-7.2, 0)
Determination of directrix 1 (x=a/c)	5.4	9.2	7.6	8.0
	6.5	11.1	9.2	9.6
Determination of directrix 2 (x=-a/e)	-5.4	-9.2	-7.6	-8.0
	-6.5	-11.1	-9.2	-9.6
Latus Rectum (2b ² /a)	2.4	3.2	2.6	3.5
	2.9	3.9	3.1	4.3

Table 5: Data for the determination of ellipse for small trefoil, high and lower temples at Mnajdra.

equation can be found from this data using the theory of ellipse equation. Table 5 shows the calculations of the ellipse geometry both in standard metric system as well as in megalithic yards identified by Thom (1971). If the megalithic yard of 2.72 feet or 0.829 metres is used the results as shown in Table 5 are achieved.

IV. Discussion of results

At Mnajdra lower temple the alignment at both equinoxes, that is, the vernal equinox and the autumnal equinox, the sun's disc appears to stand on a point on the slope exactly opposite the main passage when viewed from the interior. The calculations for the year 2875 BC show that the sun faced exactly the main passage of the temple which is in fact the vernal and autumnal equinoxes. This is still true and is still occurring.

A solar calendar at Mnajdra lower temple.

The cross quarter and eighth days at Mnajdra lower temple bring sunlight image projections that hit the megalithic stones inside the solar calendar, at other important dates. The cross-quarter days are midway between the solstices and equinoxes whilst the eighths days are mid-way between the cross-quarter days and equinoxes and between the summer and winter solstices and the cross-quarter days. The dates of the 'eighths' are 14th April, 1st June, 12th July, 31st August, 16th October,

30th November, 10th January and 25th February. The edge of the shaft of light from the rising sun hits the inside of the temple at important positions inside the temple as shown in Figure 7.

In fact the declinations of the sun during the summer and winter solstices, the autumnal and vernal equinoxes and cross quarter and eighth days at Mnajdra solar temple, fit Thom's hypothesis (Table 6). Thom statistically studied more than three hundred sites, and came up with the most important sun declinations (Wood, 1978 c). Thom's calendar is distributed in eleven months of 23 days, four months of 22 days and one month of 24 days. If Thom's hypothesis is correct, then the people of Early Bronze Age had indeed accomplished a very important system.

In a different study on the investigation of prehistoric solar calendars, MacKie, (1988 a), concludes, that by using the sequence of solar 'months' deduced by Thom, and by using the 'megalithic' rather than the true equinoxes, one could obtain the following 16 'month' dates with the cross-quarter days, the solstice dates and the equinox dates being shown in italic: 22 March, 14 April, 7 May, 31 May, 23 June, 16 July, 8 August, 30 August, 21 September, 13 October, 4 November, 27 November, 20 December, 11 January, 4 February, 27 February and 22 March (Micallef, 1990). One should



Figure 7: The Mnajdra solar calendar (Photo: Maurice Micallef)

note that the dates which Thom proposed, and subsequently MacKie, agree with our Mnajdra temple calendar.

On a separate investigation that I performed using Redshift IITM astronomical program, the following dates were deduced, with the eighths being shown in italics: 21 March, 14 April, 5 May, 1 June, 21 June, 12 July, 8 August, 31 August, 23 September, 16 October, 8 November, 30 November, 22 December, 10 January, 5 February and 25 February. These dates were arrived at after scrutinising different sun declinations which Thom used as his basis to arrive at these important dates, and comparing them with those derived for Mnajdra lower temple. Again one should notice the similarity in dates between those proposed by Thom and MacKie and the ones derived for the Mnajdra solar temple.

The moon path facing the lower temple.

The realization of the observer's position (known as the back-sight) was in itself adjusted in relation to a specific peak or point on the horizon. The aim was to create a long alignment giving fine measurements of the sun's position, moon or stars. The high precision involved is by far greater than that required in a simple agricultural calendar and must surely have involved full-time professional observers.

According to MacKie (1988 a), it would have been

necessary to spend many years first searching for suitable sites and then patiently observing to establish the exact position of the required back-sights. These back-sights are usually marked by a standing stone, (Hoskin et al, 1992). This points to the existence of a more developed Neolithic society rather than the rural primitive societies, that archaeologists believe in. The main question here is, what is happening to the moon at the most important dates during autumnal and vernal equinoxes as well as the summer and winter solstices rising sun and other celestial bodies?

The motion of the moon is quite complex, so for reasons of simplicity it will be assumed that the moon's orbit is in the same plane as the Sun. The aim is to have the moon's orbit in the same plane as the ecliptic. At the spring equinox (21st March), the sun sets very close to due west or due west. This means that the northern half of the ecliptic is therefore above the horizon at sunset at this time. Thus the first quarter moon is on the meridian in the same place as the sun at summer solstice. When full moon is attained it rises due east at sunset, and its position is at the autumnal equinox. The last quarter moon is at the winter solstice.

During the summer solstice (21^{st} June) , the sun sets in the north-west, and the first quarter moon, is now at the autumnal equinox position and follows the celestial equator. The full moon, now at the winter solstice, rises

Month No.	Number of days	Number of days from the spring equinox to the beginning of the month	Declination of the sun at the beginning of the month at 1800 BC (degrees). Thom's analysis.	Correspondi ng date in our calendar (Thom's analysis).	Number of days	Declination of the sun at the beginning of the month. Author's analysis for Mnajdra solar temple for 2875 BC. (Degrees, minutes, seconds).	Correspondi ng date in our calendar for 2875 BC (Author's analysis).	Number of days	Declination of the sun at the beginning of the month. Author's analysis for Mnajdra solar temple for 1999 AD. (Degrees, minutes, seconds)	Correspondi ng date in our calendar for 1999 AD. (Author's analysis)
1	23	0	0.44	20-Mar	0	(-)00.04.06	21-Mar	0	00.05.12	21-Mar
2	23	23	9.16	12-Apr	24	9.01.01	14-Apr	24	09.15.47	14-Apr
3	24	46	16.67	05-May	21	15.59.46	05-May	21	16.07.58	05-May
4	23	70	22.06	29-May	26	22.04.56	31-May	27	21.59.34	1-June
5	23	93	23.91	21-Jun	22	24.00.13	22-Jun	20	23.26.06	21-Jun
6	23	116	22.06	14-Jul	24	22.06.47	16 -J ul	21	22.01.21	12-Jul
7	23	139	16.97	06-Aug	25	16.03.47	10-Aug	27	16.15.03	08-Aug
8	22	161	9.17	28-Aug	21	09.00.53	31-Aug	23	08.46.51	31-Aug
9	22	183	0.44	19-Sep	21	00.06.41	21-Sep	23	00.04.27	23-Sep
10	22	205	-8.46	11-Oct	20	(-)08.03.54	11-Oct	23	(-)08.44.58	16-Oct
11	23	227	-16.26	02-Nov	22	(-)16.00.15	02-Nov	23	(-)16.27.56	08-Nov
12	23	250	-21.86	25-Nov	28	(-)22.38.24	30-Nov	22	(-)21.35.08	30 Nov
13	23	273	-23.91	18-Dec	20	(-)24.00.15	20-Dec	22	(-)23.26.21	22-Dec
14	23	296	-21.86	10-Jan	23	(-)21.57.51	11-Jan	19	(-)22.00.13	10-Jan
15	23	319	-16.26	02-Feb	24	(-)16.13.25	04-Feb	26	(-)16.01.23	05-Feb
16	23	342	-8.46	25-Feb	21	(-)9.11.13	25-Feb	20	(-)09.13.13	25-Feb

Source: Wood (1978)

after analysing declinations at Mnajdra solar temple.

Table 6: Bronze Age calendar proposed by Thom compared to Mnajdra lower (solar) temple.

well south of east. The last quarter moon is at the spring equinox and appears higher in the sky than in spring when it crosses the meridian.

On 23rd September (autumnal equinox), the sun's setting point shifts back due west and at sunset the whole of the south part of the ecliptic is above the horizon. The first quarter moon, occupies the full moon's position at the winter solstice. The full moon, whose position is now at the vernal equinox is higher when on the meridian than when in summer. The last quarter moon, is now at the summer solstice position.

On 22^{nd} December (winter solstice), the first quarter moon at sunset, climbs higher in the sky, as it is now located in the vernal equinox position. The full moon is now at the summer solstice position. The last quarter moon is at the vernal equinox position and rises due east. Table 7 shows the position of the moon during the most important seasonal dates.

Different phases of the moon could have also been observed along the main axis of Mnajdra lower temple, as Table 3 shows. It is interesting to note that alignment along the main axis of the lower temple with the full moon occurred four times throughout the year on 19^{th} March, 17^{th} August, 15^{th} September and 10^{th} October

2875 BC. No alignments were found for the moon for the high and small trefoil temples. This is not surprising given the declinations of the temple.

Summer solstice positioning slab	Equinox main altar	Winter solstice positioning slab
21 st MARCH EQUINOX		
First quarter moon	Full moon	Last quarter moon
21 st JUNE SUMMER SOLSTICE		
	First quarter moon	Full moon
	Last quarter moon	
23 rd SEPTEMBER EQUINOX		
Last quarter moon	Full moon	First quarter moon
22 nd DECEMBER WINTER SOLSTICE		
Full moon	First quarter moon	
	Last quarter moon	

Source: Observations at the Mnajdra lower temple.

Table 7: The moon's position during important seasonal dates at the Mnajdra lower temple.

Alignments with planets

It was also interesting to find that Mnajdra lower temple planets. Again this is not is aligned towards the surprising, and at this point one should ask, whether these alignments were intentional or not? Towards the end of the year 2876 BC, Mars was aligned (and still is), towards the main axis of the lower temple (Table 3). This was followed by Saturn, Venus, Mercury and Venus. No alignments with planets were found for the high and small trefoil temples at Mnajdra. This is not surprising given the declinations of the high and small trefoil temples.

Interpretation of sun, moon and planets alignments at Mnajdra temple.

Interesting enough is MacKie's hypothesis for megalithic sites in England and Scotland, used for Mnajdra lower temple. It seems that the beginning of each 'megalithic' month could have been marked by the rising of celestial bodies along the temple's main passageway (axis), which indicates their use by megalithic man to mark their own calendar. The fact that the cross quarter days are still important today, and until recently they marked important seasonal and other festivities, suggests that they might have been inherited from a very old solar calendar with well established feast days and religious ceremonies on the eight major subdivisions of the year (MacKie, 1988 b). MacKie also states that the intermediate eighths were useful seasonal indicators. MacKie believes that the eighths marked the beginning of spring, hence the time for sowing, the beginning of summer, the beginning of autumn, hence harvest time and the beginning of winter. This could be the same case for a latitude which is different from Malta's.

The rising of the sun on the first day of the four seasons, the cross-quarter days and the eighth days as seen by an observer in the temple, as well as the positioning of planets, and moon, leaves nothing to the imagination or speculation. Thus it seems that megalithic man could have constructed the Mnajdra lower temple to predict the solar and lunar motion during the year, although one must mention that the situation could be sheer coincidence as to date there is no evidence of intent. It was due to this connection of the sun's rising at different positions with the lower temple that Micallef (1992) had re-named the lower temple as the solar temple. The high temple and small trefoil temple also predict the rising and setting of certain stars. The rising of celestial bodies, as seen by an observer in the small trefoil temple, high temple and To an accuracy of 1 decimal place the angle F1OA is lower temple makes us more aware of the capacity and knowledge that these sky watchers possessed in ancient times.

The ellipse forms at Mnajdra complex

It is interesting to note the eccentricity of the ellipses at Mnajdra. The eccentricity is 0.86 for the small trefoil temple and 0.9 for the inner ellipse of the high temple. The other ellipse of the high temple bears an eccentricity of 0.9 also, whilst the solar temple's eccentricity is again 0.86. The same cannot be said when one compares the Mnajdra site with other megalithic structures. Wood

claims that the eccentricities of megalithic ellipses are generally between 0.3 and 0.7 (Wood, 1978 d). The stone settings at Postbridge. Devon is the least elliptical with an eccentricity of 0.29, at Penmaenmawr, Gwynedd the megalithic ellipse has an eccentricity of 0.31, whilst at Machrie Moor on the Isle of Arran the megalithic stone ellipse has an eccentricity of 0.5.

During the construction of these stone ellipses, it is supposed that two posts were hammered into the ground. The posts represent the foci of the ellipse. A loop of rope was made to go round both simultaneously. A stake in the loop will eventually mark out an ellipse. As the posts are marked further away from each other, this produced an elongated type of ellipse, as in the case of the Mnajdra temples. An investigation was carried out on other measurements pertaining to the small trefoil temple, the high temple and the lower temple (Table 8).



From Pythagoras Theorem $AF_1^2 = AO^2 + OF_1^2$ $AF_1^2 = 2.35^2 + 3.954^2$ $AF_1^2 = 21.16$ $AF_1 = 4.5996 \text{ m} \approx 4.6 \text{ m}$

Hence the length of AF_1 is equal to the length of half the major axis as the theorem suggests. This makes the above triangle incorporated in the small trefoil temple a right-angled triangle. Let us investigate the angles of the above triangle AOF₁ using trigonometric principles.

Tan	AF ₁ O	= opposite/ad	jacent = A	AO / OF_1	= 2.35 / 3	.654 =	32.7°.
Tan	F ₁ AO	= opposite/ad	jacent = (OF_1 / AO	= 3.654 /	2.35 =	57.3°.

exactly 90°.

If one is willing to believe the reason why the megalithic builders set out these temples in special geometric shape, then one might ask, why did they do it in the first place? Why did they have to go through such complications when a stone circle would have sufficed? Thom (1971) suggests that these structures have perimeters equal to 21/2 times a whole number of these units (Wood, 1978 e). An interesting item inscribed on the right upon the threshold of the Mnajdra's high temple is interpreted as the universal numerical building

Mnajdra Temple	AF ₁	Angle AF ₁ O	Angle F ₁ AO
Small Trefoil	4.6m	32.7°	57.3°
High (larger ellipse)	8.25m	26.3°	63.7°
High (smaller ellipse)	6.9m	25.8°	64.2°
Lower	6.9m	30.5°	59.5°

Table 8: Measurements at Mnajdra megalithic ellipses.

				e
Criteria	Small trefoil temple	High temple (larger ellipse)	High temple (smaller ellipse)	Lower temple
Triangle based dimensions (metres)	2.35 3.95 4.60	3.65 7.40 8.25	3.00 6.21 6.90	3.50 5.95 6.90
Triangle based dimensions (megalithic yards)	2.84 4.77 5.55	4.40 8.93 9.95	3.62 7.49 8.32	4.22 7.17 8.32
» Perimeter of ellipse (metres)	9.57	16.33	13.56	14.34
» Perimeter of ellipse (megalithic yards)	11.55 » 11½	19.69 » 19½	16.36 » 16¼	17.29 » 17¼
Perimeter of ellipse as a multiple of 2.5 MY	11.5/2.5 = 4.6	19.5/2.5 = 7.8	16.25/2.5 = 6.5	17.25/2.5 = 6.9
Perimeter of ellipse (megalithic rod)	» 4 ¼	» 8	» 6 ½	»7
Major axis of ellipse (m)	9.2	16.5	13.8	13.8
Major axis of ellipse (megalithic yards)	11.098 » 11	19.903 » 20	16.647 » 16 ½	16.647 » 16 ½
Major axis of ellipse (megalithic rod)	4.438 » 4	7.959 » 8	6.66 » 6½	6.66 » 6½
Distance between foci of ellipse (metres)	7.908	14.798	12.428	11.892
Distance between foci (megalithic yard)	9.539 » 9½	17.85 » 18	14.99 » 15	14.345 » 14¼
Distance between foci (megalithic rod)	3.815 » 4	7.138 » 7	5.995 » 6	5.738 » 6

Table 9: Investigation of primary data of ellipse characteristics for Mnajdra complex.

unit. According to Formosa (1975), it looks like a large L inscribed on the stone that acts as a threshold. Maelee Thomson Foster (1999) measured the incised unit which fits Thom's megalithic building unit.

As an approximation the circumference of an ellipse can be expressed as $\pi \sqrt{ab}$ where *a* stands for the semimajor axis and *b* stands for the semi-minor axis. Therefore it is quite possible to get the major axis of an ellipse and its circumference to be simultaneously whole numbers in any unit. According to Thom (1971), the megalithic builders set themselves the difficult task to make all the three sides of the right-angled triangle incorporated in the stone ellipse, whole numbers of megalithic yards all at once. It is impossible to do this exactly, and very difficult even to do it approximately. Thom (1971) states that it was not always possible to be as close as this to whole numbers. In general, the ellipse was based on a Pythagorean triangle and the perimeter was allowed to depart from a multiple of 2 $\frac{1}{2}$ MY, or the length of the perimeter was kept 2 $\frac{1}{2}$ times a whole number of megalithic yards and modified the triangle (Wood, 1978 f). In the latter case megalithic man let the minor axis with an awkward length, but made the major axis and the distance between the foci equal to a whole number of yards. The objective was to ensure that the length of the rope used for laying out the ellipse integral

> in megalithic yards. Table 9 shows the above mentioned criteria for the small trefoil temple ellipse, the high temple ellipses and the lower temple ellipse.

> Several interesting facts arise from the calculations derived from Table 9. The triangles within the ellipses derived for the Mnajdra temples are not Pythagorean numbers. Therefore it seems that these megalithic builders adjusted the eccentricity of the ellipse by moving the foci of the ellipse on the major axis with the objective of getting whole unit numbers for the perimeter and major axis of the ellipse.

The perimeter of the ellipses, in terms of whole number of units, was achieved for the high temple (larger ellipse) and the lower (solar temple) in terms of the megalithic yard. For the small trefoil, and the high temple (smaller ellipse), half a megalithic unit (Table 9). For the major axis of the ellipse in terms of megalithic yards, accurate results, in whole unit numbers, were achieved for the small trefoil temple and the larger ellipse of the high temple. The same cannot be said for the smaller ellipse of the high temple and the solar temple and

probably half a megalithic unit was used. Quite accurate results were attained for the small trefoil, high and lower temple when measurement in terms of the megalithic rod is considered for the major axis of the ellipses. Another discrepancy is the distance between foci for the lower temple ellipse. The error involved is 2.4% unless the megalithic builders used a quarter of a megalithic rod in their construction. Quite accurate results in terms of whole number units were achieved for the ellipses of the high temple. The results derived for the Mnajdra complex positively show that the distances between the foci and the major axis and the perimeters of the elliptical temples were achieved by means of some megalithic unit (Table 9).

The question still remains: did megalithic man possess mathematical knowledge in the construction of these elliptical figures. Further investigations on other megalithic temples with this elliptical shape in the Maltese Islands may eventually shed more light and knowledge in relation to Maltese megalithic temples building unit.

Conclusion

The daily slit images produced at the Mnajdra solar temple and alignments with celestial bodies may qualify this megalithic monument to be seriously considered as an astronomical observatory since the images hit important stones inside the solar temple throughout the year. The alignments at Mnajdra's lower temple with planets, especially the red planet Mars, is an intriguing fact. It seems that after all it was not only the Mayans or the Egyptians who either made reference or aligned some of their temples with either Venus or Mars respectively. The yearly alignment of Mars along the main passageway of the lower temple could have served as a means to indicate the beginning of a new-year. The other alignments of Venus, Mercury, the Moon and the Sun could have served as seasonal indicators or for festive religious ceremonies. The conversion of the measurements of the lengths of the temple apses into integral or near integral values taken at the Mnajdra temples, is more indicative of an ancient Maltese unit used during the construction of the temple, as shown by the calculations and results obtained.

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Research Article

Preliminary report on β -carotene production in the halotolerant microalga *Dunaliella bardawil*

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Summary: Algae of the genus Dunaliella are among the most widely studied for mass culture and for use as a source of food and nutraceuticals. In this paper we review the information available on this important microalga, together with some of the results we have obtained in our laboratory.

Keywords: β-carotene, Dunaliella bardawil, microalga

Introduction

The use of *Dunaliella* and other microalgae for biotechnology purposes is not new. Countries with hot, dry summers and mild winters such as Australia and Israel have long been exploiting the organism as a source of the pigment β -carotene. The Molecular Biotechnology section of the Laboratory of Molecular Genetics at the University of Malta has been involved in studies of this microorganism since 1998. In this preliminary report we summarise some of the key aspects of the biotechnological use of *Dunaliella*.

Dunaliella is a unicellular bi-flagellate green alga of the class Chlorophyceae. It inhabits a wide variety of habitats including fresh, euryhaline and hypersaline waters as well as saline soils. In most of these habitats, the organism is a fairly minor component but in brines two species, Dunaliella salina (Dunal) Teodoresco and Dunaliella bardawil, predominate. Both organisms show interesting and, in certain instances, unique mechanisms to withstand the salt fluctuations, dessication and intense solar radiation encountered in their ecological niche. Not surprisingly, these strains have been described as the dominant species in the hypersaline brines of the Dead Sea, Israel (Volcani, 1944), the Great Salt Lake, Utah (Brock, 1975) and the Pink Lake in Western Australia (Borowitzka and Borowitzka, 1988). Both strains are known to tolerate salt fluctuations between 3.5% and 35% (Borowitzka and Borowitzka, 1988), although in our laboratory tolerance to salinities as low as 0.6% was regularly recorded. This makes them one of the most halotolerant organisms known.

For many years, there was strong debate about whether *D. salina* (Dunal) Teodoresco and *D. bardawil* were in fact two different species. Using the Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) analysis of DNA coding for the 18SrRNA, Olmos and co-workers, (2000) were able to

demonstrate clearly that these are in fact two separate species.

Conditions in Malta, especially the hot dry summers, would be expected to provide ideal conditions for this organism to flourish. However, random samples taken from the Salina saltern in the summer of 1997 failed to yield any specimens, although a number of interesting halophiles such as the multicellular blue-green algae Spirulina, which is widely used as a dietary supplement, were identified. Local records of Dunaliella salina are in fact limited to a single sighting in a rock pool at Manoel Island (Lanfranco, 1974). The increase in the use of this area for recreational purposes, however, has probably contributed to the disappearance of the microalga from the site since then. To the best of our knowledge there have been no other recorded sightings since. As a result, stocks of the microalga had to be imported for this work. The strain used in our laboratory was D. bardawil and was purchased from the American Type Culture Collection (ATCC).

Uses of β-carotene

Dunaliella is best known as a source of the orange pigment β -carotene. The pigment is in wide use in the nutraceutical and pharmaceutical industries as a natural colourant and as a vitamin supplement in its own right (Mirasol, 1998). The world market for both the natural and the synthetic forms is worth in excess of 180 million dollars and is growing at a fast rate.

The uses of β -carotene in the human body are various. It is the precursor of vitamin A and it is important for cellular differentiation, vision, bone growth, erythropoeisis and the integrity of the immune system. It can improve the absorption of non-heme iron from rice, wheat and corn (Garcia-Casal *et al*, 1998). Epidemiological and oncological studies also indicate that normal to high levels of dietary β -carotene may protect against atherosclerosis (Tornwall *et al*, 2000) and may lower the risk for a number of cancers

including colo-rectal, adenocarcinoma, gastric and cardiac cancers (Ekstrom *et al*, 2000). Lung cancer is an exception, as raised levels of β carotene are associated with an increased risk of illness (Goodman, 2000; Ratnasinghe *et al*, 2000).

β-carotene production

In the natural habitat, β -carotene acts as a sun shield, effectively protecting Dunaliella from the damaging light energy in the blue region of the spectrum. In fact, under conditions of high light intensity and high temperatures between 26-36°C, the pigment collects as oily globules within the interthylakoid spaces of the chloroplast giving the whole organism a distinctive orange colour. The pigment alone can account for more than 10% of the dry weight of each organism (Ben-Amotz et al, 1982), making Dunaliella the highest known natural producer of β -carotene. By comparison, the β -carotene content n a typical leaf or alga is only around 0.3% (Ben-Amotz and Avron, 1990). The β -carotene produced in Dunaliella is a mixture of two stereoisomers, the 9-cis and all-trans forms (Figure 1), although a minor amount of α -carotene is also present. Both the amount of the accumulated β -carotene and the 9-cis to all-trans ratio depend on the intensity of light incident on the cell. Higher light intensities induce the production of more cellular β carotene and increase the 9-cis to all-trans ratio. The physicochemical properties of 9-cis βcarotene differ from those of the all-trans. The cis form is much more soluble in hydrophobic solvents (Ben-Amotz and Avron,



Figure 1. Typical high pressure liquid chromatogram of a total pigment extract from a Dunaliella bardawil strain containing high levels of b-carotene. Smaller amounts of a-carotene and the usual complement of chlorophylls found in green algae also occur (Source: Ben-Amotz and Avron, 1990)



Figure 2. The β -carotene biosynthetic pathway.

1990). It is therefore more easily absorbed into tissue and consequently may account for the greater protective role suggested in various studies.

The steps in the biosynthesis of β -carotene were first identified by Shaishi *et al*, (1992). They are similar to those existent in plants where carotenoids are derived from the general isoprenoid pathway (Figure 2).

The isoprenoids are built from a common precursor

isopentenyl diphosphate (IPP), which in plastids is believed to be formed from pyruvate and glyceraldehyde-3-phosphate. The condensation of two molecules of geranyl geranyl diphosphate (GGPP) to produce phytoene is the first committed step in the synthesis of β -carotene. GGPP is then dehydrogenated to lycopene and finally lycopene is cyclized to form β carotene (see Guerinot, 2000). Synthesis of β -carotene appears to be triggered by a number of stressors, including high solute concentration, metals such as lead and copper (Pace et al, 1977) and nitrate deficiency (Ben-Amotz and Avron, 1983). The reason for the effect of each of these stressors on the pathway is as yet unexplained, but the effect of the heavy metals may reflect an activating effect on some of the key enzymes involved in the biosynthetic pathway.

Although β -carotene can be chemically synthesised by a patented process that starts with acetone and is owned by the chemical company Badische Anilin- & Soda-Fabrik AG (BASF), the synthetic form consists of mainly trans β -carotene and much lower levels of the healthier-perceived cis-form. This difference in composition between the natural and the synthetic forms is being exploited in a successful yet aggressive marketing campaign in favour of the natural form. In particular, as consumer demands for natural products in Europe and Japan continues to increase, the market should continue to expand (Mirasol, 1988). There is certainly room for further small to medium sized culture plants that can supply the European niche.

The real challenges in supplying this growing market actually lie in harnessing the biosynthetic \beta-carotene pathway. Unlike the *Penicillium* fungi where a variety of conventional mutagenic techniques have been repeatedly used to produce high producing antibiotic strains, there have been no such programmes for Dunaliella. To date, attempts to increase the levels of β-carotene in production have centred around searches for high producers already existent in nature. This has been carried out with efficiency by the Australian, American and Israeli research HHHcorporations involved in enclosed conditions. Most of these attempts utilise a this area. It is therefore logical to predict that the next step would be to carry out mutagenic studies to isolate higher producing β-carotene strains. Genomics assisted strain improvement programmes are another way by which carotene production could be improved, although the absence of basic molecular biology protocols for Dunaliella could hinder success in this area.

Dunaliella cultivation

The halophilic strains of Dunaliella are particularly attractive candidates for mass culture. Firstly, they flourish in poor quality water that is not of agricultural use. Secondly, they possess the ability to survive in high salinities. As a result, they can be cultivated outdoors with few potential predators or competitors. Even under extensive culture, the difficulties with sterility that are encountered with the usual organisms used in fermentation such as Escherichia coli, Saccharomyces or fungal hosts are low. Axenic cultures can be maintained fairly easily without the need of antibiotics, insecticides or pesticides. In any case, studies in our laboratory have shown that Dunaliella is insensitive to most antibiotics. This result was also seen to a lesser extent in studies with the closely related

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microalga Chlamydamonas *reinhardtii* (Harris¹, personal communication). It would appear that to survive in its environmental niche, Dunaliella has developed stringent methods for limiting the intake of material across its membranes. Cultivation is also comparatively cheap since it is based on autotrophic growth in media containing carbon dioxide and inorganic nutrients. Supplementation of media with organic substrates is unnecessary, as Dunaliella does not show heterotrophic use to any extent (Ben-Amotz and Avron, 1990).

Current methods of cultivation are geared towards maximising incident sunlight and high temperatures. An open pond extensive mode of culture is most often used in the majority of plants. The principal advantages of such systems include a relatively small capital investment and a free source of energy in the form of natural sunlight. Yields of around 10mg of β-carotene per square meter of culture per day have been recorded (Ben-Amotz, 1993). Simple improvement to this system by, for example, culturing the alga in long raceways and supplementing the system with aeration by means of slow moving paddles can improve yields of β -carotene 40-fold to about 400mg per square meter per day.

However, both extensive and intensive cultures suffer from the disadvantage that they are sensitive to any sudden adverse changes in weather. Production also decreases during the night and the cooler months of the Attempts are being made to overcome these vear. disadvantages by experimenting with growing cultures variety of electric light and sunlight driven photobioreactors (Ben-Amotz, 1993). To date, the electric light driven models are capital intensive and bear high operating costs. The greater outlay could be justified by the reduction in cost of downstream processing and by the production of a sufficiently high value product. The genetic modification of Dunaliella to result in such a product may be one way in which the use of a photobioreactor may be justified. Currently no such bioengineered strain exists.

The second option is to use sunlight-driven These offer the advantages of photobioreactors. increased control and reduced costs of downstream processing. In our laboratory we have had some success using transparent tubular plastic bioreactors, where up to fifty litre culture bags and a two step salinity growth system were utilised. Cultures were initially grown at low salinities of around 12% to optimise biomass production and then salinity was rapidly increased to approximately 23% to induce β -carotene production. Throughout this process, maximum cell concentrations ranged from around 1.8×10^6 cells/ml to 4.0×10^6 cells/ ml in the lower salinities to about 30 to 80x10⁴ cells/ml for the higher salinities. These results were comparable to the readings recorded by Moulton and Burford (1990)

using the related species *Dunaliella viridis* in open ponds. Although further work needs to be carried out to improve the separation technique, more than a 10-fold increase in total β -carotene was recorded with the bioreactor derived cells compared to an equal amount of packed cells derived from a non induced culture.

It is unlikely that much higher quantities of β -carotene could be induced from larger volumes based on the same bioreactor design, as the level of sunlight incident on the inner core of the culture would be expected to become a limiting factor. However, these preliminary results indicate that such sunlight-driven photobioreactors could have a good potential in areas where land is at a premium, to generate seed cultures prior to inoculating in larger ponds. This method could also be used to ensure axenic cultures during the initial stages of culture when biomass generation is a priority.

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Article

The Newton-Leibniz Priority Dispute

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Summary: In the early eighteenth century a priority dispute erupted. The President of the Royal Society, Sir Isaac Newton, and one of the foremost continental philosophers at the time, Gottfried Wilhelm Leibniz, both claimed priority in the discovery of the differential and integral calculus. We now credit Newton as the first discoverer of the calculus, Leibniz being a close second. This review, based on a public lecture delivered by the author in October 2000, will examine the historical and mathematical aspects of the dispute.

England in the early eighteenth century saw the beginning of perhaps the most famous dispute in the history of Mathematics. In truth, its seeds were sown in the previous century. One of the contenders, Sir Isaac Newton, then President for Life of the Royal Society, had an important matter in hand and delayed the dispute by nearly three decades. But before we go into the dispute let us familiarise ourselves with the two main characters.

Two lives

Sir Isaac Newton was born prematurely in a manor house in Woolsthorpe, Lincolnshire on Christmas Day 1642 (Julian calendar), the same year Galileo died. His father died before he was born. His mother remarried the Reverend Barnabas Smith, an elderly rector. Newton was born into a family of some means, which were certainly increased by this marriage. However, Newton was sent to live with his maternal grandparents. He returned to his maternal home when his stepfather died eight years later. At the age of 12, Isaac attended the grammar school at Grantham, where he lodged with an apothecary, Mr. Clarke. That may have been his source of interest in alchemy.

He joined Trinity College, Cambridge, in 1661 at the age of 19. Three years later he was elected a scholar of Trinity, a year after which he became a Bachelor of In 1665 the plague reached Cambridge and Arts. Newton temporarily returned to his native Lincolnshire to avoid it. The three years he spent in his family home in Lincolnshire are known among Newtonian scholars as the anni mirabiles. There he discovered the Binomial Theorem to any power and in November 1665 the Differential Calculus, or as he referred to it, the Method The Integral Calculus, or Quadrature of of Fluxions. Curves, he discovered in May 1666, after discovering the Theory of Colours. The Law of Gravity and the orbits of planets followed.

After his return to Trinity College in the spring of 1667 he was elected a Fellow. Two years later, at the age of 26, he became the Lucasian Professor of Mathematics. Newton secretly pursued the study of alchemy and theology, apart from mathematics and dynamics.

Meanwhile his discoveries were as yet unpublished. He sent to John Collins, a mathematical impresario, the *De Analysi*, a document in which he set out his results on infinite series and the calculus. We owe the belated printing of Newton's *magnum opus* to the astronomer Dr. Edmund Halley. He visited Newton in 1684 and asked him what course a planet would follow if the force of attraction between sun and planet followed an inverse square law. Newton answered "an ellipse", and sent him the proof the following November in a paper called *De Motu*. This paper was expanded into the *Principia* which was published on the 5th of July1686. Significantly, he did not derive any of his results by means of his calculus.

In 1696 Newton became Warden of the Mint. He undertook his new position with zeal and was an able administrator. His duties included the persecution of forgers. In 1700 he was appointed Master of the Mint. In 1701 he gave up the Lucasian Chair. In November 1703 he was elected President of the Royal Society. And here we leave Newton for a while and proceed with a brief life of Leibniz¹.

Gottfried Wilhelm Leibniz was born in Leipzig in 1646. His father Friedrich, was a professor of moral philosophy at Leipzig. He died when Gottfried was six. His mother Catharina Schmuck, was Friedrich's third wife. He entered school when he was seven. There he learned Latin and Greek, the knowledge of which he supplemented by further reading. At the age of fourteen he entered the University of Leipzig where he studied philosophy and mathematics. He was also taught Latin, Greek and Hebrew. He was awarded a bachelor's degree in 1663.

Leibniz then proceeded to Jena. There the professor of mathematics, Erhard Weigel, who was also a philosopher, influenced Leibniz. Back in Leipzig he obtained a master's degree in philosophy. A few days later his mother died. After being refused a doctorate in law, ostensibly because of his youth, he went to the University of Altdorf. He received his doctorate in 1667.

He then went to Frankfurt, where he lived for a few years under the employ of Baron Johann Christian von Boineburg. His task included that of a secretary, librarian, and a lawyer. There he pursued various projects of a scientific, literary and political nature. He also served as lawyer to the courts of Mainz.

In 1671 he published a book on Physics, *Hypothesis Physica Nova*. In 1672 Boineburg sent Leibniz on a diplomatic mission in Paris. While in Paris he availed himself of the opportunity to make contact with French mathematicians and other intellectuals. Christian Huygens, astronomer and mathematician, was perhaps the most important contact.

The following year, Leibniz accompanied Boineburg's son and nephew on a mission to London. While in London, Leibniz met many English intellectuals, including Robert Hooke, Robert Boyle and John Pell. He was also elected a member of the Royal Society after giving a demonstration of his as yet unfinished calculating machine. It was at this time that Leibniz became acquainted with John Collins and Henry Oldenburg

Back in Paris, Leibniz studied extensively mathematics under Huygens – his visit to London convinced him that his knowledge of the subject was not adequate. After various efforts in formulating the differential and integral calculus in November 1675 he wrote a manuscript where he used the $\int f(x)dx$ notation for the first time. In autumn 1676 he discovered the now familiar result $d(x^n) = nx^{n-1}dx$ for integral values of *n*.

In 1676 Leibniz revisited London. He visited Collins and, without the author's knowledge, was shown Newton's paper De analysi. Although he took thirteen pages of notes on series he did not jot one note on the calculus. It is possible that he may have already discovered his method of calculus. The President of the Royal Society, Henry Oldenburg and Collins, who marvelled at Leibniz' mathematical abilities, persuaded Newton to correspond with Leibniz in the same year. He sent two letters, the Epistula prior and the Epistula posterior. In the former Newton expounded his theory of infinite series and the binomial theorem. In the second letter Newton expounded further the theory of infinite series. He "discussed" the calculus in a discrete way and in anagrams. He revealed nothing but simply indicated that he had a method of finding tangents and areas. Then Oldenburg died and the correspondence ceased.

In 1676 Leibniz was offered the post of librarian by the Duke of Hanover. He reluctantly left Paris in October

to Hanover via London and Holland, where he met Spinoza. Hanover remained his home until his death, though he travelled extensively.

Another important achievement by Leibniz is the invention of the binary system and arithmetic in 1679. He also discovered determinants while trying to solve systems of linear equations. The latter discovery remained unpublished. Throughout the 1680's he composed and published several philosophical works.

In 1684 he published Nova Methodus pro Maximis et Minimis, itemque Tangentibus... in Acta eruditorum, a German journal he helped to found. In this paper he described the Differential Calculus, but gave no derivations for his results. In this paper Leibniz uses differentials dx, etc. rather than derivatives. Two years later in the same journal he published De Geometria Recondita et Analysi Indivisibilium atque Infinitorum where he expounded his Integral Calculus. There was no reference to Newton in either papers. But Newton was at the time busy with the publication of his Principia and the dispute was postponed.

The seeds of contention are sown

It is relevant to a history of the Newton-Leibniz dispute to examine how Newton came to allow his De analysi out of the confines of Cambridge. In 1669 Isaac Barrow, who at the time was the Lucasian professor of mathematics, received a book from John Collins. He showed Newton the book, which was entitled Logarithmotechnica by Nicholas Mercator. In the book was the series for $\log_{e} (1 + x)$, a series which Newton had already found. Barrow, without Newton's knowledge, sent Collins the De Analysi. The paper dealt with infinite series, quadratures and his method of fluxions. Collins sent back the paper only after he had the manuscript copied. Collins, without the author's permission, sent the manuscript to various mathematicians both in England and abroad. This is the very document which Leibniz saw on his second visit to Collins in 1676.

Newton and Collins met for the first time in November 1669. The two men kept up a correspondence until 1672. Newton then became more interested in alchemy. Although Newton would not have approved of Collins spreading his work throughout Europe, this worked in his favour. Collins died in 1683 after suffering for seven years from a terrible illness. In 1708 his papers were passed to William Jones. The *De analysi* was among them. While the priority dispute raged these papers were used by Newton to support his claim.

As mentioned above, Leibniz published two papers on the differential and integral calculus in 1684 and 1686, respectively. His first paper was referred to by his allies Johann and Jacob Bernoulli as 'an enigma rather than an explication'. He did not refer to Newton's work at all. He mentioned neither the *Epistula prior* nor the *Epistula* *posterior.* The *De Analysi*, which he perused at leisure when he visited Collins in London in 1676, he conveniently forgot to mention. But Halley's visit to Newton and the preparation for publication of the first edition of the *Principia* delayed the dispute.

Nicolas Fatio de Duillier (1664 – 1753) was a Swiss mathematician and a protégé of Newton. Fatio, whom Newton had known since 1689, was probably the closest friend Newton ever had. Their relationship cooled somewhat by 1693, the year when Newton had a mental breakdown. Yet in his treatise *A Double Geometrical Investigation into the Line of Quickest Descent* published in 1699, after asserting his independent discovery of the calculus, he wrote that Newton was the first to discover the Calculus and accused Leibniz of taking advantage of Newton's modesty

Leibniz was not provoked. He wrote that learned men should not fight like fishwives, and that Newton would not approve of such rubbish that Fatio wrote. He added that Newton and himself were original masters of the calculus, as their success in solving problems on maxima and minima has shown, and which Newton had already demonstrated in 1687, that is, after Leibniz' 1684 paper!

Earlier in 1693 Leibniz sent a letter to Newton which was very courteous. Newton answered in similar terms, and wrote that he valued very highly his friendship with "one of the leading geometers of this century" and begged his censure on any point, since "I value friends more than mathematical discoveries." He also sent him a general solution using fluxional notation. This does not mean that Newton was not anxious about losing priority in the discovery of the calculus. In the first edition of the Principia (July 1686), Newton, perhaps troubled by the knowledge that Leibniz had also discovered the calculus, asserted that he corresponded with "that most excellent geometer, G.W. Leibniz" and that Leibniz communicated his method which hardly differed from Newton's "except in his forms of words By the third edition, as the dispute and symbols". progressed, all reference to Leibniz in the Principia disappeared.

In 1699 Leibniz criticised, anonymously, David Gregory's demonstration of the catenary. He found an error and claimed that the fault in the demonstration lay in the shortcomings of Newton's fluxional method. Leibniz clutched to every straw at hand to defend his claim to priority. His habit of launching his attacks anonymously, however, only earned him derision.

In 1704 Newton finally published *De Quadratura* as an appendix to the *Opticks*, although in 1693 John Wallis published a brief account of fluxions in his book *Geometry*. In the Advertisement he mentions a letter which he wrote to Leibniz in which he describes "a

Method by which I had found some general Theorems about squaring Curvilinear Figures". The following year, in the journal Acta eruditorum, Leibniz reviewed under cover of anonymity Opticks in which he compared Newton to Honoré Fabri, a man known for plagiarism. Fabri composed his geometry Synopsis geometrica (1669) by using a work by Francesco Cavalieri and by substituting different terminology claimed to have developed a new method. Similarly, according to Leibniz, Newton had used fluxions rather than differentials. Despite Leibniz' denial that he had no intention of accusing Newton of plagiarism his intention was obvious.

In 1710 John Keill in a paper on centrifugal forces in the *Philosophical Transactions* asserted Newton's priority and charged Leibniz with plagiarism. This accusation ushered in the second phase of the controversy. However, before we delve straight into an account of the controversy, let us examine our protagonists' respective methods.

Fluxions and differentials compared ⁴

Newton's approach to the calculus was a dynamical one. Isaac Barrow, who in turn was familiar with the works of Bonaventura Cavalieri, probably influenced Newton. Cavalieri thought of the tangent at a point on a curve as the direction which a particle was following while at that point. The word *fluxion* is derived from the Latin word fluxus which means flowing. Newton imagined a particle having two components of velocity - one parallel to the x-axis and another parallel to the y-axis tracing a curve in the xy-plane. He denoted these velocities \dot{x} and \dot{y} , and called them the *fluxion* of x and the *fluxion* of y, respectively. Note that Newton involved time in his calculus. He called x and y the *fluent* of \dot{x} and the *fluent* of \dot{y} , respectively. In other words, the fluxion is the inverse of the fluent and vice versa. Then he introduced the letter o to signify an infinitely small (or infinitesimal) interval of time. Hence, in the case $y = x^n$, in an interval of time o, x becomes $x + \dot{x} o$ and y becomes $y + \dot{y} o$. Newton discovered the binomial theorem and so he had no problem in expanding $(x + \dot{x} o)^n$. Then he eliminated the terms without o (namely, y and x^n) and divided throughout by o. Since o is considered as infinitely small, we then neglect terms with o, o^2 and higher powers and obtain $\dot{y} = nx^n \dot{x}$. Newton was uncertain as regards the last step, which he referred to as "blotting out the o's". He admitted that his method is "shortly explained rather than accurately demonstrated." Newton relied heavily on intuition. However he got close, as the following extract from the Principia (Vol I, Sect. I, Lemma I.) shows:

Quantities, and the ratio of quantities, which in any finite time converge continually to equality, and before the end of that time approach nearer the one to the other than by any given difference, become ultimately equal. We have here the concept of limit presented in such a way that led to confusion and to criticism. However, Newton emphasised that fluxions are never considered alone but in ratios.

The fact that o, initially non-zero and then practically set to zero led George Berkeley, an Irish dean in the Church of England, to criticise Newton and called infinitesimals "the ghosts of departed quantities" in his book *The Analyst*. Thus, concluded Berkeley, "he who can digest a second or third fluxions ... need not, me thinks, be squeamish to accept anything in divinity".

Leibniz employed *differentials*, that is differences, and hence the letter d in the calculus. He did not involve time at all. His approach was geometrical and is in fact the one we now employ. The change in variable x was denoted by dx. In Newton's notation this is equivalent to \dot{xo} . He called the quantity dx the *differential of x*. His analysis was based on the *differences between the coordinates* of two neighbouring points. Note that as yet there is no notion of a derivative. Like Newton, he neglected terms with products of infinitesimals. He justified this by appealing to intuition. In his 1684 paper he gave, correctly, various results including the product and quotient rule, without any derivations.

Both Leibniz and Newton had problems in explaining away the disappearance or neglect of terms involving products of infinitesimals. Such explanations had to wait a hundred years. Leibniz appealed to the then philosophical concept of continuity to justify the limit when the infinitesimals become zero. Nowadays we define continuity by means of limits and not vice-versa. Newton was no more nearer to an adequate explanation. Because of such problems the calculus was not at first universally accepted. Christian Huygens did not accept it but he did not attack it.

Another problem, which beset Leibniz, was the notion of higher differentials. Leibniz did not regard the ratio

$$\frac{dy}{dx}$$
, which is equivalent to $\frac{\dot{y}}{\dot{x}}$ in terms of Newton's

fluxions, as fundamental, and hence he could not give a satisfactory definition of d^2y . Leibniz appealed to an analogy: if we picture motion as a line, then the velocity is represented by an infinitely smaller line, and the acceleration by a doubly infinite smaller line. In a letter from Johann Bernoulli to Leibniz in which expressions

such as $\sqrt[3]{d^6 y} = d^2 y$ are used liberally, shows the

extent of the lack of clarity in the concepts.

The study of the convergence of series, essential to the idea of limit, which in turn is essential in both the differential and integral calculus, was lacking. Leibniz, in fact, seriously considered whether the series 1-1+1-1+... converged to $\frac{1}{2}$. Having proved that $d(x^n) = nx^{n-1}dx$ for integral values of *n* he then assumed the result for rational values of *n*. Newton expanded $(x + o)^n$ for fractional values of *n* as an infinite series. He assumed convergence without any proof. Leibniz

thought of $\frac{dy}{dx}$, as a ratio rather than as a limit.

It is unfortunate that both men lacked rigour. But despite this there was no lack of results This was especially true on the continent, where Leibniz method was used with advantage in preference to fluxions. The Bernoulli's produced many useful results.

Newton and Leibniz differed from others before them in that their methods were general. As stated above, rigorous proofs were lacking. Newton and Leibniz were the first to recognize the relation between the problem of tangents and that of quadratures (areas) – that one was the inverse of the other. This is the fundamental theorem of the calculus. However, we must not forget that others before Newton and Leibniz did important work concerning tangents and areas. John Barrow, and Fermat were close to the discovery of the calculus. Thus Newton and Leibniz "stood on the shoulders of giants".



Fig. 1 Newton's fluxions and Leibniz' differentials compared.

Before the Calculus

We shall first examine briefly the work Fermat and Barrow, two figures who influenced both Newton and Leibniz. The idea of changing the independent variable of a function by a small amount and then setting that amount equal to zero was not something new at the time of Newton and Leibniz. Fermat employed it in his method for finding maxima. To find the maximum area of a rectangle with sides x and (a - x) with x < a, he altered x by a small amount E. Fermat then argued that near the maximum the areas of both rectangles should be nearly equal, and by neglecting E, he obtained correctly x = a/2. It may be said that infinitesimals were accepted after Fermat applied them with success to this and other problems.

His method of finding tangents is illustrated in Fig. 2. We first find the subtangent TQ as follows. Let OT = a, OQ = x and $QQ\phi = E$. We note in passing that E is Newton's \dot{x} o and Leibniz' dx. We shall illustrate this method for the parabola $y = x^2$. We note that for nearby points P and P' we have $QP = x^2$ and $Q'P\phi' < (x + E)^2$. Hence we have



$$\frac{(x+E)^2}{TQ'} > \frac{P'Q'}{TQ'} = \frac{PQ}{TQ} \implies \frac{(x+E)^2}{x+E-a} > \frac{x^2}{x-a}$$

After cross-multiplying, opening brackets and simplifying, we obtain

 $2x(x-a)E + E^2(x-a) = x^2E$

After dividing by E and simplifying we end with

 $x^2 + xE - aE = 2xa.$

Fermat then argued that equality holds if we set E = 0and hence we obtain $a = \frac{1}{2}x$. Hence the tangent to the curve at P is

$$\frac{PQ}{TQ} = \frac{x^2}{x-a} = \frac{2x^2}{x} = 2x$$

Note that the idea of a limit does not occur in the argument. Fermat did not have the binomial theorem, and hence he failed to obtain general results. Barrow's method (see Fig. 3) was essentially similar to that of Fermat. But the rules he gave were more general. They could be applied for implicit equations. He laid down the following rules:

- 1. Let MR = a and NR = e;
- 2. Substitute the values x e and y a for x and y, respectively, in the equation;
- 3. Reject those terms with powers of a and e or terms with ae, etc.;
- 4. Equate the known terms (i.e. terms without a or e) to zero:
- 5. Substitute *MP* for *a* and *TP* for *e*, and hence determine *TP*.

Here, of course, in stating (5) Barrow makes the assumption that the points M and N are close. The idea of a limit, although not expressed, is inherent in this assumption. The letters a and e are our more familiar



 Δy and Δx , respectively. Barrow did not mention

Format, so we may perhaps assume that he was not familiar with Fermat's method.

We now consider the Fermat's method of quadrature for the equation $y = x^{p/q}$. Consider the points on the x-axis x, ex, e^2x , e^3x , ..., where e < 1. We have thus a set of ever-diminishing intervals. If we construct rectangles from these points, as shown in Fig. 4, we can approximate the area under the curve by the sum of terms of the form

$$(e^{n-1}x)^{\frac{p}{q}} \cdot (e^{n-1} - e^n) x = x^{\frac{p+q}{q}} e^{\frac{p+q}{q}(n-1)} (1-e)$$

Hence, summing to infinity starting from n = 1, we obtain p+q

 $x^{\frac{p+q}{q}}(1-e) / \left[1-e^{\frac{p+q}{q}}\right]$

The nearer e is to 1 the more accurate will this expression for the area be. Before doing this Fermat made the substitution $e = E^q$. Noting that $1 - e = 1 - E^q = (1 - E) (1 + E + E^2 + ... + E^{q-1})$

1- $e^{\frac{p+q}{q}} = 1 - E^{p+q} = (1 - E) (1 + E + E^2 + ... + E^{p+q-1})$ we substitute this in the equation. Setting E = 1, we

obtain the area
$$\frac{p}{p+q} x^{\frac{p+q}{q}}$$

One is almost tempted to attribute the discovery of the calculus to Fermat. But one must bear in mind that Fermat saw no connection between the problem of tangents and that of quadrature. Furthermore, he did not recognise "differentiation" and "integration" as operators in themselves independent of geometrical applications.

The dispute erupts

In 1711 the Secretary of the Royal Society received a letter from Leibniz, who was also a member. Leibniz complained that in a paper to the *Philosophical Transactions*, the author Dr. John Keill insulted him. In



Fig. 4 Fermat's method of quadrature.

this paper Keill gave Newton precedence in the discovery of the calculus. Newton saw Leibniz' letter as a warning – his priority in the discovery was at stake. Newton chose his champions. Foremost among these were Keill himself, the Secretary Hans Sloane, Roger Cotes, and Edmund Halley. In 1712 the Society set up "a numerous Committee of Gentlemen of Several Nations" whose aim was to investigate Leibniz' accusation. The nations represented in this, ostensibly, impartial committee were England, Scotland and Ireland. A Prussian ambassador and a Huguenot émigré were also thrown in.

In 1713 all the relevant correspondence between Newton and Collins was published in a volume known as *Commercium epistolicum*. The outcome from this committee the following year was as expected. Keill was exonerated by the report and Leibniz was accused of plagiarism.

Both Newton and Leibniz worked behind the scenes. In 1713 an anonymous printed sheet known as the *Charta volans* spread quickly throughout the continent. This paper emphasised the fact that Newton published nothing about the calculus before Leibniz. It quoted a "leading mathematician" who said that in 1670 Newton invented only his method of infinite series. The "leading mathematician" was Johann Bernoulli, who asked Leibniz not to mention him. Johann and his nephew Nikolaus were prominent, albeit reluctant, allies of Leibniz throughout the dispute. They were not aware that Leibniz saw the *De analysi* when he was in London.

In the same year the inaugural issue of *Journal Literaire* carried an anonymous letter by Keill in defence of Newton. A French translation of the report by the Royal Society was included. Leibniz responded in the same journal by publishing (anonymously) a translation of the *Charta Volans* and a treatise on the difference between Newton's and Leibniz' methods. The author argued that in the *Principia* Newton did not make use of the calculus.

The response was another communication by Keill in the same journal published in 1714. The January and February issue of the *Philosophical Transactions of the Royal Society* was devoted, except for three pages, to Newton's cause. The dispute was, of course, officially between Keill and Leibniz. In 1716 a number of foreign ambassadors assembled at the Royal Society to examine the documents which comprised the *Commercium epistolicum*. They recommended that Newton and Leibniz should communicate directly. Newton was thus compelled to answer Leibniz' letter. In his reply to Leibniz' first letter he called the "leading mathematician" quoted in the *Charta volans* as a "pretended mathematician". Leibniz showed the letter to Bernoulli in an attempt to provoke him. The correspondence lasted five rounds of letters, the length of which increased with every round. The correspondence came to an end with Leibniz' death in December.

The matter did not end with Leibniz' death. Six years after his death The Royal Society published a review, the *Recensio*, in which the dispute was recapitulated in Newton's favour. Needless to say the author was Newton. In 1722 the *Commercium epistolicum* was republished with revisions and footnotes that were unannounced in any preface.

Newton had his enemies on English soil. Dr. John Woodward informed Leibniz that whatever was done against him "proceeded solely from Mr. Newton" and hoped that he would not blame the Royal Society. He also promised to get him a copy of the *Commercium epistolicum*. The Astronomer Royal, John Flamsteed, who at the time of the dispute was immersed in one himself with Newton, sent Leibniz a list of mistakes in Newton's lunar theory.

Consequences

Newton's victory in the dispute was, in a sense, unfortunate. His method of fluxions was cumbersome. His notation and method were still in use in England in 1816. There was confusion between fluxions and infinitesimals as is apparent in Joseph Raphson's book *The History of Fluxions* published in 1715. He unjustly criticised Leibniz method and notation as "less apt and laborious" and as a "far-fetched symbolizing" and "insignificant novelties". Meanwhile, Leibniz' notation and method were adopted with great success on the continent.

Just as a clear language is essential to good literature, a clear mathematical notation is essential to the development of mathematics. The Newton-Leibniz dispute shows us that mathematicians may allow politics to influence their adoptions and impair their judgement. When the calculus was put on a firm footing in the 19th century, Leibniz notation was universally adopted. All that remains of Newton's notation is as the derivative of x with respect to time. But no modern teacher refers to as the fluxion of x.

The dispute occurred at a time when scientific societies under royal patronage came into existence. An ambitious man accepted and honoured by members of an established society meant instant fame which in turn guaranteed employment. His papers would be published and read widely. These societies had a controlling influence on intellectual life – they could make or break an aspiring intellectual. When Newton established himself as President of the Royal Society he became arrogant and autocratic. Newton controlled the membership and even cut short debates. We have already seen how Newton used the society for his own ends in his dispute with Leibniz.

It appears that Newton was more interested in forming a "Newtonian" school rather than furthering the advancement of science. He failed to recognise the superiority of Leibniz method over his own. Both Newton and Leibniz unashamedly used journals to further their own cause. However, it must be acknowledged that the *Acta eruditorum*, unlike the *Philosophical Transactions*, published papers from both sides of the divide. Although printing was advanced enough for the spread of knowledge, yet communication between individuals was still precarious. No postal system existed and one had to find travellers to act as couriers. When Oldenburg died communication between Newton and Leibniz ceased.

One cannot end this section without commenting on Newton's reluctance to publish. One of the reasons he avoided publishing was to avoid controversy. When he published a paper on corpuscular theory of light, it was challenged by the adherents of the wave theory. This controversy (not by any means a dispute) occupied him for some time and distracted him from his other pursuits, mainly alchemy. Newton, unlike Leibniz, stood to lose only fame by not publishing. Leibniz, on the other hand, was not a man of means. Fame meant employment, which is essential for financial security. Hence his unwillingness to share the discovery of the calculus with others. Newton, on the other hand, felt that his reputation was at stake. Newton could not take accusation of plagiary lightly. an In a letter to Bernoulli, after accepting his denial of attacking him personally, claimed that though he never sought fame among foreign nations, yet he had to preserve his character.

As we saw, others before Newton and Leibniz were close to the discovery of the Calculus. The time was ripe for its discovery. It is not inconceivable that Leibniz discovered the Calculus independently. In fact we now credit him with the independent, albeit later, discovery of the Calculus. There comes a time when a major theory simply begs discovery. At such times more than one individual may qualify as a discoverer.

Two deaths

Newton devoted a considerable time in his old age revising his scientific works for publication as new editions. His *Opticks* and *Principia* were bestsellers. The controversy with Leibniz did not diminish his stature or acceptance of his scientific works on the continent. Newtonianism took the known world by storm.

Newton pursued his theological researches as assiduously as his other interests. It is estimated that he wrote more than a million words on the subject. He wrote books on chronology, prophesies and on superstitious nonsense like the Cabala and Numerology. He believed in Hermes Trimegistus, a mythical ancient figure who passed on to Mankind scientific knowledge. He accepted 4004 BC as the year of creation, a date calculated by James Ussher, later Bishop of Armagh. He also gives the date of the Argonauts' expedition!

Newton died on 20th March 1727 at one o'clock in the morning. Among the pallbearers were the Lord Chancellor, and members of the Committee of the Royal Society. He was interred in Westminster Abbey.

The last years of Leibniz' life were dedicated mostly to the dispute. But he still remained creative and in 1710 he published *Théodicée*, a philosophical work in which he tackled the problem of good and evil. In 1714 he published *Monadologia*, perhaps his most influential philosophical work. On 14th November 1716 he died in Hanover after long suffering from arthritis and gout. Only his secretary, Eckhart, attended his funeral.

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¹ For the Leibniz biography I have relied heavily on the Internet site

² William Jones introduced the symbol π for the ratio of the circumference to the diameter of a circle.

³ Manuel, F. E. 1968, p.260

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Commentary

Reflections On The Coming Era of Artificial Intelligence

Nicholas J. Sammut

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Summary: The quest of every species in the attempt to overcome its bounds and limits to preserve itself for eternity has existed from the very beginning. If one were to analyse all the activity of any sort of life, one may suggest that these efforts point to one primary scope: 'To get an egg from an egg'.

From the Darwinian theory of evolution it may arise that this self-preservation not only propagates the species itself, but also probably spawns other species if part of that population over time is separated from the main one and thus could it could become different. However, as organisms became more complex and sophisticated, the physical propagation through genes in the gene pool from body to body and generation to generation via sperms or eggs was not enough. As an addendum, these complex organisms utilised virtual propagation in the form of memes or "mental patterns" (Dawkins 1976). Memes in theory, virtually propagated themselves in the meme pool by leaping from brain to brain via a process which in the broad sense is called imitation.

Meme virtual propagation is seen as instinct and/or social behaviour in animals, but in humans, this is seen in many other ways including ideas, tunes, jingles, quotes and theories. However, in the process of the evolution of these memes, an adaptation occurred. The desperate search of these memes to self-propagate better and to expand more with greater efficiency has resulted in the birth of a better medium to host them- The Machine. Thus the birth of Artificial Intelligence.

Intelligence may be defined as " the mental ability to learn and understand things and information that is relatively of value" while Artificial means "something not originating naturally". While the individual definitions of these words is plausible, their meaning does change slightly when they are fused together. Of course, the general trend is to define something artificial, as being something man-made. But this does not necessarily mean that it is not natural. Artificial intelligence is the new natural process utilised by memes to ensure their self-replication.

As described by Russell and Norvig (1995) this virtual meme sowing, propagation, and expansion includes the imitation of humans as they think and as they act, and, on a more noble level, the evolution of rational thoughts and acts in the absolute, ideal way (assuming humans do not adopt this absolute, ideal way of thinking and acting). As expected, there are many ways of going about artificial intelligence. Neuromorphic systems, expert systems, fuzzy systems, statistical pattern recognition, machine learning and computational learning are to mention a few. Although it is practically impossible to predict how AI will affect the future, clouded insights to what it may achieve can be speculated upon. The potential of intelligent machines is enormous. Human resources are currently the most precious resource in our evolution. Imagine having a machine that is much better than a human: more efficient, more precise, faster, safer, more intelligent better at communication and interaction with others of its kind ... the improvements on humans are innumerable. A machine can be defined as "any system or device that performs or assists in the performance of a human task". Machines, as have always been, are extensions of humans. They are our tools as extensions of our limbs, and now, with AI, they will be our tools as extensions to our brain.

But many humans are sceptical and scared; and they have reason to be so. What if AI is used as a weapon against human interests in general? What if machines become self-aware and just take over? Is AI nature's way of disposing of us? Are we self-destructive beings? Are we going to provide the world with the greatest mass extinction of life, as we know it, dawning with a new era of a new life form? Could be. But the evolution of AI cannot be stopped. No organism ever managed to stop evolution, and humans are unlikely to be an exception.

Man was always scared of his own power to destroy himself. Yet it is frequently ignored, that if man is capable of creating machines that are more intelligent than himself, it is equally possible that man is capable of increasing his own intelligence using artificial techniques.

The only fundamental criterion is: Not to create the monster before creating its cage.

Artificial Intelligence is extremely revolutionary, especially if it is combined with other cutting-edge

technologies. Nanotechnology (the study of the synthesis of molecular-based machines) is opening doors to new concepts that up to a few years ago were only talked about in science fiction. Indeed nanomachines may perform tasks, which are performed by present larger machines. Instead of having big lumps of molecules (man's current machine) working on other big erratic lumps of molecules (what the machine is working on), nanobots, being so small, may work on molecules one at a time assembling perfect precision tools and extremely fast nanocircuits. Indeed, in the limit, these extremely small machines may be used not only to assemble extremely small tools but could also be used to assemble other nanomachines like themselves. In other words, the nano-assemblers self-replicate. Combining nanotechnology with artificial intelligence gives man the intelligent nanomachine, and the intelligent nanomachine gives man the key to a lock that he could never open before; the key to the mystery of life.

Artificial intelligence together with nanotechnology will totally change life the way man knows it. Intelligent nanomachines could be used to enter cells and repair them, marking an end to disease and in the extreme, an end to aging. They could be used to manipulate molecules to manufacture perfect materials, which are light and extremely strong (for example manufacturing diamond to use as girders, car bodies and plane fuselages). They could be used to create natural resources from very common elements. They may be used to eliminate the environmental problems we are currently suffering from by disassembling (using disassemblers rather than assemblers) the poisonous molecules and transforming them to useful compounds. The possibilities are infinite.

AI and nanotechnology may provide a vertical solution to aging by literally stopping it (as mentioned above). But AI may also provide a lateral way of increasing the time that man lives. Combining AI with neuroanatomy and psychology may result in a machine (or agent) in the form of a sophisticated robot that directly communicates with a person's brain. Thus, it could be the case that a particular intelligent machine would interact directly with a human's mind, would store that person's characteristics (clone his memes) and be a mechanical representation of the person. The machine would then go on and perform the tasks that that person hates doing (such as going to work) and later on, would reconnect and re-upload (to the person's brain), the information gathered by the machine. In this way, the machine would do the work instead of the person, yet still in the particular way that that man would have done the work had he gone to do it personally. Moreover, the person would still know exactly what would have happened at work after the re-upload and would be confident enough that

whatever the machine would have done was exactly what he would have done in exactly the same way he would have done it. Thus the intelligent agent virtually would give the opportunity to the person, of being at two places at the same time (hence a lateral way of increasing time). Naturally, many agents could be employed by one person allowing him to do only the things that he would enjoy doing most, whilst using his talents to the full (by means of the agent) in his contribution to humanity. Many other applications of such an agent may spawn from this idea such as the machine version of dead people (provided eternal life through nanotechnology is not achieved) the experience of people living as other forms of life (provided the agents are manufactured not only as humanoids but also as replicas of animals) and an addition of memory or computational power of the human brain.

Indeed the doors that artificial intelligence would open to the future are innumerable, though many still think that some doors should remain shut. Yet the insatiable quest of man and of all living things (man being their dominator) to find means of self-preservation, of gaining the unlimited, of spreading beyond all bounds and of reaching the eternal was, is, and will always be, an obsession providing an unstoppable driving force. Artificial intelligence is currently one of these means, and once man has insights into it, it will grow, no matter how much it is condemned, no matter how much it is prohibited, no matter how many difficulties are in its course. For once man can dream it, man will do it, and where there is a will (and the will there is) there is a way. Remember Jules Verne?

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Abstracts

Studies on the melanomacrophage centres of seabass, *Dicentrarchus labrax*, and seabream, *Sparus aurata*, from two fish farms in Malta

Ahmed Nfati

The distribution and histochemical nature of melanomacrophage centres in the spleen, kidneys and liver were studied in clinically abnormal sea bass (Dicentrarchus labrax), and gilthead sea bream (Sparus auratus), collected from two fish farms in Malta. Starvation experiments were also conducted. The results demonstrate that melano-macrophage centres in sea bass which survived a VNN outbreak were not significantly affected, possibly due to the acute nature of the disease. In sea bream, the results suggest that tissue catabolism is a major factor contributing to the formation of the pigments within melano-macrophages. During the starvation experiments, the melano-macrophage centres had increased after 6 weeks and 10 weeks of complete deprivation of food. These results indicate that increased deposition of melano-macrophage centres was clearly associated with cachexia; there was less clear correlation with other clinical symptoms such as fin erosion and swollen abdomen. After ten weeks of starvation, haemosiderin is observed mostly in the melano-macrophage centres of the spleen, with no deposition being noticed in the kidneys.

This work has confirmed that the splenic melanomacrophage centres of sea bream are the sites where iron derived from effete red blood cells is stored as haemosiderin for possible eventual re-utilisation.

The melano-macrophage centres were entirely absent from the liver of all specimens.

In vitro culture of *Pistacia terebinthus* – a shrub indigenous to the Maltese Islands

Alain Camilleri

Pistacia terebinthus (Family Anacardiaceae) is a small deciduous tree, native to the central and western Mediterranean. The genus *Pistacia* consists of 10-11 species, all important in providing rootstock material

resistant to pests, diseases and environmental stresses. They are also tolerant to drought and are able to thrive in poor soil conditions. All species of *Pistacia* are threatened with genetic erosion due to the destruction of natural habitats, and also because the modern commercial varieties are preferred over the wild/local varieties.

The local *P. terebinthus* populations are very restricted, the largest occurring in Wied Bloq (l/o Qormi) near the St. Sebastian Fireworks Factory. It appears that this population could be native, unlike other populations such as that occurring at Buskett. It is therefore vital to preserve this population as the remaining natural gene pool of *P. terebinthus* in the Maltese Islands.

The *in vitro* culture of this shrub is one way to propagate the species. It has the advantage over conventional vegetative propagation in being independent of season and climate. The trees grown from *in vitro* cultured specimens are also free from pathogens.

Buds and seeds collected from shrubs growing in Wied Bloq were used as starting material for culture. Buds were not suitable as explants because they should ideally be collected in the Spring, and also due to the ever-present fungal contamination. Seeds were successfully surface sterilised using ethanol (70%) and, either 5% sodium hypochlorite or 10% household bleach solutions. Best results of germination resulted when the embryos were liberated from their seed coats before placing them into the media in the culture vessels.

After successfully germinating *in vitro*, the effect of various BAP concentrations (1.0, 1.5. 2.0 and 2.5 mg/l) on multiplication was studied. The optimum BAP concentration was found to be 2.5 mg/l. However, browning of the leaves and stem, and shoot and leaf tip necrosis became a problem due to the presence of oxidised polyphenolic compounds produced by the explants.

Successful measures taken to reduce the effect of polyphenols included the addition of antioxidants to the medium (ascorbic acid, polyvinylpyrrolidone, and activated charcoal), and reducing the incubation light intensity. Increasing the calcium concentration was beneficial. Frequent subculturing (15-21 days) was found to most suit P. terebinthus explants.

The structure, composition and seasonal changes of a *Cystoseira spinosa* v. *tenuior* assemblage at Delimara (S.E. Malta)

Carmela Mifsud

This study is an investigation of the structure, typology and seasonal changes of an assemblage characterised by the dominance of *Cystoseira spinosa* Sauvageau var. *tenuior* (Ercegovic) Cormaci, *et. al.*, occurring on hard substrate in the shallow infralittoral. Sampling was carried out between October 1997 and August 1998. This assemblage was found growing in the area of Delimara known as Kalanka/Qala t-Tawwalija. The reproductive phenology and the general morphology of the dominant species were also studied.

This particular assemblage was chosen because Cystoseira spinosa v. tenuior is itself a rather poorly known taxon and there have been no detailed studies especially on its morphometry. For the purposes of morphometric and phenological investigations, samples were collected, where possible, every month. The morphological parameters investigated were length of the main axis, length of branches and length and diameter of tophules, while the reproductive state of the plants was also noted. This particular population was observed to have a vegetative period between October and July and undergoes full dormancy in August, when all the branches fall. The largest branch length (mean branch length being 6.3cm) occurs between March and May. Abscission (in August) follows after the fertile stage of the plant, which is mostly between March and May. Abscission coincides with the largest values for tophule length and width, since this is the time when the accumulated reserves are mostly essential. The average tophule length was 8.9mm and the mean diameter for these tophules was found to be 4mm.

For the floristic investigation, samples were studied seasonally in order to make as full a list as possible of all species that may be present within this assemblage throughout the year. A total of 132 species were recorded (72 rhodophytes, 25 phaeophytes, 25 chlorophytes and 10 cyanophytes). Only 10 species were found to be exclusively growing as undergrowth or as accompanying species. From all the different life forms present, the Ephemerophyceae represented the highest percentage: 58%.

The epiphytes were examined, throughout the year, in relation to four different parts of the host plant, namely the attaching disc (d), main axis (a), tophules (t) and the branches (p). The preferred microhabitat for most species was found to be the branches, probably due to their dendritic morphology and to the better opportunity for light acquisition. However, marked seasonal variations were noted, particularly in relation to the fact that the branches are shed in August. Moreover, the branches are the structures most exposed to violent hydrodynamism characteristic of autumn and winter, thus delicate species would be easily dislodged. The main axis, which is a perennial part of the *Cystoseira*, supports a more stable epiphyte component, since most epiphytes growing on this part are perennials.

The number of epiphytes was highest in winter, and from the increase in certain groups (some types of chlorophytes) it was deduced that this increase could have been due to subtle traces of pollution in the bay. No direct pollution measurements were carried out for the purpose of this study. However, data available in the literature (Monitoring Programme for Coastal Waters, 1997; 1998) indicate that traces of pollution namely from the Wied Ghammieq sewage outfall, from the nearby fish farm and from the outlet of the Delimara power station, may exist near to the study area.

The protective effects of *Opuntia* extract against stress conditions *in vitro*: methodologies for the determination of biological activity and for isolation of the active molecule responsible for such effects.

Claire Angele Baluci

The heat shock response is an immediate, transient response to a wide variety of physiological stresses. Heat shock proteins (HSPs) are synthesised when cells are exposed to heat as well as other types of cellular injury. They are evolutionarily highly conserved, and have an essential function in the survival of organisms, protecting them from trauma. Unfortunately, the stress response in the human body is not so immediate, and it can only react after a period of time, when the stress damage is already done, by repairing the damaged tissue. The prickly pear cactus (Opuntia) holds a secret deep in its genes that enables it to induce and amplify the rapid synthesis of heat shock proteins produced by the body under conditions of stress, thus minimising the harmful effects of the stress condition. This explains why such interest has been shown in this area of study.

In the first part of the study, different techniques enabling the efficient and accurate biological analysis of *Opuntia* extracts (TEX-OE[®]) *in vitro* were evaluated. Using a dye exclusion method for the determination of cell viability, it was found that heat stress of non-treated HL60 cultures induced a significant decrease in cell viability (31%), whereas cultures previously treated with TEX-OE[®] were shown to be somewhat resistant to

death induced by hyperthermia (5.5%). cell Morphological evaluation of the HL60 cells confirmed these results, where cultures treated with TEX-OE® prior to heat stress showed a clear-cut resistance to apoptotic cell death. The same response was exhibited in HaCaT cultures subjected to heat stress and tested using the XTT viability assay, where heat stress of nontreated cells also induced a significant decrease in cell viability in comparison with the TEX-OE® treated cultures (53.6% and 10.7% respectively). Results obtained for the XTT assay were similar to those obtained from the dye exclusion method, thus indicating that the latter procedure represents a suitable model for monitoring biological activity of TEX-OE®. Studies carried out to show the effect of recurrent heat stress on HL60 cultures demonstrated a dramatic decrease in cell viability for non-treated cells over the three-day experimental period (78.4%), and a significant resistance to cell death in TEX-OE® treated cultures (12.1%). It was also shown that the duration of protection after heat shock achieved by TEX-OE® treatment is three days. Observations for cell viability were related to HSP72 levels, where a significant difference in the expression of HSP72 in response to each heat stress was observed between treated and nontreated cultures. A rapid increase in HSP72 levels (17fold) which were maintained at high values throughout the experiment were observed for treated cultures, whereas non-treated cells showed a relative inability to respond immediately to the stress, with higher levels (<2-fold) only being achieved after the second day of experimentation. These results thus indicate that the protective role of Opuntia against stress induced lesions and cell death can be related to the heat shock response.

The second part of the study involved the development and assessment of efficient methods for the extraction, separation, and purification of the active components for Opuntia. For preparative separation, medium pressure liquid chromatography on a silica gel column was most suitable. Using a hexane-acetone solvent system, the active components were efficiently separated into one main fraction, the activity of which was confirmed in vitro using the technique for the determination of HSP72 levels. Further purification of this fraction was achieved by the application of a normal-phase 12µm silica column eluted with heptaine-diethyl ether (95:5%). The active molecule responsible for the biological effects of Opuntia was isolated using revered-phase high-performance liquid chromatography on a Kromasil 5 μ m C₁₈ *n*-octyldecyl siloxane column. Isolation of the active molecule was found to be most complete with the use of 95:5% methanol-water solvent system. Identification of the active molecule remains to be determined. Various methods and techniques in order to achieve this are currently under investigation.

Biological study of Maltese hedgehogs (subfamily-Erinaceinae) for conservation

Clifford Borg

In order to undertake long-term conservation management programmes for any organism and habitat, some knowledge is required about the biology of the species and the habitat it needs for survival. No detailed scientific work has been carried out on hedgehogs in Malta prior to this study. Thus it was considered necessary to obtain biological knowledge of this group for the legal protection to be backed with realistic conservation management possibilities.

From the 63 hedgehog individuals sampled in different localities in Malta, two colour forms (pale and dark) were found to show significant difference in the spine band sizes, demonstrating a clear distinction among the hedgehogs inhabiting Malta. To assess this distinction using another tool, the large toe or Hallux of 10 individuals was also measured, but no statistical difference was obtained between the Hallux size of the two types of hedgehogs in Malta.

A study of hedgehog diet, habitat preference, distribution and abundance, sex ratio, body measures, seasonality in diet and behaviour and incidence of ectoparasites infestation were all considered in this research project.

Dietary studies indicated that the preferred item was beetles (Order Coleoptera). Hedgehogs were mostly found near agricultural areas. Hedgehog abundance at Ghajn Tuffieha was found to be around 0.64 per hectare. Ectoparasite infestation occurred mainly near urban areas.

A detailed questionnaire targeting Maltese farmers, hunters and bird watchers added valuable insights and information on Maltese hedgehogs.

From hedgehog road casualty analysis undertaken in this research project, it became evident that more local awareness needs to be implemented in particular to areas of high road density and close to areas with greater hedgehog abundance. Other aspects found to affect hedgehog survival include habitat degradation and extensive use of pesticides and insecticides.

Variation in *Posidonia oceanica* (L.) Delile meadow parameters in relation to location and meadow type

Graziella Tonna

Around the Maltese Islands, the seagrass Posidonia oceanica forms two types of meadows: "reticulate", consisting of patches of seagrass interspersed with barren areas of sand or rock, and "continuous", consisting of homogenous seagrass beds. Analysis of the variation of plant morphology and sediment characteristics was carried out. Samples were collected from four roughly equidistant sites of similar water quality: Mellieha Bay, White Rocks, St Thomas Bay and Ramla l-Hamra, to study large-scale variation and from four zones within Mellieha Bay to study smallscale variation. For each station, samples were collected from each of the two types of meadows. The morphological parameters investigated were shoot density, leaf area index, leaf biomass and epiphyte load per shoot. The sediment characteristics analysed were weight ratio of roots and rhizomes to sediment, organic content and grain size scatter.

One-way and two-way ANOVA were used to test for statistical significance. For such a complex analysis, the one-way ANOVA was easier to interpret.

Small-scale variation within Mellieha Bay was significant for leaf biomass, epiphyte load and leaf area index. Such variations are attributed to differences in nutrient availability, water clarity and grazing pressure at different localities within the bay. Large-scale variation was very evident in the continuous meadows, which were found to differ significantly in all the The reticulate meadows parameters investigated. differed between the sites only in leaf area index. This phenomenon is explained through "edge effects". The differences between continuous meadows may be due to various environmental parameters such as nutrient and light availability, sediment load, currents and biotic and anthropogenic influence. Continuous meadows varied from the reticulate ones in sediment characteristics but not in shoot parameters.

Seed dormancy, seed germination and *in vitro* propagation of Hawthorn (*Crataegus monogyna* Jacq.) and Wild Pear (*Pyrus syriaca* Boiss.)

Matthew Borg

Micropropagation refers to the propagation in culture by axillary or adventitious means. It is a general term for vegetative *in vitro* propagation and allows the culturist to grow clones of a mother plant that would have been selected for its advantageous traits. The plants produced are free from bacteria, fungi and possibly viruses. They can be produced in very large numbers over a very short period of time and can thus be ideal to propagate rare or endangered species.

Two such cases are the wild pear, *Pyrus syriaca* Boiss. and the wild hawthorn, *Crataegus monogyna* Jacq. These were micropropagated using young seedlings as primary explants. Both plants are of great importance and have great potential in sectors ranging from pharmaceuticals to rootstocks. Both constitute an important part of the local flora with hawthorn being an important food source for migratory birds such as the song thrush. Some hawthorn varieties are considered endangered by the IUCN and the wild pear is considered rare.

For *C. monogyna* the best seeds were chosen from three localities, whilst for *P. syriaca* a number of trees from a single valley were used to obtain seeds. Attempts were made to micropropagate these plants from buds, but these were already dormant due to the approaching winter months.

The seeds of both species were put into a sterile culture and allowed to germinate on Murashige and Skoog basal medium (MS+A). On germinating, the stems were excised at the epicotyl. They were then placed on one of media that was considered suitable for two multiplication. The first medium was a MS+A medium that also contained two plant growth substances, 1.0 mgl⁻¹ 6-benzylaminopurine (BAP), a cytokinin, and 0.1 mgl⁻¹ naphthalene acetic acid (NAA), and auxin. This was coded IF01. The second multiplication medium was coded SG02 and was based on a Lepoivre medium containing 0.1 mgl⁻¹ BAP. The effectiveness of both media for shoot multiplication was then compared using both plants. SG02 was found to be the preferable medium for multiplication, and thus produced the most plants over a specified time span.

Callus, originating from leaves, roots and cotyledons was obtained on a medium containing $1 \text{ mgl}^{-1} 2,4$ -dichloro-phenoxyacetic acid (2,4-D), another auxin, and an attempt was made to obtain indirect organogenesis and embryogenesis. Tissues produce embryos by a

process known as somatic embryogenesis. These are the equivalent of zygotic embryos, but are produced not by the fusion of an egg and sperm nucleus, but from somatic cells. Somatic embryogenesis and organogenesis were obtained for *C. monogyna* using a medium identical to IF01 with 5 times the amount of vitamins (ET08). The former is of importance as it seems to be a new phenomenon for Hawthorn that has not previously been reported.

Experiments were also set up, *in vivo* and *in vitro*, to study the effects of the seed coat (testa), the embryo sac, and light, on the delay in seed germination in *C. monogyna*. Germination in this plant is renowned for its delay and could take up to 2 years to occur. In these experiments this has been reduced to about 2 days both *in vivo* and *in vitro*. Removal of the testa did speed up germination but it was only when the embryo sac was totally removed that germination was reduced to 2 days. Partial removal of the embryo sac was not as effective as its complete removal, thus giving an indication of the presence of dormancy inducing hormones in the aleurone layer or endosperm.

The effect of light on the germinating seeds was studied so as to obtain information on the growth of roots, hypocotyl and stems in light and in total darkness. Ideal growth occurred in the dark, where root and hypocotyl growth was favoured. Thus, this procedure would make the plant available not only by micropropagation but also by regular cultivation.

A rooting protocol was also set up using MS+A containing the auxin indole acetic acid (IAA). Concentrations of 1.5 mgl⁻¹ and 3.0 mgl⁻¹ were used. Neither was successful. *C. monogyna* stems did root in peat, thus allowing one to bypass the *in vitro* rooting stage.

Aspects of the potential impact of marine aquaculture on the environment

Mohamed Treesh

Ecological changes due to coastal aquaculture depend on the pattern and levels of farming as well as the physical, chemical and biological characteristics of the site. In this study a preliminary baseline survey, was first carried out, on the more relevant environmental parameters of two fish farms, one land based and the other an offshore cage farm. Water samples of different origins were then mixed in varying protocols, placed in clear plastic bags and monitored for the more relevant parameters in order to gain some insight into the potential effect of farm effluent water on the environment.

At the offshore site, temperature, salinity, dissolved oxygen and pH were, as expected, not significantly affected by the presence of the fish farming activities. On the other hand, levels of nitrates were significantly higher at the cage site when compared with the control site. Phosphates were significantly higher at the cage site on two occasions but identical on the remaining three. The chlorophyll-a levels at the cage site were also significantly higher on three occasions. This implies that the nutrients released from the fish farming activities have a long enough half-life to allow for phytoplankton to take advantage of this localised enrichment. In turn, the nutrient and chlorophyll-a values at the control site are noticeably higher than those normally encountered in Maltese open coastal waters. This is presumably due to the fact that, at the particular site under study, it takes more than 300 meters for the released nutrients to be diluted enough to fall below the threshold level for affecting phytoplankton growth. It is also possible that there are other nutrient inputs into the area.

On the other hand, land based farms are normally much more susceptible to fluctuating environmental conditions than their open sea based counterparts as they do not enjoy the stabilising effect of a large body of water such as the open sea can offer.

When open sea and fish pond water were mixed in a number of combinations, the mixture containing 60% fish pond water exhibited the highest peak in chlorophyll-a. However, when the experiment was part repeated it was the bag containing only fish pond water that exhibited the highest chlorophyll-a value. In all bags containing combinations of these two water sources, however, the peak in chlorophyll-a was, without exception, recorded four days from initiation of the experiments. This confirms that in open waters it is the diluting factor of the water movements that prevent enhanced primary productivity from increased nutrient levels from fish farm effluents.

When bore hole water was mixed with open seawater the increase in chlorophyll-a concentrations was more gradual and continued throughout the duration of the experimental period. This possibly reflects the relatively lower starting concentration of both phosphate and phytoplankton cells and results in a longer latent period taking much longer for cell proliferation to take place. In contrast, when effluent pond water was mixed with open seawater, the chlorophyll-a peaked much quicker, that is, after two days possibly reflecting the higher starting concentration of phytoplankton cells.

By and large, in all experiments, chlorophyll-a peaks coincided with decreasing nutrient levels and increasing oxygen and pH values. In all cases it was the phosphates that became limiting and led to population declines, leaving relatively abundant concentrations of nitrates in the water. This is in keeping with all previous reports on Mediterranean seawater, that without exception conclude that phosphate is the limiting factor.

Studies on species composition show that, as expected, cell counts peaked concomitantly with chlorophyll-a levels. Species composition did not change in the course of the experiments and three diatom species dominated, *viz.*, *Biddulphia aurita*, *Achnantes longipes* and *Navicula* sp.

Monitoring urbanisation in Malta via satellite remote sensing

Neville Micallef

In a densely populated island like Malta, urban sprawl leads to significant impacts on environmental quality and on biodiversity through habitat loss. Locally, the urban sprawl of about 1% every five years is concentrated mainly in the outskirts of towns and villages. This urban sprawl gives rise to habitat degradation and loss of space. Moreover, this will eventually give rise to or help to endanger or even exterminate biologically important species. The objective of the present work was to investigate the urban patterns using satellite data and identify any urban change over a period of five years (January 1995 till March 2000). Landsat 5 and Landsat 7 satellite imagery were utilised to monitor this change. Aerial photographs available at the Planning Authority showed that in the five year period under investigation there was an approximate urban increase of 1%.

Various classification methods were investigated using different sizes and types of training sites to find the best classifier for the various land types found on the Maltese Islands. A range of urban areas could be detected and classified using both unsupervised and supervised classification. Furthermore, maximum likelihood classifier (an example of supervised classification) proved to be the best methodology to detect urban areas. Results obtained tend to be have a degree of subjectivity and repeatability is low because there is no fixed procedure or standard method of classification.

A number of different cases of false positives and false negatives were identified and documented. These include the 'Spectral Confusion', 'Isolated Pixels', 'Edge Effect' and 'Pixel Noise'. The main cause of these wrongly classified pixels was the limited spatial resolution of thirty metres by thirty metres of the Landsat satellite images.

Image overlay did not yield any useful results mainly due to the false positives and edge effects. The Landsat spatial resolution was found to be acceptable in larger test areas. This present study demonstrated that this was not the case for the Maltese Islands. The ideal resolution to carry out a similar study on a small island like Malta should be of five metre by five metre or less.

Sections and results from the satellite images were compared by ground truth data and field observation and specific cases of increase in urban coverage were documented and compared visually.

The value of 1% urban sprawl over five years could not confirmed by using satellite remote sensing as applied in the present study. However, in some areas (e.g. Marsascala in Malta and Nadur in Gozo) urban sprawling was detected and documented.

Aerial imagery proved to be the best for Malta if very accurate results are required, however, costs are very high and the process is time consuming. The results obtained during this work show that satellite imagery is a fast and cost effective way of monitoring the urban areas, although several disadvantages and problems were encountered.

In the near future, with the improvement of the spatial resolution of the new satellites (such as the IKONOS with a very low spatial resolution), monitoring of urban sprawling for environmental purposes, in Malta and Gozo will be much more effective and efficient.

Parasitological studies on farmed sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata* in Malta

Rabia El-Sherif

The objectives of this study were twofold:

i) to compare the parasitic fauna of gilthead sea bream *Sparus aurata* which were being reared in offshore cages with those which were being reared in land-based ponds.

ii) to compare the parasites of gilthead sea bream with those of sea bass *Dicentrarchus labrax*, which species were both being reared in offshore cages.

The correlation between the incidence and prevalence of

parasites and consequential pathology was also studied.

A total of 310 fish distributed between two species were sampled from different systems.

Upon parasitological screening, 37 different species of parasites were identified; viz., 18 protozoans, 8 monogeneans, 2 digeneans, 3 crustaceans, 2 cestodes, 2 metacercariae (eye fluke), 1 unclassified nematoda and epitheliocystis (*Chlamydia* – like organisms).

In a survey of the parasites of 155 gilthead sea bream from the offshore sea cages in local waters in Malta, seventeen different ectoparasites were identified, with the most commonly occurring being Furnestinia echeneis. Lamellodiscus ignoratus, Cryptocaryon irritans and Amyloodinium ocellatum. Eight different species of endoparasites were recorded, with Ceratomyxa sparusaurati, being the most common. In a survey of the parasites of 64 gilthead sea bream from land-based ponds, fourteen ectoparasites were identified, of which Furnestinia echeneis, Amyloodinium ocellatum and Lamellodiscus ignoratus, were the most commonly encountered. Eight species of endoparasites were recorded, with Diplostomum sp., Diplostomum-like, Myxidium leei and Certomyxa sparusaurati species being the most common; the incidence level of endoparasites was, however, generally low.

In a survey of the parasites of 91 cultured sea bass twelve species of ectoparasites were identified, with *Diplectanum aequans, Diplectanum laubieri, Cryptobia* sp., and *Trichodina* sp. being the most common. Four endoparasite species were isolated, with *Ceratomyxa labracis* and *Ceratomyxa diplodae* being very common.

When results from identical sampling periods (June to August 1998) for pond and cage gilthead sea breams were analysed it was observed that parasite diversity did not differ substantially. In terms of incidence the parasite counts were, in the main, higher in pond fish as compared with cage fish. This was particularly evident for Gyrodactylus sp. However, these differences were not found to be statistically significant.

When results from identical sampling periods (October 1998 to March 1999) for gilthead sea bream and sea bass from cages were analysed, it was observed that parasite diversity did not differ substantially. In terms of incidence the parasite counts were, in the main, higher for sea bass than for sea bream. This was particularly evident in the case of Trichodina sp. and Cryptobia sp. which were much more abundant in sea bass than in sea bream resulting in the overall level of parasites being significantly higher in sea bass than in sea bream.

A significant number of unusual findings are also described. For instance, the parasite Diplectanum laubieri was commonly found on the gills of sea bass sampled from the offshore cage farm. From the published information that exists so far it was expected to be a rare find.

By and large the observations resulting from the examination of the histological sections corroborated those from fresh tissue smears. Wherever high parasite levels had been observed in fresh smears, the same was observed in tissue section with accompanying histopathological changes. It is well documented that many parasites abandon the host fish soon after the latter die and this therefore has to be taken into consideration.

Histopathological changes in the gills consisted largely of depletion of the mucus layer and damage to the primary and secondary lamellae. The changes in the secondary lamellae ranged from mild hyperplasia largely at their bases such as in Amyloodinium infections to virtually total destruction such as in Cryptobia infections. More extensive hyperplasia throughout the entire length of the secondary lamellae led to the complete fusion of these lamellae, as was observed in infestations by Furnestinia echeneis and Chlamydia-like parasites. Clubbing of the periphery of the secondary lamellae was also fairly commonly observed as in Amyloodinium ocellatum infections. Another frequent observation was the detachment of the lamellar epithelium presumably due to the rupture of the pillar cells such as was observed in some cases of Amyloodinium infections. Obviously some of the above could well have represented different stages of one and the same pathological process.

Histological observations also permitted the study of certain parasites that would otherwise prove difficult if not impossible to study in smear preparations such as (Chlamydia-like organisms). It is also possible to pick up parasites which for some reason or other may have been missed in fresh smear preparations as was the case with Henneguya sp. in this study.

News

Science Fair - 6th to 8th April 2000. Malta Chamber of Scientists

This is the second consecutive year that the Fair has been organised and its main aim is to demonstrate that in each of our daily activities Science impacts on the way we behave and make decisions. It will do so even more in the years to come - for example just imagine life without computers or for that matter the internet and the world wide web. A better understanding of the science and technology about us will improve our quality of life without having to become a professional scientist to enjoy its fruits.

The Science Fair in essence is also the composite of a number of activities that culminate with this specific event. The first of these is the Science Forum, organised by the NSTF and the Junior Chamber of Scientists, in which students from V and VI forms and 1st year University students, have debated topical issue's in Science held over 5 sessions over the past months. The debates have been heated and have covered such topics as the use of Artificial Intelligence and the recent entry of genetically modified foods onto our menu. Sessions have also been illuminated by top scientists in the area

from the University and further afield through the kind sponsorship of the Italian Cultural Institute and the British Council. The winners of this debating series will in turn be rewarded for their efforts. They will travel to London, to join some 300 hundred or so other students from all over the world to take part in the London Science Forum.

The second event, that in turn was judged by local and international experts over the days of the Fair was the result of several months hard work that were displayed on the stands throughout the three days. These young aspiring scientists participated in our local young scientist of the year competition and the winner and runner up, Mr. Nicholai Sultana and Ms Anita Cardona will represent Malta in the EU young scientist of the year competition to be held in Amsterdam later on in September. This is the third time that we are taking part in such an event and by all accounts we have fared well on the previous two occasions that were hosted by Portugal and Greece, respectively. All the participating students whether in our local event or the international

Wendy and Mike Gluyas demonstrating the ability of a slinky spring to march down stairs to the tune of "Land of Hope and Glory". One of the many demonstrations during their interactive session entitled

"Musical Squares". (Budding young scientists watching the demonstration)



contest remark that the experience is well worth it even though one at times seems to be on a roller coaster ride of frustrations and joys encountered on a voyage that ultimately is part and parcel of seeing a scientific project through to its end.

A Science fair in many ways would be incomplete without some hands on fun - the exhibits provided by the Malta Council for Science and Technology are based on some fundamental scientific concepts that have been brought to life by the students who have assembled them. These in many ways give some insight into the ways in which science is applied in our daily lives. The MSCT is also to organise a Science week in which it is envisaged that more of these exhibits will be on show accompanied by lectures demonstrating several scientific principles.

At this juncture it is rather pleasing to see that such organisations as NSTF, and the Junior Chamber of Scientists come together with the MCST and bring their unique know how to put together such events. I would hope that such occurrences may become the norm in the future.

Back to the Fair, over the three days students were able to enjoy the wonders of chemistry. To most, chemistry appears to be that dull subject in which one has to learn complicated structures and formulae - but during the Fair we had a true to life demonstration by two University chemists, namely, Dr. Henk Frey and Dr. Martin Erdtmann, of the wonderful way chemicals, some of which may be found in every Maltese kitchen, behave under specific circumstances. Most enjoyed the display of the novel way in which every day chemicals may be combined to produce some wonderful effects such as "fireworks".

We were also pleased to have back with us, Mike and Wendy Gluyas, after their first baptism of fire in last year's edition. We all, students and adults alike, enjoyed your demonstration or more appropriately I should say - sound show - called "Musical Squares".

In addition, we were also privileged to have with us Professor Edward Mallia from the Physics Department at the University who enthralled the students with his understanding of the possible uses of solar energy excluding sunbathing!! He also made it back up the hill in his solar powered cinque cento !!

All in all, it would appear that over the three days of the Fair there was something to excite most minds even those of the faint hearted. As is becoming very evident, over the next 10 years or so science is to become more important in our daily activities. If we want to enjoy the advantages of such it is imperative that we look at science as not that esoteric thing that some people do in laboratories out of the public eye, but knowledge to be used in improving our quality of life. It should in essence become part and parcel of our character like a duck taking to water or the young taking to computers.

The NSTF, the Malta Junior Chamber of Scientists and the MCST must be congratulated for their efforts in putting together an exciting couple of days. However, special thanks are due to Ms. Ruth Bajada, who without doubt has been heart and soul of this event.

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Scilink

Scilink is the junior section of the Malta Chamber of Scientists, run by a group of young scientists. Scilink wants to attract young people with an interest in science and technology, anything from computing to chemistry. Undergraduates and sixth-form students who are taking science and science related subjects can join Scilink.

The aim of scilink is to promote science amongst young people who want to know more about science. The main activities will include:

- Attendance at Chamber seminars;
- National Science Competitions;
- Participation in national and international scientific events;
- Excursions to science laboratories at University and industry;
- Practical courses and demonstrations;
- Interaction with other science organisations in Malta and abroad;
- SciLink Net Avenue: The Official Web Site with all the information online and regularly updated; http://www.cis.um.edu.mt/~scilink
- A regular Newsletter to keep all members informed of both Chamber and Scilink activities.

For further information please mail or fax your details to:

Scilink, The Malta Chamber of Scientists, P.O. Box 45, Valletta B.P.O., Valletta. Tel:/Fax: 343535

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