Slide 1 : Good day everyone, it's great to see so many people attending this talk on my research, which studies the possibility of short chain chlorinated paraffins having endocrine disruptor effects on freshwater zooplankton *Brachionus calyciflorus.*

Slide 2: This talk will show that, indeed, SCCPs do seem to cause endocrine disrupting effects on this species at low concentrations, affecting the mRNA expression levels of endocrine receptors as well as demographic parameters both associated with sexual reproduction. The effects varied when tested at two different temperature ranges.

Slide 3: Briefly, for those unfamiliar with zooplanktonic models, rotifers are small zooplankton which feeds by filtering the water to catch phytoplankton in their vicinity. They are a key species for aquatic food chains and are able to reproduce asexually very rapidly, producing their first offspring less than a day after being born, with a lifespan of a dozen days.
Their small size, ubiquitous distribution, short life span, and complex sexual reproductive cycle, with different sensitivities to toxicants at each step, make them good models for ecotoxicology.

Slide 4: Meanwhile, short chain chlorinated paraffins, or SCCPs, are aliphatic alkanes with many industrial applications which have come under increased scrutiny these past few years, after they were banned internationally for most usages by being added to annex 2 of the Stockholm convention. They are found on every continent, in surface waters as well as in the air, soil and biota.

Slide 5: These molecules are classified as PBT, persistent bioaccumulable and toxic, making them particularly concerning. They undergo long distance atmospheric transportation across thousands of kilometers and are continuously released from electronic waste, landfills, and old industrial machinery.
In aquatic environments they are known to disrupt the thyroid hormones in zebrafish as well as affect embryo development in the amphibian *Xenopus laevis*. There are few data on invertebrates, although our research found they lead to modified mRNA levels of genes associated with molting in the crustacean *Gammarus pulex* at environmental levels, as well as in genes from the antioxidant defense system.

Slide 6: Thus, knowing that SCCPs can have toxic and endocrine disrupting effects at environmentally relevant concentrations, which are typically lower than or equal to around 1µg/L, that their effects on invertebrates are not well known, and that most of these available data cannot be extrapolated to population level effects, how can we combine assays at multiple levels of biological organization to get a fuller picture of their effects on *B. calyciflorus*? And how to incorporate these assays into a framework which takes climate change environmental effects into account?

Slide 7: To this end we have exposed *B. calyciflorus* to 3 contaminant treatments, from 10 to 1000 ng/L of highly chlorinated SCCPs at two temperature ranges. The treatments mimicked a day/night cycle with daily fluctuations of temperature two 2°C above and below the mean values of 20 or 25°C. For molecular biology, organisms were exposed between 6 and 96 hours then isolated, and their mRNA was extracted and reverse-transcribed into cDNA which was measured via RT-qPCR. The three studied genes are endocrine receptors known to react to endocrine disruptors such as pesticides or hormones. In the demographic level assay, parameters were also measured until 96 hours.

Slide 8: As we can see, fold change levels for the receptors varied considerably after SCCP exposure over a 96 hour period, with the largest changes occurring directly after exposure at 6 hours, and then at 72 hours. The stars indicate significant changes compared to controls. We notice that RAR and RXR react in tandem, and all significant effects took place in either the 100ng/L or 1000ng/L

SCCP treatments. This alternance of increasing and decreasing levels could be due to compensatory mechanisms or feedback loops following a disruption.

Slide 9: At a higher temperature, which is more suitable for fast reproduction in this species, the observed effects are somewhat different. We still see a significant RXR increase at 6 hours, and generalized mRNA increases at 72 hours, but RXR and RAR differ in their reactions to the treatment, and most significant changes take place in the 10ng/L or 100ng/L treatments, possibly indicating a temperature mediated potency of the endocrine disrupting effect.

Slide 10: These results may indicate that there is a short initial period where the organisms are affected by the treatment, but overall the early period during which asexual reproduction is prevalent shows few impacts. Later on, when population density has increased and sexual reproduction becomes widespread we begin to see large effects, suggesting that this life stage is susceptible to SCCP treatment.
RXR and RAR are complementary receptors which are able to form heterodimers when RAR is exposed to small lipophilic molecules. Thus, the effects on RAR are notable as SCCPs are such molecules. As for the decoupling of RAR and RXR at 25°C, it can be explained by the fact that RXR can also associate with other receptors, forming homo or heterodimers, indicating other endocrine pathways may also be affected.
The MAPR gene has been found to influence the development of sexuality in rotifer offspring, with knockdown of the gene decreasing sexual generations of organisms (mixis).

Slide 11: Now before looking at demographic parameters, a quick recap of rotifer sexual cycles is in order. The first generation of rotifer hatched from resting eggs, usually at low population densities, reproduce quickly by parthenogenesis. Once a certain population threshold is reached, partially signaled by the mixis inducing protein, their offspring become sexualized, either producing numerous small male offspring (if unfertilized) or resting eggs (if fertilized) with long term survivability. These parameters are all sensitive to toxic or endocrine disruptor effects and can be used for ecotoxicological assays. Their names are all fairly self explanatory, except for mixis, but this simply refers to the proportion of rotifers in the population which are currently in the sexual part of the cycle.

Slide 12: While treatment by SCCP 100ng/L at 20°C had a very small significant decreasing effect on population growth, it had quite noticeable effects increasing mixis at 72 hours, while decreasing fertilization rates in the short term. However these effects did not carry over to 96 hours, nor did they affect resting egg production or hatching rates. The effect was far more marked at 25°C than at 20°C.

Slide 13: While the effect of temperature on mRNA was not a simple antagonism or synergism, here we see a clear enhancement of SCCP effects on sexual reproduction after a temperature increase. Increased mixis in rotifer is a known effect of PFOS treatments, these molecules having structural similarities to SCCPs. This may be linked to the significant increase in MAPR RNA expression we saw earlier. Since MAPR knockdown causes lower sexuality, increase in MAPR could have the opposite effect.
In theory higher mixis rates should lead to higher fertilization and resting egg production, however this was not the case. Thus, it is possible that SCCPs inhibit one of the elements of sexual reproduction, such as mating or sperm production for example. Overall the lack of many significant effects after 96 hours, and especially the lack of effects on resting eggs compared to controls, indicate that environmental SCCP concentrations don't prevent the population from perpetuating itself in the environment, although their population structure is altered.

Slide 14: >e see the usefulness of combining different approaches and environmental conditions, as although the 20°C treatments caused more changes to mRNA expression, at the whole-individual and population level the 25°C treatments had the biggest impact. The effects seem to take place especially in the first 72 hours of exposure, however multigenerational effects are possible and should be investigated. Overall we find that environmental concentrations of SCCPs affect rotifer sexual reproduction and endocrine genes.

Slide 15: Thank you for attending this talk, I'd also like to thank everyone from my lab who helped me as well as the FNRS for funding this research.