Microalgal diversity in the Indian River Lagoon system: how little we know

Paul E. Hargraves

Harbor Branch Oceanographic Institute
Florida Atlantic University
Fort Pierce FL 34946
and
Smithsonian Marine Station
701 Seaway Drive
Fort Pierce, FL 34949

Abstract The diversity of microalgae and related protists in the Indian River Lagoon system is exceedingly rich, intimately tied to microbial loop processes, and presumably highly productive. The scant evidence available suggests that primary production can be equivalent to, or exceed, that of seagrasses and seaweeds, yet the alpha diversity of species involved is poorly known. Environmental parameters modifying this diversity, which likely exceeds 2000 species, in a wide variety of known and cryptic classes, are often overlooked in monitoring. Of particular social and economic concern are the 80+ species that do, or potentially can, cause harmful algal blooms.

Key words diversity, diatoms, dinoflagellates, cyanobacteria, harmful algal blooms

Introduction

The IRL System. The Indian River Lagoon system (IRL) is over 250 kilometers long, delimited usually as the Ponce de Leon Inlet (Volusia County) in the north, and the Jupiter Inlet (northern Palm Beach County) in the south, with a surface area of 900+ square kilometers and a basin size of 5,650+ square kilometers. Biogeographically the IRL is transitional between the warm temperate Carolinian Province and the subtropical Floridian Province, but among the microalgae are many ephemeral representatives of both the cooler Virginian Province and the warmer West Indian (Caribbean) Province. Open coastal and oceanic microalgae often find their way into the IRL depending on oceanographic and meteorological conditions, and the numerous intrusions of fresh water introduce their own microalgal diversity with species-specific levels of salinity tolerance.

Microbial Loop. The importance of the so-called Microbial Loop and its processes has only been recognized in recent decades (Azam et al. 1983). These processes and the organisms that perform them are fundamental to life on earth as we know it. Originally, focus was on the recycling of complex organic molecules

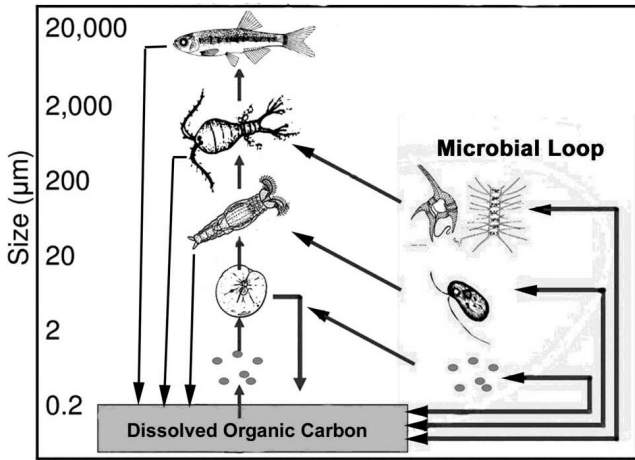Corresponding author: Paul E Hargraves, dr.pharg@gmail.com

Figure 1. Generalized Microbial Loop Structure (modified from Koh et al., 2012)

by bacteria into their constituents, and their reinjection into food webs, but more recently (Fenchel et al. 2008) research has focused on the diversity and function of prokaryotic and eukaryotic components and their contribution to microbial processes. All trophic components of a marine food web contribute organic compounds to the environment. Directly or indirectly these compounds are metabolized by bacteria or microalgae and transformed into particulate organic compounds, and these are introduced, living or dead, into the 'traditional' pathways of the food web. In the water column, metabolic processes are primarily conducted by picoplankton (0.2-2.0 µm), nanoplankton (2-20 µm), and microplankton (20-200 µm) (Sieburth et al. 1978). This is the microbial loop (Figure 1, modified from Koh et al. 2012).

The biotic diversity of organisms in microbial loop processes is high in the Indian River Lagoon system and this diversity allows for the adaptability and success as environmental conditions change on both short and long time frames. Here, I discuss diversity of both obligate and facultative autotrophs (for short, 'microalgae') of the Indian River Lagoon system, including species that actually and potentially can cause harmful algal blooms (HABs). A nearly equivalent term (excepting Cyanobacteria) would be 'protists', but most of the heterotrophic protists (e.g., ciliates, foraminifera, etc.) are excluded from this discussion.

Results and Discussion

Present and Projected Microalgal Diversity. The alpha diversity of the IRL system is poorly known. The more reliable early estimates are based on Mahoney and Gibson (1983), who listed 227 diatoms and 37 dinoflagellates, and Navarro (1982), who listed only diatoms on mangrove prop roots and are weighted strongly toward diatoms and dinoflagellates, ignoring the many other microalgal classes. In recent decades, more geographically restricted, but taxonomically more expansive

Table 1. Microalgal diversity (number of species) reported from the IRL system over time, by Class

Microalgal class	1994	Current	Likely present
"Diatoms"*	232	500+	1000+
Dinophyceae	25	142	250-300
Cyanobacteria	10	18?	30+
Dictyophyceae	2	4?	6-10?
Ebriophyceae	1	2	2
Bicosoecophyceae	0	3?	5?
Bolidophyceae	0	0	?
Chlorarachniophyceae	0	0	?
Chrysophyceae	0	3	?
Cryptophyceae	0	4	10?
Eustigmatophyceae	0	0	?
Mamiellophyceae	0	2?	?
Pedinellophyceae	0	5?	10+?
Pelagophyceae	0	2	?
Pinguiophyceae	0	1	?
Prasinophyceae	0	6	15?
Prymnesiophyceae	0	11	15-20?
Raphidophyceae	0	3	5?
Trebouxiophyceae	0	3	10+?

  * 3 classes

or more complete lists have appeared, e.g., Badylak and Phlips (2004), Hargraves (2002). The Smithsonian Marine Station at Fort Pierce lists 445 species of diatoms and 128 species of dinoflagellates in the IRL Species Inventory in 2011 but the inventory is incomplete both floristically and nomenclaturally (https://naturalhistory2.si.edu/smsfp/irlspec/Protists.htm, accessed 20 August 2020).

   Under presently accepted taxonomic categories, there are 21 classes of microalgae, assuming that the Cyanobacteria are, for present purposes, considered a class. The term microalgae has a fuzzy definition, but approximately includes the cell size ranges of picoalgae, nanoalgae and microalgae (Sieburth et al. 1978). Visible filaments and chains of cells are common among some species. The current roster of species among the classes is shown in Table 1. Under the accepted taxonomic scheme the diatoms are apportioned in three classes: Mediophyceae, Coscinodiscophyceae, and Bacillariophyceae, and summarized here simply as 'diatoms'. At present diatoms and dinoflagellates (Dinophyceae) are the best known and most studied of the microalgae in the IRL system, excepting some of the harmful algal bloom (HAB) species. Nevertheless the total numbers of species of these two groups are likely underestimated. Diatoms alone would total well over one thousand species and the dinoflagellate species would nearly double those presently reported.

   The Cyanobacteria are a phylum rather than a class. These are gram-negative photosynthetic prokaryotes. They are widely acknowledged for their prolific production of secondary metabolites, some of which are toxic to humans and marine life (Percival and Williams 2014). In general they are divided into 5 classes, and may be unicellular, filamentous, or pseudothalloid. The IRL system has at least
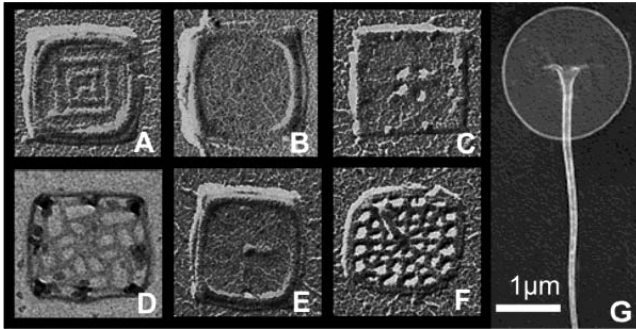
Figure 2. Body scales of unknown nano- and microflagellates, sample collected January 2007, IRL at Vero Beach. Body scales in A-F are all circa 200 nanometers per side. Body scale G, as indicated.(A-F, TEM C-replica; G, TEM.)

18 species that may form benthic or floating mats (Littler et al. 2008). The naming of Cyanobacteria species based on morphology is inaccurate without molecular and biochemical confirmation: the recent expansion of identified *Lyngbya* species in the IRL being one example (Engene et al. 2018). The filamentous *Spirulina subsalsa* is found in the IRL: it is not the common edible health food marketed as *Spirulina*, (which is in fact *Arthrospira platensis*, Szubert et al. 2018). Globally the dominant unicellular forms of Cyanobacteria are attributed to *Synechococcus* and *Prochlorococcus*. The former is common in the IRL, the latter is oceanic and not presently confirmed, though other unnamed picocyanobacteria appear to be present (Phlips et al. 2020). In addition, several species of fresh water Cyanobacteria, including the toxic species *Microcystis aeruginosa*, can be introduced to the IRL via discharges of freshwater ecosystems such as Lake Okeechobee (Phlips et al. 2012, Schaefer et al. 2020). The taxonomy of Cyanobacteria is perpetually in flux, but the few that have been recognized locally (as HABs, water discolorations, or benthic mats) have a substantial ecological impact, in contrast to more obscure classes.

In 1994 a summary of microalgal diversity in the IRL consisted basically of diatoms, dinoflagellates, and cyanobacteria (Woodward-Clyde Consultants 1994), with all other groups unknown or ignored. Twenty-five years later the number of species has grown to about 700, distributed among 19 classes (Table 1). Each water sample taken, when closely examined, has the potential to expand the diversity of the microflora. As an example, a single sample taken from the Indian River near Vero Beach several years ago (Figure 2), examined with electron microscopy, included the box scales of six unidentified species of Prasinophyceae (probably *Pyramimonas*: many Prasinophyceae have five layers of organic scales: two on the flagella and three on the cell surface) and, later, one siliceous scale of Chrysophyceae, probably *Paraphysomonas*. Many of these nano- and pico-sized species are undescribed and unrecognized. The examination of any IRL sample, whether by classical morphological methods or genetic sequencing, will invariably turn up previously unrecognized species as new records or novelties. New species

often appear in the IRL system simply upon close inspection, some toxic (*Alexandrium monilatum*, Norris 1983; originally as *Gonyaulax*); others abundant but innocuous (*Thalassiosira livingstoniorum*, Prasad et al. 2011); still others innocuous and rare (*Haslea clevei*, Sterrenburg et al. 2015). Cryptic species, those that can be differentiated genetically but perhaps not morphologically, prove to be common among microalgae, especially in widespread and species-rich genera such as *Chaetoceros* (DeLuca et al. 2019), *Skeletonema* (Smayda 2011) and the sometimes toxic *Pseudo-nitzschia* (Lundholm et al. 2012). Considering the expansion of the flora upon increasing observation and the likelihood of unrecognized, novel, and cryptic species, it is probable that the totality of microalgal diversity reaches or exceeds 2,000 microalgal species.

   Although currently recognized microalgal species in the IRL system total about 700 taxa, it is almost certain that the actual number of species is more than double (and probably triple) that number. The reasons for this estimated high number are several. First, upon examining a water sample from almost anywhere in the IRL, it is almost a given that microalgae will be seen that are unfamiliar to even the most experienced analyst – that is, a new record or a novel species. This suggests a cryptoflora that needs closer attention. Secondly, when an experienced analyst looks closely at a particular group of interest, the number of verified species will increase. This has been the case with such common species such as the diatom species *Skeletonema costatum* (Sarno et al. 2005) or the diatom genus *Haslea* (Sterrenburg et al. 2015). Third, the advent of molecular genetic analysis has frequently uncovered the presence of cryptic species not readily identified by conventional microscopy (among many examples, deVargas et al. 2015, Egge et al. 2015). Fourth, the rapid pace of global change (both environmental and biological) presents opportunities for invasive modifications to diversity via range extensions. As our knowledge increases, the number of described species increases but the number of purported cosmopolitan species decreases (Sarno et al. 2005).

   While the number of species in the well-known and comparatively familiar classes may be estimated, in Table 1 there are eight classes of substantially obscure diversity whose presence, distribution, and/or familiarity in the IRL system (and in the world ocean) are very poorly known. These classes include the following:

Bicosoecophyceae. These are heterotrophic, nanoplanktonic biflagellates currently of low known diversity but with a wide global distribution (Schoenle et al. 2020). Formerly they were included in the class Chrysophyceae, but are phylogenetically related (distantly) to the diatoms. They are widespread in the IRL, and frequently appear as contaminants in isolated microalgal cultures. At least one species belongs to the oddly named genus *Cafeteria* but whether there are others is unknown. They are voracious bacterivores (Boenigk and Arndt 2005) and since they are subject to fatal viral infections (Massana et al. 2007), their population fluxes are reputed to have an effect on microbial loop processes, including carbon recycling and nutrient dynamics.

Bolidophyceae. These are unicellular eukaryotes that contain species with cells surrounded by 5 or 8 silica plates (Parmales) or as naked flagellated species (formerly Bolidomonadales). They share a common ancestor with diatoms, one of

the most successful groups of phytoplankton (Kuwata et al. 2018). They are primarily found in oceanic and open coastal waters; theoretically they could be found in the IRL system via the five inlets connecting the IRL to the ocean, but have not yet been identified. Despite their rather small contribution to marine phytoplankton communities (on average less than 0.1%), Bolidophyceae are very widespread throughout marine systems from the tropics to the polar oceans. The number of species worldwide is unknown but probably less than 20.

Chrysophyceae. There is an evolving census as to which organisms should be included in this class. Species in the Bicosoecophyceae, Dictyophyceae, and Pedinellophyceae were all formerly included in Chrysophyceae. At present, only two genera are found in the IRL: *Paraphysomonas* and an estuarine species of the loricate colonial form *Dinobryon*. It is likely there are other members of this class to be found.

Chlorarachniophyceae. These are microscopic mixotrophic algae that have alternate ameboid and uniflagellate stages. They are found mostly in temperate and tropical coastal waters. They ingest bacteria and small protists, and photosynthesize with chlorophyte plastids originating from endosymbionts via secondary endo-symbiosis (Hirakawa et al. 2011, Ishida et al. 2007). Ten genera have been described; none have yet been identified in the IRL system.

Eustigmatophyceae. There are about 100 species in the Eustigmatophyceae, most of which are found in freshwater or soil. They are all unicellular and coccoid, about 3 - 5μm in size. Species distinctions usually require phylogenetic analysis (Kryvenda et al. 2018). At least some of the species, mostly non-marine, may have a motile stage via a uniflagellar zoospore. The genera *Nannochloropsis* and *Microchloropsis* (together, about 10 species) are the only known marine representatives – both are globally distributed in coastal waters and estuaries and are known from the US East coast (RI, CT, NY, NC) but have not been confirmed from the IRL system. Both genera are easy to cultivate and are frequently used as food in aquaculture facilities and as sources for biofuels and fatty acids production (Fawley et al. 2014)

Mamiellophyceae. Members of this class are currently divided into three orders with a total of about 30 species. Based on pigments they were formerly included in the Prasinophyceae; genetically they were separated by Marin and Melkonian (2010). Many of the marine genera are globally distributed in coastal waters (Tragin and Vaulot 2019). The genus uniflagellate *Micromonas* is very common in the IRL, sometimes blooming in restricted coves with slow water exchange. Another genus, the coccoid nonmotile *Ostreococcus*, is the smallest known eukaryote (0.8 μm), and is probably present but unconfirmed. Likely other species in this class are present but unrecognized.

Pinguiophyceae. This is a very small class of picoplankton-sized algae characterized by their ultrastructure, and differentiated genetically from other stramenopiles (Kawachi et al. 2002). There are less than 10 known genera and about as many species. Most are coccoid, but motile stages are known in some species. Their distribution and ecological dynamics are mostly cryptic; marine forms are mostly confined to warm water in both oceanic and coastal areas. They

may have significant economic importance as prolific producers of fatty acids. At least one species in present in the IRL system: *Pinguiococcus pyrenoidosus* was isolated from the Harbor Branch boat basin, now kept as several strains (CCMP1144, 2078, and 2188) at NCMA, Bigelow Lab, Maine.

Trebouxiophyceae. There are many taxa in this group, the majority of which are small filaments or endosymbionts in lichens, or unicells in a wide variety of terrestrial, freshwater, and marine habitats. They are green in color (excepting non-photosynthetic parasitic species) and include many diverse genera whose interrelatedness is suspect (Proschöld and Leliart 2007). Currently the common and widespread form-genus *Chlorella* is included in this class, as is the widely distributed *Nannochloris*, both of which occasionally appear in algal lists from Florida without taxonomic verification. Localized blooms of nonmotile 2 - 5µm spherical green cells are likely to be placed in one of these two genera. One species, *Stichococcus bacillaris*, has also been identified, and has been reported from the Florida coast, but the name *Stichococcus* has been indiscriminately applied to small elongated cells of few distinguishing features and questionable affinity, so the correct name for these cells, with highly diverse genetic lineages, and the name of *Stichococcus* itself, is taxonomically questionable (Proschöld and Darienko 2020). At least some records of *Stichococcus* are probably referable to *Picochlorum*. Also uncertain is the extent to which any of the tiny green cells should be in the poorly defined class Chlorophyeae.

Modifying Parameters to Microalgal Diversity. The major environmental parameters affecting microalgal diversity are well known and are among the ones measured with more or less greater facility than others, including temperature; salinity; light (intensity, wavelength & duration); major nutrients (N, P, Si); grazing; and competition (Reynolds 2006). Other parameters potentially can exert major influence on diversity but are rarely comprehensively examined, except in more restricted formats. These are some examples.

1. Enhancing or inhibitory trace metals: Co, Se, and Fe are critical for growth in many microalgae; the IRL is probably sufficient in most micronutrients. But Zn, Hg, Cd, Pb, etc., from terrestrial, atmospheric, and aquatic anthropogenic sources could be inhibitory in very small amounts.
2. Biochemical enhancers: vitamins and other organic growth substances produced by bacteria and other biota are necessary or advantageous to most microalgae, including mixotrophic species.
3. Biogenic antagonists: exopolymers acting as allelopathic chemicals are produced by many microalgal species and their competitors. Biological warfare among the microalgae appears to be a common but poorly understood survival strategy (Gutierrez et al. 2018).
4. Anthropogenic enhancers and antagonists: a strong influence, particularly in the IRL system with its reduced exchange rate. Pesticides, medicines, road/agricultural runoff, industrial byproducts (anything that might be called 'pollutants') have been detected in the IRL system. Conversely, some chemicals

Table 2. Actual or potential HAB microalgal species in the IRL system.

Taxonomic group	Harmful to mammals	Harmful to marine life	Decay/deoxygenation
Diatoms	8	8	3
Dinophyceae	22	14	4
Cyanobacteria	3	2	1
Haptophyceae/other	1	8	1

    may serve as enhancers to growth of microalgal species – e.g., synthetic plant hormones.

5. The IRL system is home to over 80 microalgal species that are known to be inhibitory or harmful somewhere on a global basis (Table 2; grouped according to their primary cause of 'harm'). Toxic and nontoxic exudates alone can modify short-term diversity and have unknown impact on long-term diversity. For example, the dinoflagellate *Margalefodinium polykrikoides* produces a variety of toxins (neurotoxins, haemotoxins) that kill fish, corals, bivalves and zooplankton. The toxins consistently exclude or repel predators (fish larvae), competitors (other microalgae) and pathogens (bacteria, fungi), and cause $100s of millions of losses in aquaculture facilities worldwide (Lopez-Cortez et al. 2020)

6. Life cycle phenomena: cysts and spores and other inactive cells introduced via inlets, canals, and streams may find suitable growth conditions in the myriad of microenvironments of the IRL.

7. Pathogens and parasites: these may eliminate or reduce resident species, opening a niche for otherwise excluded competitors.

8. Unnoticed and/or unknown interactions among any or many of the above.

    In many cases the causes of introduction or evanescence of a particular microalga are unknowable and complex, though there are some starkly apparent examples where the cause of a species appearance is apparent: the occasional introduction of the toxic *Karenia brevis* for example into the IRL is attributed with confidence to tidal and current hydrodynamics from the Gulf of Mexico.

Changes to diversity over time. Microalgal diversity is a constantly and fluctuating feature. In multidecadal time, the consequences of anthropogenic climate change and eutrophication will modify species composition in terms of thermal, nutrient, and sea level changes, with their varied subsidiary effects, both internal and external to the lagoon system, will probably have the most impact. The niche requirements of each species will modify the diversity pattern in both uncertain and unpredictable ways.

    On a shorter and more localized time scale, one must consider the potential for microalgal biodiversity changes in the context of demographic projections of human population. Florida's population is predicted to grow from 21.5 million in 2020 to 26.4 million in 2040 (EDR 2020). There can be little doubt that a 20% increase in human population will have a major impact on microalgal diversity in the IRL system. The IRL system includes five fast-growing counties (from North to South, Volusia, Brevard, Indian River, St. Lucie, and Martin). One might argue that

the overwhelming and frequently unforeseen addition of anthropogenic physical, chemical and biological materials will diminish or eliminate stenotolerant species to the advantage of eurytolerant species: i.e. a loss of sensitive species in competition with generalists. Microalgal diversity may also go down as the increase intensity and frequency of HAB blooms increase. Conversely, the possible opening of new niches resulting from climate change, and the consequent appearance of invasive opportunistic species might increase the overall diversity. In addition, more intensive research into the breadth of species across different classes would surely reveal new records and novel species, increasing diversity. Advances in quantitative genetics of species and populations are a rapidly expanding research area (Laruson et al. 2020, Listmann et al. 2020) that should reveal cryptic species and recognize genotypic redundancy. So there are potential pathways for an increase in biodiversity. It is also possible that perceived diversity remains fairly constant. This could come about in two ways: first, species are lost or gained independently among the many classes of microalgae with the total in relative stasis; and secondly, research on microalgal diversity stagnates, expanding only when harmful blooms intrude on the human population. The challenges have recently been discussed for HAB microalgae in particular (Wells et al. 2020). New research techniques make it possible to determine how past biodiversity has changed over the centuries, using spore proxies phenotypically and genotypically (Dzembekova et al. 2020). The shallowness of the IRL might make accurate sampling problematic, but if the past is prologue, both prior and future anthropogenic impacts on diversity might be intuited. In the final analysis, changes in microalgal diversity, and in particular the distribution and intensity of HAB events, requires continued monitoring of the organisms and their environment (Tester et al. 2020)

References

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. 1983. The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10:257–263.

Badylak S, Phlips EJ. 2004. Spatial and temporal patterns of phytoplankton composition in a subtropical lagoon, the Indian River Lagoon, Florida, USA. Journal of Plankton Research 26:1229–1247.

Boenigk J, Arndt H. 2005. Particle handling during interception feeding by four species of heterotrophic nanoflagellates. Journal of Eukaryotic Microbiology 47:350–358.

De Luca D, Kooistra WHCF, Sarno D, Gaonkar CC, Piredda R. 2019. Global distribution and diversity of *Chaetoceros* (Bacillariophyta, Mediophyceae): integration of classical and novel strategies. PeerJ 7:e7410. doi:10.7717/peerj.7410.

deVargas C, Audic S, Henry N, 45 others. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348 doi:10.1126/science.1261605.

Dolby GA, Bedolla AM, Bennett SEK, Jacobs DK. 2020. Global physical controls on estuarine habitat distribution during sea level change: Consequences for genetic diversification through time. Global and Planetary Change 187:1–18. doi.org/10.1016/j.gloplacha.2020.103128.

Dzembekova N, Rubino F, Nagai S, Zlateva I, Slabakova N, Ivanova P, Slabakova V, Moncheva S. 2020. Comparative analysis of morphological and molecular approaches integrated into the study of the dinoflagellate biodiversity within the recently deposited Black Sea sediments – benefits and drawbacks. Biodiversity Data Journal 8:e5172. doi:10.3897/BDJ8.e55172.

EDR 2020. Demographic Overview & Population Trends. Florida Office of Economic and Demographic Research. http://edr.state.fl.us

Egge S, Eikrem W, Edvardsen B. 2015. Deep-branching novel lineages and high diversity of haptophytes in the Skagerrak (Noray) uncovered by 454 pyrosequencing. Journal of Eukaryotic Microbiology 62:121–140.

Engene N, Tronholm A, Paul VJ. 2018. Uncovering cryptic diversity of *Lyngbya*: the new tropical marine cyanobacterial genus *Dapis* (Oscillatoriales). Journal of Phycology 54:435–446. doi: 10.1111/jpy.12752.

Fawley KP, Elias M, Fawley MW. 2014. The diversity and phylogeny of the commercially important algal class Eustigmatophyceae, including the new clade *Goniochloridales*. Journal of Applied Phycology 26:1773–1782. doi.org/10.1007/s10811-013-0216.

Fenchel T. 2008. The microbial loop – 25 years later. Journal of Experimental Marine Biology and Ecology 366:99–103.

Gutierrez T, Teske A, Ziervogel K, Passow U, Quigg A. 2018. Editorial: Microbial exopolymers: sources, chemico-physiological properties, and ecosystem effects in the marine environment. Frontiers in Microbiology 9:1–3. doi:10.3389/fmicb.2018.01822.

Hargraves PE. 2002. Diatoms of the Indian River Lagoon, Florida: an annotated account. Florida Scientist 65:225–244.

Hirakawa Y, Howe A, James ER, Keeling PJ. 2011. Morphological diversity between culture strains of a chlorarachniophyte, *Lotharella globosa*. PLOS One doi:10.1371/journal.pone0023193.6:e23193.

Ishida K, Ybuki A, Ota S. 2007. The chlorarachniophytes: evolution and classification. Pp. 171–182 *in* Brodie J, Lewis J, eds. Unraveling the Algae: the past, present, and future of algal systematics. CRC Press, Boca Raton Florida.

Kawachi M, Inouye I, Honda D, O'Kelly CJ, Bailey JC, Bidigare RR, Andersen RA. 2002. The Pinguiophyceae *classis nova*, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. Phycological Research 50:31– 47.

Koh ER, Martin AR, McMinn A, Ryan KG. 2012. Recent advances and future perspectives in microbial phtototrophy in Antarctic sea ice. Biology 1:542–556.

Kryvenda A, Rybalka N, Wolf M, Friedl T. 2018. Species distinctions among closely related strains of Eustigmatophyceae (Stramenopiles) emphasizing ITS2 sequence-structure data: *Eustigmatos* and *Vischeria*. European Journal of Phycology 53:471–491. doi:10.1080/09670262.2018.1475015.

Kuwata A, Yamada K, Ichinomiya M, Yoshikawa S, Tragin M, Vaulot D, Lopes dos Santos A. 2018. Bolidophyceae, a sister picoplanktonic group of diatoms – a review. Frontiers in Marine Science. 5:370. doi:10.3389/fmars.2018.00370.

Landsberg JH, Hendrickson J, Tabuchi M, Kiryu Y, Williams BJ. 2020. A large-scale sustained fish kill in the St. Johns River, Florida: A complex consequence of cyanobacteria blooms. Harmful Algae 92:101771. doi.org/10.1016/j.hal.2019.101771.

Laruson AJ, Yeaman S, Lotterhos KE. 2020. The importance of genetic redundancy in evolution. Trends in Ecology and Evolution 35:809–822. doi.org/10.1016/j.tree.2020.04.009.

Listmann L, Hattich GSI, Matthiessen B, Reusch TBH. 2020. Eco-evolutionary interaction in competing phytoplankton: nutrient driven genotype sorting likely explains dominance shift and species responses to CO2. Frontiers in Marine Science 7:1–13. doi.org:/103389/fmars.2020.00634.

Littler DS, Littler MM, MD Hanisak. 2008. Submerged Plants of the Indian River Lagoon. Offshore Graphics Inc., Washington DC.

Lopez-Cortez DJ, Nunez-Vasquez EJ, Dorantes-Aranda JJ, Band-Schmidt CJ, Hernandez-Sandoval FE, Bustillos-Guzman JJ, Leyva-Valencia I, Fernandez-Herrera LJ. 2019. The state of knowledge of harmful blooms of *Margalefidinium polykrikoides* (a.k.a. *Cochlodinium polykrikoides*) in Latin America. Frontiers in Marine Science 20. doi.org/10.3389/fmars.2019.00463.

Lundholm N, Bates SS, Baugh KA, Bill BD, Connell LB. 2012. Cryptic and pseudo-cryptic diversity in diatoms with descriptions of *Pseudo-nitzschia hasleana* sp. nov. and *P. fryxelliana* sp. nov. Journal of Phycology 48:436–454.

Mahoney RK, Gibson RA. 1983. A checklist of the phytoplankton of the Indian River, near Vero Beach, Florida. Florida Scientist 46:212–232.

Marin B, Melkonian M. 2010. Molecular phylogeny and classification of the Mamiellophyceae class. nov. (Chlorophyta) based on sequence comparisons of the nuclear-and plastid-encoded rRNA operons. Protist 161:304–336.

Massana R, Del Campo J, Dinter C, Sommaruga R. 2007. Crash of a population of the marine heterotrophic flagellate *Cafeteria roenbergensis* by viral infection. Environmental Microbiology 9:2660–2669.

Navarro JN. 1982. Marine diatoms associated with mangrove prop roots in the Indian River, Florida. Bibliotheca Phycologica 61:1–151.

Norris DR. 1983. The occurrence of a toxic dinoflagellate in the Indian River system. Florida Scientist 46:150–153.

Percival SL, Williams DW. 2014. Cyanobacteria. Pp. 79–88 *in* Percival SL, Yates MV, Gray NF, eds., Microbiology of Waterborne Diseases, 2d edition. Academic Press, New York.

Phlips, E. J., S. Badylak, J. Hart, D. Haunert, J. Lockwood, H. Manley, K. O'Donnell, D. Sun, P. Viveros and M. Yilmaz. 2012. Climatic influences on autochthonous and allochthonous phytoplankton blooms in a subtropical estuary, St. Lucie Estuary, Florida, USA. Estuaries and Coasts 35:335–352.

Phlips EJ, Badylak S, Nelson NG, Havens KE. 2020. Hurricanes, El Niño and harmful algal blooms in two sub-tropical Florida estuaries: direct and indirect impacts. Scientific Reports 10:1910. doi. org/10.1038/s41598-020-58771-4.

Prasad AKSK, Nienow JA, Hargraves PE. 2011. Plicate species of the diatom species *Thalassiosira* (Bacillariophyta) from the Atlantic and Gulf coasts of southeastern United States, with the description of *T. livingstoniorum* sp. nov. Proceedings of the Academy of Natural Sciences of Philadelphia 161:1–34.

Pröschold T, Leliaert F. 2007. Systematics of the green algae: conflict of classic and modern approaches. Pp.123–153 *in*: Brodie J, Lewis J, eds. Unraveling the Algae: the past, present, and future of algal systematics. CRC Press, Boca Raton Florida.

Pröschold, T. & Darienko, T. 2020. The green puzzle *Stichococcus* (Trebouxiophyceae, Chlorophyta): New generic and species concept among this widely distributed genus. Phytotaxa 441:113–142.

Reynolds, C. 2006. The Ecology of Phytoplankton. Cambridge University press, Cambridge. doi.org/10. 1017/CBO97805542145.

Rosen BH, Loftin KA, Graham JL, Stahlhut KN, Riley JM, Johnston BD, Senegal S. 2018. Understanding the effect of salinity tolerance on cyanobacteria associated with a harmful algal bloom in Lake Okeechobee, Florida. USGS Scientific Investigations Report 2018-5092. 32 pp. doi.org/10.3133/sir20185092.

Russill C. 2015. Climate change tipping points: origins, precursors, and debates. WIREs Climate Change 6:427–434. doi: 10.1002/wcc.344.

Sarno D, Kooistra W, Medlin LK, Percopo I, Zingone A. 2005. Diversity in the genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S. costatum*-like species with the description of four new species. Journal of Phycology 41:151–176.

Schaefer AM, Yrastorza L, Stockley N, Harvey K, Harris N, Grady R, Sullivan J, McFarland M, Reif JS. 2020. Exposure to microcystin among coastal residents during a cyanobacterial bloom in Florida. Harmful Algae 92:101769. doi.org/10.1016/j.hal.2020.101769.

Schoenle, A, Hohlfeld, F, Rosse, M, Filz P, Wylezich C, Nitsche F, Arndt H. 2020. Global comparison of bicosoecid *Cafeteria*-like flagellates from the deep ocean and surface waters, with reorganization of the family Cafeteriaceae. European Journal of Protistology 73:1–21.

Sieburth JM, Smetacek V, Lenz J. 1978. Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology & Oceanography 23:1256–1263.

Smayda TJ. 2011. Cryptic plankton diatom challenges phytoplankton ecologists. Proceedings of the National Academy of Sciences 108:4269–4270.

Sterrenburg F, Tiffany MA, Hinz F, Herwig WE, Hargraves PE. 2015. Seven new species expand the morphological spectrum of *Haslea*. A comparison with *Gyrosigma* and *Pleurosigma* (Bacillariophyta). Phytotaxa 207:143–162. doi.org/10.11646/phytotaxa.207.2.1.

Szubert K, Wiglusz M, Mazur-Marzec H. 2018. Bioactive metabolites produced by *Spirulina subsalsa* from the Baltic Sea. Oceanologia 60:245–255. doi.org/10.1016/j.oceano.2017.11.03.

Tester PA, Litaker RW, Berdalet E. 2020. Climate change and harmful benthic microalgae. Harmful Algae 91:1016555. doi.org/10.1016/j.hal.2019.101655.

Tragin, M., Vaulot, D. 2019. Novel diversity within marine Mamiellophyceae (Chlorophyta) unveiled by metabarcoding. Scientific Reports 9:1–14. doi 10.1038/s41598-019-41680-6.

Wells ML, Karlson B, Wulff A, Kudela R, Trick C, Asnaghi V, Berdalet E, 12 others. 2020. Future HAB science: Directions and challenges in a changing climate. Harmful Algae 91:101632. doi.org/10. 1016/j.hal.2019.101632.

Woodward-Clyde Consultants. 1994. Biological Resources of the Indian River Lagoon. IRL-NEP, Vol.1. Project 92F274C. Melbourne, FL.