

EU-DOCS for SmUCS

CO-SUPERVISED SUBJECT PROPOSAL FOR A DOCTORAL CONTRACT

Title of the thesis project Interactions between contaminants and parasitic helminths in coastal predators: insights from a declining population of common terns (<i>ParaConT</i>)	
La Rochelle University Research Unit Centre d'Etudes Biologiques de Chizé (CEBC, UMR7372 CNRS-La Rochelle University) Abbreviation: <u>LRUniv/CEBC</u>	Partner university University of Oldenburg Cotutelle research unit Institute of Avian Research Abbreviation: <u>UO/IAR</u>
Name of the LRUniv supervisor Dr. Alice Carravieri	Name of the co-supervisor Prof. Sandra Bouwhuis
Non-academic partner Non Governmental Organisation (NGO) 'Bündnis Naturschutz in Dithmarschen e.V.' – mission: "Species conservation project for the gull-billed tern"	
Main research field Ecotoxicology Secondary research field(s) Trophic Ecology, Parasitology	
Keywords Bulk and compound-specific stable isotopes, life-history traits, metallic trace elements, migration, per- and polyfluoroalkyl substances (PFAS), seabirds	
Scientific description of the research project Scientific context <i>Understanding how stressors interact to affect wildlife is critical to mitigate the ongoing biodiversity crisis. Two very important stressors in this context are contaminants and parasites.</i> Human activities have increased the circulation of natural trace elements, and have produced a plethora of synthetic chemicals to which species have not previously been exposed¹. Several of these contaminants resist degradation and can biomagnify, <i>i.e.</i> their concentrations can increase at each step of the food web through trophic transfer¹. These contaminants have multiple toxic properties (endocrine disruption, immunosuppression, reprotoxicity, neurotoxicity²⁻⁴), and can be especially harmful when associated with other stressors, such as extreme climatic events, disease, poor food availability or habitat loss¹⁻⁵. Studying multiple stressors in the wild is challenging, and one particular threat has been somewhat overlooked: parasitism. Parasite infections are ubiquitous in nature, and act as powerful selective forces on host life histories⁶. Helminths (<i>i.e.</i>, trematodes, cestodes, acanthocephalans, and nematodes) are macroparasites that are mostly trophically transmitted to vertebrate hosts⁶. Many helminth species are found in the internal organs of the host, where they can consume ingested food, host tissues, or host digestive products⁷. Helminths compete with the host for nutrients, and trigger expensive behavioural and immune responses, which can result in a disruption of host metabolism, fertility and survival^{6,7}.	
Fig. 1. Diagram of drivers and effects of contaminants and helminths in animals. <pre> graph LR TT[Trophic transfer] --> H[Helminths] TT --> C[Contaminants] H <--> C H --> HE[Health effects] C --> HE HE --- WS{Warning Symbol} </pre>	

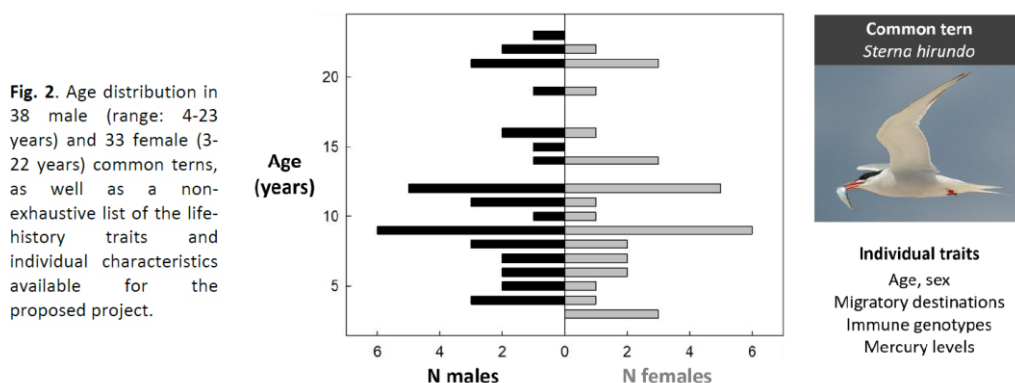
Contaminants and helminths not only have similar uptake routes and health effects^{7,8}, but also high potential to interact (Fig. 1). Parasite transmission and multiplication have, for example, been found to be exacerbated in polluted environments, as a result of contaminant-related immunosuppression⁸, and pollution effects on health have been shown to be worse in infected than in uninfected hosts^{7,8}. However, beneficial effects of helminths in polluted environments have also been described, possibly as a result of their contaminant accumulation capacity, which can decrease contaminant exposure to the host^{9,10}. As such, the interaction between contaminants and helminths could have positive and/or negative consequences for the host, depending on factors such as host and parasite species, parasite load and pathogenicity, contaminant type and concentration, and host health status^{7,10}. So far, studies in environmental parasitology, the science addressing multiple-stressor effects in host-parasite systems, have mostly focused on trace elements in freshwater fish, amphibians and invertebrate hosts^{7,8}, while studies in marine host-parasite systems, and especially on the contaminant accumulation capacity of helminths parasitising marine birds and mammals are still extremely rare¹⁰.

Marine coastal environments are the final sinks of several natural and synthetic chemicals, which can persist years to centuries in food webs and coastal sediments¹. Long-lived marine predators such as coastal seabirds are thus particularly vulnerable to their bioaccumulation and toxic effects¹¹. Seabirds have been used widely as indicators of marine pollution, and are also exceptional, yet underused, models for studying helminths⁶, many of which are yet to be described (85-95% of helminths parasitising vertebrates are still unknown¹²). Factors underpinning the occurrence and characteristics of helminth infections in seabirds, as well as the trophic links between host and parasites, are poorly known^{6,10}. Additionally, interactions between contaminants and helminths have rarely been addressed in seabirds^{13,14}, even though they could affect populations.

General aim

The main aim of *ParaConT* is to quantify contaminant-helminth interactions and their drivers in a wild coastal seabird population breeding on the German North Sea coast – the common tern *Sterna hirundo*. **Terns are particularly relevant to address the interactive drivers and effects of pollution and parasitism, because they feed at the interface between marine, freshwater and terrestrial habitats and perform intercontinental migrations. Terns can thus encounter, and disperse, contaminants and helminths of very diverse origins^{15,16}.**

Helminth species will be extracted and identified from a large sample of terns of known sex, age, and life history (Fig. 2), and dietary tracers (stable isotope analysis, SIA) and contaminants will be quantified in the tissues of both the helminths and the tern hosts to better understand nutrient and contaminant transfer. Seabirds are one of the most threatened groups of animals and the common tern is red-listed in Germany as critically endangered¹⁷, with outbreaks of avian influenza in the breeding seasons of 2022 and 2023 having exacerbated the situation. Unravelling the drivers and cumulative effects of pollution and parasitism is crucial to understand current and future threats to this declining species.



Scientific objectives and expected results

Thesis objective 1 – quantify and explain parasite and contaminant levels in common terns

Contaminant and helminth burdens in seabirds can be governed by intrinsic factors such as sex and age. Females have an additional elimination route for contaminants compared to males (egg transfer^{1,18}), and males may have a higher susceptibility to parasites than females through testosterone-induced immunosuppression^{6,7}. Age can drive differences in concentrations of trace elements such as mercury and cadmium in internal organs through long-term accumulation¹, and in the intensity of parasite infections, because young and senescent individuals tend to have weak immune systems⁶.

Extrinsic factors, such as diet, feeding habitat, and trophic level can also drive variation in both contaminant exposure and loads of trophically-derived parasites^{1,13}. If prey items are heavily contaminated and have high numbers of infective stages of parasites, co-exposure to the two stressors can take place^{13,14}, which needs to be accounted for to disentangle combined effects on host health.

To meet objective 1, the PhD student will quantify:

- 1) helminthic infections,
- 2) host tissue-specific distribution of two classes of contaminants (trace elements, per- and polyfluoroalkyl substances (PFAS)),
- 3) the most influential drivers of both contaminant and helminth burdens among intrinsic (age, sex, immune genotype) and extrinsic (migratory destinations, feeding habitat and trophic position) factors.

Predictions:

- 1) Given previous parasitological work in terns^{15,19}, we expect the birds to be infected with all major groups of helminths.
- 2) Host tissue distribution of contaminants depends on their physicochemical properties and on tissue-specific functions¹. We expect the liver to accumulate a large diversity of contaminants, while muscle or brain should concentrate a few predominant pollutants.
- 3a) Contaminant concentrations may increase with age in tissues such as liver, and differ between males and females, as it has been shown to be the case in blood¹⁸. Older and younger terns could also present higher burdens of helminths than mid-aged terns.
- 3b) Because higher variability in immune gene sequences has been associated with lower prevalence of blood parasites²⁰, we expect terns with a stronger variability of immune genotypes (inferred from major histocompatibility complex genes) to exhibit reduced prevalence and/or richness of helminths.
- 3c) Spatial behaviour and diet (inferred from geolocator data and SIA, respectively) are expected to be key drivers of between-individual variation in tissue contaminant concentrations and helminth richness.

Thesis objective 2 - quantify the transfer of contaminants and nutrients between parasites and tern hosts

All major helminth groups have been shown to bioaccumulate trace elements and some organochlorine compounds (synthetic chemicals)⁹. Arsenic, cadmium or lead can be found in helminths at concentrations hundreds of times higher than in their host tissues^{7,8}. In addition, infected hosts can have lower tissue concentrations than non-infected individuals because helminths can reduce host contaminant levels⁹. Helminths can also modify host contaminant exposure and/or health through indirect mechanisms, such as disruption of host intestinal uptake of nutrients²¹. However, helminth-contaminant interactions have been quantified almost exclusively in fish and amphibian hosts under controlled conditions, with very little work on other taxa or in the wild^{10,13,19}. In addition, the mechanisms by which helminths derive energy from the host are still elusive. Some helminths can feed on specific host tissues or consume partially digested or undigested gut content, *i.e.*, nutrients that are not strictly host derived^{22,23}. Identifying nutrient uptake mechanisms of helminths and their trophic position is essential to understand how they could interfere with contaminant uptake and effects in the host.

To meet objective 2, and by using the same samples of objective 1, the PhD student will quantify:

- 1) the concentrations of two classes of contaminants (trace elements and PFAS) in helminth tissues,
- 2) dietary proxies in both host and helminth tissues,
- 3) whether infection with helminths affects contaminant distribution in host tissues.

Predictions:

- 1) Differences in contaminant concentrations are expected in helminths belonging to the different major groups (*i.e.*, trematodes, cestodes, acanthocephalans, nematodes), as they have different morphologies affecting nutrient uptake strategies. Specifically, a greater diversity and quantity of contaminants are expected in the tissues of acanthocephalans and cestodes, because they absorb nutrients through their tegument by passive mechanisms, and lack a digestive tract⁶.
- 2) The trophic level of acanthocephalans and cestodes is expected to be equal to, or lower than, that of the host, while nematodes and trematodes, which have a digestive track, could present a trophic position that is higher than (or similar to) the host if they feed on host tissues (or its prey)^{6,23}.
- 3) Hosts with high parasite loads are expected to have lower contaminant concentrations than hosts with few or no helminths.

Scientific challenges and methods

A central challenge when studying helminth biology, but also contaminant distribution in internal organs of wild animals, is to have access to fresh carcasses of known cause of death, and information on individual traits. **This project relies on a unique sample of 71 individually marked and pedigreed**

common terns that died of avian influenza at their breeding colony in 2022.

Study system

The common tern is a Holarctic colonially breeding and migratory seabird, usually laying a clutch of three eggs each year in late spring/early summer. At the Banter See in Wilhelmshaven, on the German North Sea coast (53°36 N, 08°06 E), UO/IAR has been monitoring a population of common terns on an individual basis following a standard protocol for 31 years²⁴. Since 1992, all locally hatched birds have been marked with a unique transponder shortly prior to fledging. The colony site is equipped with antennae that record the presence of all transponder-marked individuals on resting boxes and at the nest. During reproduction, nests are checked three times weekly to record reproductive parameters and mark chicks, which are sampled for feathers for molecular sex determination²⁵. Breeding adults are blood sampled to collect data on immune genotype and mercury contamination^{18,26}, and a subset is equipped with light-level geolocators to study migratory movements and behaviour^{16,27}.

In 2022 and 2023, the Banter See common tern population was heavily hit by avian influenza (H5N1): over two months of four-times-daily searches, 511 and 111 birds (26% and 9% of the overall population) were found freshly dead and stored in a freezer (-20°C) immediately upon discovery, their corpses being available for dissection and study. Seventyone of these birds were pedigreed, geolocator-tracked and blood sampled before the onset of influenza. As such, sex, age, migratory destination, immune genotype and blood mercury contamination are available for these individuals.

Helminthic infection characterisation

To identify helminth species, and thus quantify parasite richness, we will use morphological identification: observation under a light microscope Olympus BX51, and a stereomicroscope Olympus SZ51 equipped with a Moticam 2300, followed by comparison with the literature; but also, molecular identification: DNA barcoding through PCR using different primer sets to amplify a part of the internal transcribed spacer, cytochrome oxidase and the 18S region. We will also quantify parasite prevalence (proportion of the samples infected) and load (number of parasites per individual host).

Targeted contaminants: trace elements (natural origin) and perfluorinated compounds (PFAS, synthetic)

Trace elements. Trace elements such as mercury, cadmium and lead are nonessential, as they have no biological function and affect human and wildlife health¹. Mercury is particularly concerning, because it is easily assimilated by organisms, accumulates in their tissues and strongly biomagnifies up food webs¹. Mercury exposure can increase the severity of seabird helminth infections, and affect seabird fecundity over the short and long-term²⁸. Trace elements such as manganese, selenium, and zinc, are essential (necessary for biological functions), and can have a protective role against other elements' toxicity (e.g., selenium against mercury¹). Yet, essential elements can be toxic at high concentrations. Both essential and nonessential elements can be accumulated by helminths⁹. Quantifying multiple trace elements is critical to get a mechanistic understanding of the interactions between contaminants and parasites¹³.

PFAS. PFAS are emerging pollutants that are used extensively in a variety of consumer products (fire-fighting foams, paints, coatings, textiles)²⁹. PFAS are persistent, biomagnifying, globally distributed in the environment, and have strong immunosuppressive properties^{29,30}. PFAS accumulate in seabirds, with fish-eating species living near industrialized areas having among the highest concentrations^{1,13}. The work of the LRUniv supervisor has shown that PFAS were negatively related to helminth load in a coastal seabird¹³. The high protein component of helminths⁶, as well as strong PFAS affinity for proteins^{29,30} suggest a high potential for PFAS accumulation in helminths, which merits urgent investigation. Only three of the thousand PFAS in circulation are regulated internationally (Stockholm Convention). Evaluating exposure and effects of PFAS on wildlife, and their interaction with other stressors, is thus a pressing environmental priority.

Trace elements and PFAS will be quantified in tern brain, muscle, liver, kidney, gut, blood (extracted from the heart atria) and in helminths (whole body; individual worms of the same species and host will be pooled to increase available mass and thus contaminant detection probability) using methods developed by partner laboratories (see relevant section below).

Dietary proxies

SIA has been widely applied in ecology to trace the source and flow of nutrients within food webs, by quantifying the ratios of heavy to light stable isotopes³¹. Specifically, the carbon stable isotope ratio in total proteins ($\delta^{13}\text{C}$) varies with habitat in primary producers at the base of food webs (e.g., oceanic and coastal algae have different $\delta^{13}\text{C}$ values), and is almost constant along the food web. In contrast, the nitrogen stable isotope ratio in total proteins ($\delta^{15}\text{N}$) has a stepwise enrichment from one trophic level to

the next. Hence, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in tissues of predators are time-integrated, individual-level proxies of feeding habitat and trophic position, respectively³². Stable isotopes can also help characterise complex links in food webs, such as those of host-parasite systems^{31,33}, notably through compound-specific stable isotope analysis (CSIA), which applies SIA to specific molecules within tissues, *i.e.*, amino acids³¹. This enables us to trace host-parasite molecular exchanges, and reveal the specific energy source of parasites and the quantity of biomass transferred.

SIA and CSIA will be carried out in host tissues with different temporal integrations (*e.g.*, feathers for long-term dietary information, blood and liver for short-term³²), and in helminths (whole body; individual worms of the same species and host will be pooled), using methods developed by partner laboratories (see relevant section below).

Statistical approach

Multivariate (principal component analysis, PCA) and univariate multifactorial analyses (*i.e.*, (generalised) linear models, (G)LM) will be used to meet the objectives of *ParaConT*. Specifically, PCA will be used to explore inter-relationships between contaminants, and select those that explain most of the variation, to use in multifactorial models. GLMs allow to model response variables with different distributions (Gaussian, gamma, binomial) and identify their relationship with intrinsic and extrinsic explanatory factors simultaneously. For each question, biologically meaningful models will be constructed and the best one(s) identified through model selection based on Akaike's information criterion corrected for small sample sizes³⁴. Both supervisors are very experienced and will provide training in these techniques, while also encouraging the PhD student to develop alternative statistical strategies if relevant.

Risk management

Given the heterogeneous distribution of helminths in nature⁶, there is a risk that the dissected birds are not infected. However, the project relies on 71 birds, and previous studies in terns with smaller sample sizes (range 6–41) have found several helminths^{15,19}. An additional 267 'back-up birds' of known sex and age (but less information on blood mercury level and migratory behaviour) are also available for helminth investigation. In the unlikely event that none of these birds have parasites, the ecotoxicological component of *ParaConT* is still very valuable for research advances and scientific publication on its own. In particular, only a handful of studies has previously quantified PFAS distribution in internal organs of birds, none of which focused on individuals of known traits such as age, and all of which were carried out on much smaller sample sizes (range 5–13^{35–38}).

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PhD student profile and skills required

- Master's degree (or equivalent) in Ecology, Ecotoxicology, Parasitology, or a relevant field.
- Proven skills in quantitative analysis of ecological data, preferably using R software.
- Knowledge of statistical methods such as Generalised Linear Models (GLMs) and PCA is desirable.
- Strong interest in ecotoxicology, parasitology, marine ecology and conservation science.
- Experience with metabarcoding techniques would be an advantage.
- Field and wet lab experience would be an advantage.
- Autonomy, rigour, scientific curiosity and ability to work in (international) teams.
- Excellent scientific writing and speaking skills in English (French and/or German would be an advantage).
- Initial experience in scientific publishing would be an advantage.

Scientific alignment with EU-DOCs for SmUCS objectives

Studying the interactive drivers and effects of multiple stressors on wildlife is particularly relevant in coastal environments, which are under pressure from several threats affecting biodiversity, such as habitat loss due to sea-level rise, urbanisation, agricultural expansion, but also air and water pollution, the introduction of invasive species (including parasitic ones), and global climate change. In this context, coastal birds such as the common tern can serve as reliable bioindicators to elucidate environmental impacts and the effectiveness of conservation measures. Monitoring these species provides essential information on ecological responses to anthropogenic pressures, thereby facilitating the sustainable management of coastal ecosystems. Importantly, the proposed thesis *ParaConT* will account for the influence of migratory destinations on the terns' contaminant and parasite burden (and thus potential transfer to their coastal breeding habitat). This will allow the PhD student to disentangle local from distant drivers of environmental stress in coastal habitats, which could help target conservation efforts to the relevant areas.

Societal and economic challenges and contributions

Societal. Seabirds are iconic species of the North Sea and capture the interest of audiences of all ages. The outcomes of *ParaConT* are therefore likely to generate broad public awareness. Should the PhD student wish to engage in outreach activities (e.g., Science Slam, Science Pub, the Long Night of Science), these can be carried out in collaboration with the NWDUG, on whose executive board the co-supervisor serves. Moreover, published results are shared with the general public on a regular basis at the recently established common tern museum at the Banter See field site. *ParaConT* addresses the first (Understand and beat marine pollution) and second (Protect and restore ecosystems and biodiversity) of the 10 challenges of the United Nation (UN) "Ocean Decade" and is embedded in the "Life below water" UN Sustainable Development Goals. At a regional scale, *ParaConT* will provide invaluable data on at least three (D1, biodiversity; D4, marine food webs; D8, marine contaminants, and potentially D2, invasive species) of the 11 descriptors of the MSFD, which aims to achieve or maintain Good Environmental Status of EU marine waters. Following the "One Health" concept of the World Health Organisation, common terns can be used as bioindicators of North Sea contamination, revealing

potential associated risks for humans. The outputs of *ParaConT* will help evaluating the efficiency of European regulations on wildlife contamination, and support actions from decision-makers to reduce contaminant emissions, such as including additional PFAS compounds in the Stockholm Convention, and restricting PFAS as a class under REACH (EC 1907/2006).

Economic. The results of *ParaConT* are not expected to have direct economic impact.

Partnership context

Non-academic partner - Secondment

The PhD student will be hosted by the nature conservation Non Governmental Organisation "Bündnis Naturschutz in Dithmarschen e.V." for a 3-month secondment, working on the "Species Conservation Project for the Gull-billed tern in Dithmarschen", funded by the German Ministry of Energy Transition, Climate Protection, Environment and Nature and Dithmarschen District Nature Conservation Authority. The aim of this project is to prevent the extinction of the last central European population of gull-billed terns *Gelochelidon nilotica*, and to ensure the protection of the large multi-species colony (gull-billed terns, black-headed gulls, avocets, oystercatchers and other ground-nesting birds) in which they breed, which is unique in Germany. The PhD student will build fences to prevent mammalian predation (foxes, raccoon dogs, rats), help with habitat management (e.g. preventing flooding of the colony area), monitor the behaviour and reproductive success of the focal, locally critically endangered species, and monitor threats and disturbances. During the secondment, the PhD student can also contribute to additional investigations within the project framework, in order to continuously improve the conservation measures. Day-to-day supervision will be provided by Dr. Markus Risch.

Academic partners

LRUniv/CEBC is an ecological research station located in Villiers-en-Bois (1h drive from La Rochelle), consisting of 38 permanent researchers, engineers and technicians, and hosting more than 30 international PhD students and postdocs, as well as approximately 70 Msc and Bsc students and field assistants each year. The supervisor at LRUniv/CEBC will guarantee integration into the LRUniv/CEBC and the Ecophy team, expertise in ecotoxicology, trophic ecology, environmental parasitology, statistics, and will provide human, material and financial contributions for day-to-day research activities at the LRUniv/CEBC, as well as training for dissections at UO. The supervisor will provide day-to-day supervision for the 24+ months planned at LRUniv/CEBC, as well as weekly supervision during the time spent with the partners in Germany.

The co-supervisor will host the PhD student at **UO/IAR** in Wilhelmshaven (Germany) for 6-9 months. During this time, the co-supervisor will be able to offer the PhD student on-site housing free of charge while (s)he is working at the institute, as well as day-to-day supervision, unlimited access to the samples and infrastructure of the institute. The co-supervisor will also provide weekly supervision for the 24+ months the PhD student will spend at the LRUniv/CEBC, as well as infrastructure and material for common tern dissections.

Mercury and PFAS will be quantified at the Institute for Chemistry and Biology of the Marine Environment of **UO**, while other trace elements and trophic tracers will be quantified at the Aquatic Ecology department of the **University of Duisburg-Essen**, in collaboration with Prof. Bernd Sures. This partner of *ParaConT* will also provide assistance in parasite morphological identification and DNA barcoding, as well as funding for the analyses (see details below).



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