The effect of an experimental decrease in salinity on the viability of the Subarctic planktonic foraminifera *Neogloboquadrina incompta*

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Abstract

Chemical signatures in the calcite of shells of polar and subpolar planktonic foraminifera have been frequently used to trace and quantify past meltwater discharge events. This approach assumes that the foraminifera can tolerate low salinity under extended periods. To obtain a first experimental constraint on salinity tolerance of Subarctic foraminifera, we carried out a culturing experiment with specimens of the subpolar species *Neogloboquadrina incompta* collected in the northern Norwegian Sea off Tromsø in October 2018. The foraminifera were exposed to a gradient of salinities between 35 and 25 PSU. Survival was monitored over 26 days by measuring the extent of the rhizopodial network. Although chamber growth only occurred in one of the observed specimens, likely due to the largely unknown dietary preference of the species, we observed a strong differential rhizopodial activity pattern along the gradient. The highest rhizopodial activity occurred at salinity between 35 and 31 PSU. The species is clearly able to survive long-term exposure to salinities as low as 28, but no rhizopodial activity and signs of cytoplasm degradation were observed in all specimens exposed to 25 PSU. These preliminary observations provide the first direct evidence for the salinity tolerance of *N. incompta*, indicating a range of salinity that could be plausibly expected to be recorded in the chemistry of fossil shells of the species.

Introduction

The chemical and isotopic composition of fossil shells of planktonic foraminifera is a well-established approach to investigate the past state of the ocean (e.g., Ravelo & Hillaire-Marcel 2007; Pearson 2012). For example, the oxygen isotopic signature ($\delta^{18}O$) in shells of *Neogloboquadrina incompta* has been used to infer the presence of meltwater injected into the surface ocean by icebergs (McManus et al. 1999; Came et al. 2007; Rashid & Boyle 2007; Voelker et al. 2009). The reconstructions are based on the assumption that the calcification of the shell and, therefore, the incorporation of the chemical signal occurred within the water layer affected by the discharged meltwater. This is particularly relevant in situations where the properties of the target water layer may be modified to a degree that is too hostile for the survival of the foraminifera. In this scenario, specimens of the species could be largely excluded from surface low-salinities habitat, and the oxygen-isotope composition of the remaining specimens dwelling deeper would be recording conditions below the meltwater layer, leading to a systematic underestimation of the surface salinity anomaly. Indeed, past meltwater injections in the North Atlantic likely had a magnitude sufficient to modify surface salinity below the range of naturally occurring values in the present ocean (Hemming 2004). Among the species of planktonic foraminifera occurring in the North Atlantic during these events, especially in the more distal part of the iceberg discharge plume, is *N. incompta* (Dickson et al. 2008; Voelker et al. 2009).

However, to date, no experimental data are available to constrain the range of salinities under which *N. incompta* survives and which it thus could potentially record.

Most existing experiments in which planktonic foraminifera were exposed to a gradient of environmental parameters have been carried out on tropical to temperate species (McCrea 1950; Bé et al. 1977; Bijma et al. 1990; Lea et al. 1999; Davis et al. 2017; Bertlich et al. 2018; Fehrenbacher et al. 2018; LeKieffre et al. 2018). High-latitude planktonic foraminifera have been rarely kept in culture (Manno et al. 2012), and standardized culturing protocols

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have not been established for the cultivation of these species under cold conditions (Kozdon et al. 2009; Schiebel et al. 2018). Here, we present the results from a preliminary laboratory experiment on the Subarctic planktonic foraminifera *N. incompta* with the purpose to constrain the salinity tolerance of the species. With this experiment, we aim to provide a first insight into the changes in the physiology and viability of *N. incompta* in response to different salinity conditions and introduce a novel way of monitoring its physiology, applicable in the absence of growth, by measuring the extent of its rhizopodial network.

Materials and methods

Sampling

The experiment and the microscope observations were performed in a cold room in one of the facilities of UiT—The Arctic University of Norway in Tromsø.

Specimens of *N. incompta* were collected during a cruise on the RV *Helmer Hansen* in October 2018 to the shelf and slope of northern Norway off Tromsø, specifically an area of the shelf known as Håkjerringdjupet. In the sampling area, surface water temperature ranged between 6 and 10°C. The encountered community of planktonic foraminifera was dominated by *N. incompta*, which gave us the opportunity to study the salinity tolerance of this species at the lower end of its thermal range, under conditions that can be expected to resemble those of past meltwater injections, with cold temperatures due to iceberg melting. Specimens were sampled from a water depth between 0 and 100 m, using a WP2 plankton net (63 µm mesh size) that was towed vertically. The retrieved specimens were picked on board and incubated in jars containing seawater at 35 PSU previously filtered through a 0.22 µm nitrate cellulose filter (Fig. 1) and allowed to recover for ca. 16 hr at treatment temperature (6°C).
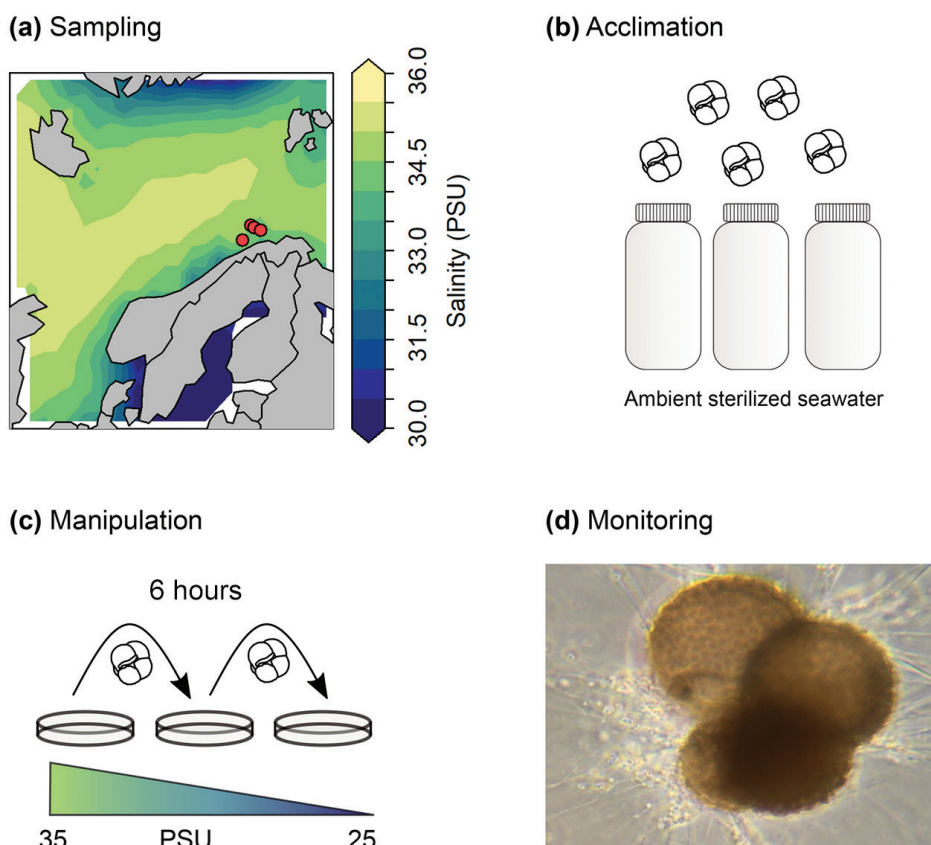
Fig. 1 Schematic representation of the different phases of the culturing procedure, from (a) the location of the sampling through (d) monitoring. Salinity values in (a) refer to the monthly average surface salinity in October, taken from World Ocean Atlas 2018 (Zweng et al. 2019).

Culture methods

Onshore, cytoplasm-bearing *N. incompta* specimens were transferred from the collection flasks into Petri dishes and, after six hours, a fraction of the specimens was transferred into new Petri dishes with a salinity lowered by 3–4 PSU to avoid osmotic shock. This acclimatization procedure was repeated from the Petri dish with lowered salinity at intervals of six hours until the minimum tested salinity of 25 PSU was reached. The tested range of salinities was chosen to reach below 30 PSU, which is the lower limit of salinity estimates in the Heinrich meltwater layers (Maslin et al. 1995; De Vernal & Hillaire-Marcel 2000). The culturing medium for the treatments (35 PSU/Control, 31 PSU, 28 PSU and 25 PSU) was obtained by consecutive dilutions of ambient seawater with MilliQ water. Salinity was measured by means of a digital refractometer. From the treatment series, cytoplasm-bearing specimens of *N. incompta* were removed and cultured individually under the treatment salinity in 75 ml Falcon flasks and constant temperature of 6°C in a cold room under eight hours light cycles (intensity of 150 µmol photons m^{-2} s^{-1} [Manno et al. 2012]). They were fed daily with 30 µl autoclaved marine microalgae *Nannochloropsis* food mix (30 µl *Nannochloropsis* concentrate: 200 ml filtered seawater), attempting to simulate a diet involving phytoplankton detritus. A population of 16 specimens in the size range of 95–203 µm was initially selected for the experiment. A larger population number was not possible with the given sampled population size and the effort associated with individual monitoring. After the introduction of the treatment gradient, one specimen was left for the individual culturing in the control (ambient) treatment (35 PSU), three for 31 PSU, three for 28 PSU and two for 25 PSU.

Analyses

The response of the individual specimens to the treatment was monitored until cytoplasm decay was observed (Fig. 2d). Cytoplasm-bearing specimens that did not display any rhizopodial net for 18 days from the start of the experiment were re-checked after day 22. The foraminifera were photographed using a digital camera attached to an inverted microscope, and the state (colour) of the cytoplasm was reported. The software ImageJ, Version 1.8.0 (Schneider et al. 2012), was used to measure the rhizopodial activity of each specimen calculated as the ratio between the maximum shell diameter and the maximum extension of the rhizopods (Fig. 3). This parameter was chosen because both measurements are largely invariant to rotation on a plane (the specimens were not floating
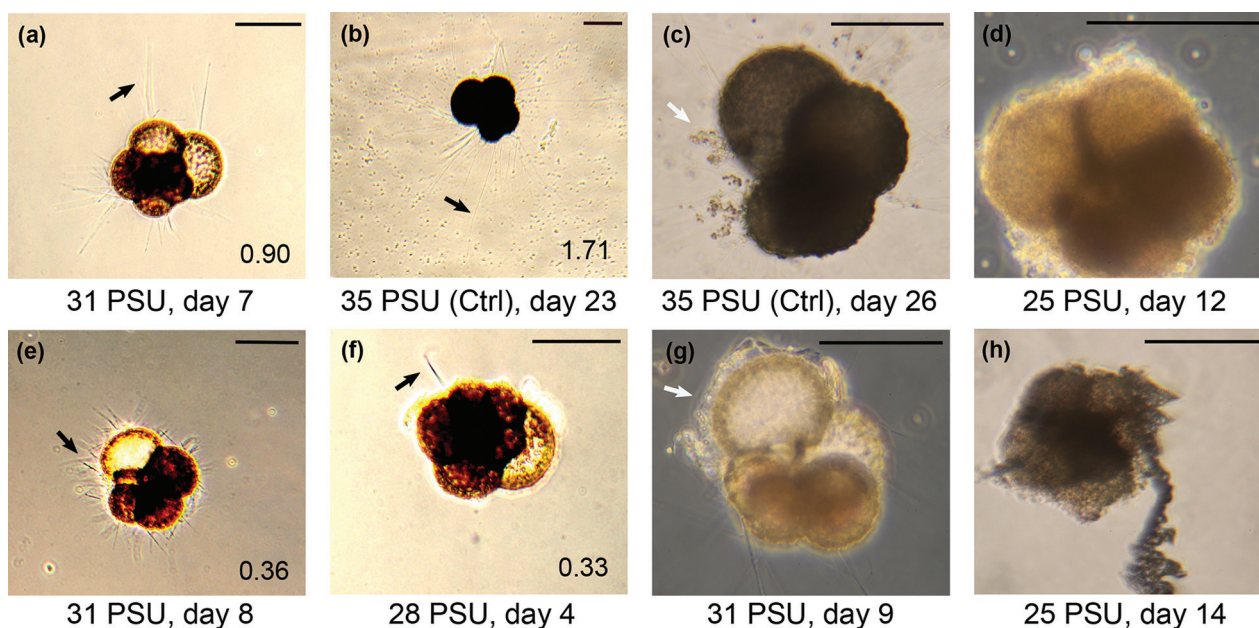
Fig. 2 Cultured specimens of *N. incompta*. Images (a), (b), (e) and (f) show specimens displaying different levels of rhizopodial activity. Black arrows indicate the r_{max} used to derive the rhizopodial activity. Contrast in the pictures has been artificially enhanced to visualize the rhizopodia. White arrows in (c) and (g) indicate respectively specimen feeding on *Nannochloropsis* and another one producing a feeding cyst. Panel (d) shows cytoplasm decaying in a specimen from the 25 PSU treatment, later followed by partial dissolution of the shell (h). Scale bars: 100 µm.
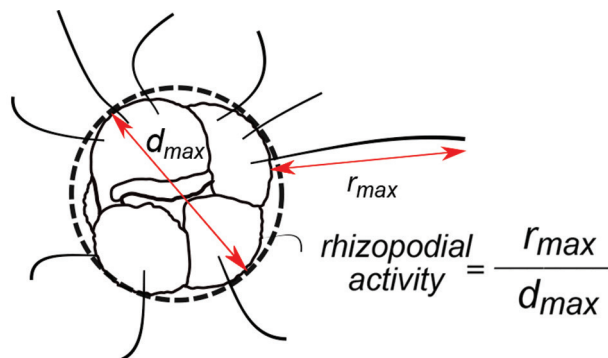
Fig. 3 Scheme illustrating the method used to derive the rhizopodial activity index in this study (see Materials and methods).

during observations) and because estimating the number of extended rhizopodial is difficult and more ambiguous than a determination of the maximum extension length. Repeated measurements on selected specimens indicate that the uncertainty on the determination of the maximum shell diameter is 3%, and assuming similar uncertainty on the rhizopodial extension, the resulting uncertainty on the index should be about 6%. After the experiment, the cultured specimens were photographed using SEM at the University of Bremen.

Given the small scale of our experiment, we decided to refrain from statistical analyses.

Results

In the control treatment, the viable *N. incompta* specimen survived for the entire duration of the experiment, displaying the highest rhizopodial activity registered (1.71; Fig. 4). In the 31 PSU treatment, one specimen survived until day 3, formed a kummerform final chamber and showing signs of shell thickening (Fig. 4). The remaining two specimens showed rhizopodial activity until days 18 and 24, respectively. The overall average rhizopodial activity was lower in this treatment than in the control (Fig. 4b). The same applies to the average activity for all observations when extended rhizopodia were observed (Fig. 4c). At the 28 PSU, two of the specimens stopped displaying rhizopodial activity after day 12 and later showed signs of cytoplasm decay. Only one specimen survived until the end of the experiment. The overall average rhizopodial activity, as well as the average activity for all observations when extended rhizopodia were observed, was the lowest (Fig. 4). None of the specimens cultured at 25 PSU showed rhizopodial activity during the experiment and both specimens showed signs of cytoplasm decay after day 15 (Fig. 2d).

Discussion

Our observations indicate that *N. incompta* rhizopodial activity decreases on exposure to salinity from 35 to 28 PSU, but survival under an extended period of time (weeks) is possible within this salinity range, whereas it appears that extended exposure to 25 PSU is lethal. There are no earlier experimental observations on the salinity tolerance of this species, and ambient salinities in the modern ocean where planktonic foraminifera occur, even in the Arctic where the lowest salinity conditions are expected, are always >29 PSU (Greco et al. 2019). However, Bijma et al. (1990) presented data on salinity limits of the related species *Neogloboquadrina dutertrei*. Although these authors used a different definition of viability based on growth, they observed that the vital processes of the tested specimens of *N. dutertrei* were completely inhibited at 25 PSU. This observation agrees with our results on *N. incompta*. It is important to note that the ability of *N. incompta* to survive under reduced salinities under laboratory conditions does not necessarily mean that it will inhabit a similarly low-saline meltwater lens in the natural environment. Indeed, laboratory experiments can only constrain the maximum range of salinities under which survival in the field may occur.

In the present experiments, one of the cultured specimens showed signs of chamber formation and thickening under the light microscope. As no calcification label was added to the culture seawater, we confirmed the observation by subsequent analyses of the recovered shell using SEM. This revealed the addition of a kummerform chamber and of shell-thickening by secondary calcification (Fig. 5). Both observations are consistent with the normal behaviour prior to gametogenesis in planktonic foraminifera (Hemleben et al. 1989). This indicates that the laboratory conditions in our experiment did not preclude growth or calcification and, therefore, the termination of its natural life cycle explains why this specimen died so early despite exposure to the non-lethal salinity level of 31 PSU. In the light of this observation, it remains unclear why the remaining specimens in our experiment survived but did not grow.

A possible explanation may lie in the low cultivation temperature of 6°C. Indeed, the few previous culturing studies on *N. incompta* grown under different temperatures reported no growth in specimens cultured at 6°C, but growth occurred at 9°C (Von Langen et al. 2005; Davis et al. 2017). Unfortunately, both culturing studies were carried out in the Pacific, which is inhabited by a different cryptic species of *N. incompta* (Darling et al. 2006), making it difficult to directly
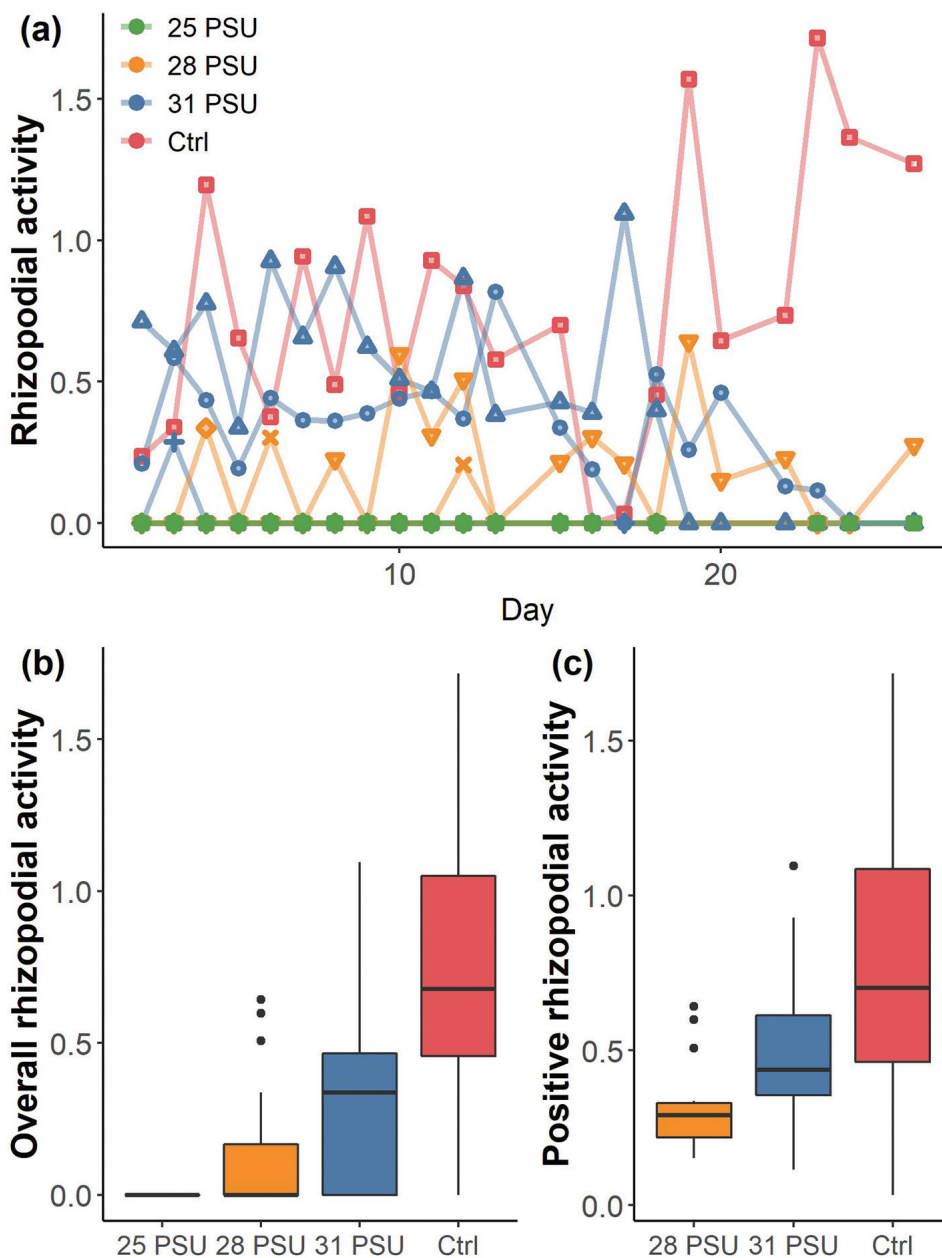
Fig. 4 (a) Individual and (b, c) overall rhizopodial activity observed during the experiment in the different treatments. Symbols in (a) refer to the different specimens; colour indicates the salinity treatment.

transfer these observations on the North Atlantic species. Alternatively, it could be that the autoclaved *Nannochloropsis* used for feeding the cultured *N. incompta* does not represent a suitable food source for this species. In the previous experiments, the cultured *N. incompta* specimens were fed with freshly killed *Artemia* (Von Langen et al. 2005; Davis et al. 2017), but recent molecular investigations revealed that this species may feed on bacteria (Bird et al. 2018). With the food preference of this species is unknown (*Artemia* cannot be the natural prey and has been taken as a substitute for marine copepods), we opted for autoclaved *Nannochloropsis*, assuming that it emulates the likely available food found below the sunlit layer (*N. incompta* is a subsurface species [Rebotim et al. 2017]) and considering that it was found to be accepted by other foraminifera (Schmidt
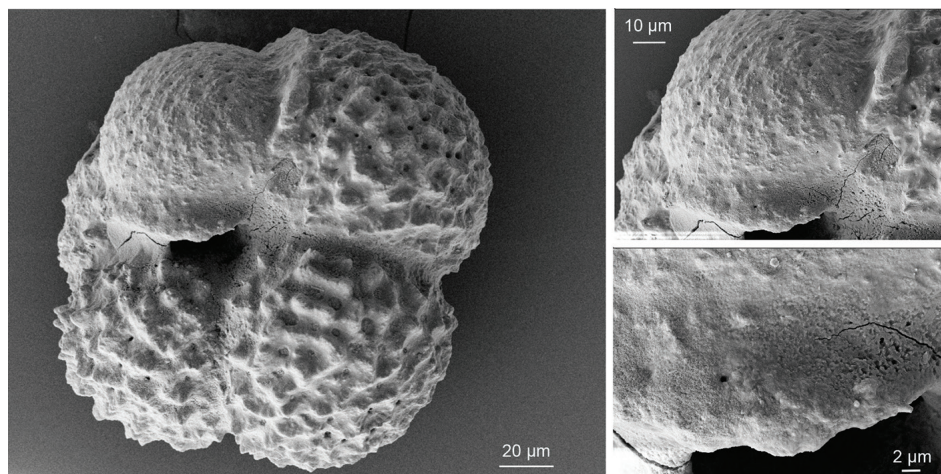
Fig. 5 Scanning electron microscopy images of the cultured specimen of *N. incompta* from the 31 PSU treatment that formed a kummerform chamber and died (presumably after completing its life cycle) after four days in culture.

et al. 2015). We observed that the autoclaved *Nannochloropsis* was accepted by *N. incompta* and collected by its rhizopodial network (Fig. 2c, g), forming a feeding cyst (Spindler et al. 1984; Hemleben et al. 1989; Heinz et al. 2005; Bird et al. 2018), but it is possible that either the quantity or quality of the food was insufficient to facilitate shell growth.

Conclusions

Our study provides first experimental and preliminary evidence for physiological stress in *N. incompta* with decreasing salinity under "polar" conditions. We show that the species survives extended chronic exposure from 35 to 28 PSU, and we interpret the complete absence of extended rhizopods at 25 PSU as evidence for physiologically lethal conditions. Our experiment indicates that quantification of the extent of rhizopodial activity may be an effective measure of physiological health, which can be used even in situations and at timescales where no shell growth occurs. Because of the small number of specimens investigated, these conclusions require validation by further experiments, but the preliminary results provide a context for assessing the salinity tolerance of this species and can serve as a basis to better interpret the palaeoclimatic reconstructions based on fossil shells of *N. incompta*.

Data availability

Data in support of the findings are available on figshare at https://doi.org/10.6084/m9.figshare.11309627.v1.

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