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Intercellular and extracellular amino acids of different bloom species in the Mediterranean Sea

by

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Abstract

The presented laboratory experiment was designed to characterize the quantity and compositional variation of algal extracellular amino acids (AAs) that may represent an alternative nutrient source in a natural environment. To resemble algal bloom scenarios, analyses were conducted in mono- and/or co-cultures of the bloom-forming species *Skeletonema costatum*, *Scrippsiella trochoidea*, *Ulva fasciata*, and *Corallina officinalis* during their active growth phase. The study revealed that *S. costatum* exhibited higher production of the dominant AAs than *S. trochoidea*. Alanine, lysine, and threonine acids are the dominant amino acids in *S. costatum* and *S. trochoidea* filtrates, which may play a role in mucus formation during mucosal phytoplankton blooms with negative ecological effects. On the other hand, aspartic, glutamine, alanine, and leucine acids are the dominant amino acids in macroalgae. In co-culture experiments, *U. fasciata* shows strong and rapid allelopathic activity against these two potentially harmful species. The AA production offers an advantage to species with the capacity to absorb them to form blooms. Thus, anthropogenic inorganic nutrient inputs may be less important for the development of algal blooms in coastal waters. A major difference that distinguishes this work from others is the use of specific multi-taxa cultures of phytoplankton and macroalgae. The study represents a new research effort in Alexandria waters.

Key words: Extracellular amino acids, *Skeletonema costatum*, *Scrippsiella trochoidea*, *Ulva fasciata*, *Corallina officinalis*

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Abbreviations:

AAs – amino acids; DFAAs – dissolved free amino acids; DOM – dissolved organic matter; HABs – harmful algal blooms; µE m^{-2} s^{-1} – micro-einsteins per second per square meter; HSD – Tukey's test

1. Introduction

Extracellular algal production is considered a significant part of the algal primary production (Myklestad 2000). Despite the fact that the pool of algal dissolved free amino acids (DFAAs) in seawater represents a small fraction of dissolved organic matter (DOM) (Thornton 2014), its exploration is critical for understanding primary producers' nitrogen demand during periods of insufficient dissolved inorganic nitrogen concentrations (Tyler et al. 2003), as well as the growth and other biological processes of living organisms (Vidoudez & Pohnert 2012) affecting the global carbon cycle (Kujawinski 2011). The release of NH_4^+ through oxidation of AAs provides a competitive advantage to algae species capable of uptake (Tyler et al. 2005; Thornton 2014). While a wide range of algal taxa can release and consume amounts of DFAAs (Hounshell et al. 2017), the extent to which algae can use DFAAs as a source of nitrogen is still unclear (Flynn et al. 2008; Li et al. 2009). Furthermore, the quantification of AAs production in nature is difficult due to the interference of several specific factors that modulate their concentration such as a more 'leaky' cell membrane (Veldhuis et al. 2001), direct cell contact that can cause interference (Vardi et al. 2002), viral infection (Bettarel et al. 2005), feeding by predators (Møller 2007), physiological status (Flynn et al. 2008), cell death (Orellana et al. 2013), short residence time and rapid turnover, growth curve (Sarmento et al. 2013), and environmental conditions (Grosse 2017).

The present study attempts to characterize the autochthonous algal AAs exudation to define its role as an alternative nutrient source that can contribute to the development of harmful algal blooms (HABs) in the environment. The experiment was designed to simulate algal bloom scenarios to characterize the extracellular AA production of different algal species from different classes during the active growth phase, i.e. the exponential phase. A set of laboratory experiments was established for: i) monocultures of both *S. costatum* Cleve (centric diatom) and *S. trochoidea* (Stein) Loeblich (thecate dinoflagellate), ii) healthy thalli of *U. fasciata* Delile (Chlorophyta) and *C. officinalis* Linnaeus (Rhodophyta), and iii) co-cultures of the tested phytoplankton species with different weights of macroalgae. This is the first research work to date in Alexandria waters aimed at expanding our understanding of bloom dynamics as a necessary step toward predicting and mitigating HABs. The selected phytoplankton species repeatedly formed blooms in Alexandria waters, for example *S. costatum* blooms were recorded there three times in 2000 (Mikhail & Labib 2014) and elsewhere (Horner et al. 2005), while *S. trochoidea* blooms were recorded in 1998 and 2014 (Labib 2002; El Shafay et al. 2019). *Skeletonema costatum* and *Scrippsiella trochoidea* can form harmful blooms. The harmful effects of *S. costatum* blooms include their large biomass, which causes various ecological disturbances such as lack of oxygen, i.e. hypoxia (Zhang et al. 2016), and water discoloration (Ibrahim et al. 2021). Moreover, the adverse effects include production of reactive aldehydes that negatively affect hatching copepods (Chen et al. 2021), as well as arsenite and dimethyl arsenic, which can block important biochemical pathways in algae, as arsenic is chemically similar to nitrogen and phosphorous (Howard et al. 1995). The first blooms of *Scrippsiella trochoidea*, documented in 1890 and 1913 under the species name *Glenodinium trochoideum*, were associated with fish kills in Sydney Harbour (Whitelegge 1891). Under unique conditions in sheltered bays, blooms become very dense and cause death of fish and invertebrates from the depletion of oxygen (Hallegraeff 2002). A red tide in China, Japan, Korea, and Russia, observed by Sakamoto et al. (2021), killed fish and caused serious economic losses. The studied macroalgae occasionally form green tide mats (Labib et al. 2015), and co-occurrence of micro-macroalgal blooms is a common phenomenon (Hosny 2016).

2. Materials and methods

Water samples and macroalgae species were collected during summer 2018 from the Qaitbey area located in the western vicinity of the Port of Alexandria, Egypt. The area has been identified as eutrophic with recurrent annual algal blooms (Labib 2002).

After isolation, cells of *S. costatum* and *S. trochoidea* were maintained in stock cultures in the f/2 medium prepared with aged and filtered offshore autoclaved seawater (Guillard & Ryther 1962) at 25°C, salinity 32–33 PSU, pH 8–8.2, under continuous light illumination of ~100 µE m^{-2} s^{-1}, and bubbled with sterile filtered air. Derrien et al. (1998) recommended the use of continuous light illumination to ensure faster growth of *S. trochoidea* rather than the light/

dark cycle. The growth was followed starting from the second day of incubation, and the daily cell density at approximately the same time was measured using a haemocytometer. Subsamples of each previously gently shaken cell suspension reaching ~1000 cells ml^{-1} for *S. costatum* and 300 cells ml^{-1} for *S. trochoidea*, which almost resemble their initial concentrations during early bloom stages recorded in the same area (Mikhail and Labib 2014), were distributed into polystyrene bottles with 150 ml of filtered seawater enriched with f/2 solution and cultured under the previously mentioned conditions. Exponential and stationary growth phases were identified daily by direct observation under a microscope (Kester et al. 1967). Microalgae samples of 3 ml were preserved with acidic Lugol's iodine; then samples were settled overnight, and counts were performed under a microscope (160 ×) in random fields using a haemocytometer to determine the phytoplanktonic algal growth (Utermöhl 1958). The early stage of the exponential growth phase was detected on day 2 in *S. costatum* cultures, and analyses were performed during four consecutive days. On the other hand, it took a relatively long time in gently shaken *S. trochoidea* cultures, which appear to require more time due to their slower growth rate (Dixon & Syrett 1988). The cell-free culture medium of both species was collected during the exponential phase after filtration through 25 mm Whatman GF/C glass fiber filters under gentle vacuum (50 mm Hg) and stored in acid-washed vials at –20°C for a maximum of 7 days (Granum et al. 2002) until analysis of extracellular amino acids. Cell division per day of both species was calculated Guillard (1973).

Fresh, adult *U. fasciata* and *C. officinalis* thalli were carefully washed in the field with seawater to dislodge attached biota and other extraneous matter and transported moist to the laboratory within 30 min. Morphological identification of species followed the checklist of Aleem (1993). In the laboratory, samples were gently brushed, rinsed with distilled water, and dried with absorbent paper at room temperature. Prior to experiments, each macroalgal species (10–15 g fresh weight) was grown for 10 h in 1 l of fresh seawater under adjusted temperature (14–27°C) and salinity (26–32 PSU), with optima at 23°C and 32 PSU (Xiao et al. 2016), and light regime, and cultures were bubbled with air at a rate of 2 l min^{-1}, followed by control cultures for each species in F/2 medium. The progressive growth during the first week was determined by biomass estimation (Li et al. 2016). Algae during the growth phase were removed from the medium by filtration, and the filtrate was kept frozen until amino acids were analyzed.

Microalgae and macroalgae co-culture experiments consisted in adding macroalgae of different weights (0.5 g, 1 g, and 2 g) to stock phytoplankton cultures obtained in the exponential phase, which were grown under the same previously mentioned conditions. After filtration of samples between days 3 and 5, the supernatant of the filtrated media with free amino acids was analyzed. The role of bacteria cannot be excluded as their existence reflects the natural environmental conditions. Dissolved free amino acids (DFAA) were analyzed in the Central Laboratory at the National Institute of Oceanography and Fisheries using High-Performance Liquid Chromatography (HPLC) according to a method published in Agilent Application Note (Henderson et al. 2000). Briefly, DFAA samples and amino acid standards solutions were derivatized with o-phthalaldehyde (OPA) and analyzed by RP-HPLC (Agilent 1260 Infinity II chromatographic system) with ultraviolet-visible detection (diode array detector, DAD). After derivatization, an amount equivalent to 2.5 µl of each sample was injected onto a Zorbax SB-C18 (5 µm, 150 × 4.6 mm, Agilent) at 40°C, and detection was performed at λ = 338 nm. Mobile phase A was 40 mM NaH_2PO_4, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was achieved at a flow rate of 2 ml/min using a gradient program that allowed 1.9 min at 0% B, followed by an increase in eluent B to 57% at 18.1 min, and then reaching 100% B at 18.6 to 22.3 min. Finally, the eluent B level dropped to 0% between 23.2 to 26 min. The concentrations of each DFAA in the samples were calculated from peak areas on chromatograms using response factors obtained from standards of amino acids (analytical grade, Sigma Aldrich, Louis Mo, USA), 17 amino acids in 0.1M hydrochloric acid, 2.5 mM each: alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and 1.25 mM cystine.

2.1. Statistical analysis

Statistical analysis was performed using software SPSS v. 26 (IBM Corporation, Armonk, NY, USA). Data were analyzed by one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$. Where applicable, multiple comparisons were performed using Turkey's test (HSD). Data are presented as mean ± standard deviation (SD) of triplicate extractions. The three-letter abbreviation code denotes amino acids. In each row, different superscript letters represent significant differences between samples ($p < 0.05$) in XLSTAT version 2014.5.03, while the same superscript letters indicate no significant differences ($p > 0.05$).

3. Results

3.1. Amino acids of phytoplankton species

A sharp reduction in the total concentration estimated at 20.33% was measured in *S. trochoidea* cultures compared with *S. costatum*. In general, the analyzed species presented similar amino acid profiles, although they showed significant differences ($p < 0.05$) in the amount and proportion of some AAs (Table 1). However, the tyrosine acid was found exclusively in *S. costatum*, accounting for 24.18–25.15% of the total AAs (Fig. 1). Of the nine AAs identified, alanine, lysine, and threonine acids were the most abundant and were responsible for the major quantity and quality fluctuations. However, the proportions of the main AAs were relatively higher in *S. trochoidea*. Other acids, such as cysteine, arginine, and methionine acids, were negligible. The average exponential division rate calculated from cell counts was 1.98 div. d^{-1} for *S. costatum* and 0.45 div. d^{-1} for *S. trochoidea*. Cell loss within 15–20% of *S. costatum* and 10–15% of *S. trochoidea* occurred during the last two days of the late exponential phase.

Table 1

Amount (mean ± SE) of amino acids in cultures of the microalgae *Skeletonema costatum* and *Scrippsiella trochoidea* (– denotes not detected)

	S. costatum	*S. trohoidea*
AAs	Amount (µM l^{-1})	
Alanine acid	3806.89 ± 0.25	894.74 ± 0.13
Arginine acid	463.47 ± 0.33	9.99 ± 0.15
Aspartic acid	408.86 ± 0.11	228.17 ± 0.35
Cystine acid	76.12 ± 0.09	2.01 ± 0.11
Glycine acid	525.17 ± 0.27	29.75 ± 0.10
Lysine acid	3035.79 ± 0.24	985.70 ± 0.09
Methionine acid	31.32 ± 0.12	16.17 ± 0.28
Threonine acid	1233.26 ± 0.21	402.65 ± 0.20
Tyrosine acid	3056.35 ± 0.17	–

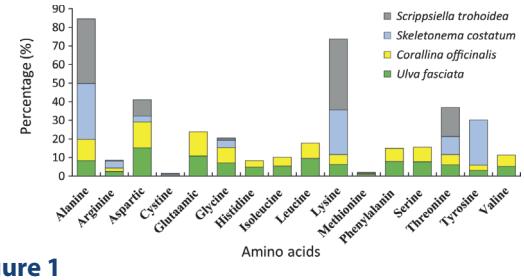

Figure 1

Percentage of amino acids in cultures of *Skeletonema costatum*, *Scrippsiella trohoidea*, *Ulva fasciata* and *Corallina officinalis*

3.2. Amino acids of macroalgae species

The amino acid profiles revealed the presence of 16 amino acids in healthy *U. fasciata* and *C. officinalis* (Table 2). Irrespective of the species and despite the similarity between the amino acids, they showed limited changes in their concentrations, with these being slightly higher in *C. officinalis* (7.64%) due to an increase in glutamine acid by 23.22% and alanine acid by 32.49%. The relative contribution indicates aspartic acid from these two species as the main acid (total 34.23% and 37.78%, respectively), while methionine, tyrosine, arginine, and isoleucine acids were of much lesser importance (Fig. 1).

Table 2

Amount (mean ± SE) of amino acids in cultures of the macroalgae *Ulva fasciata* and *Corallina officinalis*

	U. fasciata	*C. officinalis*
AAs	Amount (µM l^{-1})	
Alanine acid	7260.56 ± 2847.28	10754.96 ± 3813.81
Argnine acid	2162.72 ± 527.49	1729.23 ± 823.44
Aspartic acid	13182.29 ± 12716.03	13116.83 ± 5123.76
Glutaamic acid	9423.27 ± 971.47	12273.30 ± 2012.02
Glycine acid	6179.2 ±1024.18	7633.91 ± 1272.32
Histidine acid	4170.96 ± 534.74	3289.75 ± 406.14
Isoleucine acid	4634.40 ± 2165.61	4512.86 ± 1504.29
Leucine acid	8187.44 ± 1320.55	7844.79 ± 1973.53
Lysine acid	5406.8 ± 1374.61	4976.80 ± 473.98
Methionine acid	669.41 ± 514.93	590.47 ± 454.21
Phenylalanin aci	6797.116 ± 1699.28	6748.21 ± 1022.46
Serine acid	6745.62 ± 775.36	7338.68 ± 1120.41
Threonine acid	5252.31 ± 172.21	5145.51 ± 1513.38
Tyrosine acid	2626.16 ± 972.65	2699.28 ± 1587.61
Valine acid	4531.41 ± 66.15	5778.15 ± 2626.43

3.3. Microalgae and macroalgae co-cultures

Nine AAs released in the co-culture of *Ulva* with *S. costatum* were identified (Table 3). However, the results revealed considerable variation in the content and relative contribution, with a consistent, rapid increase in production corresponding to increased *Ulva* weight. This increase was estimated to be 21.22 and 31.76 times higher with the addition of 1 g and 2 g compared with the lowest addition of *Ulva*. The AAs composition as a function of added weight exhibited a distinct change. Tyrosine and arginine acids were detected only with 0.5 g of *Ulva*, as were aspartic and cystine acids with 1 g of *Ulva*, methionine acid, although not detected with 0.5 g of *Ulva*, contributing the major fraction with 1 g and 2 g of *Ulva* (47.45% and 59.12%), and lysine and threonine acids were common

Table 3

Amount (means) and relative % of amino acids in co-cultured *Skeletonema costatum* and *Ulva fasciata*. Different letters indicate statistically significant differences between the treatments using Tukey's test (– denotes not detected).

Amino acid	0.5 g *Ulva*		1 g *Ulva*		2 g *Ulva*	
	Amount	%	Amount	%	Amount	%
Alanine acid	1143.99 ± 0.31 ^{C}	32.3	2207.81 ± 0.55 ^{B}	2.94	2710.52 ± 0.59 ^{A}	2.4
Arginine acid	334.73 ± 0.42 ^{A}	9.45	–	–	–	–
Aspartic acid	–	–	357.45 ± 0.25 ^{A}	0.48	–	–
Cystine acid	–	–	92.60 ± 0.13 ^{A}	0.12	–	–
Lysine acid	952.80 ± 0.51 ^{C}	26.91	3101.87 ± 0.13 ^{A}	4.13	2494.39 ± 0.29 ^{B}	2.22
Methionine acid	–	–	35663.01 ± 0.49 ^{B}	47.45	66497.70 ± 0.36 ^{A}	59.12
Phenyllalanin acid	–	–	33087.24 ± 0.06 ^{B}	44.03	40254.47 ± 0.50 ^{A}	35.79
Thereonine acid	1091.39 ± 0.44 ^{A}	30.82	643.31 ± 0.07 ^{B}	0.85	528.31 ± 0.59 ^{C}	0.47
Tyrosine acid	18.29 ± 0.47 ^{A}	0.52	–	–	–	–
Total AAs	**3541.2**		**75153.29**		**112485.39**	

with *Ulva* additions, contributing most with 1 g of *Ulva*. The relative contribution changed dramatically as alanine, threonine, and lysine acids dominated with 0.5 g of *Ulva*, while methionine and phenylalanine acids were the significant major contributors with the other two additions.

Consistent with the previous experiment, co-cultured *U. fasciata* and *S. trochoidea* (Table 4) showed the same production trend and almost unchanged compositional structure, with varying contribution. On the other hand, AAs yield was found to be about 10 times higher with 0.5 g of *Ulva* and much reduced with 1 g and 2 g of *Ulva* (56% and –45%). The composition of AAs exhibited an interesting feature characterized by the overwhelming dominance of isoleucine, with its highest contribution at the highest *Ulva* addition. Further, a trace proportion of proline acid was determined for the first time.

The effect of the red alga *C. officinalis* on the AAs production was tested in co-culture with *S. costatum* (Table 5). Compared with the *Ulva* culture with *S. costatum*, clear differences were observed such as a more diverse AAs composition (14 AAs), a lower relative content, a relatively lower effect of increasing weight and corresponding concentrations, and varied compositional structure of the major constituents. Except for the increased concentrations with 0.5 g of *C. officinalis* compared with the same addition of *Ulva* (2.35 times), the sharp reduction in content reaches 55.7% and 96.92% after the addition of 1 g and 2 g, respectively. As for the AAs distribution according to *C. officinalis* weights, most of the detected AAs appeared with 1 g, and the least with 0.5 g. The results also indicated specific AAs composition with specific *C. officinalis* weights, leucine and isoleucine acids with 1 g, and glutamine acid with 2 g. The relative

Table 4

Amount (mean ± SE) and relative % of amino acids in co-cultured *Scrippsiella trochoidea* and *Ulva fasciata*. Different letters indicate statistically significant differences between the treatments using Tukey's test (– denotes not detected).

Amino acid	0.5 g *Ulva*		1 g *Ulva*		2 g *Ulva*	
	Amount	%	Amount	%	Amount	%
Alanine acid	1028.73 ± 0.59 ^{C}	3.03	2476.80 ± 0.41 ^{B}	5.00	3590.29 ± 0.70 ^{A}	7.1
Arginine acid	1166.86 ± 0.46 ^{A}	3.44	908.10 ± 0.48 ^{B}	1.83	624.11 ± 0.29 ^{C}	1.23
Cystine acid	287.89 ± 0.39 ^{A}	0.85	3.32 ± 0.13 ^{B}	0.01	–	–
Glutamine acid	58.90 ± 0.16 ^{B}	0.17	1861.31 ± 0.33 ^{A}	3.76	–	–
Glycine acid	135.11 ± 0.48 ^{B}	0.4	4310.54 ± 0.56 ^{A}	8.70	113.29 ± 0.24 ^{C}	0.22
Isoleucine acid	28372.77 ± 0.12 ^{C}	83.64	35824.50 ± 0.36 ^{B}	72.31	46009.11 ± 0.16 ^{A}	91
Lysine acid	759.44 ± 0.51 ^{B}	2.24	3085.09 ± 0.25 ^{A}	6.23	74.46 ± 0.61 ^{C}	0.15
Phenylalanine acid	170.57 ± 0.41 ^{A}	0.5	38.63 ± 0.54 ^{B}	0.08	–	–
Proline acid	–	–	14.30 ± 0.08 ^{B}	0.03	129.81 ± 0.32 ^{A}	0.26
Thereonine acid	212.16 ± 0.23 ^{B}	0.63	1022.56 ± 0.33 ^{A}	2.06	16.59 ± 0.10 ^{C}	0.03
Tyrosine acid	1730.80 ± 0.08 ^{A}	5.1	–	–	5.76 ± 0.06 ^{B}	0.01
Total AAs	**33864.33**		**49545.15**		**50563.42**	

Table 5

Amount (mean ± SE) and relative % of amino acids in co-cultured *Skeletonema costatum* and *Corallina officinalis*. Different letters indicate statistically significant differences between the treatments using Tukey's test (– denotes not detected).

Amino acid	0.5 g *Corallina*		1 g *Corallina*		2 g *Corallina*	
	Amount	%	Amount	%	Amount	%
Alanine acid	95.69 ± 0.49 ^{C}	1.15	1160.92 ± 0.67 ^{A}	3.49	446.02 ± 0.54 ^{B}	12.86
Arginine acid	–	–	518.58 ± 0.21 ^{A}	1.56	357.46 ± 0.43 ^{B}	10.31
Aspartic acid	505.76 ± 0.13 ^{A}	6.07	439.55 ± 0.42 ^{B}	1.3	208.77 ± 0.20 ^{C}	6.02
Cystine acid	24.89 ± 0.23 ^{B}	0.30	62.02 ± 0.10 ^{A}	0.19	–	–
Glutamine acid	–	–	–	–	343.02 ± 0.13 ^{A}	9.89
Glycine acid	10.76 ± 0.37 ^{C}	0.13	709.10 ± 0.09 ^{A}	2.13	372.17 ± 0.62 ^{B}	10.73
Histidine acid	–	–	16414.45 ± 0.28 ^{A}	49.29	53.14 ± 0.55 ^{B}	1.53
Isoleucine acid	7645.42 ± 0.39 ^{A}	91.72	–	–	–	–
Leucine acid	–	–	4755.04 ± 0.72 ^{A}	14.28	–	–
Lysine acid	33.78 ± 0.65 ^{C}	0.41	475.03 ± 0.53 ^{A}	1.43	187.20 ± 0.16 ^{B}	5.4
Phenylalanine acid	–	–	7700.81 ± 0.54 ^{A}	23.12	–	–
Proline acid	6.05 ± 0.08 ^{B}	0.07	469.75 ± 0.47 ^{A}	1.41	–	–
Thereonine acid	–	–	598.52 ± 0.08 ^{A}	1.8	233.98 ± 0.21 ^{B}	6.75
Tyrosine acid	13.01 ± 0.47 ^{B}	0.16	–	–	1266.52 ± 0.68 ^{A}	36.52
Total AAs	8335.36		33303.77		3468.28	

contribution indicated significant dominance of isoleucine acid with 0.5 g, histidine and phenylalanine acids with 1 g, tyrosine acid with 2 g, and proline acid was still the smallest contributor.

4. Discussion

During the active (exponential) growth phase, the AA analysis is an attempt to resemble an algal bloom situation, where such information is still scarce. Determining the specific growth phase where the largest production of AAs occurs is still a matter of debate and the mechanisms of AAs excretion by algae are still largely unknown (Franklin et al. 2006). The exponential phase is the fastest growing phase among others (Bruckner et al. 2011; Orellana et al. 2013; Sarmento et al. 2013). *S. costatum* grew faster, exhibited a shorter exponential phase, and showed higher production of the dominant AAs compared to *S. trochoidea*, estimated at 4.25-fold for alanine and 3.06-fold for each lysine and threonine. The composition of AAs was uniform, more or less stable, but tyrosine acid was found exclusively in *S. costatum*, accounting for about one-fourth of total AAs (Martin-Jezequel et al. 1988). As previously reported (Vidoudez and Pohnert 2012; Sarmento et al. 2013), the variation in the amount and proportion of the released AAs is mainly determined by the species age and culture conditions. However, the AA relative frequency sequence differs from that reported for *S. costatum* by Martin-Jezequel et al. (1988), where glycine, aspartic, and glutamine acids dominate, respectively. However, these results are consistent with Mannino and Harvey (2000), indicating the significant contribution of alanine and threonine acids and lesser importance of aspartic and glutamic acids. Our data indicate a significant contribution of threonine acid in *Skeletonema*, which contradicts the data reported by Sarmento et al. (2013), similar to that for arginine acid in *S. trochoidea* (Meksumpun et al. 1994). Since arginine acid is a precursor to saxitoxin and gonyautoxin acids (Anderson & Harrison 1988), considering its rapid, lethal effects on shellfish and larvae (Tang & Gobler 2012), the current changes in the composition may offer an advantage in preventing dinoflagellate red tide outbreaks.

The study shows more diverse AA composition in macroalgae, homogeneity, predominance of aspartic, glutamine, alanine, and leucine acids, and limited changes in concentrations, slightly higher in *C. officinalis* (7.64%). The dominance of aspartic and glutamic acids was proven in Alexandria waters for the same examined macroalgae (Mustafa and Eladel 2015; Ismail 2017), previously documented elsewhere (Akgül et al. 2015).

The main difference that distinguishes this work from others was the determination of AA production in multi-taxa cultures of phytoplankton and macroalgae species, where, to our knowledge, no experimental or field comparative studies are available for this combination of taxonomic groups.

This study revealed considerable variation in the content, composition, and relative contribution of the released AAs compared with cultures of individual taxa. The substantial changes were similar to those reported for *Ulva intestinalis* by Lourenco et al. (2004). The sharp reduction in the number of AAs compared with monocultures of each species during their exponential phase (control) was estimated at 43.75% for *U. fasciata*, 25% for *S. costatum*, and 47.06% for *S. trochiodea*. A positive trend was observed between the weight increase in *Ulva* and *C. officinalis* in co-cultures with *S. costatum*, while it was less pronounced in co-cultures of *Ulva* with *S. trochoidea*, which may reflect some resistance. Kolmakovaa & Kolmakov (2019) reported significantly higher AA content in macroalgae compared to phytoplankton species. The co-culture experiments lasted only five days in an attempt to reduce and/or avoid potential causes responsible for the observed variations, which often remain elusive. Another cause may be the allelopathic effect of *U. fasciata* on the growth of *S. costatum* and *S. trochiodea* (El-Sheekh et al. 2010; EL Shafay et al. 2019), as *U. fasciata* is characterized by strong and quick allelopathic activity against these two potentially harmful species, and the growth inhibition occurred after six days of co-incubation. Since the algal cultures used were not axenic, the variation in AA production was probably modified by co-occurring prokaryotes, which is consistent with Bruckner et al. (2011) for diatom and bacteria cultures. The differential uptake and selectivity of amino acids proved similar among macroalgae and phytoplankton (Tyler et al. 2003), which may also be a significant cause (Kolmakovaa & Kolmakov 2019). Furthermore, the expected decrease in inorganic nitrogen content in the medium with no additional supply may also be responsible for the observed variation (Granum et al. 2002). Thus, such variation is mainly a direct result of the balance between uptake and release of AAs, however, the mechanisms affecting the release of dissolved AAs in such multispecific cultures need further studies.

4.1. Ecological significance: Emphasis on bloom formation

Consistent with ecological significance, all the algal species tested have been reported to form recurrent red/green tides in some estuaries and coastal waters of Alexandria and elsewhere, with subsequent undesirable ecological effects. Most previous studies worldwide focused on the major contribution of eutrophication in coastal systems affecting the increased frequency, magnitude, or extension of HABs (Malone & Newton 2020). However, the processes driving the formation of blooms remain unclear, and a positive relationship between HABs and increased inorganic nutrient inputs of anthropogenic origin has never been convincingly demonstrated (Zingone & Waytt 2012). Recently, Mikhail et al. (2020) presented new evidence and alternative interpretations for Alexandria waters, which do not support the conclusions of previous studies. The authors concluded that the potential for bloom formation occurs even with low to intermediate values of inorganic nutrients (surface nitrate occasionally reaches 0.2 µM) when relative availability of dissolved organic matter (DOM) is high. This is supported by a significant correlation between DOM concentrations and abundance of bloom species, hence DOM is considered as an alternative nutrient source that may enhance the bloom formation, in particular in the case of dinoflagellates (Glibert et al. 2001), which is consistent with other studies (Tas & Yilmaz 2015; Liu et al. 2020). Phytoplankton autochthonous exudations, such as DFAA, have been identified as important contributors of DOM (Yamamoto et al. 2004; Thornton 2014; Burpee et al. 2016). While their biological role in aquatic organisms and ecosystems is significant and diverse (Wu 2009), very little is known about their characteristics and accumulation during the development of algal blooms (Suksomj et al. 2009). The extracellular alanine, lysine, and threonine acids, present in *Skeletonema* and *Scrippsiella* filtrates during the active growth phase, can be released during their massive blooms in the natural environment. Given the high turnover rate of AAs in the water column and consequently the production of ammonia, AAs represent another pathway of nutrient acquisition and a substantial contributor to algal N demand in the environment. This process is important seasonally in estuaries (Bronk & Glibert 1993) to stimulate phytoplankton blooms (Hounshell et al. 2017) of specific causative species (Mulholland et al. 2002) and may provide, through selectivity of AAs, a significant portion of the total N demand in estuarine macroalgal blooms (Tyler et al. 2005). These data may therefore help shed light on bloom dynamics, leading to better implementation of optimal bloom prevention and control measures. Dissolved organic matter, which is released with the development of phytoplankton blooms, has a negative ecological impact in areas affected by algal blooms (Sellner & Nealley 1997), because it not only contributes, along with alanine, lysine, and threonine acids, to mucus formation during mucosal phytoplankton blooms, but also affects nutrient cycling, as already reported by Metaxatos et al. (2003).

5. Conclusion

Since the coastal waters of Alexandria and elsewhere are affected by recurrent massive monospecific and/or multispecific blooms of phytoplankton and macroalgae, it seems likely that the release of AAs in the water column and the production of ammonia may contribute to the existence and long duration of these recurrent blooms. Alanine, lysine, and threonine acids released in monocultures of *Skeletonema costatum* and/or *Scrippsiella trochoidea* may contribute to the formation of mucilaginous phytoplankton blooms, with negative ecological consequences. Therefore, research on bloom dynamics is very important for coastal zone management. During the exponential phase in the co-culture of *Skeletonema costatum* and *Scrippsiella trochoidea* with *Ulva fasciata* and *Corallina officinalis*, a sharp reduction in the number of amino acids and inhibition growth of microalgae occurred, while weights of macroalgae increased, which implies that macroalgae are characterized by a strong and rapid allelopathic activity against these two potentially harmful species. Thus, these data can shed light on bloom dynamics, consequently leading to better implementation of optimal bloom prevention and control measures. Our findings may potentially have far-reaching ecological implications.

Authors' contributions:

Sh.H. participated in the design of the study, fieldwork, collection of samples, and taxonomic identification; M.E. helped to draft the manuscript, approved the final version of the manuscript, revised and edited; W.L. participated in the design of the study, coordination, and helped to draft the manuscript.

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References

Akgül, R., Bayram, K., Füsun, A., & Ormanc, H. B. (2015). Crude Protein Values and Amino Acid Profiles of Some Red Algae Collected from Çanakkale. *International Journal of Molecular Sciences, 44*(4), 527–530.

Aleem, A. A. (1993). The marine algae of Alexandria, Egypt, University Alexandria. pp 139. Anderson, D.M. & Cheng, T. P.-O. (1988). Intracellular localization of saxitoxins in the dinoflagellate *Gonyaulax tamarensis. Journal of Phycology, 24*, 17–22. https://doi.org/10.1111/j.1529-8817.1988.tb04451.x

Bettarel, Y., Kan, J., Wang, K., Williamson, K. E., Cooney, S., Ribblett, S., Chen, F., Wommack, K. E., & Coats, D. W. (2005). Isolation and preliminary characterization of a small nuclear inclusion virus infecting the diatom *Chaetoceros* cf. *gracilis. Aquatic Microbial Ecology, 40*, 103–114. https://doi.org/10.3354/ame040103

Bruckner, C.G., Rehm, C., Grossart, H.P. & Kroth, P.G. (2011). Growth and release of extracellular organic compounds by benthic diatoms depend on interactions with bacteria. *Environmental Microbiology* 13(4), 1052-1063. https://doi.org/10.1111/j.1462-2920.2010.02411.x.

Bronk, D. A., & Glibert, P. M. (1993). Application of a 15 N tracer method to the study of dissolved organic nitrogen uptake during spring and summer in Chesapeake Bay. *Marine Biology, 115*(3), 501–508. https://doi.org/10.1007/BF00349849

Burpee, B., Saros, J. E., Northington, R. M., & Simon, K. S. (2016). Microbial nutrient limitation in Arctic lakes in a permafrost landscape of southwest Greenland. *Biogeosciences, 13*(2), 365–374. https://doi.org/10.5194/bg-13-365-2016

Chen, T.-C., Ho, P.-C., Gong, G.-C., Tsai, A.-Y., & Hsieh C.-H. (2021). Finding Approaches to Exploring the Environmental Factors That Influence Copepod-Induced Trophic Cascades in the East China Sea. *Diversity (Basel), 13*(7), 299. https://doi.org/10.3390/d13070299

Derrien, A., Coiffard, L.J., Coiffard, C. & De Roeck-Holtzhauer, Y. (1998). Free amino acid analysis of five microalgae. *Journal of Applied Phycology* 10(2), 131-134. https://doi.org/10.1023/A:1008003016458

Dixon, G. K., & Syrett, P. J. (1988). The growth of dinoflagellates in laboratory cultures. *The New Phytologist, 109*, 297–302. https://doi.org/10.1111/j.1469-8137.1988.tb04198.x

El-Sheekh, M. M., Khairy, H. M., & El-Shenody, R. A. (2010). Allelopathic effects of cyanobacterium *Microcystis aeruginosa* kützing on the growth and photosynthetic pigments of some algal species. *Allelopathy Journal, 26*(2), 75–290. https://doi.org/10.1007/s10457-010-9321-z

El Shafay, S. M., Abo Shady, A. M., Abdel-Fatah, L. M., Hosny, Sh., & Labib, W. (2019). Allelopathic effect of the green macroalga *Ulna fasciata* (Delile) on species forming potentially harmful algal blooms. *The Egyptian Journal of Experimental Biology (Botany)*, *15*(2), 251–260. https://doi.

org/10.5455/egyjebb.20190505044917

Flynn, K. J., Clark, D. R., & Xue, Y. (2008). Modeling the release of dissolved organic matter by phytoplankton(1). *Journal of Phycology, 44*, 1171–1187. https://doi.org/10.1111/j.1529-8817.2008.00562.x PMID:27041714

Franklin, R.B. & Mills, A.L. (2006). Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. *Microbial Ecology* 52(2): 280-288. https://doi.org/10.1007/s00248-006-9033-0

Glibert, P. M., Magnien, R., Lomas, M. W., Alexander, J., Fan, C., Haramoto, E., Trice, M. & Kana, T. M. (2001). Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events. *Estuaries, 24*(6), 875–883. https://doi.org/10.2307/1353178

Granum, E., Kirkvold, S., & Myklestad, S. M. (2002). Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: Diel variations and effects of N depletion. *Marine Ecology Progress Series, 242*, 83–94. https://doi.org/10.3354/meps242083

Grosse, J., Burson, A., Stomp, M., Huisman, J. & Boschker, H.T. (2017). From ecological stoichiometry to biochemical composition: variation in N and P supply alters key biosynthetic rates in marine phytoplankton. *Frontiers in Microbiology* 8: 1299. https://doi.org/10.3389/fmicb.2017.01299

Guillard, R. R. L. (1973). Division rates. In R. Stein (Ed.), Handbook of phycological methods: culture methods and growth measurements (pp. 290–311). Cambridge University Press.

Guillard, R. R. L., & Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. *Canadian Journal of Microbiology, 8*, 229–239. https://doi.org/10.1139/m62-029 PMID:13902807

Hallegraeff, G. M. (2002). Aquaculturists' Guide to Harmful Australian Microalgae. School of Plant Science, 2nd ed. University of Tasmania, Hobart, Tasmania Australia. 136. ISBN 1-86295-049.

Henderson, J., Ricker, R., Bidlingmeyer, B., & Woodward, C. (2000). Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids. In Application bulletin 5980–1193. Agilent Technologies., https://www.agilent.com/cs/library/chromatograms/59801193

Hounshell, A. G., Peierls, B. L., Osburn, C. L., & Paerl, H. W. (2017). Stimulation of phytoplankton production by anthropogenic dissolved organic nitrogen in a coastal plain estuary. *Environmental Science & Technology, 51*(22), 13104–13112. https://doi.org/10.1021/acs.est.7b03538 PMID:29083877

Hosny, Sh. (2016). Ecological and Physiological Studies on the Interaction between Macroalgae and Phytoplankton off Alexandria Mediterranean Coast (Egypt). Thesis (Ph.D), Faculty of Science, Tanta University.

Horner, R. A., Postel, J. R., Halsband-Lenk, C., Pierson, J. J., Pohnert, G., & Wichard, T. (2005). Winter-spring phytoplankton blooms in Dabob Bay, Washington. *Progress in Oceanography, 67*, 286–313. https://doi.org/10.1016/j.pocean.2005.09.005

Howard, A. G., Comber, S. D. W., Kifle, D., Antai, E. E., & Purdie, D. A. (1995). Arsenic speciation and seasonal changes in nutrient availability and micro-plankton abundance in Southampton Water, U. K. *Estuarine, Coastal and Shelf Science, 40*(4), 435–450. https://doi.org/10.1006/ecss.1995.0030

Ibrahim, E. A., Zaher, S. S., Ibrahim, W. M., & Mosad, Y. A. (2021). Phytoplankton dynamics in relation to Red tide appearance in Qarun Lake, Egypt. *Egyptian Journal of Aquatic Research, 47*(3), 293–300. https://doi.org/10.1016/j.ejar.2021.07.005

Ismail, G. A. (2017). Biochemical composition of some Egyptian seaweeds with potent nutritive and antioxidant properties. *Food Science and Technology,* 37, 294-302. https://doi.org/10.1590/1678-457X.20316

Kester, D., Duedall, I., Connors, D., & Pytkowicz, R. (1967). Preparation of artificial seawater. *Limnology and Oceanography, 12*, 176–179. https://doi.org/10.4319/lo.1967.12.1.0176

Kolmakova, A. A., & Kolmakov, V. I. (2019). Amino Acid Composition of Green Microalgae and Diatoms, Cyanobacteria, and Zooplankton [Review]. *Inland Water Biology, 12*(4), 452–461. https://doi.org/10.1134/S1995082919040060

Kujawinski, E. B. (2011). The impact of microbial metabolism on marine dissolved organic matter. *Annual Review of Marine Science, 3*, 567–599. https://doi.org/10.1146/annurev-marine-120308-081003 PMID:21329217

Labib, W. (2002). Phyto-zooplankton coupling off Alexandria (Egypt). *Egyptian Journal of Aquatic Biology and Fisheries, 6*(3), 59–84. https://doi.org/10.21608/ejabf.2002.1751

El Shafay, S., Abo Shady, A., Abdel-Ftah, L. M., Ali, S. H., & Labib, W. (2019). Allelopathic effect of the green macroalgae *Ulva fasciata* (Delile) on potentially harmful algal bloom. *The Egyptian Journal of Experimental Biology (Botany), 15*(2), 169–180. https://doi.org/10.5455/egyjebb.20190505044917

Li, P., Mai, K., Trushenski, J., & Wu, G. (2009). New developments in fish amino acid nutrition: Towards functional and environmentally oriented aquafeeds. *Amino Acids, 37*(1), 43–53. https://doi.org/10.1007/s00726-008-0171-1 PMID:18751871

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., & Legendre, L. (2016). Growth responses of *Ulva prolifera* to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports, 6*, 26498. https://doi.org/10.1038/srep26498 PMID:27199215

Liu, Q., Tian, Y., Liu, Y., Yu, M., Hou, Z., He, K. & Jiang, Y. (2020). Relationship between dissolved organic matter and phytoplankton community dynamics in a human-impacted subtropical river. *Journal of Cleaner Production*, 125-144. https://doi.org/10.1016/j.jclepro.2020.125144

Lourenço, S. O., Barbarino, E., Lavín, P. L., Marquez, U. M. L., & Aidar, E. (2004). Distribution of intracellular nitrogen in marine microalgae. Calculation of new nitrogen-to-protein conversion factors. *European Journal of Phycology, 39(1)*, 17–32. https://doi.org/10.1080/0967026032000157156

Malone, T. C., & Newton, A. (2020). The globalization of cultural eutrophication in the coastal ocean: Causes and consequences. *Frontiers in Marine Science, 7*, 670. https://doi.org/10.3389/fmars.2020.00670

Mannino, A., & Harvey, H. R. (2000). Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. *Organic Geochemistry, 31*(12), 1611–1625. https://doi.org/10.1016/S0146-6380(00)00099-1

Marsot, P., Cembella, A. D., & Colombo, J. C. (1991). Intracellular and extracellular amino acid pools of the marine diatom *Phaeodactyllum tricornutum* (bacillariophyceae) grown on unenriched seawater in high-cell-density dialysis culture. *Journal of Phycology, 27*(4), 478–491. https://doi.org/10.1111/j.0022-3646.1991.00478.x

Martin-Jézéquel, V., Poulet, S. A., Harris, R. P., Moal, J., & Samain, J. F. (1988). Interspecific and intraspecific composition and variation of free amino acids in marine phytoplankton. *Marine Ecology Progress Series, 44*, 303–313. https://doi.org/10.3354/meps044303

Meksumpun, S., Montani, S., & Okaichi, T. (1994). Changes in Some Chemical Components of *Alexandrium catenella* and *Scrippsiella trochoidea* during Their Growth Cycles. *Fisheries Science, 60*(2), 207–212. https://doi.org/10.2331/fishsci.60.207

Metaxatos, A., Panagiotopoulos, C. & Ignatiades, L. (2003). Monosaccharide and amino acid composition of mucilage material produced from a mixture of four phytoplanktonic taxa. *Journal of Experimental Marine Biology and Ecology*, 294(2), 203-217. https://doi.org/10.1016/S0022-0981(03)00269-7.

Mikhail, S., Khalil, N., & El-Hadary, M. (2020). A new recorded of red tide forming species, *Heterocapsa triquetra, Gymnodinium impudicum, Heterosigma akashiwo* and *Thalassiosira rotula* in Alexandria Waters, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries, 24*(6), 207–223. https://doi.org/10.21608/ejabf.2020.117258

Mikhail, S., & Labib, W. (2014). Invasion-allelopathy interaction in the eutrophic coastal waters of Alexandria: Case of the invaders raphidophyte *Chattonella antiqua* and the dinoflagellates *Alexandrium ostenfeldii* and *Gymnodinium catenatum*. —International Congress On Estuaries & Coastal Protected Areas, ECPA 2014, 04 - 06 November 2014, Izmir – Turkey.

Miralto, A., Barone, G., Romano, G., Poulet, S. A., Ianora, A., Russo, G. L., Buttino, I., Mazzarella, G., Laabir, M., Cabrini, M., & Giacobbe, M. G. (1999). The insidious effect of diatoms on copepod reproduction. *Nature, 402*, 173–176. https://doi.org/10.1038/46023

Møller, E. F. (2007). Production of dissolved organic carbon by sloppy feeding in the copepods *Acartia tonsa, Centropages typicus*, and *Temora longicornis. Limnology and Oceanography, 52*, 79–84. https://doi.org/10.4319/lo.2007.52.1.0079

Moustafa, Y. T. A., & Eladel, H. M. H. M. (2015). Amino acids profile of *Ulva* sp. macroalgae collected from Alexandria coast in Egypt: A potential food resource *Point Journal of Botany and Microbiology Research,* 1(2): 015-022. https://www.researchgate.net/publication/285235218

Mulholland, M. R., Gobler, Ch. J., & Lee, C. (2002). Peptide hydrolysis, amino acid oxidation, and nitrogen uptake in communities seasonally dominated by *Aureococcusanopha gefferens. Limnology and Oceanography, 47*(4), 1094–1108. https://doi.org/10.4319/lo.2002.47.4.1094

Myklestad, S. (2000). Dissolved organic carbon from phytoplankton, p. 111–148. In: P. Wangersky [ed.]. The handbook of environmental chemistry. In *Marine Chemistry*. V. 5D. Springer

Orellana, M. V., Pang, W. L., Durand, P. M., Whitehead, K. & Baliga, N. S. (2013). A role for programmed cell death in the microbial loop. *Plos One*. 8(5): e62595. https://doi.org/10.1371/journal.pone.0062595

Sakamoto, S., Lim, W. A., Lu, D., Dai, X., Orlova, T., & Iwataki, M. (2021). Harmful algal blooms and associated fisheries damage in East Asia: Current status and trends in China, Japan, Korea and Russia. *Harmful Algae, 102*, 101787. https://doi.org/10.1016/j.hal.2020.101787 PMID:33875176

Sarmento, H., Romera-Castillo, C., Lindh, M., Pinhassi, J., Sala, M. M., Gasol, J., M., Marrase, C., & Taylor, G. T. (2013). Phytoplankton species-specific release of dissolved free amino acids and their selective consumption by bacteria. *Limnology and Oceanography, 58*(3), 1123–1135. https://doi.org/10.4319/lo.2013.58.3.1123

Sellner, K. G., & Nealley, E. W. (1997). Diel fluctuations in dissolved free amino acids and monosaccharides in Chesapeake Bay. *Marine Chemistry, 56*(3-4), 193–200. https://doi.org/10.1016/S0304-4203(96)00069-2

Suksomjit, M., Nagao, S., Ichimi, K., Yamada, T., & Tada, K. (2009). Variation of Dissolved Organic Matter and Fluorescence Characteristics before, during and after Phytoplankton bloom. *Journal of Oceanography, 65*, 835–846. https://doi.org/10.1007/s10872-009-0069-x

Tang, Y. Z., & Gobler, C. J. (2012). The toxic dinoflagellate *Cochlodinium polykrikoides* (Dinophyceae) produces resting cysts. *Harmful Algae, 20*, 71–80. https://doi.org/10.1016/j.hal.2012.08.001

Tas, S., & Yilmaz, I. N. (2015). Potentially harmful microalgae and algal blooms in a eutrophic estuary in

Turkey. *Mediterranean Marine Science, 16*, 432–443. https://doi.org/10.12681/mms.1042

Thornton, D. C. O. (2014). Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *European Journal of Phycology, 49*(1), 20–46. https://doi.org/10.1080/09670262.2013.875596

Tyler, A. C., McGlathery, K. J., & Anderson, I. C. (2003). Benthic algae control sediment-water column fluxes of organic and inorganic nitrogen compounds in a temperate lagoon. *Limnology and Oceanography*, *48*, 2125–2137. https://doi.org/10.4319/lo.2003.48.6.2125

Tyler, A. C., McGlathery, K. J., & Macko, S. A. (2005). Uptake of urea and amino acids by the macroalgae *Ulna lactuca* (Chlorophyta) and *Gracilaria vermiculophylla* (Rhodophyta). *Marine Ecology Progress Series, 294*, 161–172. https://doi.org/10.3354/meps294161

Utermöhl, H. (1958). Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen, 9(1):1-38. https://doi.org/10.1080/05384680.1958.11904091

Vardi, A., Schatz, D., Beeri, K., Motro, U., Sukenik, A., Levine, A., & Kaplan, A. (2002). Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. *Current Biology, 12*(20), 1767–1772. https://doi.org/10.1016/S0960-9822(02)01217-4 PMID:12401172

Veldhuis, M. J. W., Kraay, G. W., & Timmermans, K. R. (2001). Cell death in phytoplankton: Correlation between changes in membrane permeability, photosynthetic activity, pigmentation and growth. *European Journal of Phycology, 36*, 167–177. https://doi.org/10.1080/09670260110001735318

Vidoudez, Ch., & Pohnert, G. (2012). Comparative metabolomics of the diatom *Skeletonema marinoi* in different growth phases. *Metabolomics, 8*, 654–669. https://doi.org/10.1007/s11306-011-0356-6

Whitelegge, T. (1891). On the recent discoloration of the waters of Port Jackson. *Records of the Australian Museum, 1*, 179–192. https://doi.org/10.3853/j.0067-1975.1.1891.1253

Wu, G. (2009). Amino acids: Metabolism, functions, and nutrition. *Amino Acids, 37*(1), 1–17. https://doi.org/10.1007/s00726-009-0269-0 PMID:19301095

Xiao, J., Zhang, X., Gao, C., Jiang, M., Li, R., Wang, Z., Li, Y., Fan, S., & Zhang, X. (2016). Effect of temperature, salinity and irradiance on growth and photosynthesis of *Ulva prolifera*. *Acta Oceanologica Sinica, 35*(10), 114–121. https://doi.org/10.1007/s13131-016-0891-0

Yamamoto, T., Oh, S. J. & Kataoka, Y. (2004). Growth and uptake kinetics for nitrate, ammonium and phosphate by the toxic dinoflagellate *Gymnodinium catenatum* isolated from Hiroshima Bay. *Japan Fisheries Science*, 70 (1): 108-115. https://doi.org/10.1111/j.1444-2906.2003.00778.x

Zingone, A. Tim & Wyatt. (2012). Harmful algal blooms: keys to the understanding of phytoplankton ecology. The Sea. *Harvard University Press, Harvard*. 13: 867-926. https://www.researchgate.net/publication/284429375

Zhang, J., Xiao, T., Huang, D., Liu, S. M., & Fang, J. (2016). Editorial: Eutrophication and hypoxia and theirimpacts on the ecosystem of the Changjiang Estuary and adjacent coastal environment. *Journal of Marine Systems, 154*, 1–4. https://doi.org/10.1016/j.jmarsys.2015.10.007