GAME - Effects of microplastic particles on the stress tolerance of marine deposit feeders

GAME ist ein internationales Trainings- und Forschungsprogramm, in dessen Rahmen in jedem Jahr Studien zu einer anderen ökologischen Fragestellung durchführt werden. Dies geschieht an bis zu neun Küstenstandorten zeitgleich auf der Nord- und Südhalbkugel, wobei die praktischen Arbeiten von unseren Teilnehmern unter Anleitung lokaler Wissenschaftler ausgeführt werden.

Final report on the 11th GAME project:
Effects of microplastic particles on the stress tolerance of marine deposit feeders

1.0 Introduction

In addition to the deposit of nutrients, heavy metals and harmful organic substances, it is primarily plastic litter that has led to high levels of pollution of the seas by humans during the last 60 years. Estimates assume that every year between 6.4 and 27 million tonnes of plastic reach the oceans (Rochman et al. 2013a). The largest proportion (approx. 80%) is of terrestrial origin and is brought to the sea in rivers and by the wind (Andrady 2011). These deposits are primarily a widespread problem in locations where litter flows are not closed off or rubbish depots are not sufficiently secured. A good 20% of plastic litter occurs on the sea itself, for example when fishing vehicles lose or abandon nets and other items of equipment (Andrady 2011). As most of the plastic polymers used have a lower density than seawater, this litter can initially remain on the water's surface for a long time and drift over long distances. This is how plastic litter also reaches those islands and sections of the coast that are a long way from any civilisation (Hidalgo-Ruz & Thiel 2013). Transport through flow systems also has the effect that the litter is not spread evenly over the seas. Whilst an estimated 15% of litter collects on the coasts, an additional, probably similar proportion is caught by the oceanic circulating currents and forms areas there with a very high density of plastic items. These are the well-known "trash vortexes" or Oceanic Garbage Patches (Martinez et al. 2009). The remaining marine plastic litter – in other words a good 70% of the total material found in the seas – has very probably already sunk and is located on the sea floor (e.g. Bergmann & Klages 2012).

In addition to horizontal and vertical transport over long distances, the plastic litter is also broken down in the sea. This occurs on the one hand through photo-oxidative and chemical decomposition processes and on the other hand through friction and the pounding of the waves. This continually fragments the plastic until large items have become microscopic particles. These are also increasingly accumulating in the seas' sediments and can now be found on many sedimentary coasts but also in deep seas (Hidalgo-Ruz et

Image 1: The locations of the 11th GAME project.

al. 2012, Cauwenberghe et al. 2013). Whilst the partly dramatic effects of macroplastic on marine organisms are already relatively well-known, the consequences of the pollution of sea sediments with microplastic on the animals living in these eco-systems are largely unclear. Some of the few investigations from the period before the start of this project had shown that benthic filter-feeders, such as mussels, but also deposit feeders, such as the lugworm Arenicola marina, ingest microplastic particles with their food (Ivar do Sul & Costa et al. 2013). What effects this has on the physiological performance of these animals, for example on their tolerance of environmental stress, and on their reproductive success was however completely unclear.

There are several scientific models on how the ingestion of very small plastic particles can harm invertebrate animals in the sea. On the one hand, if the latter occurs in high densities, they can clog the filter mechanisms, gills, siphons and/or gastrointestinal tract of these organisms and lead to death through starvation or choking. It is also suspected that plastic fragments can collect in the stomachs of such animals and lead to a feeling of satiety, which means that the animals stop feeding. This would also lead to them becoming weaker and ultimately death from starvation. In addition, it is well-known that harmful organic substances such as oil residues or pesticides accumulate on plastic materials. They accumulate there because plastic represents a water-repellent environment in an otherwise polar medium. Depending on the harmful substance and type of polymer, the size of the accumulation can reach 1:1 million compared with the surrounding seawater (Mato 2001). This is how plastic fragments become harmful substance vectors. If these are ingested by animals, part of the harmful substance load can desorb in the gastrointestinal tract and then reach tissue (Bakir et al. 2014). An observational study of seabirds, which are known to ingest large amounts of macroplastic with their food, has shown that they display a sharp rise in pollutant loads (Teuten et al. 2009). This indicates that this mechanism does in fact play a role.

This scenario and the fact that there is hardly any empirical data on the effects of microplastic on marine invertebrates, were the starting point for the 11th GAME project. The pollution of the seas by microplastic primarily affects organisms that are at the bottom of the food chain. This points to the fact that this phenomenon can have great ecological relevance. In addition, numerous important ecosystem functions such as water purification or the aeration of sediments often depend on the bioactivity of benthic invertebrates. Everything which has a detrimental effect on these performances has the potential to permanently change marine ecosystems.

In an experimental approach the students participating in the 11th GAME project investigated the influence of contaminated microplastic on various species of deposit feeders at 8 locations. Special emphasis was placed on ensuring that the experiments reflected realistic conditions and did not overestimate the contamination by microplastic as well as the latter's level of pollution. In preparation for this, a literature study was carried out, which summarised the existing knowledge about the pollution of coastal sediment with microplastic particles.

2.0 Materials & Methods

2.1 Locations

The 11th GAME project was undertaken at a total of 8 locations worldwide. These were Coquimbo in central-northern Chile (South Pacific), Niterói on Guanabara Bay in Brazil (South Atlantic), Puerto Morelos on the Yucatan Peninsula in Mexico (Caribbean), Funchal on Madeira, Portugal, Menai Bridge/Menai Strait in Wales, Hanko in southern Finland, Akkeshi on Hokkaido, Japan, and Bogor on the Island of Java, Indonesia (Image 1). The binational student teams were supervised at these stations by local scientists. In 2013 the working group with Prof. Dr. Vivianne Solís-Weiss from the Universidad Autónoma de México (Autonomous University of Mexico) participated in a GAME project for the first time. At each site various species of deposit feeders from different groups of organisms were investigated initially to see whether they ingested the plastic material selected for the experiments (see below). One species was then selected per site from this pool of possible organisms for the experiment.

2.2 Microplastic monitoring

In order to obtain an impression of the pollution with microplastic already in existence at the locations, sediment samples were taken at all the stations and investigated through optical methods for plastic fragments. For this purpose in the habitats, from which the experiment organisms that were used later also originated (see below), 3 x 5 short core samples were taken. Depending on the site and type of the experiment organism, these habitats were located in sub- or intertidal areas (Image 1). In addition, at all the locations the same number of cores was taken at the high tide line in order to obtain a comparative value. The sediment cores were then washed in the laboratory with a hyper-saline salt solution (1.2 g NaCl per ml of tap water) and the remainder then put in a 500 µm sieve in order to retain all the components up to a size fraction of 500 µm (see Hidalgo-Ruz et al. 2012). This residue was then viewed under a stereo microscope and all the plastic fragments it contained with a size of 500 – 5000 µm were counted and assigned to one of 6 categories: fibres, foils, foams (Styrofoam), fragments, pellets or balls (Image 3).

2.3 Plastic material and harmful substances

Before the start of the practical work in April 2013 all the participating students met for a one-month introductory course at GEOMAR in Kiel, as part of which the methodology for the global experiment was laid down. Already in the preparatory stage one plastic material suitable for the experiments was identified, namely polystyrene pellets, as used for the production of Styrofoam. They have a diameter of 700-900 µm and contain inclusions of pentane, which serves as a gas propellant when the material expands. Polystyrene was particularly suitable for the intended experiment as it has a greater density then seawater and therefore sinks and accumulates in sediment. At the same time the pellets are of a size corresponding to that of the food spectrum of many marine deposit feeders.

Image 2: Collecting sediment samples at the Coquimbo site in Chile. The samples were filled into transport containers on site and processed further in the laboratory.

In order to load the plastic material with harmful substances, the pellets were incubated in seawater at the various stations for two weeks. An unpolluted site and a site polluted anthropogenically both close to the marine biological stations were selected for this in each of the participating countries. This selection was made on appearance, whereby locations close to leisure boat or industrial ports were in principle classified as polluted. The comparable locations were often in protected areas, which are largely free of human influence. The plastic pellets were then filled into sealable tea strainers made of stainless steel and briefly fixed under the surface of the water by means of a floating structure and guy lines.

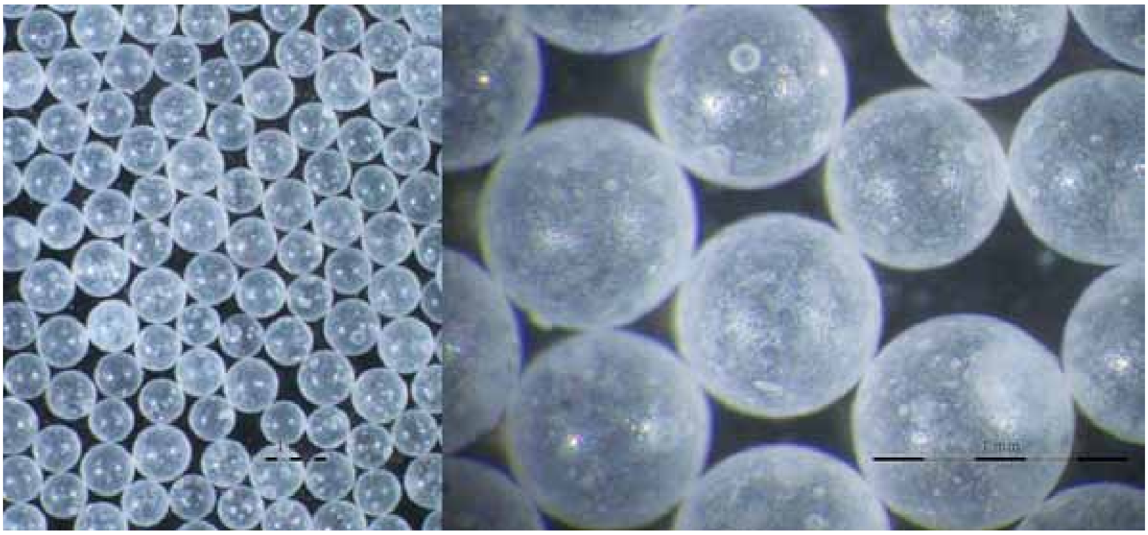

Image 3a+b: Small polystyrene balls, the initial material for Styrofoam production, were used in the experiments as model microplastic. They had a size of 700-900 µm and contained gas inclusions.

2.4 Chemical analysis

Evidence of harmful organic substances, above all in low concentrations, is methodically very laborious and therefore evidence was provided of only one single compound in the laboratory as part of GAME. This is phenanthrene, an aromatic hydrocarbon, which can be found in crude oil as well as oil or fuel residues. It is a widespread harmful substance, which is known to have a negative effect on aquatic organisms. The concentration of this substance in seawater, on the plastic pellets, as well as in the tissue of the experiment organisms was determined at the end of the study in the laboratories of the Institute for Toxicology and Pharmacology for Natural Scientists at Christian-Albrechts-Universität zu Kiel (CAU - University of Kiel). In terms of methodology, this took place via high pressure liquid chromatography (HPLC) with evidence of fluorescence.

2.5 Experiment organisms

Overall in pre-experiments 29 types of deposit feeders from a total of 9 taxonomic major groups were tested to see whether they ingest the plastic pellets selected for the experiments. This was the case for twelve species, and 9 of these were finally selected for the experiments. These were the lugworm Arenicola marina (Wales), the Pacific lugworm Abarenicola pacifica (Japan), the sea cucumber Holothuria sanctori (Madeira), the Baltic tellin Macoma balthica (Finland), the two types of echiuran worms Ochetostoma baronii and Urechis chilensis (Chile), the serpent star Ophiocoma echinata (Mexico), the mangrove whelk Terebralia palustris and the mudflat fiddler crab Uca rapax (Brazil). On the one hand, these species are differentiated by the particle size that they ingest, and on the other hand with regard to their selectivity. Whilst most of these species tend to feed rather unselectively, the tellin, which pipettes particles with its siphons from the sediment surface, grabs particles in a very targeted manner. Also the mangrove whelk appears to feed rather selectively. All these species assume important ecosystem functions in their habitats and an adverse effect on their performance through pollution of the environment is likely to have far-reaching consequences for the entire ecosystem.

2.6 Exposure experiments

In the exposure experiments individual specimens of the various animal species were exposed to a density of 400 polystyrene pellets per kilogramme of sediment dry mass in the laboratory over a period of two months. In accordance with the various pre-treatments, there were two experimental groups: a) sediment with pellets, which were incubated in seawater free of harmful substances and b) sediment with pellets that were incubated in polluted seawater. There was also a reference group, in which the animals were husbanded in sediment without microplastic. The husbandry of the animals took place individually, in basins whose dimensions were adjusted to the size of the relevant animals. The latter were supplied with oxygen via air pressure systems and air outlets and the water in the experiment basins was also either replenished continuously – via a circulation filter or a through-flow system - or manually on a regular basis. In order to rule out that metabolic waste products accumulated in the basins, despite changing the water, the ammonium content of the water was checked regularly.

Image 4: The experiment structure in the laboratories of the School of Ocean Sciences in Wales. There was a lugworm in every container.

During the exposure experiment the animals were fed in accordance with their nutritional requirements. The nature and quantity of the feed was geared to the relevant animal species and size of the experiment individuals. At the start of the experiment the animals were also acclimatised to the laboratory conditions over a period of one week. In order to prevent the harmful substances bound to the plastic in the basins declining over time through desorption or decomposition, the plastic pellets in the basins were replaced on a regular basis. New sediment filled with plastic was added to the basins for this, merely by adding a layer of new sand over the existing sand. Such an event was well-tolerated by the animals because it corresponds to the effect of a storm, during which sediment is moved about and deposited in a different place. At the start of the exposure experiments the wet weight of all the individual animals was determined and if possible the length of the animals (maximum elongation). During the experiments the production of faeces was also determined for all the species where this was possible.

2.7 Tolerance to oxygen deficiency

The main response variable for this study, which was recorded at all 8 stations and for all species, was the tolerance of the animals to oxygen deficiency. This is a stress factor, which occurs periodically in many benthic systems on earth. As a result, the area affected by this worldwide is constantly increasing, which in most cases is based on rising nutrient loads. This means that a lack of oxygen is another facet of the global change in marine ecosystems. Lethal effects of microplastic or the harmful substances bound up with it were not expected during the short period of time available for the experiment and with the extremely realistic concentrations of plastic and harmful substances. Therefore the aim was to draw conclusions about the negative effects of pollution indirectly through changes to the stress tolerance of the animals. The harmful substances adhering to the plastic can lead to poisoning to which the animals react with detoxification processes. The energy used for this should then no longer be available for the physiological response to the oxygen deficiency stress and the oxygen deficiency tolerance should decline. In order to record such an effect, the survival period of the animals under oxygen deficiency was used as the main response variable. Oxygen was removed from the surrounding water through ventilation with nitrogen until the concentration fell below 2 mg/l. The experiment containers were then sealed. In order to prevent the accumulation of metabolic waste products, the water was regularly replaced with seawater that had already been deoxygenated.

3.0 Results

3.1 Microplastic monitoring

The density of microplastic particles varies both between as well as within the various investigation areas. The lowest densities were found in the experiment sample sites in Finland, Japan and Portugal; here the densities found were always below 10 particles per kilogramme of dry weight of sediment. In Brazil, Mexico and Wales the density varied between 10 and 50 particles per kilogramme of sediment, whilst it was highest in Indonesia with over 70 parts per kilogramme of sediment. Almost everywhere the samples from the sub- or intertidal area contained more plastic than at the high tide line, only in Mexico was the subtidal zone completely free of plastic (Image 5a). The data from Chile was not yet available at the time the report was compiled. Overall the density of microplastic particles correlated very well with the abundance of macroplastic: at relatively clean locations such as Finland, Japan and on Madeira (Portugal) where there is also little plastic litter, pollution with microplastic was low. Also the type of microplastic particle varied between the different regions and like the density of the particles seems to be heavily dependent on the local circumstances. For example in Indonesia foil residues were found in particular, which probably originate from packaging materials, whilst in Japan, Portugal and Wales fibres from fishing nets and ropes dominated. On the other hand in Brazil and Mexico, where there is a lot of plastic litter that has been thrown away carelessly on the beaches, the most common form of microplastic was pieces of plastic bottles and other containers (Image 5a+b).

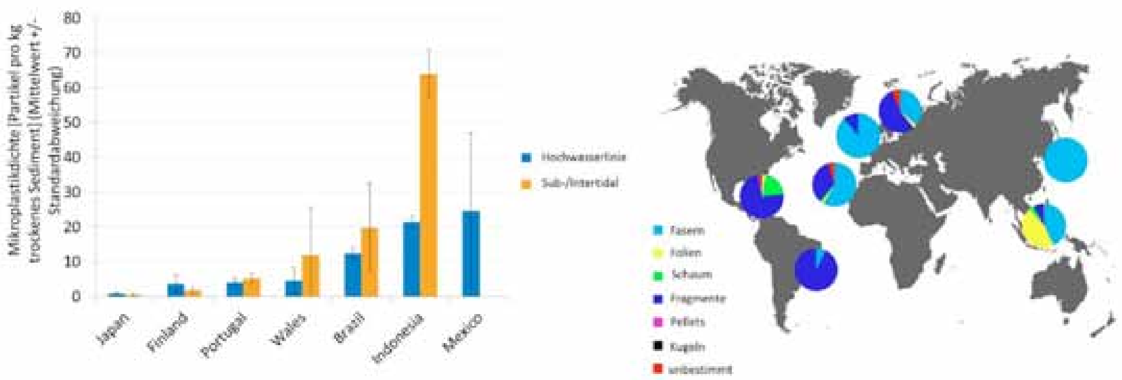

Image 5a+b: Results of microplastic monitoring. Left the density of the microplastic and right its composition at the individual locations.

3.2 Influence of microplastic on oxygen deficiency tolerance

All the species investigated as part of the project continually ingested microplastic particles during the 2-month exposure tests. This was verified by regular checks of faeces. The precise rates of ingestion could however not be determined for logistical reasons. However, as the plastic balls used were distributed evenly in the sediment and most animals did not display selective food ingestion, it can be assumed that the ingestion of microplastic was proportional for all species to the total amount of sediment ingested.

In the final experiments on oxygen deficiency tolerance there were no statistically significant effects of plastic pollution on the survival of the animals. There is also no role played in this regarding where the plastic was incubated previously. Neither the plastic incubated at an unpolluted location nor the material from the polluted location probably loaded with more harmful substances had negative effects on the stress tolerance of the animals investigated. Although some species showed trends – these were however not homogenous and indicated a possible positive or negative effect of the plastic depending on the species. Correspondingly the joint analysis of all the data collected also shows no pointed effect. The Kaplan-Meier survival curves of the various experiment groups (Image 6a+b) run uniformly and no differences can be observed. The statistical assessment was also correspondingly insignificant.

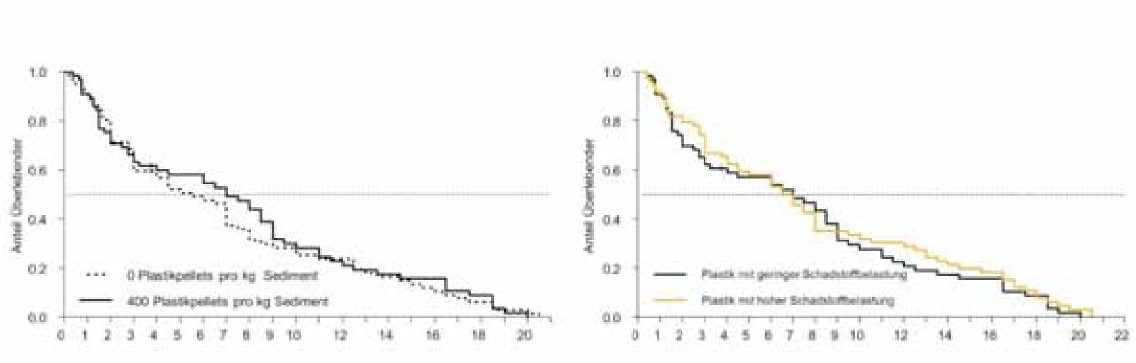

Image 6a+b: Results of the stress tolerance experiments. After the animals had been subjected to an environment polluted with plastic for 2 months, their survival was investigated under an oxygen deficiency.

4.0 Discussion

The monitoring carried out by the GAME participants again showed clearly that microplastic is extremely widespread in marine habitats. Only at the site in Mexico could no evidence of microplastic be found in the subtidal zone sampled there. This may be associated with the fact that the deposit of macroplastic here takes place above all via the beaches and less from the sea. However, microplastic existed at all the other locations, but the densities were partly well below the values that were found as part of other studies (e.g. Carson et al. 2011). For example the density of 400 particles per kilogramme of sediment used as the basis of the exposure experiments owing to literature values was higher by a factor of 4 to 40 than the microplastic densities, which were actually found at the various locations. It must be stated in connection with this that no direct comparison is possible with other studies as the locations sampled by us have previously never been investigated with regard to their contamination with microplastic. In terms of methodology, the procedure selected for GAME was not very different to the methods used in the other studies, so that it is not likely that the particle densities were systematically underestimated. The abundance and composition of the microplastic correlated very well with the overall pollution of the locations. This indicates that the particles found are of autochthonous origin and were not carried over long distances by the sea. The latter scenario is known from deep sea locations such as Hawaii or Easter Island, where large amounts of allochthonous plastic material can be found which was carried over long distances by the ocean currents (Carson et al. 2011, Hidalgo-Ruz & Thiel 2013). Overall pollution with microplastic can be forecast very accurately for the coastal locations selected for this study.

There was no evidence for any of the species used by us of any natured effect of microplastic on physiological performance (tolerance to oxygen deficiency). The environment in which the microplastic was previously incubated also played no role in this. Our assumption was that plastic, which had been incubated for two weeks at a polluted location, subsequently has a higher load of organic harmful substances than plastic had been incubated at an unpolluted location. A direct verification of this assumption by using chemical evidence was possible only for phenanthrene. However, these analyses showed that the assumption is in principle correct: phenanthrene could be found more frequently on the material from the polluted locations than on the plastic from the unpolluted locations. The quantities were also greater. In addition, the balls from the polluted locations often displayed considerable colouration, whilst the material from the unpolluted locations had not changed in colour (Image 10).

How should the absence of effects in our experiments be interpreted? The data from the monitoring suggests that pollution with microplastic particles in the experiments has overestimated the actual levels of pollution. However, it must be remembered that the experiments with a duration of only 8 weeks were relatively short. Most of the species investigated by us live for several years and are subject to continuous contamination with microplastic throughout their lives. It can therefore not be deduced from our experiments that pollution of benthic habitats with microplastic causes no problems for the organisms living there. Various other short-term studies, which were published last year, have found evidence of negative effects of contaminated microplastic on fish and also on benthic invertebrates (Rochman et al. 2013b, Wright et al. 2013). When interpreting these results it must however be noted that the plastic densities selected by the investigators were many times higher than those used in the GAME study. Our experiments have therefore shown that under conditions that are as realistic as possible no such effects are to be expected over a period of 8 weeks. However, this does not rule out the existence of long-term effects. For example as part of this study, owing to the limited technical facilities, it could not be clarified whether in the test period damage had already occurred at cellular level. This could have an effect on the physiological performance, growth and ability to reproduce of the animals in the long term. It would therefore be desirable to undertake tests which cover as much of the lifespan of benthic invertebrates as possible and which record the possible effects both at a cellular as well as an organic level. From the data that has been available so far it is very difficult to derive forecasts whether and how the current pollution of coastal habitats by microplastic will have an adverse effect on the animals living there in the long term. A work published at the end of last year on the influence of microplastic on the energy reserves of the lugworm Arenicola marina (Wright et al. 2013) indicates that negative effects on the bioactivity and reproductive success of these animals can be expected, at least with high levels of pollution of sediments with microplastic. However, it should be considered that the particle density selected for this study was higher by a factor of 500 than that selected by us. It therefore overestimates the actual pollution through microplastic – at least if based on the particle density found as part of the GAME project – by a factor of 2,000 to 20,000. This makes it clear that at present studies are above all required which investigate the influence of realistic particle densities over a longer period.

Image 7: After the microplastic balls had been incubated for 2 weeks in the sea, the material at the polluted locations mostly showed considerable colouration.

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Image 8: Participants in the 11th GAME project in front of the pilot house in Schleimünde: 1st row from the left: Sarah Piehl, Vanessa Rüttler, Angelica Amaya Márquez, Yuliana Syamsuni, Ulrike Grogoll, Jenni Grossmann, Shasha Wang (Guest), Erica Ferreira; 2nd row from the left: Jonas Martin, Valeria Hidalgo-Ruz, Kento Matsuo, Dennis Brennecke, Filipa Paiva, Markus Franz, Juliane Jacob.

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