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Haematococcus: a successful air-dispersed colonist in ephemeral waters is rarely found in phytoplankton communities

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Abstract: In a literature search, the presence of *Haematococcus* in phytoplankton communities and its biogeography were investigated. *Haematococcus*, although showing a wide biogeographical distribution, has been rarely found in phytoplankton communities. Simultaneously, the colonization potential of air-dispersed *Haematococcus* in ephemeral waters and its interactions with coexisting phytoplankton taxa were examined by microscopy and molecular methods. *Haematococcus* was a successful colonist, appearing among the first taxa in the experimental containers. According to principal component analysis, *Haematococcus* growth rate was negatively correlated with the abundance and species richness of the other autotrophs. Furthermore, a negative correlation between *Haematococcus* and *Chlamydomonas* and a positive one between *Haematococcus* and *Chlorella* were found. Overall, *Haematococcus* appears to be an effective air-dispersed alga that can successfully colonize and establish populations in small ephemeral water bodies. However, its absence from phytoplankton in larger permanent water bodies could be related to its high light requirements, its competitive disadvantages against other algae, and the grazing pressures from predators. The results of our study suggest a life strategy based on adaptation to higher light intensities in very shallow waters compared with optical dense lakes. Therefore, ephemeral waters are the regular habitat for *Haematococcus* instead of being "stepping stones" for the colonization of lake phytoplankton.

Key words: *Haematococcus,* airborne, colonists, biodiversity, biogeography

1. Introduction

Haematococcus has received considerable attention as a microalga with high commercial potential. The genus comprises several freshwater species according to morphological classification (Peebles, 1909). The most recent taxonomic analysis places them in the order Volvocalles (Massjuk et al., 2011), class Chlorophyceae. A recent phylogenetic analysis by Buchheim et al. (2013) suggested that *Haematococcus* might not be a monophyletic genus and proposed that *H. pluvialis* is the only species within the genus. During its life cycle, *Haematococcus* changes rapidly from nonmotile resting cells to flagellated cells and vice-versa (Borowitzka et al., 1991). The resting cells quickly appear when environmental conditions are unfavorable for normal cell growth, including high UV irradiance and low nutrient concentrations (Boussiba and Vonshak, 1991).

The resting cells of *Haematococcus* can accumulate considerable amounts of ketocarotenoid astaxanthin (maximum 2% of the dry weight; Pignolet et al., 2013), possibly as a form of protection against high light and oxygen radicals (Kobayashi et al., 1992). This strong antioxidant has many commercial applications in human health, nutrition, aquaculture, and cosmetics (Guerin et al., 2003). The financial and medical significance of astaxanthin has resulted in high interest in this natural producer of astaxanthin, with numerous studies investigating the potential (Lorenz and Cysewski, 2000), financial advantages (Li et al., 2011), and theoretical background (Saei et al., 2012) of natural astaxanthin production in *Haematococcus* cultures. This ample knowledge contrasts with the limited published information about the presence and abundance of *Haematococcus* in natural systems, and specifically in phytoplankton, and its ecology and biogeography.

Among the few publications available, it has been suggested that *Haematococcus* can be commonly found

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in small freshwater bodies such as ponds, birdbaths, and temporal water collections (Proctor, 1957). Furthermore, investigations of airborne algae in urban environments have identified it as a successful air-disperser by means of the resting cells (Genitsaris et al., 2011a). The occurrence of *Haematococcus* in the air and its widespread presence in small temporary rather than large permanent water bodies (phytoplankton's habitat) can be attributed to its ability to withstand expeditious and extreme fluctuations in light, temperature, dryness, and nutrient concentrations (Proctor, 1957). In addition, phytoplankton lives in an optical dense medium, where only a fraction of the light penetrating the water can be utilized for photosynthesis (Tilzer, 1983). Moreover, there is evidence that *Haematococcus* growth is inhibited and it has competitive disadvantages when cooccurring with common, cosmopolitan phytoplankton genera such as *Scenedesmus* and *Chlamydomonas* (Proctor, 1957).

In the present study, initially a literature search was performed to investigate the presence of *Haematococcus* in natural phytoplankton communities and its biogeography occurrence. Next, all *Haematococcus*-related sequences deposited in GenBank were retrieved in order to explore the biodiversity of its phylotypes from culture and environmental samples. Following a previous study on microalgae air-dispersion and colonization in experimental water containers in an urban area (Genitsaris et al., 2011a), we repeated the experimental set-up in order to examine the colonization potential of air-dispersed *Haematococcus* in ephemeral waters throughout the entire seasonal cycles and its interactions with coexisting phytoplankton taxa. In particular, the following hypotheses were examined: 1) *Haematococcus* is among the first colonizers of ephemeral waters through air dispersal; 2) *Haematococcus* is negatively influenced by other established species; and 3) ephemeral waters are the regular habitat for *Haematococcus* instead of being "stepping stones" for the colonization of optically dense lakes.

2. Materials and methods

2.1. Literature search

In parallel with the experiment (see below), a literature search was performed in order to retrieve all scientific references mentioning *Haematococcus* in samples from natural systems and phytoplankton until October 2014. Relevant studies, including available anecdotal reports, were identified by searching the Web of Science (http://apps.isiknowledge.com), Scopus (www.scopus.com), and Google.Scholar (http://scholar.google.com) electronic databases with the MeSH and textword string "(Haematococcus) OR (Haematococcus AND phytoplankton) OR (Haematococcus AND ecology) OR (Haematococcus AND ecosystem) OR (Haematococcus AND natur*) OR (Haematococcus AND astaxanthin).

2.2. Study area

The sampling site for collecting air-dispersed algae was located in the city of Thessaloniki, on the northernmost coast of the Aegean Sea, Greece. The experimental set up was arranged on the rooftop of a tall building (~ 50 m) at the center of the city in 2013, in an open area surrounded by local urban vegetation. Although regular measurements of airborne particulate matter (Samara et al., 2014), allergenic pollen (Damialis et al., 2007), and fungal spores (Gioulekas et al., 2004) have been published, only sporadic reports on air-dispersed algae in the area are available (Genitsaris et al., 2011a, 2011b; Genitsaris et al., 2014), which showed that *Haematococcus* was among the most common algae detected, being identified with both microscopy and molecular analysis.

2.3. Experimental set-up

Passively dispersed airborne microorganisms were collected in tap water containers filled with 10 L of tap water (length: ca. 50 cm, width: ca. 30 cm) during the summer of 2013 (from 20 June to 1 August) and autumn/winter period of 2013 (from 12 November to 23 December), as in Genitsaris et al. (2011a). Specifically, a set of duplicate tap water containers along with a control container covered with transparent plastic sheet were placed in the sampling site for 6 weeks. This experiment served as a repetition of a previous one (Genitsaris et al., 2011a), in which the same experimental procedure in the same sampling site was implemented during three periods (autumn 2007, winter 2007/2008, and spring 2008). Meteorological data (air temperature, relative humidity (RH), wind speed, total precipitation, and total sunshine/total solar radiation) for the study periods were provided by the Department of Geology, Aristotle University of Thessaloniki (Table 1).

2.4. Laboratory analysis

Laboratory and microscopic analyses were performed as in Genitsaris et al., (2011a) with focus on *Haematococcus* individuals and cooccurring phytoplankton taxa. Briefly, fresh and Lugol-preserved water subsamples were taken weekly from the water containers and examined in sedimentation chambers using an inverted microscope (Nikon Eclipse TE2000-S). Air-dispersed plankton was identified using standard taxonomic keys, while microscopic counts were made according to the inverted microscope method (Utermöhl, 1958). Average abundance values between the duplicates were calculated for each week.

Samples for the molecular identification of air-dispersed microeukaryotes, based on sequencing of the 18S rRNA gene, were collected from the duplicates in the final (6th) week of each experimental period and integrated into one sample. Approximately 100 mL of water were filtered through membrane filters of 0.2-μm pore size (Whatman). DNA was extracted using

Table 1. Meteorological data (air temperature, relative humidity (RH), wind speed, total precipitation, and total sunshine/total solar radiation) in the city of Thessaloniki during the 5 study periods.

Period	Average air temperature (°C)	Average RH (%)	Average wind speed (m/s)	Total precipitation (mm)	Total sunshine(min)*/ solar radiation (w/m^2)
Oct–Dec 2007	12.36	72.54	2.01	93.90	9980.96*
Jan–Feb 2008	8.38	68.82	1.68	32.10	12,870.40*
Mar–Apr 2008	13.91	63.52	1.94	79.95	9733.57*
Jun–Aug 2013	26.92	53.83	4.43	1.80	422,411.84
Nov–Dec 2013	10.99	75.60	3.12	1.40	56,067.24

*Total sunshine (min) values correspond to the periods Oct–Dec 2007, Jan–Feb 2008, and Mar–Apr 2008. Total solar radiation values (w/m^2) correspond to Jun–Aug 2013 and Nov–Dec 2013.

the PowerSoil DNA isolation kit (Mo Bio Laboratories) according to the manufacturer's protocol after slicing the filters under sterile conditions. Tag-pyrosequencing of the V4-V6 region of the 18S rRNA gene was amplified by using the eukaryote-specific primer pair Euk528F (5'- CCGCGGTAATTCCAGCTC -3') (Zhu et al., 2005) and EukR18R (5'- CGTTATCGGAATTAACCAGAC -3') (Hardy et al., 2011). Sequencing was performed as described in Dowd et al. (2008) with Roche 454 FLX titanium instruments and reagents, following manufacturer's guidelines at the MR DNA Ltd. sequencing facilities. Data processing and quality control were performed with the Mothur software (v 1.30) (Schloss et al., 2009). Sequences with ≥200 bp, no ambiguous pairs, and no homopolymers ≥8 bp were included for further analysis. These sequences were aligned using the SILVA SSU database (release 108, Pruesse et al., 2007) containing 62,587 eukaryotic SSU rRNA sequences. All sequences were binned into operational taxonomic units and were clustered (with the average neighbor algorithm) at 97% sequence similarity (Kunin et al., 2010). Taxonomic classification was based on the GenBank database (http://www.ncbi.nlm.nih.gov/genbank).

2.5. Data analysis

The retrieved pyrosequences were compared with the BLAST function against GenBank and the sequence with the closest resemblance (99%) to *Haematococcus* sequences was depicted. Furthermore, the *Haematococcus* sp. clone KORDI03 (accession number: FJ877140) was selected as a reference *Haematococcus* sequence and was also compared against GenBank. All sequences with >98% resemblance were selected for phylogenetic analysis in order to explore the phylogenetic relationships among all known cultured and environmental sequences associated with *Haematococcus* species. Sequence data were compiled using the MEGA 4 software (Tamura et al., 2007) and aligned using the CLUSTALX aligning utility. The phylogenetic tree was constructed based on maximum likelihood analysis using the Jukes–Cantor substitution model in MEGA 4 (Tamura et al., 2007). Bootstrapping under parsimony criteria was performed with 1000 replicates.

Population net growth rates (r) of the air-dispersed autotrophs identified in the water containers were calculated. The growth rate was determined based on the population abundance between 2 consecutive samples (weeks), divided by 7, in order to calculate the value per day, according to the equation:

$$r = \left(\frac{\ln B_i - \ln B_{i-1}}{t_i - t_{i-1}}\right)/7 \ [d^{-1}]$$

where B_i and B_{i-1} are the abundances at weeks t_i and t_{i-1}.

Regression analysis (IBM SPSS statistics 22.0) and redundancy analysis (CANOCO 4.5; Ter Braak and Smilauer, 2002) were performed in order to examine the relationship between *Haematococcus* abundance and meteorological parameters (air temperature, RH, wind speed, total precipitation, and total sunshine/total solar radiation). The average and maximum values of *Haematococcus* abundance for every period were used for both analyses. Correlations between *Haematococcus* abundances and environmental parameters were tested by Monte Carlo permutation tests.

Furthermore, principal component analysis (PCA) (Legendre and Legendre, 1998) was used to determine relationships: (a) between the growth rate of *Haematococcus* sp. and the species richness of the cooccurring autotrophs found in the water containers and their total abundance; and (b) among the growth rate of each of the dominant cooccurring autotrophs found in the water containers during the 5 periods investigated. When necessary, log transformation of values was conducted to achieve normality.

3. Results

3.1. *Haematococcus* in natural systems

Only a small proportion (<1%) of studies on *Haematococcus* species related to *Haematococcus* natural populations (Table 2). In half of these studies, *Haematococcus* individuals were identified at the genus level. In the rest, species identification was provided for the following species: *H. pluvialis* (10 studies), *H. lacustris* (9 studies), *H. buetschlii* (3 studies), *H. draebakensis* (1 study), and *H. thermalis* (1 study) (Appendix). The natural systems in which *Haematococcus* species were documented were located in all biogeographic regions (Figure 1) and consisted mainly of small, temporary freshwater bodies such as ponds, phytotelmata, thermal waters, water tanks, and artificial reservoirs and canals (in 28 out of 49 studies). In few cases, they were found as rare species in phytoplankton communities of larger permanent freshwaters such as rivers and lakes (in 18 studies). In 3 studies, *Haematococcus lacustris* was reported from air samples (Appendix).

The sequences derived from the molecular analyses of these air samples include only *Haematococcus*-related sequences (>98%) from natural systems deposited in GenBank. All sequences with >98% similarity with a reference *Haematococcus* sequence (see also Materials and Methods section) are presented in Figure 2. Based on phylogenetic analyses, the environmental clones attributed to *Haematococcus* are grouped with *H. lacustris* and *H. pluvialis.* According to the 18S rRNA gene, these two species are phylogenetically very similar. On the other hand, *H. zimbabwiensis* is grouped with other chlorophytes rather than *Haematococcus*-related sequences (Figure 2).

3.2. *Haematococcus* as an air-dispersed colonist

Among the air-dispersed colonists that were identified in the water containers in 2013 and those that were taken into account from a previous study in the area (2007–2008), 28 unicellular autotrophs and several heterotrophic protists were identified (Table 3). During the cold months (January–February 2008 and November–December 2013) the lowest number of taxa was detected (14 and 13, respectively), while 16 autotrophic species were documented during October–December 2007 and June–August 2013. While 11 out of 28 autotrophic taxa were found only in a single experimental period, *Haematococcus* sp. was the only autotroph that was constantly present in all periods/seasons. Furthermore, both regression and redundancy analyses showed no significant correlation among the environmental parameters and *Haematococcus abundance* ($P > 0.05$ in all cases).

In all experimental set-ups, *Haematococcus* reached high abundances (up to 4×10^4 cells mL^{-1}) except during November–December 2013, when the maximum abundance was only 300 cells mL^{-1} (Figure 3). In the 2007 and 2008 water containers, *Haematococcus* abundance was similar to the total abundance of all other autotrophs. During June–August 2013, *Haematococcus* dominated the autotroph community as it reached 10 times higher abundance values than the rest of the autotrophs (Figure 3). The highest net growth rate (0.9 d^{-1}) for *Haematococcus* was calculated during March–April 2008. The highest observed growth rate among the other dominant autotrophs was reached by *Limnothrix* sp. (1.4 d^{-1} in January–February 2008) and *Scenedesmus* sp. (1.4 d^{-1} in March–April 2008) while the other dominant autotrophs, *Chlorella* sp. and *Chlamydomonas* sp., reached growth rates of maximally 1.3 and 1 d^{-1}, respectively (Figure 4).

Figure 5a shows the ordination of the five experimental periods with respect to the two principal component axes (Axis I and Axis II) arising from PCA analysis based on *Haematococcus* growth rate, species richness, and autotroph abundance. Axis I and Axis II accounted for 54% and 33% of the total variation, respectively. Differences between the five periods were not evident because of the high overlap between the groups. The opposing direction between the vector of *Haematococcus* growth rate and the vectors of the autotroph abundance and species richness indicates a negative correlation. As expected, species richness and abundance increased in the final weeks of the experiments as indicated by the direction of the respective vectors and the position of the samples. The correlations between the growth rates of *Haematococcus* sp. and the growth rate of the cooccurring autotrophs *Chlamydomonas* sp., *Chlorella* sp., *Limnothrix* sp., and *Scenedesmus* sp. are given in the PCA of Figure 5b. The first two axes with the largest eigenvalues explain 37% and 23% of the total variance in the data, respectively.

Table 2. *Haematococcus* species citations in the literature.

References	Google Scholar	Scopus	Web of Science
Haematococcus in phytoplankton	48	2	6
Total	11400	592	1042
Percentage (%)	0.42	0.34	0.58

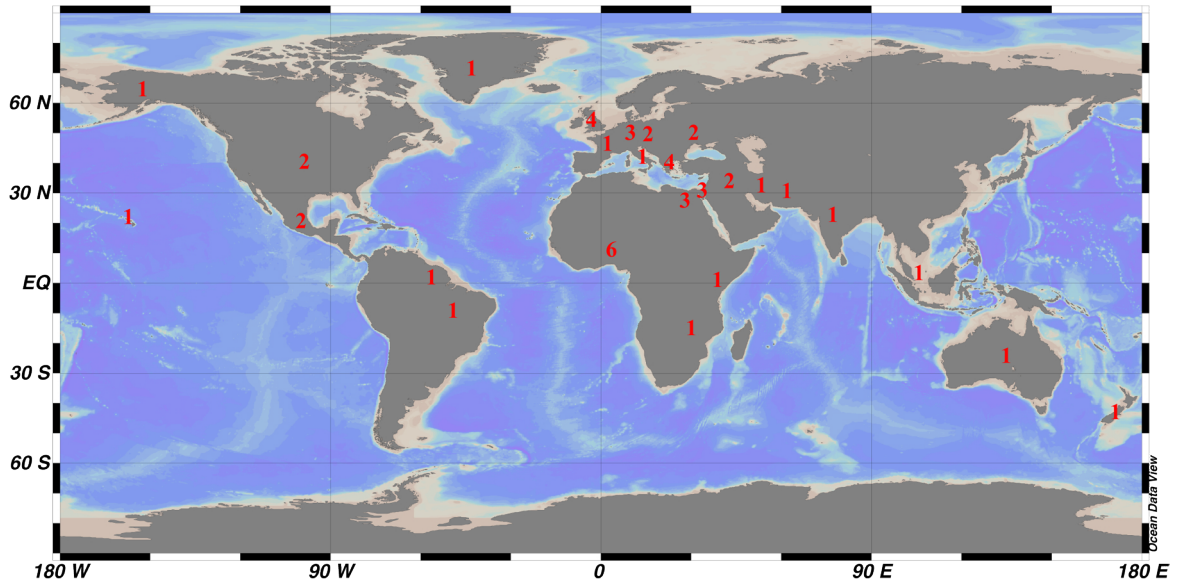

Figure 1. Map of the biogeographic regions that *Haematococcus* species were identified in natural systems. The number indicates the number of studies from the specific region.

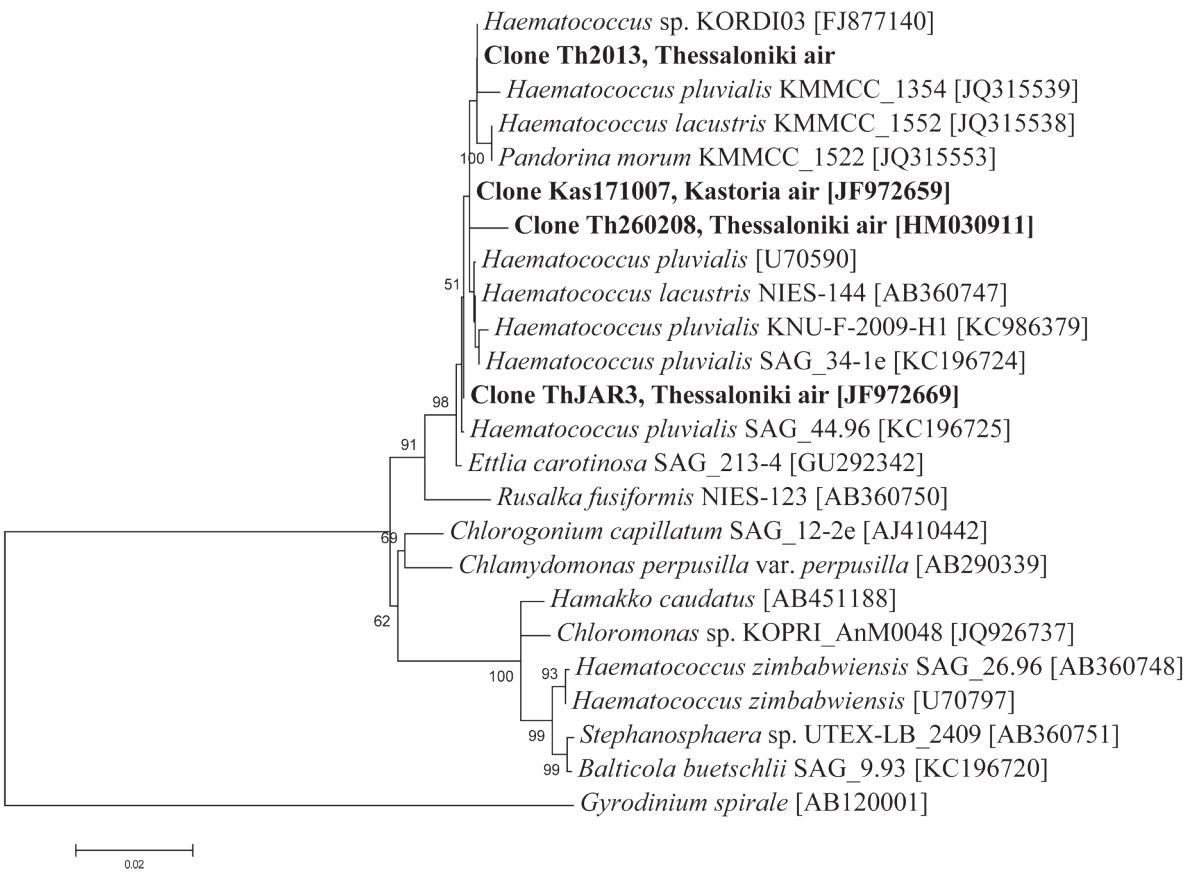

Figure 2. Phylogenetic tree of environmental (**in bold**) and cultured *Haematococcus*-related 18S rRNA sequences based on the maximum likelihood method as determined by Jukes–Cantor distances. One thousand bootstrap analyses were conducted. GenBank accession numbers are shown in parentheses. Scale bar represents 2% estimated distance.

Table 3. Air-dispersed morphospecies identified in the experimental containers in the city of Thessaloniki during five different periods comprising all seasons. The 2007 and 2008 data were acquired from Genitsaris et al. (2011a).

Morphospecies	October–December 2007	January–February 2008	March–April 2008	June–August 2013	November–December 2013
Autotrophs					
Aphanizomenon sp.				+	
Chlamydomonas sp.	+	+	+		
Chlorosarcinopsis bastropiensis	+				
Chlorella sp.	+	+	+		+
Cosmarium sp.				+	
Haematococcus sp.	+	+	+	+	+
Fusola sp.				+	+
Gleotrichia sp.	+				
Gleotila sp.	+				+
Grammatophora sp.	+	+	+		
Komvophoron sp.	+			+	
Limnothrix sp.	+			+	+
Lobosphaera tirolensis			+		
Meridion circulare				+	
Monoraphidium sp.				+	
Mougeotia sp.	+	+	+		
Nitzschia frigida					+
Nitzschia linearis				+	
Nitzschia sp.	+		+	+	
Oscillatoria sp.	+		+	+	+
Pediastrum duplex				+	
Planktolyngbya sp.				+	
Pseudanabaena sp.	+			+	+
Scenedesmus sp.	+	+	+	+	+
Stichococcus sp.	+	+	+		
Tabellaria floculosa				+	
Trebouxia sp.			+		
Zygnema sp.	+	+	+		
Grazers and Heterotrophs					
Amoeba radiosa				+	+
Amoeba spp.	+	+	+		
Ciliophora sp.	+	+	+		
Stentor coeruleus				+	+
Sorogena sp.	+	+	+	+	+
Podohedriella falcata	+				
Pattersionella vitiphila	+	+	+		
Eimeriidae		+			
HNFs	+	+	+	+	+

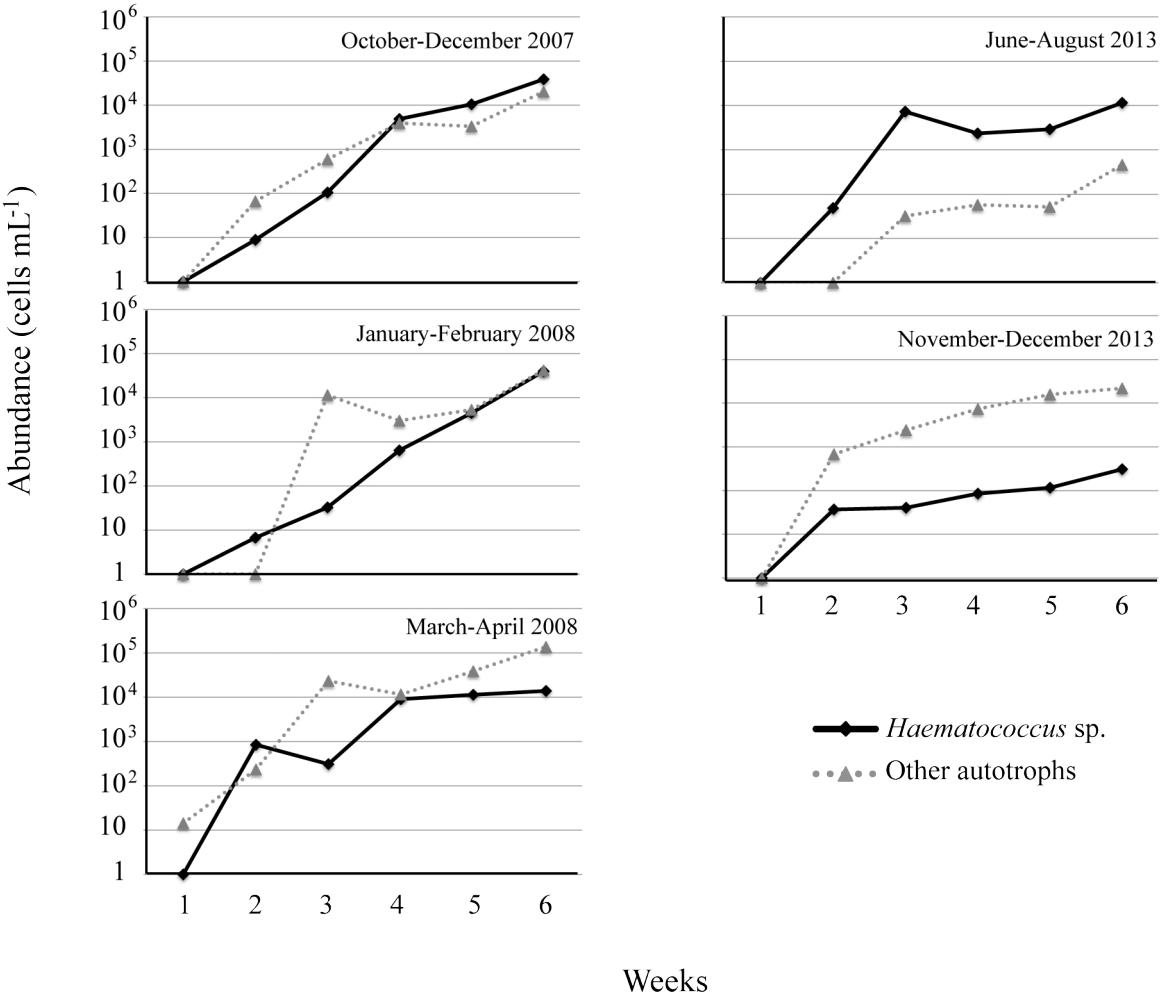

Figure 3. Abundance (cells mL^{-1}) of *Haematococcus* (black line) and other autotroph species (grey dashed line) in the water containers during the experiment periods.

According to the vectors direction, the *Haematococcus* growth rate seems to have a negative correlation only with *Chlamydomonas* growth rate. *Chlorella*, *Scenedesmus*, and *Haematococcus* growth rate was clearly higher during the early stages of the experiments (weeks 2 and 3), in contrast to the growth rate of *Chlamydomonas*, which was higher through the final weeks of the experiments (weeks 4, 5, and 6).

4. Discussion

Our literature search indicated that less than 1% of the studies mentioning *Haematococcus* relate to its occurrence in natural systems and even less in phytoplankton communities of lakes, reservoirs, and rivers. On the other hand, the vast majority of published information relates to astaxanthin production (Ambati et al., 2014 and references within), commercial applications (Guedes et al., 2011 and references within), and culturing techniques for increased productivity (Del Campo et al., 2007 and references within). Among the natural systems in which *Haematococcus* species were identified, approximately 60% (28 studies) were ephemeral water bodies, including basins, canals, ponds, pools, and artificial reservoirs (see Appendix), widespread throughout all biogeographic regions. Fewer studies report *Haematococcus* from larger permanent freshwater bodies (e.g., lakes and permanent reservoirs), and no study has identified it in saline waters (for detailed information see Appendix). In these few studies of larger freshwater ecosystems, *Haematococcus* was among the rare phytoplankton taxa, while it was usually found to thrive in ephemeral small water bodies (Proctor, 1957).

Among the studies that identified *Haematococcus* species in natural systems, three reported its occurrence in air samples, identified both by microscopy and molecular analyses (Genitsaris et al., 2011a, 2011b; Genitsaris et al.,

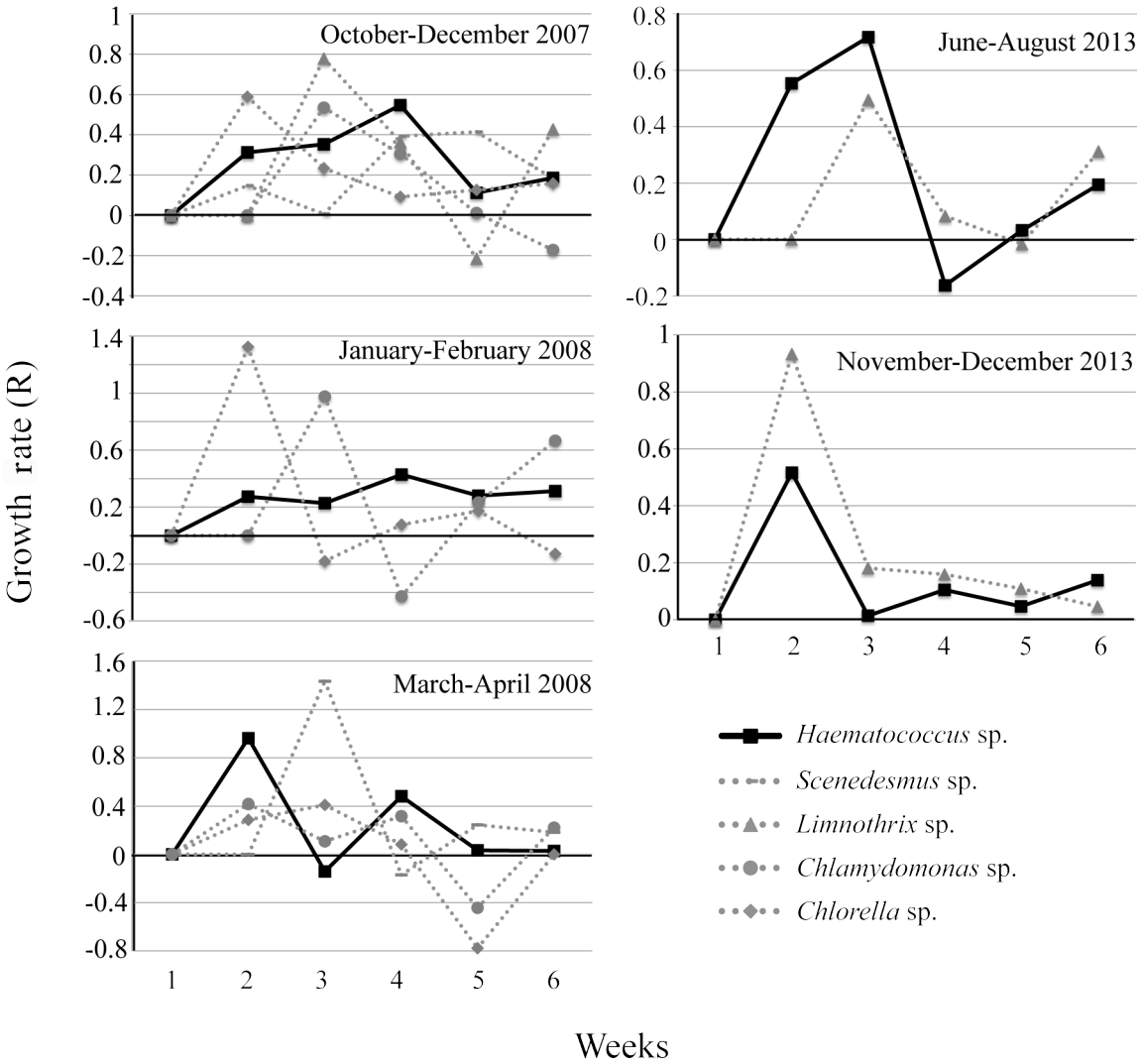

Figure 4. Growth rates of *Haematococcus* (black line) and the dominant autotroph species (grey dashed lines) in the water containers during the experiment periods.

2014). Interestingly, the only environmental samples in which *Haematococcus*-related sequences are detected are the above-mentioned air samples, while no *Haematococcus*-related sequences have been reported in plankton samples. *Haematococcus* individuals identified microscopically and by molecular techniques in the present study were identical to the individuals identified in previous studies of this air-dispersed taxon at the same site (Genitsaris et al. 2011a; Genitsaris et al., 2014). In addition, they were in all cases among the first microorganisms colonizing the water containers through air-dispersion. Genitsaris et al. (2011a) suggested that the success of *Haematococcus* in air dispersal and colonization could be explained by its remarkable life cycle, which includes resistant cysts and a swift transition to reproduction stages when encountering favorable conditions. Indeed, it has been shown that microorganisms inhabiting inland water systems have developed resting stages to achieve dispersal and colonize new environments (Incagnone et al., 2014). The ability of *Haematococcus* to withstand both drying and heating for long periods and its rapid germination under favorable conditions of ephemeral waters (Proctor 1957) appears to be decisive for being a successful colonist.

When a potential colonizer reaches a new habitat, it has to pass through a series of "filters" before it becomes successfully established (Muirhead and MacIsaac, 2005). For example, these filters may include abiotic local factors as well as the biological features of the existing species and the community structure (Florencio et al., 2014). Specifically, the traits that a pioneer microorganism needs in order to establish in ephemeral water bodies include: 1) mechanisms for the arrival (e.g., resting cysts); 2) fast

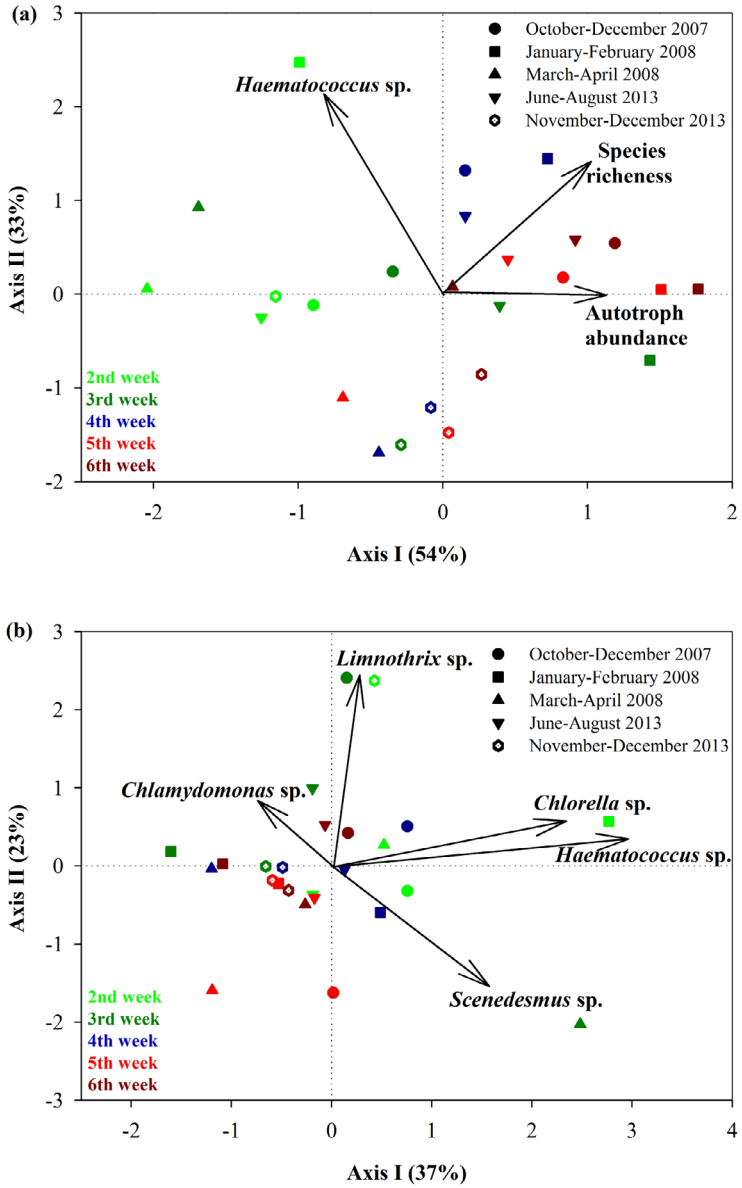

Figure 5. Ordination of the principal components analysis (PCA) for the water samples taken weekly (for six weeks) from the mesocosms during the five experimental periods based on: a) the *Haematococcus* growth rate, species richness and autotroph abundance; and b) the growth rate of each of the dominant species (*Chlamydomonas* sp., *Chlorella* sp., *Haematococcus* sp., *Limnothrix* sp., and *Scenedesmus* sp.). Parameters are indicated as vectors.

germination; 3) tolerance of extreme light conditions, as the ephemeral water bodies are usually shallow and provide no self-shading by plankton; and 4) a wide thermal tolerance. *Haematococcus* fulfills all the above requirements. It is characterized by swift production of resting stages under stress (Boussiba and Vonshak, 1991), fast germination when conditions become favorable, and high UV and thermal tolerance (Kobayashi and Okada, 2000). Moreover, it has been detected in all biogeographic regions under a wide range of climatic factors and environmental conditions (see Figure 1 and Appendix), indicating that abiotic features (such as solar irradiance, temperature, UV light, and dryness) are generally not inhibiting factors for the establishment of the motile life-stage of *Haematococcus* in aquatic systems. In addition, in the present study, *Haematococcus* abundance was not

significantly correlated with any of the meteorological parameter tested in all experimental periods. A possible explanation is that, following shifts to unfavorable environmental conditions, including unsuitable nutrient availability, *Haematococcus* simply switches into its dormant stage until the conditions are favorable again (Pringsheim, 1966). Another factor facilitating the establishment of *Haematococcus* during the early stages of our experiment was the very limited number and low abundance of autotrophic and heterotrophic species with low abundances inhabiting the water containers. Thus the potential unfavorable effects of the biological components against *Haematococcus* establishment were minimal.

On the other hand, the traits that contribute to the disappearance of pioneer species or the absence of a species in permanent/deep water bodies, like lakes' phytoplankton, include competitive disadvantages in comparison with other algae, high sensitivity to grazing, and high light requirements, which would lead to sensitivity against shading by other phytoplankton species (Harris, 1978; Falkowski and Owens, 1980). During the later stages of the experiments (5th and 6th week), when the species richness and abundance of air-dispersed autotrophic colonists increased, the growth rate of *Haematococcus* dropped (Figure 4), although it retained its high abundance. The growth rate of *Haematococcus* was negatively correlated to both species richness and autotroph abundance (potential competitors), suggesting that it has higher growth rates where/when it is free from competition by other algae/cyanobacteria. While *Haematococcus* showed positive correlations with *Chlorella* and *Scenedesmus*, it showed a negative correlation with *Chlamydomonas* (Figure 5). In contrast, Proctor (1957) showed that *Haematococcus* is inhibited by *Scenedesmus* and *Chlamydomonas* and proposed that it may be inhibited by other chlorophytes as well. Our results do not support the inhibition by all chlorophytes, but are consistent with an inhibition by *Chlamydomonas* (Figure 5). Another explanation for the *Haematococcus* growth rate decrease could be that this organism is suggested to be a nutritious prey for many predators. In a temporary food web, Genitsaris et al. (2011a) observed ciliates and amoebas to feed mainly on *Haematococcus* individuals and proposed that their astaxanthin content makes them an ideal prey. Finally, motile cells of *Haematococcus* have been shown to have light saturation coefficients between 260 and 320 μmol m^{-2} s^{-1} (Jianguo and Jingpu, 2000), placing it among the most high-light adapted algae, as shown by the range of saturation coefficients form 20 to 300 μmol m^{-2} s^{-1}, compiled by Harris (1978). The evolution of the light harvesting system that is more effective at higher light intensities is generally adaptive to shallow waters, where shading from other species is avoided (Falkowski and Owens, 1980). The positive correlation between *Haematococcus* and *Chlorella* in our study suggests a similar to *Chlorella* strategy for adaptation to generally higher light intensities than those prevailing in optical dense lakes.

Overall, *Haematococcus* appears to be an effective air-dispersed microorganism resting almost everywhere, which can quickly and successfully colonize newly formed systems and establish populations in small ephemeral water bodies, with low competition and predation pressures. The successful colonization and establishment can be primarily attributed to the resting cysts, which it rapidly produces under unfavorable conditions. On the other hand, its absence from larger permanent water bodies could be related to the competitive disadvantages against cooccurring taxa and the grazing pressures from higher predators. An analogous contrast between a typical, often dominant plankton alga and a colonizer of shallow ponds has also been shown in salt water by comparing the light requirements of the shade-adapted diatom *Skeletonema* and the high-light-adapted chlorophyte *Dunaliella* (Falkowski and Owens, 1980). Thus, ephemeral water bodies may serve as a temporal "pit-stops", a means for *Haematococcus* to increase its abundance and thrive before biotic pressures force it to migrate or abiotic pressures drive it to rest. However, laboratory controlled experiments using lake water are needed in order to establish competition patterns with other phytoplankton species and to investigate predator-prey relationships, which could provide more information about the ecology and biogeography of *Haematococcus* species.

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Appendix. *Haematococcus* morphospecies in natural systems.

Morphospecies	Natural System	Region	Reference
Haematococcus sp.	Freshwater basin	High Arctic	Kim et al. (Arctic 64: 25-31; 2011)
Haematococcus sp.	Freshwater canal	UK	Moss et al. (J Appl Ecol 40: 782-792; 2003)
Haematococcus sp.	Freshwater pond	USA	Johnson (Freshwater Biol 25: 51-59; 1991)
Haematococcus sp.	Small water body	Nigeria	Chia et al. (Oceanol Hydrobiol St 41: 39-47; 2012)
Haematococcus sp.	Reservoir	Nigeria	Tanimu et al. (International Symposium on Environmental Science and Technology; 2011)
Haematococcus sp.	Artificial pond	Nigeria	Chia et al. (Afr J Aquat Sci 36: 35-46; 2011)
Haematococcus sp.	Reservoir	Greece	Katsiapi et al. (Environ Monit Assess 181: 563-575; 2011)
Haematococcus sp.	Lake	Malaysia	Kutty et al. (Pak J Biol Sci 4: 309-313; 2001)
Haematococcus sp.	River	Kenya	Ojunga et al. (Water Qual Res J Can 45: 235-250; 2010)
Haematococcus sp.	Sewage treatment plant	Egypt	Shehata & Badr (Environ Manage Health 7: 9-14; 1996)
Haematococcus sp.	River	Iraq	Abed et al. (International Journal of Advanced Research 2: 895-900; 2014)
Haematococcus sp.	Lagoon	Iran	Noroozi et al. (Int J Algae 11: 276-288; 2009)
Haematococcus sp.	Lake	New Zealand	Chapman (Book chapter in Hypertrophic Ecosystems, Developments in Hydrobiology; 1980)
Haematococcus sp.	River	Iraq	Hassan et al. (Basrah Journal of Science 28: 273-288; 2010)
Haematococcus sp.	Artificial pond	UK	Hitchman & Jones (Freshwater Biol 43: 231-241; 2000)
Haematococcus sp.	Artificial reservoir	Zimbabwe	Basima et al. (Phys Chem Earth, Parts A/B/C 31: 821-831; 2006)
Haematococcus sp.	Freshwater	Israel	Masyuk & Lilitskaya (Int J Algae 3: 48-61; 2001)
Haematococcus sp.	Freshwater	Israel	Masyuk et al. (Int J Algae 1: 71-85; 1999)
Haematococcus sp.	River	Australia	Kobayashi et al. (Mar Freshwater Res 47: 1025-1036; 1996)
Haematococcus sp.	Water body	Ukraine	Masyuk & Lilitskaya (Book chapter in Algae of Ukraine: diversity, nomenclature, taxonomy, ecology and geography; 2005)
Haematococcus sp.	Freshwater pools	French Guiana	Carrias et al. (Plant Biol 16: 997-1004; 2014)
Haematococcus sp.	Lake	France	Le Jeune et al. (Aquat Toxicol 83: 223-237; 2007)
Haematococcus sp.	Pond	UK	Kelcey (Urban Ecol 9: 99-142; 1985)
Haematococcus sp.	Lake	USA	Stewart & Lowe (Ohio J Sci 108: 82-94; 2008)
Haematococcus spp.	Freshwater	Brazil	Menezes & Bicudo (Hoehnea 35:435-468; 2008)
Haematococcus pluvialis	Lake	Greece	Chrisostomou et al. (J Plankton Res 31: 877-884; 2009)
Haematococcus pluvialis	Rainwater pond	Germany	Schwarz et al. (International Symposium on Growing Media and Hydroponics; 2004)
Haematococcus pluvialis	Nuclear fuel-storage pond	UK	Groben (report; 2007)
Haematococcus pluvialis	Lake Tovel	Italy	Cavalca et al. (Ann Microbiol 51: 159-177; 2001)
Haematococcus pluvialis	Lake	Mexico	Navarro et al. (Hidrobiológica 14: 91-103; 2004)
Haematococcus pluvialis	Waste stabilization pond	Egypt	Ghazy et al. (Am J Environ Sci 4: 316-325; 2008)
Haematococcus pluvialis	Lake	Israel	Pollingher et al. (Israel J Plant Sci 46: 155-168; 1998)
Haematococcus pluvialis	Freshwater	Ukraine	Gorbulin (The Journal of Karazin Kharkiv National University, Series: Biology 16: 63-76; 2012)

Appendix. (Continued).

Haematococcus pluvialis	River	Egypt	El-Gohary et al. (Aust J Basic Appl Sci 2: 1345-1355; 2008)
Haematococcus pluvialis	Irrigation reservoir	Germany	Schwarz et al. (Agr Water Manage 71: 145-166; 2005)
Haematococcus lacustris	Air	Greece	Genitsaris et al. (Sci Total Environ 478: 70-79; 2014)
Haematococcus lacustris	Lake	India	Senthilkumar et al. (J Environ Biol 29: 747-752; 2008)
Haematococcus lacustris	Air	Greece	Genitsaris et al. (Aquat Microb Ecol 62: 139-152; 2011a)
Haematococcus lacustris	Air	Greece	Genitsaris et al. (Front Biosci (Elite Ed) 3: 772-787; 2011b)
Haematococcus lacustris	Freshwater	Pakistan	Ali et al. (Pak J Bot 42: 3457-3462; 2010)
Haematococcus lacustris	Artificial pond	Nigeria	Chia et al. (Braz J Bot 34: 285-295; 2011)
Haematococcus lacustris	Water tank	Nigeria	Chia et al. (Eur J Sci Res 110: 501-510; 2013)
Haematococcus lacustris	River	Nigeria	Zakariya et al. (*J Environ Sci* 2: 31-37; 2013)
Haematococcus lacustris	Reservoir	Mexico	López-López & Serna-Hernández (Rev Biol Trop 47: 34-44; 1999)
Haematococcus buetschlii	River	Slovakia	Hindák & Hindáková (Bulletin Slovenskej Botanickej Spoločnosti, Bratislava 26: 9-17; 2004)
Haematococcus buetschlii	River	Czech Republic	Hasler et al. (Fottea 7: 49-68; 2007)
Haematococcus buetschlii	Phytotelmata	Germany	Gebühr et al. (Plant Biol 8: 849-860; 2006)
Haematococcus draebakensis	Sewage oxidation pond	Alaska	Hilliard (Report; 1965)
Haematococcus thermalis	Thermal waters	Hawaii	MacCaughey (I Bot Gaz 65: 42-57; 1918)