Dark nutrient uptake at low temperature and subsequent light use efficiency by the dinoflagellate Heterocapsa triquetra

Karin Ojamäe, Anneliis Peterson & Inga Lips

To cite this article: Karin Ojamäe, Anneliis Peterson & Inga Lips (2016) Dark nutrient uptake at low temperature and subsequent light use efficiency by the dinoflagellate Heterocapsa triquetra, Marine Biology Research, 12:9, 978-985, DOI: 10.1080/17451000.2016.1216570

To link to this article: http://dx.doi.org/10.1080/17451000.2016.1216570

Published online: 05 Oct 2016.
Original Article

Dark nutrient uptake at low temperature and subsequent light use efficiency by the dinoflagellate *Heterocapsa triquetra*

Karin Ojamäe, Anneliis Peterson and Inga Lips

Marine Systems Institute at Tallinn University of Technology, Tallinn, Estonia

**ABSTRACT**

For population growth in stratified environments, flagellates are known to migrate to the nutrient-rich water layers below the thermocline to take up inorganic nutrients and ascend to the nutrient-deficient euphotic water layer to photosynthesize. The present study investigated dark nutrient uptake at 4°C (characteristic water temperature below the thermocline in the Baltic Sea) by the dinoflagellate *Heterocapsa triquetra*. The recovery of photosynthetic performance and the improvement of cell growth after dark nutrient acquisition and meeting suitable light conditions were also studied. On average, the consumption of NO$_3^-$ and PO$_4^{3-}$ in the absence of light at 4°C was 0.04 and 0.003 µM h$^{-1}$, respectively. N:P uptake ratios were similar during dark, cold incubation and a following light-dark cycle. In the nutrient-deficient cultures, the effective photochemical yield was lowered to 36%, while it recovered to 64% after simultaneous dark incubation and nutrient addition. Increased photosynthetic efficiency yielded a 34% higher cell concentration after incubation in dark, cold, nutrient-enriched conditions in comparison to the parallel N-deficient cultures that received no nutrients. The results suggest that *H. triquetra* can successfully compromise between dark nutrient acquisition and the use of the internal nutrient storage for photosynthesis later in the light field.

**Introduction**

During spring and summer the development of temperature stratification is common in temperate seas. In April–May, a seasonal thermocline starts developing in the Baltic Sea. The upper mixed layer with a typical depth of 10–20 m and temperature of 15–20°C becomes separated from the cold intermediate layer of 2–4°C by mid-June. After the spring bloom, the inorganic nutrients are depleted in the euphotic layer, and a density gradient prevents mixing between the nutrient-poor and nutrient-rich water layers. In late July, during the period of strongest stratification and inorganic nutrient deficiency in the surface layer, high biomass concentrations of the dinoflagellate *Heterocapsa triquetra* (Ehrenberg) Stein are often found. Over the same period, layers of subsurface and deep chlorophyll a maxima (DCM) are reported in different areas of the Baltic Sea (Pavelson et al. 1999; Kononen et al. 2003; Lips et al. 2010, 2011; Hällfors et al. 2011; Lips & Lips 2014). These phytoplankton biomass maxima have mostly been detected at the depth of the nitracline (15–35 m), which usually coincides with the base of the seasonal thermocline (e.g. Lips et al. 2010). In the Gulf of Finland in July/August, DCM are mostly dominated by the dinoflagellate *H. triquetra*, although *Dinophysis* spp. and the photosynthetic ciliate *Mesodinium rubrum* (Lohmann) may also contribute to these maxima on some occasions (Kononen et al. 2003; Hällfors et al. 2011; Lips & Lips 2014).

The DCM can often be an indication of phytoplankton migration patterns. Migrations to the deep nutrient-rich layers to harvest inorganic nutrients have been shown in flagellates to overcome nutrient limitation (Cullen & Horrigan 1981; Heaney & Eppley 1981; Doblin et al. 2006; Jephson & Carlsson 2009). At night, cells may descend to the dark and deep nutrient-replete layers below the thermocline to take up inorganic nutrients. Cells ascend to the nutrient-deficient euphotic layer during the day, where they use the incident solar radiation for photosynthesis. Vertical sampling and high-resolution autonomous vertical profiling have demonstrated diel and bi-diurnal migration patterns for a phytoplankton community dominated by *H. triquetra* (Lips & Lips 2014). Migrations to the deeper nutrient-rich layers have been hypothesized to explain the high biomass values of this species in the nutrient-depleted surface layer in July in the Gulf of Finland (e.g. Lips et al. 2011; Lips & Lips, 2014). In fact, a cost–benefit analysis by Raven &
Richardson (1984) hypothesizes that dinoflagellates win energetically from the vertical migration behaviour even when the energy and nitrogen costs (synthesis and operation of the flagellar apparatus, transport and storage capacity for nitrate) have been taken into account. Moreover, nutrient assimilation in the dark has been shown to support the growth of many dinoflagellates (Cullen & Horrigan 1981; Olsson & Granéli 1991; MacIntyre et al. 1997). The question arises – does _H. triquetra_ perform vertical migrations for nutrient acquisition, and does it take up sufficient nutrients in the deep, dark, cold waters to allow it to survive and bloom in the nutrient-deficient Baltic Sea surface layer in late summer?

In laboratory conditions, the dark nutrient uptake by _H. triquetra_ has only been documented in a warm growth environment of 20°C (Paasche et al. 1984). At the same time, the maximum nutrient uptake rate of phytoplankton is known to be a function of molecular diffusivity, which is a linear function of temperature – molecular diffusivity being slower at lower temperatures (Baird et al. 2001). The literature also does not provide any direct evidence indicating the extent to which _H. triquetra_ benefits from dark nutrient uptake in a way that would manifest in either higher growth rate, biomass increase or improved photosynthetic performance later in the light field. In the present study, our experimental conditions were aimed to simulate some of the main environmental factors met in the Gulf of Finland that influence nutrient uptake and photosynthetic efficiency in phytoplankton: (i) light availability, (ii) temperature, and (iii) nutrient preconditioning. The primary objective of this study was to indicate, in a laboratory experiment, the ability of _H. triquetra_ to take up inorganic nutrients (nitrate and phosphate) in cold and dark conditions and to demonstrate that nutrients previously taken up in the dark improve cell growth and photosynthetic capacity later in the light field.

### Materials and methods

**Culturing conditions and experimental design**

A non-axenic monoculture of _Heterocapsa triquetra_ was established from single cells isolated from the Gulf of Finland in 2012. The culture was maintained in T2 medium (Spilling et al. 2011), which is modified f/2 medium (Guillard 1975), where the molar nutrient ratio, N:P, is adjusted to 16:1. The culture, with salinity around 6, was grown at 15°C and an irradiance of 200 μmol m⁻² s⁻¹ with a light–dark cycle of 16:8 hours. Ten days prior to the experiment, a small amount of culture (1–2 ml) was inoculated into two 250 ml Erlenmeyer flasks prefilled with 120 ml filtered and autoclaved seawater. Nutrients were added directly into the cultures according to Spilling et al. (2011), with a final nitrate concentration of 24 µmol l⁻¹ and a phosphate concentration of 6 µmol l⁻¹. Phosphate was added in excess (N:P = 4) to simulate the natural environment of N-limitation in the Gulf of Finland.

To initiate the experiment 500 ml flasks (n = 3; Figure 1) were filled with the above cultures at 820 cells ml⁻¹ and maintained at the light and temperature conditions above. Once cell density was > 1000 cells ml⁻¹ (day 2), cultures were diluted with sterile seawater. The dilution was necessary to avoid CO₂ limitation and consecutive pH rise as a result of high cell numbers in the late exponential phase. By day 6 of the experiment, when _H. triquetra_ cells had reached the stationary growth phase, the cultures were split

---

**Figure 1.** The timeline of the experiment with information on subculture volume, growth conditions and cell densities in different experimental phases. On day 6 cultures were split into two sets of subcultures: one set was left unmodified until the end of the experiment (arrow pointing up), and the second set received nutrients and went through the 48 hour dark (ID) incubation period at 4°C (arrow pointing down) before being brought back to the initial light and temperature conditions.
in two and poured in triplicate into 500 ml Erlenmeyer flasks up to 230 ± 2.5 ml. One set of flasks was maintained at the light and temperature conditions described above until the termination of the experiment; additional nutrients were never added. The other set of flasks was wrapped in foil, and then a nutrient-rich medium was added. Nutrient concentration increased to a final concentration of nitrate of 4.53 μM and phosphate of 0.91 μM (N:P = 5) in the nutrient-amended cultures. Similar nutrient concentration levels are found in the surface layer of the Gulf of Finland before the formation of the thermal stratification in the water column in spring. Nutrient concentrations at the upper part of the nutriclines (15–35 m) in the cold intermediate layer are usually lower. However, we aimed to avoid nitrate being depleted from the dark-incubated cultures because it would not be possible to show the maximum growth yield in that case. The flasks were incubated in the dark at 4°C for 48 h (I0) and after that brought back to the previous culturing conditions (I1) described above. Samples for measurements of cell concentration were fixed with acid Lugol solution and cells counted using a Sedgewick–Rafter counting chamber and an inverted microscope. The specific growth rates (μ), between consecutive time points, were calculated during exponential growth as:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

where $N_1$ and $N_2$ denote the abundances at time steps $t_1$ and $t_2$, respectively. Culture pH was monitored daily directly in the flasks and was regulated with a 1 M HCl solution during the first days of the experiment (Mettler Toledo SevenEasy pH meter).

**Variable chlorophyll fluorescence analysis**

Standard variable chlorophyll fluorescence measurements were done using the MULTI-COLOR-PAM chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) following the saturation pulse method (Schreiber 2004). Parameters used for the assessment of the photosynthetic capacity of *Heterocapsa triquetra*, obtained with the saturation pulses, were: effective photochemical quantum yield ($\phi_{II}$), regulated non-photochemical energy conversion ($\phi_{NPE}$), non-regulated heat dissipation and fluorescence emission ($\phi_{NO}$), maximum photochemical quantum yield ($F_v/ F_m$) and photochemical fluorescence quenching ($qP$; Table I). These parameters provide basic information on the fate of absorbed light energy (Kramer et al. 2004; Klughammer & Schreiber 2008). Parameters for the light regimes used were: measuring light = 1 μmol photons m$^{-2}$ s$^{-1}$ at 440 nm (30 s), saturating light = 5710 μmol photons m$^{-2}$ s$^{-1}$ at 440 nm (30 ms), actinic light = 200 μmol m$^{-2}$ s$^{-1}$ at 440 nm (30 s). All fluorescence parameters were calculated after subtraction of the blank fluorescence, measured in filtered (GF/F glass-fibre filter) and autoclaved seawater. Before fluorescence measurements, cells were incubated at in situ temperature in quartz cuvettes for 10–20 minutes in the dark to allow full oxidation of the primary electron acceptor, quinone A ($Q_A$). Cells were stirred in the cuvette during measurements.

**Inorganic nutrient measurements**

Nutrient analysis was carried out from filtered and autoclaved seawater prior to use in the experiment. During the experiment, samples for nutrient analysis were collected at five different time steps: on day 2 after culture dilutions, on day 6 before and after nutrient additions prior to the dark incubation period, on day 8 from nutrient-enriched cultures after the dark incubation period, and on day 9 from nutrient-enriched cultures. Samples were analysed using the Lachat QuickChek 8500 Series 2 (Lachat Instruments, Hach Company) for $NO_3^-$ and $PO_4^{3-}$ according to the ISO 15681–1 method for $PO_4^{3-}$ and the ISO 13395 method for $NO_3^-$. The lower detection range for $PO_4^{3-}$ and $NO_3^-$ was 0.06 and 0.07 μM, respectively.

The nutrient uptake rates were calculated from the decreases of the respective nutrient concentrations. To compare the rates in units of μM h$^{-1}$, nutrient uptake was assumed to be constant over the measured periods, which differed in length. The dark incubation period at 4°C (I0) of 48 hours was compared to the

| Table I. Chlorophyll fluorescence parameters used in this study. Abbreviations, see text. |
|------------------|------------------|------------------|
| **PSII fluorescence parameter** | **Calculation** | **References** |
| $F_v/F_m$ | $F_{m} - F_{0}/ F_{m}$ | Kitajima & Butler (1975) |
| $\phi_{II}$ | $(F_{m} - F)/ F_{m}$ | Genty et al. (1989) |
| $\phi_{NO}$ | $F/F_{m}$ | Hendrickson et al. (2004) |
| $\phi_{NPE}$ | $F/F_{m} - F/F_{m}$ | Hendrickson et al. (2004) |
| $qP$ | $F/F_{m} - F/F_{m}$ | Schreiber et al. (1986) |
| $F_{0}'$ | $F/F_{m} - F/F_{m}$ | Oxborough & Baker (1997) |


period of a light–dark cycle (I_{LD}) of 24 hours. Hence, the amount of nutrients taken up during I_D or I_{LD} was divided by the respective hours of the measured period.

Results

Nutrient and cell concentrations

The dynamics of the nutrient levels in the cultures and the amounts of nutrients taken up in different experimental phases by *Heterocapsa triquetra* are shown in Figure 2(a). The concentration of NO_3^− in the non-amended cultures was stable at 0.44 ± 0.02 µM (open circles; last day of measurement in non-amended cultures was day 6, and no measurements were made on day 9), whereas the level of PO_4^3− decreased by 26% down to the concentration of 0.67 ± 0.1 µM by day 6 (open rectangles).

In the nutrient-amended cultures, the concentrations of both NO_3^− and PO_4^3− decreased during dark, cold (4°C) incubation (I_D) (group means before and after I_D were significantly different for both nutrients; paired t-test, \( p < 0.05 \)). The average consumption rate of NO_3^− during I_D was 1.04 µM day\(^{-1} = 0.04 \) µM h\(^{-1}\) and in the following warm (15°C) light:dark cycle (I_{LD}) it was 1.69 µM day\(^{-1} = 0.07 \) µM h\(^{-1}\). The consumption rate of PO_4^3− in I_D was 0.075 µM day\(^{-1} = 0.003 \) µM h\(^{-1}\) and in the following I_{LD} 0.14 µM day\(^{-1} = 0.006 \) µM h\(^{-1}\). However, the differences between the consumption rates of both nutrients in I_D and I_{LD} were not statistically significant (t-test, \( p > 0.05 \)), most probably due to the small data set. Nutrients were consumed in similar N:P ratios of 14:1 and 12:1 during I_D and I_{LD}, respectively.

The cell concentration in the non-amended cultures was mostly stable around 1000 cells ml\(^{-1}\) during the whole experiment (Figure 2b). Cell abundance decreased slightly after relocating the nutrient-amended cultures from I_D to I_{LD} (negative growth rate of −0.11 day\(^{-1}\) by day 9). After that, the growth rate in these cultures increased to 0.22 day\(^{-1}\) in two days, yielding a 34% higher cell concentration (1390 cells ml\(^{-1}\)) by day 11 when compared with the non-amended cultures at the same day (1040 cells ml\(^{-1}\)).

Variable fluorescence dynamics

The maximum PSII quantum yield (F_v/F_m) for the non-amended *Heterocapsa triquetra* cultures declined as a function of time from 0.79 to 0.60 during the experiment (Figure 2c). The mean F_v/F_m in the nutrient-amended cultures after the I_D (the last three days of the experiment) was 0.77 ± 0.01 (mean ± SE, \( n = 9 \)), being on average significantly higher than the mean in the non-amended cultures (0.66 ± 0.03) during the same period (t-test, \( p < 0.01 \)).

Measurements of photophysiological variables yielded more insight into the photochemical and non-photochemical quenching processes in the non-amended *H. triquetra* cultures. The effective photochemical yield (\( \phi_P \)) for the non-amended cultures declined with time from 0.71 to 0.36. During the first five days,
the decline mainly resulted from compensatory changes in antenna downregulation, $\phi_{\text{NPQ}}$ (the latter increased from 0.02 to 0.21; Figure 3a). Under these conditions, the PSII reaction centres were essentially completely open (high qP), and photosynthetic yield was primarily determined by changes in non-photochemical quenching. Onwards from day 5, the capacity of $\phi_{\text{NPQ}}$ to regulate light capture was saturated, leading to the gradual decline of open reaction centres (qP). However, from day 8 until the end of the experiment, $\phi_{\text{II}}$ was mainly influenced by non-light-induced (basal or dark) quenching processes ($\phi_{\text{NO}}$ increased from 0.25 to 0.42; Figure 3a). Increased energy dissipation as $\phi_{\text{NPQ}}$ and $\phi_{\text{NO}}$ caused a photoregulative change in the non-amended $H$. triquetra cultures on days 10–11, i.e. $\phi_{\text{II}}$ was lowered down to 0.36.

In the nutrient-amended cultures, $\phi_{\text{II}}$ recovered up to 0.64 and was on average higher when compared to the non-amended cultures (t-test, $p = 0.01$). The decline of $\phi_{\text{II}}$ resulted mainly from the increasing $\phi_{\text{NPQ}}$ (0.06–0.17) but also from a slight decrease in qP (0.90–0.83; Figure 3b). Energy dissipation as $\phi_{\text{NO}}$ was stable (range 0.29–0.31; Figure 3b).

**Discussion**

The present study documents that the dinoflagellate $Heterocapsa$ triquetra can take up both inorganic nitrogen and phosphorus in a dark and cold (4°C) environment. Similar physical conditions are characteristic of the water layers below the seasonal thermocline in summer in the Baltic Sea. This documented ability for dark nutrient uptake supports our hypothesis that $H$. triquetra migrations from warm surface layers to the deeper cold layers could be aimed at acquiring available inorganic nutrients that are at that time lacking in the surface layer. The described migration pattern (Lips et al. 2011) together with dark nutrient uptake presented in this study explains the competitive advantage of $H$. triquetra populations in the nutrient-limited surface layer and the species’ ability to reach bloom concentrations in the summer when the Gulf of Finland is strongly stratified. As the dark uptake of NO$_3^-$ was demonstrated to be accompanied by the uptake of PO$_4^{3-}$, the importance of nutrient-retrieval migrations to the nutrient-limited surface layer are even more emphasized by the present study. The observed N:P uptake ratios by $H$. triquetra were similar in the dark, cold (4°C) and illuminated, warm (15°C) environments, ~14:1 and 12:1, respectively. This is noteworthy because a significantly lower N:P uptake ratio in the dark or no difference in light–dark uptake of P was reported in previous studies (e.g. Rivkin & Swift 1985; Riegman et al. 2000; Müller et al. 2008).

While the influence of single environmental factors on nutrient uptake in dinoflagellates have been studied rigorously, studies with combined environmental factors on nutrient uptake are lacking. In fact, little attention has been paid to the dark uptake rates of nutrients at different temperatures or dark nutrient uptake by cells previously grown in an illuminated but nutrient-poor environment. Often, higher uptake rates of nitrates in the light compared with dark uptake rates are reported (e.g. Dortch & Maske, 1982; Riegman et al. 2000; Jauzein et al. 2011). $Heterocapsa$ niei (Loeblich III) Morrill & Loeblich III takes up 60% less nitrate nocturnally compared to the uptake

![Figure 3](image-url)
during the day (Cullen 1985), whereas *H. triquetra* is able to take up nitrate more or less equally in light and dark conditions at 20°C (Paasche et al. 1984). In general, nutrient uptake decreases with temperature (Smyad 1997; Reay et al. 1999). In the present study, potentially half the nutrients were taken up in the dark, cold conditions compared with the uptake in the warm, illuminated conditions. However, as the uptake correlations were found not to be statistically significant, most probably due to the small data set, this issue needs to be studied further. Additionally, the cells’ history of the nutrient supply can play an important role. Dinoflagellates differ widely in their ability to take up nitrogen when grown in N-sufficiency (Paasche et al. 1984). Nitrate uptake and reduction relies on carbohydrates that are used up immediately in the light if nitrate is available, but are stored in N-starved cells and could be used for dark nitrate assimilation (Cullen 1985). Some earlier laboratory studies have shown that N-deprived phytoplankton cells have greater dark uptake rates of inorganic nitrogen compared with N-replete cells (Dortch & Maske, 1982; Cochlan et al. 1991).

The present data also provide evidence that the nutrient uptake in the dark was followed by improved performance of the photosynthetic apparatus of the cells later in the illuminated conditions. The maximum PSII quantum yield (Fv/Fm) was higher after nutrient additions (up to 0.76), and the majority of the absorbed light energy was channelled into photochemical processes (φII). In dinoflagellate cultures, an Fv/Fm of 0.6 has been considered to be an indication of cells with a well-functioning photosynthetic apparatus (Suggett et al. 2009; López-Rosales et al. 2014). In the present experiment, Fv/Fm declined in the non-amended cultures but was never lower than 0.6. Relatively high values of Fv/Fm together with the long duration of the stationary growth phase in the non-amended *H. triquetra* cultures (~9 days) may indicate that *H. triquetra* cells are able to acclimate to low nutrient conditions and survive for relatively long periods in a nutrient-depleted surface layer in the sea. The described ability may be achieved by the use of intracellular nutrient storages, as dinoflagellates can store significant amounts of inorganic and organic nitrogen forms (e.g. the review by Dagenais-Bellevillee & Morse 2013). It is thought that in the stationary growth phase nutrients are reallocated to the functions where they are most needed, and the balance between the light harvest and electron transport are adjusted in such a way as to maximize efficiency (Parkhill et al. 2001).

The decrease in maximum photochemical efficiency (φII) in the nutrient-limited cells has been reported (Lippemeier et al. 1999; Roberts et al. 2008), as well as the recovery following nutrient re-addition after starvation (e.g. Greene et al. 1992; Lippemeier et al. 2001; Young & Beardall 2003). Fluorescence, as opposed to cell division, responded faster to the nutrient additions in our experiment, which is in agreement with nutrient spiking found in other algae (Lippemeier et al. 2001, 2003). Decreasing φII and complementarily increasing φNO and φNPO in the non-amended *H. triquetra* cultures was related to the damage in the reaction centres to a great part. The operating efficiency of PSII (φII) during the final days in the non-amended cultures was sufficient (0.36–0.45) to maintain the population size at a steady level. The sum of the φNPO and φNO contributed to a substantial fraction (0.55–0.64) of the absorbed light energy lost non-photochemically. Although, the concentration of the light-harvesting pigments in *H. triquetra* have been shown to decrease heavily under nutrient starvation and only slight changes in their relative concentration to chlorophyll *a* take place (Latasa & Berdalet 1994). The latter suggests that the general structure of the photosynthetic apparatus does not change, and in the case of nutrient resupply, faster recovery of the cells may be expected. In the present study, the φII recovered up to 64% after nutrient addition. Enrichments with limiting nutrients often restore the values of φII implying that nutrient limitation on photosynthetic energy conversion is common in the sea (Falkowski et al. 1991; Falkowski 1992).

In conclusion, evidence of phosphate and nitrate uptake by the migratory dinoflagellate *H. triquetra* in a cold environment in the absence of light was found. Some signs of improved photophysiology (after nutrient uptake in the dark) and cell growth was also apparent. A recovery of the effective photochemical efficiency after the end of the dark incubation period indicated the potential use of intracellular nutrient reserves to improve the efficiency of PSII reaction centres. Nearly constant cell concentration and relatively high effective photochemical quantum yield in the potentially nitrogen-limited cultures indicated the ability of the *H. triquetra* population to survive for extended periods in low nutrient conditions. The presented data show that *H. triquetra* populations indeed win from compromises between nutrient acquisition in deep, dark, cold layers at night and subsequent photosynthesis in the warm euphotic layer during the day in the Baltic Sea. In order to estimate the importance of upward vertical flux of inorganic nutrients induced by the migration of *H. triquetra* in comparison to the contribution of hydrophysical processes (as turbulent mixing, transport by upwelling events, etc.), further laboratory and field studies are needed.
Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by Institutional Research Funding (grant no. IUT (19-6)) from the Estonian Ministry of Education and Research; and the Estonian Science Foundation (grant no. 8930).

ORCID

Karin Ojamäe http://orcid.org/0000-0003-1809-4052
Inga Lips http://orcid.org/0000-0001-6556-6281

References


