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Why Cyanobacteria Produce Toxins? Evolutionary Game Theory Suggests the Key

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Abstract

Cyanobacteria are a source of potent toxins among which the microcystin (a hepatotoxic peptide encoded by the *mcy* gene cluster of *Microcystis spp.*) is a frequent cause of poisoning in inland waters worldwide. Although the molecular basis of microcystin production is known, its role is still unknown. It was suggested that microcystin production have a metabolic cost that could be offset by some benefit (e.g. protection from grazing). We check that: i) microcystin-producing and non-producing strains occurs simultaneously in the *Microcystis spp.* blooms, ii) evolutionary forces (mutation, genetic drift) control frequencies of microcystin production and non-production strains, and iii) microcystin producing strains have diminished fitness compared with non-producing strains. We employ evolutionary game theory to explain the maintaining of microcystin-producing genotypes in natural populations of *Microcystis spp*. A two-strategy (to produce or not microcystin), two-players game of cooperators (microcystin-producing genotypes) and cheaters (non-producing genotypes) explains the coexistence of both genotypes in the same bloom. A bloom composed mostly by the microcystin-producing "cooperators" genotype, the "temptation of defection" (increase of non-producing genotypes) is counteracted by kin selection, which enable that natural selection can favour the cooperators. The closest related individuals occur within cyanobacteria blooms, cyanobacteria reproduces asexually providing sets of clones.

Keywords: toxic cyanobacteria, microcystin, kin selection, game theory, cooperators-cheaters

1. Introduction

Cyanobacteria are often competitively superior to other phytoplankton (Dokulil & Teubner, 2000) and recurrently form dense surface blooms in inland waters worldwide (Bartram, Carmichael, Chorus, Jones & Skulberg, 1999), a risk that could increase in frequency as a result of global change (Huertas, Rouco, López-Rodas & Costas 2010; Huertas, Rouco, López-Rodas & Costas, 2011; Rouco, López-Rodas, Flores-Moya & Costas, 2011). Many species of cyanobacteria can produce potent cyanotoxins that threaten humans, livestock and wildlife (Carmichael, 2001), but it has long been known that *Microcystis aeruginosa* Kützing is the most frequent cause of toxicity affecting humans and animals (Sivonen et al., 1990). *M. aeruginosa* produce cyclic heptapeptides called microcystin, which are potent hepatotoxins (Carmichael, 1994; Dawson, 1998). Since microcystins are the most commonly encountered cyanotoxins they are a matter which is of increasing concern. For instance, many people died of acute liver failure as the result of intoxication by microcystin in Brazil (Jochimsen, Carmichael, An, Cardo & Cookson, 1998) and numerous toxic episodes were also reported in China (Chen & Xie, 2005; Zhang, Xie, Liu, Chen & Liang, 2007). Exposure to low concentrations of microcystin through drinking water increases significantly the risk of liver and colorectal cancer (Martínez-Hernandez, López-Rodas & Costas, 2009). Also, microcystin cause catastrophic mass mortalities of fauna even in pristine national parks of wildlife (Alonso-Andicoberry, García-Villada, López-Rodas & Costas, 2002; López-Rodas, Maneiro, Lanzarot, Perdigones & Costas, 2008).

It has long been known that a complex mixture of microcystin-producing and non-producing strains occurs simultaneously during the *M. aeruginosa* blooms (Codd, Bell, & Brooks, 1989; Shirai et al., 1991; Kaebarnick & Neilan, 2001; Vezie et al., 1998). For example, around one-third of strains isolated from lakes and reservoirs in Spain were found to be non-toxic (Alvarez, Basanta, López-Rodas & Costas, 1998; Martin, Carrillo & Costas, 2004; Carrillo et al., 2003).

Over the last years, the molecular basis of how *Microcystis* produce microcystin has been discovered. Microcystin is a peptide synthesised non-ribosomally via a thio-template mechanism (Arment & Carmichael, 1996) by the

microcystin synthetase enzyme complex, which is encoded by the microcystin (*mcy*) gene cluster (Kurmayer & Christiansen, 2009). The *mcy* gene cluster of *Microcystis* has been already sequenced (Nishizawa et al., 2000; Tillett et al., 2000). Molecular analysis shows that evolution of the *mcy* genes occurs through asexual reproduction and horizontal gene transfer, with ancestral wild type microcystin-producing genotypes and strains with non-functional genes (Bjørg et al., 2003).

Unfortunately, the molecular basis of the *myc* gene cluster does not explain the biological role of microcystin. The classic approach assumes that microcystin production has a metabolic cost, but generates some advantages that compensate the cost. In this sense, numerous evidences suggest that microcystin production prevent grazing of *Microcystis* by zooplankton, filter feeders and others. Toxin-producing *Microcystis* often have lethal effects upon zooplankton, or significant reduce the growth of these primary consumers (De Mott, Zhang, & Carmichael, 1991; Kinder, 1995; Rohrlack, Henning, & Kohl, 1999; Trubetskova & Haney, 2000). *Daphnia* species actively evade *Microcystis* blooms through vertical migrations (Kinder, 1995). Filter-feeding animals as zebra mussels employ a sophisticated mechanism based on the selective alimentation eliminating living *Microcystis* in pseudo-feces to avoid microcystin producers (Vanderploeg et al., 2001). It is known that animals that consume *Mycrocystis* scum die due to the toxic effect of cyanotoxins (Alonso-Andicoberrt et al., 2002; López-Rodas et al., 2008; Francis, 1878; Galey et al., 1987; Soll & Williams, 1985; Matsunaga et al., 1999). Thus, the protection against grazing will compensate the metabolic cost of microcystin production. Other useful functions for microcystin have been suggested such as binding iron (Utkilen & Gjølme, 1995) or involvement in quorum sensing (Dittmann et al., 2001). Despite this, experiments with cultures of *Microcystis* that had the *myc* gene knockout showed no apparent differences with *m*+ functional cultures (Dittmann et al., 2001). Producing microcystin does not seem to be a substantial cost under laboratory conditions. Additionally, other laboratory experiments show that the values of heritability of microcystin production were significantly higher than those found in fundamental physiological characters (López-Rodas et al., 2006). It is generally assumed that only the quantitative traits with little or no selective advantage show high values of heritability (Falconer & Mackay, 1996).

Surely, microcystin production is more complex than which can be explained from the current knowledge of the molecular basis of the myc gene complex, because microcystin production seems to be a complex quantitative trait. In fact, laboratory studies have shown that changes in environmental conditions induce changes in microcystin concentration within a strain by a factor of three to four times, whereas microcystin production between strains grown under identical culture conditions may vary in the three or more orders of magnitude (reviewed by Sivonen & Jones, 1999; Carrillo et al., 2003; Lopez-Rodas et al., 2006).

Consequently, the role played by microcystin production in the biology of *Microcystis* is still far from clear. Einstein said once that you cannot solve a problem with the same mentality it has been posed, but rather you must change the way you approach it. So, we decided to address this controversy in a different perspective. The key question is to analyse why usually microcystin-producing and non-producing strains appear together in the same *Microcystis* bloom and neither of the strains prevail over the other. A population genetics approach could find out what kinds of evolutionary forces are acting (i.e. mutation, natural selection, genetic drift...) on microcystin-producing and non-producing strains and calculate their genotypic frequencies and their evolution (throughout of fitness values, selection coefficients, effective population sizes...).

Consequently, first we performed a classic population genetics experiment to estimate the role played by the evolutionary forces on the frequency of microcystin-producing and non-producing strains. Afterwards, the results obtained were analysed employing evolutionary game theory.

Evolutionary game theory could explain the cases in which producing microcystin has an evolutionary advantage in a population of *Microcystis aeruginosa* and viceversa. Since von Neumann & Morgenstern (1944) and Nash (1950) presented game theory as a mathematical approach to evaluate the results of the different strategic decisions among the complex interactions of self-interested individuals, this discipline has been able to successfully resolve complex problems of population genetics. The application of game theory to evolving populations of life organisms (i.e. evolutionary game theory) is focused on the effect of the frequency with which various competing strategies are found in the population. Evolutionary game theory has been particularly useful to explain complex aspects of biology that had not been explained within the context of Darwinian process by classic population genetics, such as the evolutionary basis of altruistic behaviours (Maynard-Smith, 1982).

The conceptual basis of our approach is based on three steps:

1) Isolate microcystin-producing and non-producing strains from the same population of *Microcystis aeruginosa* (because the evolutionary phenomena occur within the populations).

2) Demonstrate that classic evolutionary forces (mutation, genetic drift and natural selection) can change the frequency of the toxin producing and no toxin producing genotypes (to prove that microcystin production is not only a physiologic phenomenon).

3) Applicate evolutionary game theory to estimate the best strategy (i.e. producing or non-producing toxins) according to the population conditions (i.e. the frequencies of the producing and non-producing cells).

2. Material and Methods

2.1 Isolation of Strains and Culture Conditions

Twenty-one different clonal cultures of *M. aeruginosa,* numbered from one to twenty-one, from the Algal Culture Collection, Veterinary Faculty, Complutense University, Madrid, were used (Figure 1). All the strains were isolated from same area of Guadalquivir basin (SE Spain), in order to compare the population genetics parameters of different strains within the same population, because the evolutionary forces (e.g. natural selection) act within populations. Each strain was isolated as follows: First of all colonies morphologically identified as *M. aeruginosa* based on morphologic characteristics according to Komarek & Anagnostidis (1998) were isolated using micro-pipettes. Only colonies within the middle of the range of cell and colony sizes with clear *M. aeruginosa* morphotypes were isolated. Afterwards, a single vegetative cell was isolated from each colony using a Zeiss-Eppendorf (Carl Zeiss, Hamburg, Germany) micromanipulator–microinjector. Since *M. aeruginosa* cells divide asexually, the clonality of each culture was assumed.

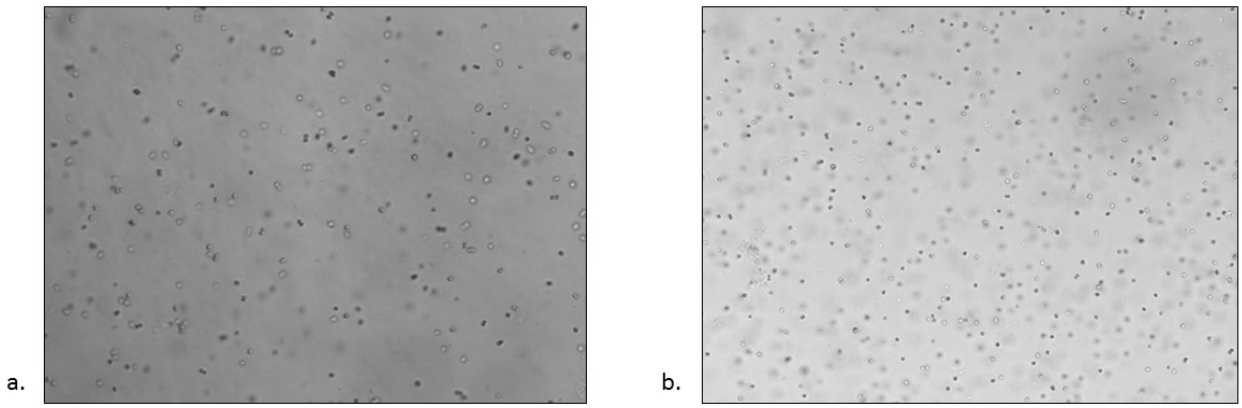

Figure 1. Microphotographs of two different clonal cultures of *M. aeruginosa*

Two microphotographs of two different strains of *M. aeruginosa* from the Algal Culture Collection, Veterinary Faculty, Complutense University, Madrid. a) a non-toxic strain and b) a toxic strain. As can be seeing in the figure *M. aeruginosa* does not make colonies in laboratory cultures.

The strains were grown in 100 mL ventilated cell culture flasks covered with a filter cap (Greiner, Bio-One Inc., Longwood, 155 NJ, USA) with 20 mL of BG-11 medium (Sigma, Aldrich Chemie, Taufkirchen, Germany) at 24ºC with a continuous photon irradiance of 80 µmol photons m^{-2} s^{-1} over the waveband 400–700 nm, supplied by daylight fluorescent tubes. Cells were maintained axenically in balanced growth (mid log exponential growth) by serial transfers of an inoculum to fresh medium (Cooper, 1991). Periodic controls (i.e. by observation under fluorescence microscope after acridine orange staining and plating in standard bacterial cultures) assure the absence of detectable bacteria. The position of each culture flask in the incubator chamber was randomly changed once every day. More details on culture of Microcystis are given in Lopez-Rodas et al. (2006) and Rouco et al., (2011). In order to allow full environmental acclimation, the strains were grown under these constant experimental conditions during two months. The maintenance of the strains under the same stable environmental conditions is necessary for disengaging environmental and genetic influences on the phenotypic variability, which ensures that differences found among strains are exclusively due to genetic variability (Brand, 1981; Costas, 1990).

2.2 Microcystin Determination

The microcystin production of the different strains was measured in mid log exponential growth cultures (i.e. acclimated, balanced growth (Cooper, 1991)) of 3 x 10^6 cell ml^{-1} using a microcystin commercial kit MicroCystest (ZEU-INMUNOTEC, Zaragoza, Spain), based on inhibition of the protein phosphatase 2A (PP2A) by microcystins, and therefore capable of detecting all potentially toxic microcystins with a detection limit of 0.08μg/L and a working range 0.25-2.5 μg/L. This test was approved by Environmental Technology Verification program of the USA Environment Protection Agency (EPA). Extraction of toxins and total microcystin (water dissolved microcystin + microcystin within cells) was measured following the manufacturer's recommendations and expressed as μgrams per litre (μg/L). Two different operators performed triplicate measurements of microcystin. The reliability, reproducibility and precision of microcystin measurements were established according to the British Standards Institute (1979) and Thrusfield (1995). Reliability was determined based on the agreement between three iterations of measurements made by the same observer on the same replicate, whereas reproducibility was determined as the agreement among three sets of observations made by two different observers. The measurements to guarantee reliability, reproducibility and precision were performed on two lots of reagents using (i) microcystin-LR controls (Sigma–Aldrich) at 1.0 and 4 μg/L, and (ii) extracts from Ma2, Ma7 and Ma14 strains containing 3 x 10^6 cell ml^{-1}. We select these three strains in order to have representation of a non-producing strain (Ma2), a strain with an average production (Ma7) and the highest microcystin-producing strain (Ma14). Precision was determined as the minimum variation in microcystin, which could be detected.

2.3 Fitness Estimation

The Malthusian parameter of fitness (m) was estimated as previously described (Costas, 1991) as the acclimated maximal growth rate from the general equation of growth:

$$N_t = N_0 e^{mt} \quad (1)$$

where N_0 is the initial cell number (after the lag period), and N_t is the cell number after 7 d; therefore, m was computed as Log e (N_7/ N_0)/ 7. Cells were counted using settling chambers, by two independent observers who counted three replicates per strain. The number of samples counted per replicate was determined using the progressive mean procedure to ensure a counting error of ≤ 5%. The Darwinian fitness (i.e. relative fitness) of each genotype was estimated as the ratio:

$$m_i / m_{max} \quad (2)$$

where m_i is the Malthusian parameter of the strain i and m_{max} is the maximum Malthusian parameter. Consequently, the maximum value of fitness will be 1. The growth rates were estimated using triplicates of each genotype. More details on estimation of fitness in *Microcystis aeruginosa* (including heritability and evolution of fitness and microcystin production) are given in López-Rodas et al. (2006) and Rouco et al. (2011). The reliability, reproducibility and precision of microcystin measurements and fitness estimations were established as previously described for *Microcystins*. Ma2, Ma7 and Ma14 strains.

Afterwards, we use linear regression to modelling the relationship between fitness (dependent variable y) and microcystin production (explanatory variable x) using InStat package (GraphPad Software, La Jolla, CA, USA).

2.4 Population Genetics Experiments

We assume the simplest theoretical model of population genetics that might well explain the observed results: i) the functional microcystin-producing genotypes (*m*+) could become non-functional (*m*-) due to occurrence of spontaneous mutation (*m*+ → *m*-); ii) conversely non functional microcystin-producing genotypes (*m*-) can become functional (*m*+) due to reversion (*m*- → *m*+). Within a microcystin-producing population should have a majority of *m*+ genotypes and perhaps a few *m*- genotypes originated by mutation. On the contrary, within a non-producing population should have a majority of *m*- genotypes and possibly a few *m*+ genotypes originated by reversion In a small population the alleles *m*+ and *m*- should be under a mutation–genetic drift balance.

We can essay this model in the laboratory using experimental populations of *Microcystis aeruginosa*. The experimental populations were laboratory cultures microcystin producing (*m*+ strains) and non-producing (*m*-) strains maintained by serial transfers of a small inoculum. In these experimental populations act mutation and genetic drift. The possibility of losing an allele by genetic drift in small populations is high. Consequently, sometimes microcystin-producing cultures could lose their toxicity by random genetic drift, because only *m*- genotypes (originated by mutation *m*+ → *m*-) were transferred in the small inoculum. And just the opposite, sometimes non-producing cultures could became toxic also by random genetic drift, because only *m*+ genotypes

(originated by reversion *m*- *m*+) were transferred in the small inoculum. To experimentally check this model, two experiments were performed simultaneously:

i) experiments maximizing random genetic drift: Twenty-one clonal cultures were grown until a cell number of about 10^8. Afterwards the cultures were transferred to fresh medium using extremely small inoculum (around 100 cells) to maximize genetic drift. The probability that some allele is lost in such small inoculum is very high. This flash-crash process was repeated periodically during 20 times.

ii) experiments minimizing random genetic drift (controls): The same twenty-one clonal cultures were grown until a cell number of about 10^8 cells. Afterwards the cultures were transferred to fresh medium using large inoculum (around 10^5 cells) to prevent genetic drift. The probability that some allele is lost in such inoculum is extremely low. This serial transfer process was also repeated periodically during 20 times.

In both experiments, the microcystin production was measured at the start of and after the 20 flash-crash (i.e. mutation- genetic drift) cycles in exactly the same conditions.

2.5 Evolutionary Game Theory Model of Microcystin Production

We assume that a bloom is a *Microcystis aeruginosa* population where *m*+ is a microcystin-producing wild type genotype, and *m*- is a non-producing mutant genotype. Relative frequency of m^+ is *p*, relative frequency of m^- is *q*. Fitness of *m*+ genotype is w_1 and fitness of *m*- genotype is w_2.

We can systematize all possible cases by considering an evolutionary game between two strategies, i) produce microcystin (m^+ genotype) and ii) not produce microcystin (m^- genotype), playing in two different populations, population 1 with predominant m^+ genotypes and ii) population 2 with predominant m^- genotypes.

The payoff matrix of both strategies is:

	playing against		(3)
	m+ genotype	*m*- genotype	
m+ genotype	w_1 (+)	w_1 (-)	
m- genotype	w_2 (+)	w_2 (-)	

The entries of the matrix denote the fitness for the row player. A *m*+ genotype obtains a payoff w_1 (+) when playing another *m*+ genotype, but payoff w_1 (-) when playing a *m*- genotype. Likewise, *m*- genotype obtains a payoff w_2 (+) when playing against *m*+ genotype, or w_2 (-) when playing against *m*- genotype. This is a case of two-players, two-strategy game, whose evolutionary dynamics has studied in detail (Rapoport & Chammah, 1965; Axelrod, 1984; Taylor & Nowak, 2009).

3. Results and Discussion

3.1 Evolutionary Forces (as Mutation or Genetic Drift) can Affect the Microcystin Production

Measurements of microcystin production were performed with high reliability (98.2%±0.7%), reproducibility (96.8%±1.0%) and precision (0.1 µg/ ml). Despite being grown under identical laboratory conditions, initially 6 strains (Ma2, Ma5, Ma6, Ma10, Ma16, Ma17) were unable to produce microcystin, whereas that the 15 strains produce 1.34 ± 0.23 µg/ml of microcystin LR equivalent (mean ± standard error) in of cultures with 3 x 10^6 cell/ml.

When the 21 clonal strains were subjected to experiments maximizing random genetic drift (the cultures were transferred using inoculum around 100 cells during 20 times), most of the strains maintained similar microcystin production (Table 1). But four clones (Ma3, Ma13, Ma20, Ma21) that initially had produced microcystin became no producing after the experiment (Table1). Inversely, two clones that had not produced microcystin at first (Ma6, Ma16) became able to produce at the end of the experiment (Table 1). In contrast, when the twenty-one clonal strains were maintained by serial transfers using large inoculum (10^5 cells) to prevent genetic drift, toxicity of each strain remained unchanged (Table 1).

Table 1. Population genetics experiments to check the role of mutation, reversion and genetic drift between microcystin-producing and no-producing strains (+ microcystin producing strain; - no-producing strain)

Strain	Initial microcystin production	Final microcystin production after experiments maximizing random genetic drift	Final microcystin production after experiments minimizing random genetic drift (controls)
Ma1	+	+	+
Ma2	-	-	-
Ma3 [a]	+	-	+
Ma4	+	+	+
Ma5	-	-	-
Ma6 [b]	-	+	-
Ma7	+	+	+
Ma8	+	+	+
Ma9	+	+	+
Ma10	-	-	-
Ma11	+	+	+
Ma12	+	+	+
Ma13 [a]	+	-	+
Ma14	+	+	+
Ma15	+	+	+
Ma16 [b]	-	+	-
Ma17	-	-	-
Ma18	+	+	+
Ma19	+	+	+
Ma20 [a]	+	-	+
Ma21 [a]	+	-	+

[a] initially microcystin-producing strains, which became no-producing after the experiments maximizing random genetic drift.

[b] initially no-producing strains, which became producing after the experiments maximizing random genetic drift.

These results suggest that *m*- genotypes could occur in strains of *m*+ genotype as a result of spontaneous mutation and also could become fixed in populations by genetic drift. Previous work show that genetic drift is an important component in the evolutionary changes affecting to microcystin production, that occur in *Microcystis* populations as response to eutrophication and temperature increase (Rouco et al., 2011) as well as in other harmful algae (Flores-Moya, Costas, & López-Rodas, 2008; Flores et al., 2012).

3.2 Darwinian Fitness of Microcystin-Producing and Non-Producing Strains

Darwinian fitness was measured with high reliability (93.3% ± 1.2%) and reproducibility (90.7% ± 0.9%), whereas precision was of 0.01, high enough to detect inter-strain variability.

Initially, a high inter-strain variation was found for the fitness measured under identical laboratory conditions (Table 2). It should be noted that no microcystin-producing strains has higher fitness than microcystin-producing. Fitness of non-producing strains ranges from 0.8 to 1.0 (mean = 0.90; sd = 0.09), whereas fitness of microcystin producing strains range from 0.4 to 0.8 (mean = 0.65; sd = 0.13). Statistically significant differences ($p = 0.0006$, $t = 4.12$, d.f. 0 19 in Student t-test) were observed between producing and non-producing strains. A regression analysis of microcystin production (measured in ppb of microcystin LR-equivalent in cultures with 3×10^6 cell/ml) and Darwinian fitness of the 21 different strains of *M. aeruginosa* also show that the genotypes that produce more amount of microcystin have less Darwinian fitness than those non-producers genotypes (statistically significant negative regression, $P < 0.001$, $R^2 = 0.71$ was observed; Figure 2).

When these strains were subjected to experiments maximizing random genetic drift most of the strains maintained similar fitness values. But two strains (Ma3 and Ma20) increase in fitness after losing the ability to produce microcystin, whereas the strain Ma20 decrease in fitness after recovering the ability to produce microcystin (Table 2). In contrast, when the twenty-one clonal strains were maintained by serial transfers using large inoculum (10^5 cells) to prevent genetic drift, toxicity of each strain remained unchanged.

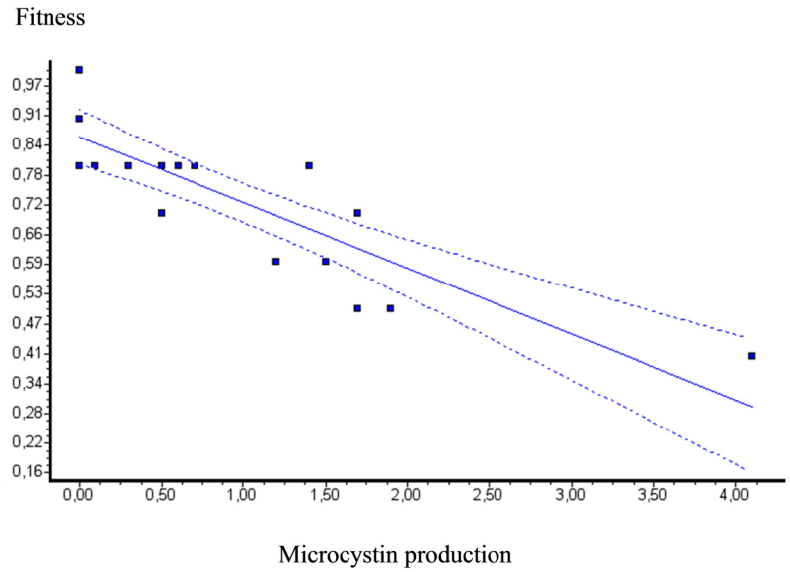

Figure 2. Linear regression

Linear regression and 95% confidence interval between Microcystin production (in ppb of cultures with 3 x 10^6 cell ml^{-1}) and Darwinian fitness of 21 different strains of *M. aeruginosa.*

Table 2. Darwinian fitness of 21 different strains of *M. aeruginosa*

Strain	Initial microcystin production	Initial Darwinian fitness	Final Darwinian fitness after experiments maximizing random genetic drift	Final Darwinian Fitness after experiments minimizing random genetic drift (controls)
Ma1	+	0.8	0.7	0.8
Ma2	-	0.8	0.9	0.8
Ma3 [a]	+	0.6*	0.9*	0.6
Ma4	+	0.8	0.7	0.8
Ma5	-	0.9	0.9	0.9
Ma6 [b]	-	0.8*	0.5*	0.8
Ma7	+	0.6	0.6	0.6
Ma8	+	0.8	0.7	0.8
Ma9	+	0.8	0.8	0.8
Ma10	-	1.0	1.0	1.0
Ma11	+	0.8	0.8	0.8
Ma12	+	0.7	0.6	0.7
Ma13 [a]	+	0.7	0.8	0.7
Ma14	+	0.4	0.4	0.4
Ma15	+	0.5	0.5	0.5
Ma16 [b]	-	0.9	0.8	0.9
Ma17	-	1.0	1.0	1.0
Ma18	+	0.7	0.6	0.7
Ma19	+	0.6	0.6	0.6
Ma20 [a]	+	0.5*	0.7*	0.5
Ma21 [a]	+	0.6	0.6	0.6

* statistically significant differences in mean of fitness between initial Darwinian fitness and final Darwinian fitness after experiments maximizing genetic drift, $p<0.01$, student t test.

[a] initially microcystin-producing strains, which became no producing after the experiments maximizing random genetic drift.

[b] initially no microcystin-producing strains, which became producing after the experiments minimising random genetic drift.

An outstanding fact that emerges from our results is that producing microcystin has a strong cost in fitness. This interesting fact had not been highlighted previously because the studies that characterize the fitness of different strains (genotypes) isolated from the same population are scarce. In the literature there are studies comparing *Microcystis* strains isolates from different populations, forgetting that natural selection acts within populations and not between populations (Lewontin, 1974; Spiess, 1989; Gould, 2002). In contrast, all strains used in our study were isolated as close as possible in space and time, so that they should belong to the same population. A recent study analysing DNA sequencing and genetic variability for physiological and morphological traits show that these strains are very close at genetic level (Lopez-Rodas, 2013).

According to classic population genetics results obtained by fitness valuation suggest that non-producing genotype (*m*-) during a *Microcystis* bloom should be more numerous than microcystin-producing genotype (*m*+) due to it higher fitness. But numerous evidences reveal that the non-toxin producing cells do not prevail massively during a bloom (reviewed in Sivonen & Jones, 1999; data for South Spain populations in Alvarez et al., 1998; Carrillo et al., 2003; Martin et al., 2004; Lopez-Rodas, 2006). Consequently, it is necessary to find an explanation for the natural maintenance of a high frequency of microcystin producing cells in a bloom population.

3.3 The Model of Population Genetics and Game Theory of m+ and m- Genotypes

We propose a theoretical approach of game theory model (two-player and two-strategy model) to explain the abundance of microcystin producing cells. Similar approach was tackle for explaining apparently low fitness behaviours as altruism (Maynard Smith, 1982; Thrusfield, 1995)

Game theory, it has long been employed to determine the best strategy for a genotype interacting with other genotypes to reach the best outcome to himself (Hamilton, 1964; Trivers, 1971; Maynard Smith & Price, 1973). In essence, an evolutionary game is related to reproductive success so that the fitness of a genotype depends on payoff of his strategy interacting in cooperative or non-cooperative strategies with other various strategies in the population (Samuelson, 1997; Hofbauer & Sigmund, 2003; Nowak & Sigmund, 2004; Nowak, 2006).

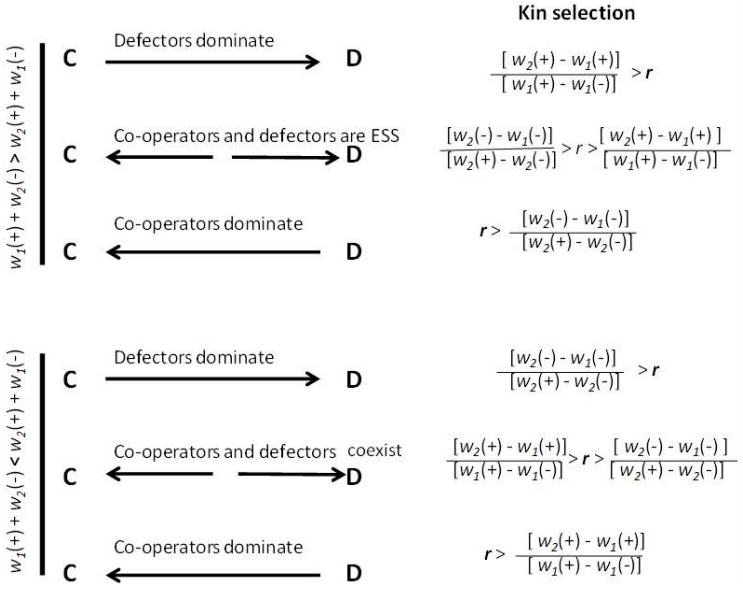

Figure 3. Kin selection can lead to the evolution of cooperation

Being the parameter r the coefficient of genetic relatedness between individuals, for this model we find $w_1(+) + w_2(-) > w_2(+) + w_1(-)$, cooperators and defectors cannot coexist; if $w_1(+) + w_2(-) < w_2(+) + w_1(-)$ cannot be simultaneously a evolutionary stable strategy (ESS).

If $w_1(+) + w_2(-) > w_2(+) + w_1(-)$ then $r_D > r_C$. Three possibilities: i) if $r_D > r_C > r$ then defectors dominate; ii) if $r_D > r > r_C$ then cooperators and defectors are a ESS; iii) if $r > r_D > r_C$ then co-operators dominate.

If $w_1(+) + w_2(-) < w_2(+) + w_1(-)$ then $r_C > r_D$. Three possibilities: i) if $r_C > r_D > r$ then defectors dominate; ii) if $r_C > r > r_D$ then co-operators and defectors are not a ESS; iii) if $r > r_C > r_D$ then cooperators dominate.

Based on the payoff matrix of two players (*m*+ and *m*-) with each of them competing against the other for the same ambient, different strategies can take place as a function of the payoff which leads to different final populations. Evolutionarily stable strategies (i.e. is stable against invasion by a fraction of mutants using the other strategy) can occur depending of payoffs (Figure 2): two strategies of the matrix are Nash equilibrium (the best strategy of a player in order to acquire the maximum possible payoff, knowing the strategy that perform the other player) (Nash, 1950): The first is if $w_1(+) > w_2(+)$ and the second if $w_1(-) < w_2(-)$. In a large mixed population (without small subpopulations), a Nash equilibrium strategy is an evolutionary stable strategy (Maynard Smith, 1982). In practice, the huge blooms of *Microcystis* composed of billions of individuals mixed by the water movements are as close as possible to a theoretical infinite, well-mixed population. Consequently, in such populations 3 different evolutionary stable strategies are possible (Figure 3):

i) If $w_1(+) > w_2(+)$ and $w_1(-) > w_2(-)$, then *m*+ dominates *m*-. In this case is better to produce microcystin (i.e. be a wild type *m*+ genotype) because the expected payoff to produce microcystin (*m*+ genotype) is greater than that of not produce microcystin (*m*- genotype) in any *Microcystis* population. Consequently, wild type the *m*+ genotypes always prevail in the population.

ii) In the reverse situation $w_1(+) < w_2(+)$ and $w_1(-) < w_2(-)$, then *m*- dominates *m*+. Obviously, in this case the expected payoff of *m*- is greater than that of *m*+. Here, *m*- mutants always prevail in the population.

iii) If both conditions of Nash equilibrium $w_1(+) > w_2(+)$ and $w_1(-) < w_2(-)$ occurs together, then both strategies can be a evolutionary stable strategy. In a population where most genotypes are wild type *m*+ it is the bests to be *m*+. In contrast, in a population where most genotypes are *m*- mutants it is the bests to be *m*-.

Since in the blooms of *Microcystis* simultaneously coexist microcystin-producing and non-producing genotypes, obviously do not occur Nash equilibrium in natural conditions. But another solution is possible when strategies generate payoff that are different to those that allow Nash equilibrium. This solution is the key to understand why wild type microcystin-producing *m*+ genotypes and non-producing *m*- genotypes always appear together in the *Microcystis* blooms.

iv) If $w_1(+) < w_2(+)$ and $w_1(-) > w_2(-)$, then there is stable co-existence between both genotypes. In a *Microcystis* bloom where most genotypes are microcystin-producing wild type *m*+, ($p > q$) it is the bests strategy to be a non-toxic *m*- genotype. Contrarily, in a *Microcystis* population where $p < q$, it is the best strategy to be *m*+ genotype. Thus, equilibrium of frequency-dependent selection is achieved. In this case, there is a stable co-existence between the both genotypes in the *Microcystis* populations. Only this theoretical solution of the payoff matrix presents a stable coexistence of both genotypes (i.e. in a *Microcystis* bloom where most genotypes are *m*+ it is the best strategy to be *m*- and viceversa). In this case, the frequency dependent selection leads to equilibrium as shown in the *Microcystis aeruginosa* blooms.

At the biological level, there is an explanation to this model of stable coexistence of both genotypes. Although producing toxins is useful to prevent grazing by zooplankton filter feeders and large animals (Alonso-Andicoberry et al., 2002; López-Rodas et al., 2008; De Mott et al., 1991; Kinder, 1995; Rohrlack et al., 1999; Trubetskova & Haney, 2000; Vanderploeg et al., 2001; Frances, 1878; López-Rodas & Costas, 1999), it is also costly in terms of fitness, whereby the toxin-producing *m*+genotypes showed diminished fitness with respect to non-producing *m*- genotypes in the absence of grazers (Carrillo et al., 2003; López-Rodas et al., 2006). Our results also show significant negative regression between fitness and toxin production.

Let us assume a *Microcystis* bloom constituted by individuals with the *m*+ ancestral genotype. In this bloom appears a new genotype *m*- by a rare spontaneous mutation, which does not produce microcystin, so it fitness is slightly above than those of *m*+ ancestral genotypes and is also protected from grazing although he do not produce microcystin. As its fitness is greater than the ancestral *m*+ genotypes, the frequency of *m*- mutants will increase gradually. In this population it is best to be *m*-, it is tempting to be a *m*- genotype. But there will come a point where if there are not enough *m*+ genotypes, the bloom would not be protected any more.

This can be interpreted as that the *m*+ genotypes forgo some of their potential for the common good of the population, performing a cooperative strategy. But the temptation for cheating (*m*- genotype) plays an important role. Under this view, the *Microcystis* blooms comprise two strategies: i) the cooperators (*m*+ genotypes), which would invest in microcystin production for the good of the entire population, and ii) the cheaters (*m*- genotypes), which would take the advantage conferred by the *m*+ cooperators to acquire the maximum profit from the situation, giving nothing in return.

Consequently, the maintenance of cooperation requires a specific mechanism to enable that natural selection can favour to the cooperators instead of to the cheaters. Kin selection could be the mechanism. Classic view of kin

selection is based on the concept that usually the evolutionary game is played among genetic relatives individuals (Hamilton, 1964; Samuelson, 1997; Cavalli-Forza & Felman, 1978). A gene encodes some kind of cooperative behaviour promotes its own survival if it is also present in the beneficiary of the cooperation. Cyanobacteria reproduce asexually. Consequently, cyanobacteria populations are formed by sets of clones (López-Rodas & Costas, 1997) of his act of cooperation becomes a clone of herself. Clones are more likely to benefit from (pseudo)altruistic behaviour than distantly related individuals.

From the seminal works of Maynard-Smith (1982), Nowak (2006) and Taylor & Novak (2009) arises an intriguing idea. Cooperators and cheaters could coexist in a population where the values of the payoff of both strategies (*m*+ cooperators and *m*- cheaters) meet the following condition:

$$\frac{[w2(+) - w1(+)]}{[w1(+) - w1(-)]} > r > \frac{[w2(-) - w1(-)]}{[w2(+) - w2(-)]} \text{ (Hofbauer \& Sigmund, 2003)} \quad (4)$$

$$r_{cooperators} > r_{total\ population} > r_{cheaters}$$

where:

a *m*+ genotype obtains a payoff $w_1(+)$ when playing another *m*+

a *m*+ genotype obtains a payoff $w_1(-)$ when playing a *m*-

a *m*- genotype obtains a payoff $w_2(+)$ when playing a *m*+

a *m*- genotype obtains a payoff $w_2(-)$ when playing a *m*-

and *r* = coefficient of genetic relatedness between individuals (an estimation of the average genetic relatedness between the playing individuals)

Apparently *r* can be high in the blooms of cyanobacteria, because experimental data show that *Microcystis* blooms are usually constituted by few different genotypes (Alvarez et al., 1998; López-Rodas & Costas, 1997; Martín-Montaño et al., 2000). In addition, the approximation described above determinates that for the coexistence of the *m*+ and *m*- must be also satisfied that:

$$r_{cooperators} > r_{total\ population} > r_{cheaters} \quad (5)$$

so is said that the coefficient of cooperators should be higher than the total coefficient of genetic relatedness of the population and in turn, the coefficient of cheaters should be lower than the other two. For this to happen, within the population the majority of the population should be the *m*+ genotype, which fit with the experimental evidences (Alvarez et al., 1998; López-Rodas & Costas, 1997; Martín-Montaño et al., 2000). Whereupon it could be said that the strategy perform by cyanobacteria depends in last term on the kinship of the cooperators genotype.

Other causes (such as group selection, graph selection, direct and indirect reciprocity, reviewed by Nowak, 2006; Taylor & Nowak, 2009) could explain microcystin production, but as cyanobacteria are very simple organisms, it seems reasonable to propose a *lex parsimoniae* explanation.

Experimental evidence allows to validate a population genetics model based on a two-strategy (to produce or not produce microcystin), two-players game of cooperators (the wild type *m*+ microcystin-producing genotypes) and cheaters (the *m*- non-producing mutants), which explain the coexistence of both genotypes in the same population and provides a clear explanation of the role played by microcystin. Our model for explaining toxin production by *Microcystis* is based on the following assumptions: i) mutation from wild type toxin-producing *m*+ genotypes to non-producing *m*- mutants producers occurs spontaneously; ii) reversion from non-producing *m*- mutants to toxin-producing *m*+ genotypes also occurs spontaneously; and iii) in the main, toxin-producing *m*+ genotypes have less fitness than non-producing *m*- mutants.

For the moment, an experimental demonstration of this game theory model of two strategies and two players not easily approachable. Instead, we have provide a list of indirect experimental evidences. In any case, our approach seems to be a "gedanken experiment" type of problem (a thought experiment, a hypothetical scenario carried out to understand a real problem). At present, there is a tendency to interpret the phenomenon of microcystin production under the reductionist perspective of what we know about its molecular basis (specifically on the *myc* gene cluster). These studies have often assumed that *myc* genes operate in a closed system in which the presence or absence of certain gene sequences directly determines microcystin production. However, cyanobacteria blooms exhibit a wide range of variability in microcystin production, which is the result of barely measurable interactions between a particular genotype and its corresponding environment. In such cases, classical models of population genetics have a great power to explain biological phenomenon under an evolutionary level. There is also a tendency to seek

new functions to explain the role of microcystin, relegating to a second place its toxic effect, despite that continuously produces countless mass mortalities among the consumers of cyanobacteria. In contrast, our model follow an Ockham´s razor type argument (among competing hypothesis, the one with less assumptions should be selected), which takes into account the environment and operates whatever the genetic basis of toxin production (because the selection unit is the complete genotype). After all, Charles Darwin masterfully explained the mechanisms of evolution nearly 100 years before the development of molecular genetics.

4. Conclusions

1. Evolutionary forces (i.e. mutation, genetic drift, selection) control the frequency of microcystin-producing and non-producing cells in a process at the population genetics level and not at the physiological level.

2. Microcystin has useful functions (i.e. grazing prevention) but its production is so costly that the non-producing *m-* genotypes have higher fitness. Consequently, non-producing genotypes should be predominant in a bloom, which contradicts observations from natural *Microcystis* blooms (usually around 60-70% microcystin producing cell, 30-40% non-producing cells)

3. A two-strategy (to produce microcystin or not), two-players evolutionary game of cooperators (microcystin-producing) and cheaters (non-producing) is the simplest explanation to the simultaneous occurrence of microcystin-producing and non-producing strains. In this game, depending on the payoff matrix could be achieved evolutionary stable strategies (such as Nash equilibrium with domain of producing or non-producing genotypes respectively), but also a stable co-existence between both genotypes if when the microcystin-producing cells dominates being non-producing is the best strategy and vice versa.

4. The maintenance of microcystin-producing cooperators requires kin selection to enable that natural selection can favour to the cooperators instead of to the non-producing cheaters. Since cyanobacteria reproduce asexually, their populations are formed by sets of clones, which is the closest kinship possible.

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References

Alonso-Andicoberry, C., García-Villada, L., López-Rodas, V., & Costas, E., (2002). Catastrophic mortality of flamingos in a Spanish national park caused by cyanobacteria. *Veterinary Record, 151*, 706-707.

Alvarez, M. J., Basanta, A., López-RodasÓPEZ-RODAS, V., & Costas, E. (1998). Identification of different serotypes during a Microcystis aeruginosa bloom in a SW Spanish reservoir. *Harmful Algae.,* 291-295.

Arment, A. R., & Carmichael, W. W. (1996). Evidence that microcystin is a thio-template product. *Journal of Phycology, 32,* 591-597. http://dx.doi.org/10.1111/j.0022-3646.1996.00591.x

Axelrod, R. (1984). *The Evolution of Cooperation*, Basic Books. http://dx.doi.org/10.1126%2Fscience.7466396.

Bartram, J., Carmichael, W. W., Chorus, I., Jones, G., & Skulberg, O. M. (1999). Chapter 1 Introduction, In, *Toxic Cyanobacteria in Water, A Guide to Their Public Health Consequences Monitoring and Management*, World Health Organization.

Bjørg, M., Boison, G., Skulberg, O. M., Fastner, J., Davies, W., & Gabrielsen, T. M. (2003) Natural Variation in the Microcystin Synthetase Operon mcyABC and Impact on Microcystin Production in Microcystis Strains. *Journal of Bacteriology, 185*(9), 2774-2785. http://dx.doi.org/10.1128/JB.185.9.2774-2785.2003.

Brand, L. E. (1981). Genetic variability in reproduction rates in marine phytoplankton populations. *Evolution, 35*(6), 1117-1127. http://dx.doi.org/10.2307/2408125

Carmichael, W. W. (1994). The Toxins of Cyanobacteria. *Scientific American, 270*, 78-86. http://dx.doi.org/10.1038/scientificamerican0194-78.

Carmichael, W. W. (2001). Health Effects of Toxin-Producing Cyanobacteria, "The CyanoHABs". *Human Ecological Risk Assessment, 7*(5), 1393-1407. http://dx.doi.org/10.1080/20018091095087

Carrillo, E., Ferrero, L. M., Alonso-Andicoberry, C., Basanta, A., Martin, A., López Rodas, V., & Costas, E. (2003). Interstrain variability in toxin production in populations of the cyanobacterium *Microcystis aeruginosa* from water-supply reservoirs of Andalusia and lagoons of Doñana National Park (southern Spain). *Phycologia, 42*(3), 269-274. http://dx.doi.org/10.2216/i0031-8884-42-3-269.1

Cavalli-Forza, L. L., & Feldman, M. W. (1978). The evolution of continuous variation III Joint transmission of genotype phenotype and environment. *Genetics, 90*, 391-425.

Chen, Y., & Xie, J. (2005). Third-Party Product Review and Firm Marketing Strategy. *Marketing Science, 24*(2), 218–240. http://dx.doi.org/10.1287/mksc.1040.0089

Codd, G. A., Bell, S. G., & Brooks, W. P. (1989). Cyanobacterial toxins in water. *Water Science Technology, 21*, 1-13. http://dx.doi.org/10.1080/09670269910001736462

Cooper, S. (1991). *Bacterial growth and division, biochemistry and regulation of prokaryotic and eukaryotic division cycles*. Academic Press.

Costas, E. (1990). Genetic variability in growth rates of marine dinoflagellates. *Genetica, 82*(2), 99-102. http://dx.doi.org/10.1007/BF00124638

Dawson, J. W. (1998). Institutions Investment and Growth, New Cross-Country and Panel Data Evidence. *Economic Inquiry*, *Western Economic Association International, 36*(4), 603-19.

De Mott, W. R., Zhang, Q., & Carmichael, W. W. (1991). Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of Daphnia. *Limnology and Oceanography, 36*, 1346-1357. http://dx.doi.org/10.4319/lo.1991.36.7.1346

Dittmann, E., Erhard, M., Kaebernick, M., Scheler, C., Neilan, B. A., & Börner, T. (2001). Altered of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC7806. *Molecular Microbiology, 147*, 3113-3119.

Dokulil, M., & Teubner, K. (2000). Cyanobacterial dominance in lakes. *Hydrobiologia, 438*, 1–12. http://dx.doi.org/10.1023/A:1004155810302

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics,* Ed 4 Longmans Green, Harlow, Essex, UK.

Flores, A., Rouco, M., García-Sánchez, M. J., García-Balboa, C., Gonzalez, R., & Costas, E. (2012). Effects of adaptation chance and history on the evolution of the toxic dinoflagellate *Alexandrium minutum* under selection of increased temperature and acidification. *Ecology and Evolution, 2*, 1251-1259. http://dx.doi.org/10.1002/ece3.198

Flores-Moya, A., Costas, E., & López-Rodas, V. (2008). Roles of adaptation chance and history in the evolution of the dinoflagellate *Prorocentrum triestinum* under simulated global change conditions. *Naturwissenschaften, 95*, 697-703. http://dx.doi.org/10.1007/s00114-008-0372-1

Francis, G. (1878). Poisonous Australian lake. *Nature, 18*, 11–12. http://dx.doi.org/10.1038/018011d0

Galey, F. D., Beasley, V. R., Carmichael, W. W., Kleppe, G., Hooser, S. B. & Haschek, W. M. (1987). Blue-green algae (*Microcystis aeruginosa*) hepatotoxicosis in dairy cows. *American Journal of Veterinary Research, 48*, 1415-1420.

Gould, J. S. (2002). The Structure of Evolutionary Theory. Harvard University Press 1433pp.

Hamilton, W. D. (1964). The genetical evolution of social behaviour II. *Journal of Theoretical Biology, 7*, 17-52. http://dx.doi.org/10.1016/0022-5193(64)90039-6

Hofbauer, J., & Sigmund, K. (2003). Evolutionary Game Dynamics. *Bulleting of the American Mathematical Society, 40*, 479-519. http://dx.doi.org/10.1090/S0273-0979-03-00988-1

Huertas, I. E., Rouco, M., López-Rodas, V., & Costas, E. (2010). Estimating the capability of different phytoplankton groups to adapt to contamination, herbicides will affect phytoplankton species differently. *New Phytology, 188*, 478-487. http://dx.doi.org/10.1111/j.1469-8137.2010.0.3370.x

Huertas, I. E., Rouco, M., López-Rodas, V., & Costas, E. (2011). Warming will affect phytoplankton differently, evidence through a mechanistic approach. *Proceedings of the Royal Society B, 278*, 3534−3543. http://dx.doi.org/10.1098/rspb.2011.0160

Jochimsen, E. M., Carmichael, W. W., An, J. S., Cardo, D. M., & Cookson, S. T. (1998). Liver failure in death after exposure to microcystin at a hemodialysis center in Brazil. *New England Journal of Medicine, 338*, 873-878. http://dx.doi.org/10.1056/NEJM199807093390222

Kaebarnick, M., & Neilan, B. A. (2001). Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiology Ecoogyl, 35*(1), 1-9. http://dx.doi.org/10.1111/j.1574-6941.2001.tb00782.x

Kinder, K. R. (1995). The effect of Microcystis aeruginosa on Daphnia feeding behavior and vertical distribution, PhD MS Thesis University of New Hampshire, Durham NH, USA.

Komarek, J., & Anagnostidis, K. (1998). *Cyanoprokaryota 1*. In H. Ettl Gärner, H. Heynig, & D. Mollenhauer (Eds.), *Teil: Chroococcales*. Süsswasserflora von Mitteleuropa 19/1, Gustav Fischer, Jena 548p.

Kurmayer, R., & Christiansen, G. (2009). The genetic basis of toxin production in Cyanobacteria. *Freshwater Review, 2*, 31-50. http://dx.doi.org/10.1608/FRJ-2.1.2

Lewontin, R. C. (1974). *The genetic basis of evolutionary change*. Columbia University Press, New York, NY, USA.

López-Rodas, V., & Costas, E. (1997). Characterization of morphospecies and strains of *Microcystis* (Cyanobacteria) from natural populations and laboratory clones using cell probes (lectins and antibodies). *Journal of Phycology, 33*, 446-454. http://dx.doi.org/10.1111/j.0022-3646.1997.00446.x

López-Rodas, V., & Costas, E. (1999). Preference of mice to consume *Microcystis aeruginosa* (toxin producing cyanobacteria), A possible explanation for numerous fatalities of livestock and wildlife. *Research in Veterinary Science, 67*, 107-110. http://dx.doi.org/10.1111/j.0022-3646.1997.00446.x

Lopez-Rodas, V., Costas, E., & Flores-Moya, A. (2013). Phenotypic and genetic diversities are not correlated in strains of the cyanobacterium *Microcystis aeruginosa* isolated in sw Spain. *Acta Botanica Malacitana, 38*, 5-12.

López-Rodas, V., Costas, E., Bañares, E., García-Villada, L., Altamirano, M., & Rico, M. (2006). Analysis of poligenic traits of *Microcystis aeruginosa* (Cyanobacteria) strains by Restricted Maximum Likelihood (REML) procedures, 2 Microcystin net production, photosynthesis and respiration. *Phycologia, 45*(3), 243-248. http://dx.doi.org/10.2216/04-31.1

López-Rodas, V., Maneiro, E., Lanzarot, M. P., Perdigones, N., & Costas, E. (2008). Cyanobacteria cause mass mortality of wildlife in Doñana National Park. *Veterinary Records, 162*, 317-318. http://dx.doi.org/10.1136/vr.162.10.317

Martin, A., Carrillo, E., & Costas, E. (2004). Variabilidad genética para la producción de toxina en poblaciones de *Microcystis aeruginosa* en dos embalses de abastecimiento de Andalucia. Limnetica, 23, 153-158.

Martínez Hernandez, J., Lopez-Rodas, V., & Costas, E. (2009). Microcystins from tap water could be a risk factor for liver and colorectal cancer: a risk intensified by global change. *Medical Hypothesis, 72*, 539–540. http://dx.doi.org/10.1016/j.mehy.2008.11.041

Martín-Montaño, A., Carrillo, E., Costas, E., & Basanta, A. (2000). Identificación de serotipos de Microcystis aeruginosa con distinto grado de toxicidad en un embalse de abastecimiento. *Tecnología del Agua, 199*, 54-59.

Matsunaga, H., Harada, K. I., Senma, M., Ito, Y., Ushida, S., & Kimura, Y. (1999). Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan, sudden appearance of toxic cyanobacteria. *Natural Toxins, 7*, 81-84. http://dx.doi.org/10.1002/(SICI)1522-7189(199903/04)7:2<81::AID-NT44>3.0.CO;2-O

Maynard Smith, J., & Price, G. R. (1973). The logic of animal conflict. *Nature, 246*, 15-18. http://dx.doi.org/10.1038/246015a0

Maynard Smith, J. (1964). Group selection and kin selection. *Nature, 201*, 145–47.

Maynard Smith, J. (1982). *Evolution and the Theory of Games*. Cambridge University Press. http://dx.doi.org/10.1017/CBO9780511806292

Nash, J. (1950). Equilibrium points in n-person games. *Proceedings of the National Academy of Science USA, 36*(1), 48-49. http://dx.doi.org/10.1073/pnas.36.1.48

Nishizawa, T., Ueda, A., Asayasma, M., Fujii, K., Harada, K. I., Ochi, K., & Shirai, M. (2000). Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. *Journal of Biochemistry, 127*, 779-789. http://dx.doi.org/10.1093/oxfordjournals.jbchem.a022670

Nowak, M. A., & Sigmund, K. (2004). Evolutionary dynamics of biological games. *Science, 303*(5659), 793-799. http://dx.doi.org/10.1126/science.1093411

Nowak, M.A. (2006). Five rules for the evolution of cooperation. *Science,* 314 (5805), 1560-1563. http://dx.doi.org/10.1126/cience.1133755

Rapoport, A., & Chammah, A. (1965). *Prisoner's Dilemma*. Ann Arbor, University of Michigan Press.

Rohrlack, T., Henning, M., & Kohl, J. G. (1999). Does the toxic effect of *Microcystis aeruginosa* on *Daphnia galeata* depend on microcystin ingestion rate? *Archiv für Hydrobiologie, 146*, 385-395.

Rouco, M., López-Rodas, V., Flores-Moya, A., & Costas, E. (2011). Evolutionary changes in growth rate and toxin production in the cyanobacterium Microcystis aeruginosa under a scenario of eutrophication and temperature increase. *Microbial Ecology, 62*, 265-273. http://dx.doi.org/10.1002/ece3.198

Samuelson, L. (1997). *Evolutionary games and equilibrium selection*. MIT, Cambridge.

Shirai, K. M., Ohtake, A., Sano, T., Matsumoto, S., Sakamoto, T., & Sato, A. *(*1991). Toxicity and toxins of natural blooms and isolate strains of *Microcystis spp* (cyanobacteria) and improved procedure for purification of cultures. *Applied and Environmental Microbiology, 57*(4), 1241-1245.

Sivonen, K., & Jones, G. (1999). *Cyanobacterial toxins*. In I. Chorus, & J. Bartram Eds.), *Toxic Cyanobacteria in Water, A Guide to Their Public Health Consequences, Monitoring and Management* (pp. 41–111). London: Spon Press.

Sivonen, K., Carmichael, W. W., Namikoshi, M., Rinehart, K. L., Dahlem, A. M, & Niemelä, S. I. (1990). Isolation and characterization of hepatotoxic microcystin homologues from the filamentous freshwater cyanobacterium Nostoc sp Strain 152 App. *Environmental Microbiology, 56*, 2650-2657.

Soll, M. D., & Williams, M. C. (1985). Mortality of a white rhinoceros (*Ceratotheratium simum*) suspected to be associated with the blue-green alga, *Microcystis aeruginosa*. *Journal of South African Veterinary Association, 56*, 49-51.

Spiess, E. B. (1989). *Genes in Populations* (2nd ed.). Wiley, New York, NY, USA.

Taylor, C., & Nowak, M. A. (2009). *How to evolve cooperation*. In S. Levin (Ed.), Games, Groups, and the Global Good (pp. 41-56). New York: Springer. http://dx.doi.org/10.1007/978-3-540-85436-4_2

Thrusfield, M. (1995). *Veterinary Epidemiology* (2nd ed.). Blackwell Science.

Tillett, D., Dittmann, E., Erhard, M., vonDöhren, H., Börner, T., & Neilan, B. A. (2000). Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806, an integrated peptide-polyketide synthetase system. *Chem Biol, 7*, 753–764. http://dx.doi.org/10.1016/S1074-5521(00)00021-1

Trivers, R. L. (1971). The Evolution of Reciprocal Altruism. *The Quarterly Review of Biology, 46*(1), 35–57. http://dx.doi.org/10.1086/406755

Trubetskova, I., & Haney, J. (2000). The impact of the toxic strain of *Microcystis aeruginosa* on *Daphnia magna Crustacean. Issues, 12*, 457-461.

Utkilen, H., & Gjølme, N. (1995). Iron-stimulated toxin production in *Microcystis aeruginosa. Applied and Environmental Microbiology, 61*, 797-800.

Vanderploeg, H. A., Liebig, J. R., Carmichael, W. W., Agy, M. A., Johengen, T. H., & Fahnenstiel, G. F. *(*2001). Zebra mussel (Dreissena polymorpha) selective filtration promoted toxic Microcystis blooms in Saginaw Bay (Lake Huron) and Lake Erie, *Can J Fish Aquat Sci, 58*, 1208–1221. http://dx.doi.org 10.1139/cjfas-58-6-1208

Vezie, C., Brient, L., Sivonen, K., Bertru, G., Lefeuvre, J. C., & Salkinoja-Salonen, M. (1998). Variation of microcystin content of cyanobacterial blooms and isolated strains in lake Grand- Lieu (France), *Microbial Ecology, 35*, 126-135. http://dx.doi.org 10.1007/s002489900067

von Neumann, J., & Morgenstern, O. (1944). *Theory of Games and Economic Behavior*. Princeton University Press.

Zhang, D., Xie, P., Liu, Y., Chen, J., & Liang, G. (2007). Bioaccumulation of the hepatotoxic microcystins in various organs of a freshwater snail from a subtropical Chinese Lake, Taihu Lake, with dense toxic *Microcystis blooms. Environmental Toxicology and Chemistry, 26*(1), 171–176. http://dx.doi.org/10.1897/06-222R.1