Project description: *Environmental impact of nanoplastics from fragmentized consumer plastics*

Aim

This project aims to fill existing knowledge gaps regarding the production of nano-sized plastic particles during the breakdown of larger plastic material in the aquatic environment and handling of plastic waste. The project will also describe the environmental impact of these naturally occurring nanoplastics and evaluate the usefulness of commercially available model nanoplastics for standardized testing. By means of detailed characterization we will provide knowledge regarding how size, shape and type of plastic material may affect the ecological consequences of exposure to breakdown nano-sized plastic particles.

Specific goals

1. Produce breakdown nanoplastics from consumer plastics and characterize their parameters.
2. Identify particle characteristics that can be related to a higher risk of causing negative biological effects.
3. Compare acute and long-term sublethal effects of breakdown plastics with commercial standard nanoplastics in relevant environmental conditions.
4. Create a toxicity map with consumer plastics that break down into nanoplastics with specific characteristics important for toxicity.

Limitations

This project will be limited to investigating effects caused by exposing freshwater organisms to nano-sized plastic particles. The project will mainly focus on nano-sized plastic particles, defined as plastic particles which have at least one dimension below 100 nm. However, the particles investigated in the project will have an overall size distribution containing also larger fractions of microplastics (defined as particles <5 mm in size) as these also will be included in the samples where plastic is broken down into smaller particles.

Research plan

This project aims at investigating current knowledge gaps regarding the exposure to lethal and sublethal concentrations of nanosized plastic breakdown products in the aquatic environment. By using nanoparticles (NPs) produced during the natural degradation of plastics at sublethal concentrations we will be able to focus on more realistic exposure scenarios as both particle characteristics and concentrations will likely be more similar to those expected to be found in nature.

Work package outline

The proposed project is divided into four work packages (WPs) in order to assess the potential breakdown of plastics into nanosized fractions and assess the biological risks these materials may pose to aquatic wildlife and other organisms exposed to nanoplastics in water.

WP 1) Characterization of nano-sized plastic particles produced during breakdown of macro plastics

Work package one focus on improving our understanding regarding the production and analysis of nano-sized plastic particles during the breakdown of macro plastics. Photochemical breakdown of polystyrene into nano-sized particles has been reported (Lambert and Wagner, 2016, Gigault et al., 2016), and we have results showing that exposing styrene foam to harsh mechanical force results in the production of nano-sized PS particles, see Figure 1 (Ekvall et al., submitted). To produce breakdown nanoplastics, plastics of different types, including e.g. polyethylene, polystyrene, styrene foam, biodegradable plastics and plastics weathered in waste deposits, will mechanically be degraded in two different ways: A harsh fast method and softer, long-term method that more resembles natural conditions. In the fast method, the plastics will be cut with blades in food blenders (see figure 1), or the plastic will be forced through narrow spaces using a homogenization apparatus. The formed nano-sized particles will be separated by filtration through 400 nm filters or smaller. The isolated nanoparticles can be further broken down or chemically modified by exposure to ultraviolet (UV) radiation. Using the softer long-term method, the plastics will be subject to constant movements and UV radiation for an extended time period, up to 12 months. Furthermore, the formed nano-sized particles will be compared with commercially available nanoplastics, commonly used in nanoparticle toxicity studies, in order to determine how well these particles reflect nanoplastics produced during plastic degradation. This knowledge will be vital for our understanding of how nanoplastics are formed in nature and will give insight in how well commercially available model nanoplastics resembles naturally degraded plastic particles.

Formed and commercially available manufactured nanoparticles will be thoroughly characterized for size, surface charge and morphology. Dynamic light scattering (DLS), Nanoparticle tracking analyses (NTA), Differentiated sedimentation centrifugation (DSC) and size exclusion chromatography coupled to DLS will be used to determine the size of the formed particles. The methods are based on different physical features and complement each other regarding the size range, concentration range, and the how well they analyze samples with complex size distribution. The surface charge will be estimated by determining the Z-potential and by classical acid base titrations. The morphology will be determined by TEM and three dimensional cryo-TEM. The composition of the formed nanoplastics as well as other chemicals released during the breakdown process will be determined by UV spectrometry and ATR-FTIR. All instruments are available and have been tested in preliminary studies performed in preparation for this proposal. This combination of methods will make it possible to develop characterization of nanoplastics beyond the state of the art. This may lead to that characterizing nanoplastics in waste plant leachate will be possible.

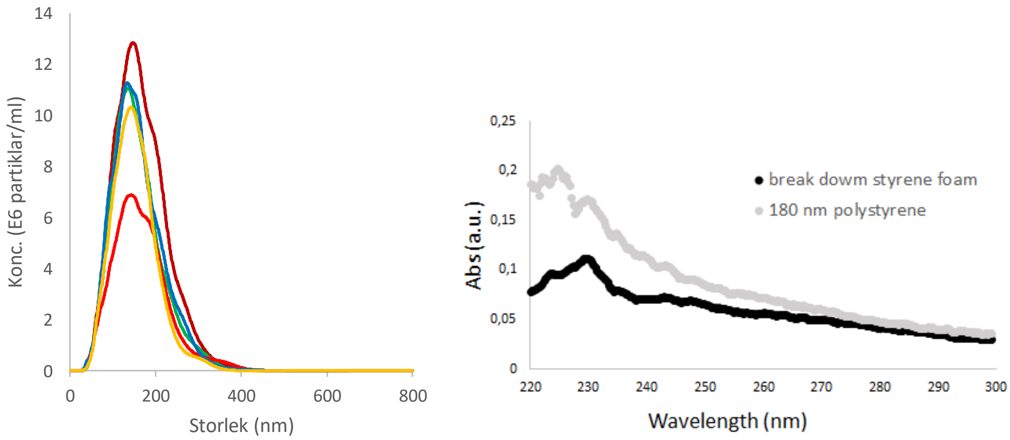

Figure 1: Left panel: The size of nanoplastics after mechanical breakdown of styrene foam determined by NTA. Right panel: Absorbance spectra of commercial 180 nm polystyrene particles and breakdown products from styrene foam. The peak around 230 nm is characteristic for polystyrene, whereas the broad should at longer wavelengths indicate the presence of other substances. Adapted from Ekvall et al., submitted manuscript.

WP 2: Acute biological effects caused by exposure to nano-sized plastic particles

All studies of the effects of nanoplastics on aquatic organisms, and as far as we know, on cells and mammals, have used model polystyrene nanoparticles. However, despite being well defined in terms of size and surface modification, it is not known how well these particles represent those that are produced during the breakdown of macro plastics. In WP2 we will use the particles formed and characterized from WP1 and the characterized commercial model nanoparticles in order to evaluate acute biological effects caused by exposure to these particles on freshwater organisms. Using particles formed from different types of plastics and of different sizes and shapes will allow us to identify factors that are important in determining the biological effects of nanoplastics. Comparisons with commercial nanoplastics will answer the question about how useful these manufactured commercial particles are in standardized acute toxicological tests.

The freshwater zooplankter *Daphnia magna* will be used as model organism and be exposed to the nanoplastics in acute toxicity tests (24-72 hours exposure). After exposure, we will evaluate acute lethal effects (mortality) as well as sublethal behavioral effects. Behavioral effects will be evaluated using a technique where *D. magna* is labelled with fluorescent nanoparticles (Ekvall et al., 2013). Labelling of the organisms allow for detailed individual behavioral tracking in 3D without affecting the natural behavior of the organisms (Bianco et al., 2013, Ekvall et al., 2013). Concentrations used in the acute toxicity test will likely be much higher compared to those we would expect to find in nature. High concentrations will affect the ultimate size distribution of the added particles due to aggregation of particles and flocculation with algae and other organic matter. The characteristics of the particles will be carefully followed with the methods described in WP1. However, using higher concentrations in an initial screening for potential toxic effects will allow us to determine which factors (i.e. plastic type, size and shape) that may be most important in determining the biological effects of plastic NPs. The data obtained in the acute toxicity test will allow us to identify which test factors that should be prioritized for further testing for sublethal and accumulation effects at lower concentrations and longer exposures, conditions resembling a natural exposure scenario (in WP3).

WP 3: Effects of long-term exposure to sublethal concentrations of nano-sized plastic particles

Although the concentrations of nanoplastics in natural ecosystems are expected to be, with few exceptions, very low, previous studies show that they are very potent and may even pass the blood-brain barrier in vertebrates, causing severe damages (e.g. Mattson et al. 2017). Hence, it is of utmost importance to now develop methodology to identify sub-lethal effects at lower concentrations of nanoplastics during longer exposure times, i.e. mimicking the exposure situation in natural ecosystems. Hence, within WP3 we will focus on exposure scenarios at low, sublethal concentrations during long time (weeks to months) which allow for accumulation of nanoplastics and reproduction studies. We focus our testing on the particles produced in WP1 and scenarios identified in WP2 to perform tests at semi-natural conditions. Tests will be performed using *D. magna* and one of its natural fish predators (Crucian carp, *Carassius carassius*).

Zooplankton is an important link in the transfer of energy in the aquatic food-web, linking primary producers and top consumers. To quantify how populations of zooplankton can be affected by exposure to nanoplastics, *Daphnia magna* will be exposed to sublethal concentrations for a period of 21 days. The nanoplastics will be administered together with alga during the feeding of the *Daphnia*. During this period, we will monitor survival, behavior and reproduction of the organisms in order to gain insight in how long-term exposure at sublethal concentrations can affect population growth in a common freshwater zooplankter. We are presently developing methods to detect accumulated nanoplastics in organisms. Effects on reproduction and hence, population growth rate, could have potential cascading effects up the food web. There is also accumulating evidence that nanoplastics can be transferred up the aquatic food web, and that this can cause both physiological and behavioral alterations in fish (Mattsson et al., 2015, Mattsson et al., 2017, Chae et al., 2018). All studies have, to our knowledge, been performed using commercial model particles, using the particles from WP1 and scenarios identified in WP2, we will expose crucian carp to plastic nanoparticles. The fish will be exposed through a natural food-chain by consuming *Daphnia* which in turn has fed on algae (*Scenedesmus* sp.) that has been incubated together with nanoparticles (Cedervall et al., 2012). After exposing the fish to nanoplastics during two months we will track the behavior of exposed and unexposed fish in artificial ponds (each about 20x40 m in size) mimicking their natural living environment. An acoustic telemetry system that is already in place close to Lund University and that is developed by VEMCO (https://vemco.com) will be used to track the fish in the ponds. This system will allow for high resolution, three-dimensional data collection during about two months. To gain understanding of how sub-lethal exposure to nanoplastics may affect food web dynamics, we will also release a natural predator, pike (*Esox lucius*), into the ponds. This will give us completely new insights on how exposure to plastic NPs may affect energy transfer in aquatic ecosystems.

WP4 Creation of a toxicity map of different characteristics of nanoplastics
Commercial polystyrene nanoparticles are widely used as model particles in nanotoxicology and in an increasing number of publications reporting toxicity of nanoplastics. There are, however, no studies evaluating how well these model particles reflect the effects of nanoplastics that are released, or broken down, from larger sized plastic material in the environment. In WP1-3 we will collect such data and in WP4 compare the effect of model particles with different characteristics i.e. size, morphology, and surface charge with the effect of breakdown nanoplastics. By using a wide range of model and breakdown nanoplastics we will be able to match the biophysical characteristics of breakdown nanoplastics with their effect on nature. Our main goal is to create a map that evaluates which characteristics of nanoparticles are most likely to be harmful to the environment. Furthermore, we will assess which plastic products have those characteristics after breakdown and therefore should be avoided, or even removed from products, or collected and recycled with great care after use of the products. This map will be of direct help for regulators and industry in their future work with plastics.

Scientific background
Plastics in the aquatic environment has recently received considerable attention and it has been estimated that every year 1.15 to 2.41 million tons of plastics reach the oceans through lakes and rivers (Lebreton et al., 2017), and disheartening photos of animals trapped by plastic debris or with the stomach full of plastics are numerous in the media flow. However, there is a growing concern that smaller plastic pieces, microplastics (< 5mm), may be a more severe threat to wildlife, and, furthermore, that even smaller particles, such as nano-sized plastic particles, are a different, and perhaps, an even more alarming threat since they may enter the cell metabolism. Nanoplastics are with today's technology difficult to detect but they have been sampled in the north Atlantic subtropical gyrus (Ter Halle et al., 2017). Nanoplastics can reach nature through the use of nano-sized products, but a more likely source is from breakdown of larger pieces of plastic by mechanical or photochemical processes (Lambert and Wagner, 2016, Gigault et al., 2016). These processes occur both on plastics discarded in nature as well as during recycling or handling of plastic waste. In laboratory environments, photochemical breakdown of polystyrene has been shown to result in the formation of nano-sized particles (Lambert and Wagner, 2016). Experiments in our group show that even simple mechanical forces applied on polystyrene products used in our daily life produce nano-sized polystyrene particles (Ekvall et al., submitted article).

The occurrence of nanoplastics in nature is not well documented as samplings of plastics usually do not include nano-sizes (Gigault, et al., 2016), suggesting that a better understanding of weathering and characterization of breakdown plastics in water is essential (Jahnke, et la., 2017). Furthermore, as the plastics are broken down the number of particles and their combined surface area increase considerably, which likely enhances the biological impact.

Nanoplastics introduced to the aquatic environment has been shown to have a detrimental effect on wildlife (Mattsson et al., 2015, Chae and An, 2017). By using fluorescently labelled particles, nanoplastics have been shown to be taken up by organisms, such as aquatic crustaceans (Lee et al., 2013, Cui et al., 2017), monogononont rotifers (Jeong et al 2016) oyster

larvae (Cole and Galloway, 2015), fish larvae (Manabe et al., 2011), and fish (Kashiwada et al.,2006, Lu et al., 2016, Skjolding et al., 2017). In addition, unlabeled polystyrene particles have been demonstrated to pass the blood-brain barrier in fish (Mattsson et al., 2017). The small size is important in itself as it gives the nanoplastics different characteristics compared to larger pieces of plastics (Mattsson et al., 2015, Chae and An, 2017). The importance of size has in some cases been reported and the toxicity of 50 nm amine modified polystyrene to zooplankton (*Daphnia magna*) is much higher than the toxicity of larger particles of the same material and surface characteristics (Mattsson et al., 2017). Likewise, 70 nm polystyrene is more toxic to rotifers (*Brachionus koreanus*), and the survival rate of fish Medaka (Oryzias latipes) larvae is lower when exposed to 50 nm polystyrene compared to exposed to 500 nm polystyrene (Manabe et l., 2011). Overall, there is evidence that nanoplastics will affect water living organisms in a different, and still largely unknown way, compared to larger pieces of plastics.
Generally, the toxicity of a substance is evaluated in acute tests on single species during which the mortality in high concentrations of nanoplastics or other plastics during a short period of time is determined. These tests do not capture all possible scenarios. For example, sublethal effects demonstrated in a short-term test may show harmful effects over time, e.g. through accumulation of nanoplastics in food chains or in individual organisms. Sublethal effects of nanoplastics may also affect the ecological systems as organism will be more prone to additional stress and predatory pressure. Several species have been reported to be affected by nanoplastics. For example, impaired reproduction in crustacean (Besseling, et al., 2014, Cui et al., 2017), metabolic changes in zebra fish (Lu et al., 2016) and Crucian Carp (Cedervall et al., 2012, Mattsson et al., 2015), and behavioral changes in brine shrimp (*Artemia fransiscana*) larvae (Bergami et al., 2016) and Crucian carp (Cedervall et al. 2012, Mattsson et al. 2015, 2017). Our group has previously shown that nanoplastics can travel through an aquatic three-level food chain consisting of algae – zooplankton – fish and, after transfer, severely affect the behavior and metabolism of the fish (Cedervall et al. 2012, Mattsson et al. 2015, 2017). The food chain transfer has recently been confirmed also in a four-level trophic food chain (Chae et al., 2018), showing that once entering a food chain the nanoplastics can stay and be transported upwards to higher trophic levels. The observed effects appear first after weeks of exposure (Mattsson et al. 2015), suggesting that the nanoplastics are accumulating in the top consumer. Ongoing research in our group include long-term effects on zooplankton of low concentrations of commercially used nanoplastics.

Our research about nanoplastics in aquatic food chains has received considerable attention also from the public and media, which we interpret as a great general concern about plastics and how it affects human health, as well as natural ecosystems. The concerns are reasonable as the amount of plastics in nature continues to grow and show up in for example our tap water. The lack of knowledge may cause unnecessary concerns and may force industry and regulators to act prematurely. Hence, it is of utmost importance to understand the sources of nanoplastics and which processes and fractions that constitute a threat, in order for industry and decision makers to direct production towards less harmful products.

Practical relevance - impact on society, industry and regulators

The role of plastics as a pollutant is becoming a common topic in everyday conversations, as well as an increasing concern for regulators, environmental organization, and industries. The fact that microplastics, and likely nanoplastics, are present in our daily life is of course a reasonable concern. For example, microplastics have been detected in our drinking water both in tap water and bottled water, most likely due to the breakdown of plastics. Not having knowledge, or answers to questions, or knowing what to do may cause worries or even fear. It is therefore of outmost importance to improve our understanding regarding environmental effects when plastic material is released, broken down and transported in the environment. It is further important to communicate new knowledge to the society to give decision makers, as well as stakeholders and end-users, the best possibilities to make good and well-founded everyday choices and decisions.

Our project will provide novel understanding about how ordinary, daily used consumer products can be broken down to nanoplastics. Moreover, the environmental impact of these nanoplastics will be studied, which in itself bring new, urgently needed, knowledge. By comparing the behavior and environmental effects of breakdown nanoplastics with commercially produced nanoplastics it will be possible to evaluate the hazards of breakdown nanoplastics using existing knowledge about commercial nanoplastics. Furthermore, this knowledge will provide the basis for a regulatory map from which the breakdown nanoplastics from different plastic products can be evaluated and to which extent commercial nanoplastics can be used to evaluate the hazards of breakdown nanoplastics in nature. Therefore, the project has the potential to directly affect decision making regarding which plastic products that has the most unwanted effects, due to the nano-sized material after breakdown, on environmental processes and organisms' behavior.

The project will be of special interests for industries producing and developing plastic products. The plastic industry is of course concerned about the increasing focus on the possible environmental threats plastic may cause. It is noteworthy that plastics are not only important in the packing and construction industry but also often a composite part of materials in high technology. For example, in water cleaning and solar cells that is regarded as technologies providing environmentally friendly solutions in the future, but that will demand a large production. We are part of the strong research environment "NanoLund" and have therefore close contact with researchers and companies developing the next generation of products. We have a large network within nanoparticle producing companies through our communication with SPIF (Svensk Plastindustriförening; http://www.svenskplast.org/).

Large amounts of plastics are reaching our waste plants for burning or deposition, and the ashes are deposited. Today nanomaterials including nanoplastics are not followed or treated in any special way, which means that we do not know what happens with plastics or nanoplastics in waste plants. It is also likely that nanoplastics are produced while burning plastic and that nanoplastics may leak from depositions. Therefore, our project is of special interest to the waste industry. This has been a long-term concern for our research. To address

this kind of questions we have initiated a very fruitful collaboration with Sysav, the main waste plant company in southern Sweden (https://www.sysav.se/).
The proposed project focuses on breakdown nano-sized plastics, the handling of information of it in society, as well as on the effects of the nanoplastics in the environment. Thereby, our project directly addresses several of the United Nation´s Sustainable Developmental Goals, including *"#3 Good Health and Well-Being*", "*#6 Clean water and sanitation*", *"#9 Industry, innovation and infrastructure*" and *"#11 Sustainable cities and communities*" and *"#12 Responsible consumption and production*".

Project organization and management

Participants

The project will be coordinated by *Tommy Cedervall,* Biochemistry and Structural Biology, Lund University. Other key persons are *Lars-Anders Hansson* at Aquatic Ecology, Lund University, and *Mikael Ekvall* and *Martin Lundqvist* at Biochemistry and Structural Biology, Lund University.

Tommy Cedervall has been involved in nanoplastic and nano safety research for more than 10 years. He has in depth knowledge about nanoparticle, including plastics, size characterization, interactions with biological matters and organisms in the aquatic environment, and how nanoplastics can change protein functions and organisms' metabolisms. He is the coordinator for the nanosafety area within NanoLund, part of the Centre of Environmental and Climate (CEC) research at Lund University, and a member of Mistra`s Environmental Nanosafety Program and are coordinating the programs Phase II application. He is an experienced communicator and has long-term experience with communicating with mass media, and the general public.

Lars-Anders Hansson is Professor in Limnology at Lund University and has during three decades performed research on environmental issues, specifically effects of climate change, estrogens and large-scale environmental drivers, such as eutrophication and brownification on freshwater ecosystem function including both studies in natural and large-scale experimental systems. During the last five years he has focused on environmental effects of nanoparticles, specifically focusing on physiological and behavioral effects at different trophic levels. Hansson has a long experience as research coordinator both nationally and internationally and have functioned as PI in several EU projects, as well as coordinator for a previous Biodiversa project (LIMNOTIP), denoted as "a success story" by BiodivERsA. In the present project Hansson will have main responsibility for coordinating and supervising the biological aspects, as well as connecting our project to the environmental scale.

Mikael Ekvall has a PhD in Biology from Lund University and currently holds a researcher position at the Department of Chemistry, Lund University, focusing on the effects caused when nanoparticles are introduced in aquatic food-chains. Ekvall has extensive experience in behavioral biology and developed the system for detail tracking of zooplankton behavior using fluorescent nanoparticles during his PhD studies.

Martin Lundqvist is a researcher at Lund University and has 20 years of research experience within the field of characterizing how proteins interact with nonaoparticles and what the interaction leads. Several of his articles are heavily referenced. During his research career, he has worked at a number of universities, both in Sweden and abroad. Martin has also worked at a high school in a special program, initiated by the Royal Academy of Sciences and funded by the Wallenberg foundations, aiming to raise the high school interest in chemistry. He set up a research lab at Klippan High School, in which high school students learned research methodology by participating in ongoing university research projects.

Synergy effects from our collaboration are manifested in the combination of thorough nano and nanoplastic knowledge, understanding of protein and metabolic mechanisms, expertise of ecological systems, and the broad knowledge of acute and sublethal tests on aquatic organisms. Such a broad chemical-biological interface is unusual and has led to break-through research and a deep understanding of mechanisms behind observed toxicological effects. Our broad expertise is also the basis for producing, characterizing and evaluating the environmental effects of breakdown plastics. As we together have expertise of all steps in the process of the WP´s described above, high quality and well-founded results, as well as methodological developments are guaranteed.

Facilities
Biochemistry and Structural Biology, and Aquatic Ecology has together all necessary facilities and equipment, including temperature rooms for cultivation of algae, zooplankton, and fish, video equipment, as well as tracking facilities for analyses of zooplankton and fish behavior. Moreover, particle size characterization instruments are also available, including dynamic light scattering plate reader, Zeta sizer, nanoparticle tracking analyses, and differential centrifugal sedimentation. TEM and three dimensional cryo-TEM is available at the Department of Chemistry. All standard biochemical equipment as centrifugation, electrophoresis, UV absorbance, fluorescence spectrometry and so on are available at Biochemistry. Likewise, standard laboratory equipment and all necessary permissions for cultivation and dissection of fish and other organisms are available at Aquatic Ecology.

Communication
Target groups
The target groups are mainly defined by the groups in Society our research will have impact on, which is described above. From our own previous outreach activities, we know that plastics and, specifically, small plastic particles are a topic of great concern for the *general society*. Microplastics in water, food, and in the environment is a common topic in everyday conversations and therefore, the *general society* is a target group for new knowledge and questions. We have during many years developed an industrial network including plastic industries, nanotechnology industries, and waste management industries. All three *industries* are for different reasons concerned about the effects of nanoplastics within their different specific areas. An additional aspect is that nano-technology is balancing between something good and bad, i.e. there is a continuous stream of novel material and products which are beneficial for society and the everyday lives of citizens, but on the other hand society cannot

neglect the severe risks that have been identified for some of these products. Finally, *authorities*, *regulators* and organizations responsible for environmental action is a third target group. Evaluation of possible risks and identification of necessary measures demand a thorough understanding of sources and effects of micro- and nanoplastics in nature. Specifically, we have, or will initiate, a dialogue with the following stakeholders:
General society: Naturskyddsföreningen, journalists, schools
Industries: SPIF (Svenska plastindustriföreningen), Swenanotech, individual companies as Trelleborgs AB, Perstorps AB, waste industry as Sysav and Sydvatten
Authorities/Regulators: Naturvårdverket, Havs och vattenmyndigheten, Kemikalieinspektionen, Swenanosafe.

Communication with the general society
Our previous results on the effects of plastic nanoparticles in food chains and especially fish have received much attention in Swedish and International media including TV, radio and newspapers. As a consequence of this attention, our research is well known and we are often contacted by organizations to give lectures or by interested high school students. Last year we were part of project combining theater, exhibition, and workshops about plastics in our waters for school children together with the theater company Teater Sagohuset. We reached about 300 high school students with the workshops. We have also supervised 12 high-school students to do laboratory projects and held many lectures for non-scientific audiences. Moreover, our research was highlighted in a TV program for teenagers called “Planet Plast” produced by NRK (2018). These out-reach activities will continue as we believe it is important to communicate our kind of results to the general society. Furthermore, we will continue to communicate our research specifically to media through press releases and personal contacts. We have ongoing communication with Orb media (US based news company) and BBC about documentary TV programs. Orb media has already visited us and made interviews for the international media. Media and journalists that has contacted us:

- SVT: Richard Lööf
- SR: Johan Lindahl
- BBC: Lizzie Bolton
- NRK (Norwegian TV): Astrid Engen
- NRK Vitenskap och teknik: Frid Kvalpskarmo Hansen
- Orb Media (USA based): Dan Morrison
- CBC (Canadian radio): Amy Joy

Scientific communication and data availability
We plan to publish all results that come out from the planned project in international, well renowned scientific journals as Open Access publications, and communicate them to the scientific community during conferences, seminars and work-shops. We also make sure that our results and outreach activities are posted on Faculty, Department, Section homepages. In addition, we will implement a separate homepage covering ongoing research in the proposed project.

We are part of Swenanosafe within Swetox that coordinate the development of nanosafety in Sweden. We have regular contacts with Swenanotech, that organize the Swedish nanotechnology industry, and with recently also with SPIF, that organize the Swedish plastic industry. Furthermore, we have a large network of individual nano and plastic industries. These contacts ensure that our results reach and are communicated with regulators, politicians and with the industry.

We have in the budget allocated funds for two workshops during the two first years and for a conference during the third year. These activities will be part of our plan to involve the target groups early in the research. The first workshop will open up a communication between researchers in the field of micro- and nanoplastics in the environment and the plastic industry authorities and regulators. The aim of this first workshop is to determine what kind of small plastics that can be expected to find in the nature and which can be expected to negatively affect the environment. The second workshop will focus on the methodology to find nanoplastics in a carbon-based biological background to provide researchers with new experimental tools and regulators with data about the presence of nanoplastics. The conference will be a forum for us to present our results in the context of new results from other researchers in the field. Part of the conference will be aimed at communicate the results to the general public, industry and regulators.
To further strengthen the involvement of target groups we want to organize a broad reference group including Naturvårdsverket, Havs och vattenmyndigheten, the regional County board, kemikalieinspektionen, and Swenanosafe. Industrial representatives can be Swenanotech, Svenska plastindustriföreningen (SPIF), and individual companies as Perstorps AB and Trelleborgs Ab. The general society may be represented by Naturvårdsföreningen, and by journalists interested in writing environmental articles, such as Orb Media (see above). The reference group will regularly be informed of our research activities and subject to questions from us about research needs and the development of plastic related questions within their area of expertise. They will also have access to our homepages, and scientific and popular scientific publications, and be invited to work shops and conferences. The reference group will be put together at the beginning of the project and have a first startup meeting within the first three months of the project. Thereafter at least one planed meeting each year in addition to being invited to the planed workshop and conference. The results from the project will also be communicated during the research days arranged by Naturvårdsverket and during meetings arranged by Naturvårdsverked and Havs och vattenmyndigheten together with other relevant projects.

Open Access – Data and Scientific articles
We plan to publish all results that come out from the planned project in international, well renowned scientific journals as Open Access publications. Collected characterization data and data on biological effects will be used to create our nanoplastics toxicity map. All data collected during the project will be made available and shared according to the policy of data handling and sharing described by Naturvårdsverket.

Time plan

Time plan outlining the project:

Tasks	**Half year**					
WP1	**1**	**2**	**3**	**4**	**5**	**6**
Fast mechanical breakdown of polystyrene and styrene foam						
Complementary characterization model nanoplastics						
Photochemical modification after fast breakdown						
Long-term mechanical and photochemical breakdown of polystyrene and styrene foam						
Characterization of breakdown nanoplastics (continuous)						
Sampling and characterization of leachate water						
WP2						
Complement acute and long-term toxicity data from model nanoplastics						
Acute toxicity and behavior after fast mechanical breakdown						
Acute toxicity and behavior after long-term breakdown						
WP3						
Long-term test on zooplankton, reproduction, mortality and behavior						
Fish in environmental tanks						
WP4						
Complete toxicity map of breakdown plastics						
Communication						
Workshop 1						
Workshop 2						
Reference group meetings						
Written information to reference group						
Homepage starting						
Data reported on homepage, and to stakeholders, including press releases						
Manuscripts submitted						
Final report						

Budget

Title of the call: Utlysning av forskningsmedel för Mikroplaster

Applicant: Tommy Cedervall

Affiliation: Lund University
Title of the project proposal: Environmental impact of nanoplastic fagmentized consumer plastics

	SEK year 1	SEK year 2	SEK year 3	Total
Project costs				
1. Salaries				
Project leader (10%)	81022,00	83209,00	85456,00	249 687,00 kr
Post doc or researcher (100%)	639170,00	656428,00	674152,00	1 969 750,00 kr
2. Travels				
Travels (e.g. participation in conferences and meetings)	50000,00	50850,00	50850,00	151 700,00 kr
3. Other costs				
Animal maintenance and aquarium	20000,00	20340,00	20340,00	60 680,00 kr
Tracking equipment		101700,00		101 700,00 kr
UV lamps for plastic break down	30000,00			30 000,00 kr
Instrument time (TEM, Cryo-tem)	30000,00	30510,00	10170,00	70 680,00 kr
Plastic nanoparticles	15000,00	10170,00	10170,00	35 340,00 kr
Common plastic and other laboratory consummables	75000,00	76275,00	76275,00	227 550,00 kr
4. Cummunication				
4.1. Open access publications	20000,00	20340,00	20340,00	60 680,00 kr
4.2. Conference and workshops organized by the project group for dissimination of results	25000,00	25425,00	101700,00	152 125,00 kr
4.3. Other communication costs Homepage	3000,00	3051,00	3051,00	9 102,00 kr
Total 1-4	988 192,00 kr	1 078 298,00 kr	1 052 504,00 kr	3 118 994,00 kr
5. Overhead costs				
Overhead costs at the university/college/institute where the funds will be administered **(%)** Use column E "Total"				60%
Total sum	1 581 107,20 kr	1 725 276,80 kr	1 684 006,40 kr	4 990 390,40 kr

References

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I. (2016) Nano-sized polystyrene affects feeding, behavior, and physiology of brine shrimp *Artemia franciscania* larvae. *Ecotox. Environ. Safety* 123, 18.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A. (2014) NP affects growth of *S. obliquus* and reproduction of *D. magna. Environ. Sci. Technol*. 48, 12336.

Bianco, G., Ekvall, M. T., Bäckman, J. & Hansson, L. A. 2013. Plankton 3D tracking: the importance of camera calibration in stereo computer vision systems. *Limnology and Oceanography-Methods,* 11**,** 278.

Cedervall, T., Lard, M., Frohm, B., Hansson, L.-H., and Linse, S. (2012) Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. PLoS One 7, e32254.

Chae, Y., and An, Y.-J. (2017) Effects of micro- and NPs on aquatic ecosystems: Current research trends and perspectives. *Marine Pollution Bulletin* 124, 624.

Chae, Y., Kim, D., Kim, S.W., and An Y.J. (2018) Trophic transfer and individual impact of nano-sized polystyrene in a four species freshwater food chain. Scientific Reports, 8, 284.

Cole, M., and Galloway, T.S. (2015) Ingestion of NPs and microplastics by pacific oyster larvae. Environ. Sci. technol. 49, 14625.

Cui. R., Kim, S.W., and An, Y-J. (2017) Polystyrene NPs inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean *Daphnia galeata. Scientific reports* 7, 12095.

Ekvall, M. T., Bianco, G., Linse, S., Linke, H., Bäckman, J. & Hansson, L. A. 2013. Three Dimensional Tracking of Small Aquatic Organisms Using Fluorescent Nanoparticles. *Plos One,* 8.

Gigault, J., Pedrono, B., Maxit, B. Halle, A.T. (2016) Marine plastic litter: the unanalyzed nano-fraction. *Environ. Sci.: Nano*, 3, 346.

Jahnke, A., Arp, H.P.H., Escher, B.I., Gewert, B., Gorokhova, E., Kühnel, D., Ogonowski., M., Potthoff, A., Rummel, C., Schmitt-Jansen, M., Toorman, E., and McLeod, M. (2017) Reducing the uncertainty and confronting ignorance about the possible impacts of weathering plastic in the marine environment. *Environ. Sci. Technol. Lett.* 4, 85.

Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S-., Hwang, U.-K., Zhou, B., Souissi, S., Lee, S.-J., and Lee, J.-S. (2016) Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*) *Environ. Sci. Technol*. 50, 8849

Kashiwada, S., Distribution of nanoparticles in see-through Medaka (*Oryzias latipes*) (2006) *Environ Health Perspect* 114, 1697.

Lambert, S, and Wagner, M. (2016) Characterization of NPs during degradation of polystyrene. Chemosphere, 145, 265.

Lebreton, L.C.M., van der Zwet, J., Damsteeg, J-W., Slat, B., Andrady, A., Reisser, J. (2017) River plastic emmisions to the world´s oceans. *Nat. Commun*. 8, 15611.

Lee, K.-W., Shim, W.J., Kwon, O.Y., and Kang, J.-H. (2013) Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicas. Environ. Sci. Technol.* 47, 11278.

Lu, y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., and Ren, H. (2016) Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. *Environ. Sci. Technol.* 50, 4054.

Manabe, M., Tatarazako, N., and Kinoshita, M. (2011) Uptake, excretion and toxicity of nano-sized latex particles on medaka (*Oryzis latipes*) embryos and larvae. *Aquatic toxicology* 105, 576

Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., and Cedervall T. (2015) Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. *Environ Sci Technol.* 49, 553-61.

Mattsson, K., Hansson, L. -A., and Cedervall, T. (2015) Nano-plastics in the aquatic environment *Environ. Science-Process & Impacts* 17, 1712-21.

Mattsson, K., Johnson, E.V., Malmendal., A., Linse, S., Hansson, L.-A., and Cedervall, T. (2017) Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. *Scientific reports* 7, 11452.

Skjolding, L.M., Ašmonaité, G., Jølck, R.I., Andresen, T.L., Selck, H., Baun, A., and Sturve, J. (2017) An assessment of the importance of exposure routes to the uptake and internal localization of fluorescent nanoparticles in zebrafish (Danio rerio), using light sheet microscopy. Nanotoxicology 11, 351.

Ter Halle, A., Jeanneau, L., Martignac., M., Jardé, E., Pedrono, B., Brach, L., and Gigauly, J. (2017) NPs in the north atlantic subtropical gyre. *Environ. Sci. Technol*. 51, 13689.