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Doctoral thesis

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Large red damselfly (Pyrrhosoma nymphula). Photo: G. Kjærstad

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Contents

Summary

Alien invasive species are regarded as one of the most serious threats to freshwater biodiversity. Once established in a new habitat, such species are generally difficult to remove. One of the few effective measures to remove invasive freshwater animals is the use of rotenone, a natural compound derived from certain tropical plants. Rotenone is commonly used to eradicate or reduce stocks of unwanted fish species, but due to negative impacts on non-target taxa its use is controversial. Although treatments with consecutive rotenone applications are common, most investigations of effects of rotenone on non-target taxa are conducted for single treatments and often on short time scales, i.e. up to one year. Accordingly, there is a need to investigate potential negative effects on non-target taxa in treatments with several consecutive rotenone applications as well as to evaluate the long-term impacts, i.e., for several years.

Despite decades of research, the impacts of rotenone treatments on invertebrates (i.e. number of individuals/taxa) are contradictory, ranging from minor to major negative effects. This could partly be explained by the huge difference in taxonomic resolution among investigations. Rotenone treatment impacts on benthic invertebrates are often reported on the genus or family level, potentially masking effects on the species level. Even if the taxa identification is done to the species level, this is generally done for well-known groups like mayflies, stoneflies and caddisflies, but rarely for species rich groups like non-biting midges, aquatic worms or water mites. There is consequently a need to develop methods able to detect species that are difficult or impossible to identify by traditional morphological identification. In addition to the taxon specific responses to rotenone, it is possible that the trophic structure of the benthic invertebrates may be altered following rotenone treatment. This could happen as a consequence of some predatory and prey invertebrate taxa being removed or reduced in abundance by rotenone and others increasing due to fish removal.

In this thesis, I investigate how freshwater ecosystems, with a focus on benthic invertebrates, respond to invasive species eradication using rotenone treatments. Specifically, the following research questions were examined:

1. What is the effect in terms of taxa composition of repeated rotenone treatments on riverine benthic invertebrates?
2. What are the long-term impacts (i.e., up to four years) of rotenone treatments on riverine and lake benthic invertebrate communities?
3. Can increased taxonomic resolution in identification by use of DNA metabarcoding give further insight into responses of benthic invertebrates to rotenone treatments?
4. Do rotenone treatments alter the trophic resource use and trophic diversity of benthic invertebrate communities?

The study sites were situated in three boreal areas, one in the southern part of Northern Norway (the Fusta and Drevja watercourses) and two in Central Norway (River Ogna and six lakes in the Bymarka area). At all sites, rotenone treated and untreated stations were sampled before and after the treatments. Several sampling methods were used, both quantitative (Surber sampling) and qualitative (kick-net and Z-sweep sampling). To increase the taxa detection, DNA metabarcoding of the preservative ethanol (peDNA) of Z-sweep samples of the Bymarka lakes was performed and compared to traditional morphological identification of the same samples before and after rotenone treatment. Stable isotope analyses of invertebrates and their basal feeding resources were used to infer rotenone-induced changes in the trophic niche of benthic invertebrates.

To investigate the effects of repeated treatments (research question 1), the benthic fauna of River Ogna was studied following three consecutive rotenone treatments (6-10 months between the treatments). While the two first treatments showed minor effects, the benthic invertebrate fauna was severely affected shortly after the third treatment, and several taxa were missing. During the two first treatments, the water temperature was relatively low compared to the third treatment. Since the toxicity of rotenone increases with temperature, the high temperatures during the third treatment were more likely the main reason for the observed results, rather than the number of treatments.

The rotenone treated River Fusta and Lake Fustvatnet and the nearby untreated River Drevja and Lake Drevvatnet were surveyed over a five-year period to evaluate long-term impacts on the benthic invertebrate community (research question 2). Only minor immediate effects (i.e. < 1 month) or long-term effects (4 years) of rotenone treatment were detected in both the lake and the river. However, samples taken 9 months after the lake treatment showed major negative effects in the outlet river, where many taxa were missing. Due to low temperatures and limited light, the breakdown of rotenone in the lake took several months, probably allowing water containing rotenone to enter the outlet river for a long period. The low-dose long-term rotenone exposure from the lake seems therefore to have a much higher negative impact on the benthic community of the river than the two previous high-dose short-term rotenone exposures of the river treatments.

Metabarcoding in combination with preservative ethanol DNA (peDNA) and traditional morphological identification methods in the Bymarka lakes were used to clarify if metabarcoding in combination with peDNA could generate further insight to rotenone treatment impacts on benthic invertebrates (research question 3). Both methods showed a significant effect of the rotenone treatment on community composition in terms of changes in Bray-Curtis dissimilarity. For the change in the number of taxa, only the metabarcoding peDNA method was able to detect a treatment effect. This was most likely due to the greater ability of this method to register more taxa, especially for several species-rich groups not easily determined by morphology.

Stable isotope analyses of benthic invertebrates were used in a before-after-control-impact (BACI) approach to study rotenone treatment effects on the trophic niche of benthic invertebrate communities in the Bymarka lakes (research question 4). We found no significant differences in the pooled $\delta^{13}C$ and $\delta^{15}N$ values of benthic invertebrates after rotenone treatment in the three treated study lakes. For the functional feeding groups *predators* (dragonflies - Odonata) and *grazers* (snails - Lymnaeidae), the $\delta^{13}C$ values increased after rotenone treatment, suggesting increased feeding on littoral benthic algae, whereas *gatherer-collectors* (mayfly larvae - *Leptophlebia*) showed decreased $\delta^{13}C$ values following fish removal in treated lakes. The sample-size corrected standard ellipse areas (SEA_C) and the Bayesian estimates of standard ellipse areas (SEA_B) slightly increased in two of the three treated lakes, due to increased range in $\delta^{15}N$. However, the changes in the resource use and trophic diversity of benthic invertebrate communities following rotenone treatments of lakes were generally minor.

In conclusion, the results in this thesis indicate that benthic invertebrate communities have a high ability to recover following rotenone treatments, even if impacts are severe, as shown for the third treatment in River Ogna and River Fusta. However, for some taxa it may take several years to reappear, which highlights the importance of long-term studies. Metabarcoding of peDNA was shown to be a useful method for detecting rotenone treatment effects but needs to be further developed to fully replace traditional identification methods based on morphology. The use of stable isotopes indicated only minor impacts of rotenone treatment on the trophic niche of benthic invertebrates, but more studies are needed before definitive conclusions can be drawn.

Rotenone treatments should be planned and conducted with caution to avoid irreversible negative impacts on freshwater ecosystems. It is a need for comprehensive research to better understand and predict potential consequences of such disturbances.

List of Papers

The thesis is based on the following manuscripts:

Paper I: Kjærstad, G., Arnekleiv, J. V. & Speed, J.D.M. 2015. Effects of three consecutive rotenone treatments on the benthic macroinvertebrate fauna of the River Ogna, Central Norway. River Research and Applications, 32: 572-582. https://doi.org/10.1002/rra.2873

Paper II: Kjærstad, G., Arnekleiv, J.V., Velle, G. & Finstad, A.G. 2021. Long-term responses of benthic invertebrates to rotenone treatment. River Research and Applications, 1-14. https://doi.org/10.1002/rra.3919

Paper III: Kjærstad, G., Majaneva, M.A., Falahati, M., Topstad, L., Finstad, A.G Arnekleiv, J.V. & Ekrem, T. DNA metabarcoding of preservative ethanol reveals changes in invertebrate community composition following rotenone treatment. Manuscript.

Paper IV: Eloranta, A.P., Kjærstad, G., Power, M., Lakka, H.K., Arnekleiv, J.V. & Finstad, A.G. Impacts of piscicide-induced fish removal on resource use and trophic diversity of lake invertebrates. Manuscript.

Introduction

Biodiversity loss is one of the world's biggest challenges threatening ecosystem functions and services (Oliver et al. 2015). Freshwater habitats, which have a disproportionately high species richness compared to area, are especially vulnerable to human activities and environmental change (Dudgeon et al. 2006). Among several emerging threats to freshwater biodiversity, alien invasive species are regarded as one of the most important (IPBES 2019, Reid et al. 2019). The term 'alien' is often used interchangeably with 'non-native', 'exotic' and 'introduced' (Warren 2021). All alien species will have impacts on their new environment simply by their presence, e.g. via affecting the availability of space, food, water, or other resources for other species. The impacts may be negative or positive or often a combination of both (Russel & Blackburn 2017). Although the term 'invasion' has been used to describe any process of colonization and establishment outside a species' native range (Reise et al. 2006), it is commonly used for alien species that may induce ecologically as well as economically negative impacts (e.g., Dittel & Epifanio 2009, Kumschick et al. 2015). When an invasive species is established in a new region, it may have great local ecological impacts by, e.g., being a decomposer, herbivore, predator or parasite (Williamson 1996), or have evolutionary consequences through hybridization (Blackwell et al. 2020). The positive impacts of invasive species may be in terms of contribution to species richness, biotic interactions and ecosystem services (Schlaepfer 2018). Alien species may even be valuable for conservation by being functional substitutes for extinct taxa (Schlaepfer et al. 2011). The negative ecological effects of many invasive species are expected to accelerate in the future due to increased global trade and transportation, as well as climate change (Rahel & Olden 2008, Hulme 2009). A wide range of methods are applied to eradicate or control invasive freshwater fish populations, including physical removal, chemicals or biological control. Chemical treatment with rotenone is one of the most effective measures in fish removal projects (Rytwinski et al. 2018).

Rotenone is a naturally occurring substance derived from tropical plants of the bean family Leguminosae and used for centuries for capturing fish in certain areas of South America and Southeast Asia (Ling 2001). The use of rotenone for fisheries management purposes started in North America in the 1930s (Finlayson et al. 2000). It has been used for several purposes, such as quantifying fish abundances, manipulating fish populations to maintain sport fisheries, treatment of rearing ponds and, increasingly, to eradicate invasive species (McClay 2000, 2005). In addition to the USA, rotenone is currently used in fish management in several countries, e.g. Canada, South-Africa, Australia and New Zealand. In Norway, rotenone treatment has been carried out since the 1960s to control or eradicate undesirable or invasive fish species, and during the latest decades also to eradicate the invasive salmon parasite *Gyrodactylus salaris* Malmberg by killing its hosts, salmonid fishes. When the host is removed the parasite will not survive for a long time. The parasite has severe impact on juvenile Atlantic salmon (*Salmo salar*) in infested rivers, with a mean estimated mortality of 86% (Johnsen et al. 1999). The numerous Norwegian rotenone treatments to eradicate invasive freshwater fish or *G. salaris* during the last two decades have generally been successful (Sandodden et al. 2018, Bardal 2019).

Since rotenone is relatively insoluble in water, other compounds like emulsifiers and solvents need to be added to enhance the mixing and dispersion of rotenone. The amount of rotenone and the composition of liquid formulations have changed over time, and the rotenone formulation currently used in Europe is CFT-Legumine with rotenone constituting 3.3 % (Bardal 2019).

Rotenone may be bound to sediments and suspended material, and natural degradation in water is dependent on water temperature (Dawson et al. 1991) and light (Schnick 1974), but also turbidity and pH (USEPA 2007). The breakdown of rotenone may last from a few days to several weeks (Ling 2001), or even months following lake treatments in late autumn (Adolfsen et al. 2014).

Rotenone affects aerobic respiration on the cellular level by blocking mitochondrial electron transport inhibiting NADH ubiquinone reductase (Singer and Ramsey 1994). Rotenone is highly toxic to fish and certain invertebrates (Fukami et al. 1969), whereas birds and mammals are known to be much less sensitive. Amphibian and fish eggs, as well as adult amphibians, are also less susceptible to rotenone poisoning due to their lower uptake of toxicants from water (Ling 2001). Zooplankton seem generally to be more rotenone sensitive than larger benthic organisms (Vinson et al. 2010, Beaulieu et al. 2021). The development and emergence of dormant copepod and brine shrimp eggs may also be negatively affected by rotenone (Covi et al. 2016, Reed et al. 2018).

Previous findings of rotenone treatment effects on benthic invertebrates range from severe (Binns 1967, Hamilton et al. 2009) to minor impacts (Dudgeon 1990, Blakely et al. 2005). Most studies report taxon-specific responses (e.g., Chandler & Marking 1982, Arnekleiv et al. 2001, Eriksen et al. 2009). The insect orders Ephemeroptera, Plecoptera and Trichoptera, commonly referred to as EPT taxa, are important components of the benthic community and often used as indicator taxa in biomonitoring programs. The EPT taxa are generally known to be rotenone sensitive (Mangum & Madrigal, 1999, Arnekleiv et al. 2001, Eriksen et al. 2009). However, there is a huge variation in sensitivity within these orders, with some taxa being relatively rotenone tolerant (Engstrom-Heg et al. 1978, Kjærstad & Arnekleiv 2011). Rotenone sensitivity may depend on the instar stage, with early instars being typically more sensitive than later instars (Gladsø and Raddum 2002, Kjærstad & Arnekleiv 2011). Gastropoda, Odonata and Coleoptera are known to be rotenone tolerant (Chandler & Marking 1982, Holocombe et al. 1987, Kjærstad & Arnekleiv 2011), whereas taxa rich groups like Chironomidae and Oligochaeta are reported to have a variable rotenone tolerance (Koksvik & Aagaard 1984, Mangum & Madrigal 1999, Pham et al. 2018). However, such groups are generally identified above the species level and the response to rotenone for most species within these groups is unknown.

Despite the negative impacts of rotenone on several benthic invertebrates, the recolonization of most taxa will often take place within a year (Binns 1967, Pham et al. 2018). For treatments covering larger parts of the watershed, recolonization may take several years (Mangum & Madrigal 1999). However, since investigations of rotenone impacts and recolonization of invertebrates often take place over a relatively short time scale, typically up to one year, the long-term effects (> 1 year) following treatment are still poorly known.

Studies of rotenone treatment effects often focus on single treatments. However, in treatments aiming to eradicate *G. salaris*, rotenone has been applied multiple times in each watershed to increase the possibility for a successful outcome (Sandodden et al. 2018). Studies of the effects of several consecutive treatments on non-target taxa are consequently needed.

The reason for the somewhat contradictory findings on the rotenone treatment effects and recovery of invertebrates may be due to differences in the spatial and temporal extent of the treatments, study approach, and taxon-specific variation in piscicide toxicity (Vinson et al. 2010). Regarding variation in toxicity among taxa, it is important to know which taxa are present at a given time. In many cases, the absolute species diversity and community-level responses are overlooked if studied with conventional, morphological identification methods that are particularly problematic with small species and instar stages. Due to coarse taxonomic resolution, species-level responses to stressors may be overlooked (Beermann et al. 2018). Consequently, there is an urgent need to improve the detection of species-level taxa to better understand the effects of rotenone treatments.

New cost-effective methods, such as DNA metabarcoding, have the potential to overcome some of the limitations of traditional taxa identification methods. DNA can be extracted from environmental samples of water, sediment or air (environmental DNA, eDNA), from sample preservatives where animals are conserved, or directly from the sampled individuals, either individually or as a sample mixture. The use of high-throughput sequencing platforms enables simultaneous sequencing of DNA from a high number of taxa. Sequences may then be associated with taxonomic names from a reference database. DNA-based methods have shown promising results in biomonitoring of benthic invertebrates (Gibson et al. 2015, Elbrecht et al. 2017), but there are challenges such as contamination in the field or laboratory, incomplete reference DNA databases, and wrong interpretation of the data (Goldberg et al. 2016).

Accurate taxa identification is of high importance for tracking changes in invertebrate communities, but it is not the only way to evaluate the effects of rotenone treatments. Following piscicide treatments, the community and food-web structure of the ecosystem may change due to the effect of fish removal and removal or reduction of rotenone sensitive invertebrates and subsequent restocking of fish. Fish may impact invertebrate communities directly by reducing the number of certain benthic invertebrates, especially of large-bodied taxa (Weyl et al. 2010, Jeppesen et al. 2017). The presence of fish can also have indirect effects by restricting the habitat and resource use of invertebrates (e.g., Bernot & Turner 2001). When fish are removed, predatory invertebrates may partly take over the fishes' role as top predator and induce marked top-down effects (Cobbaert et al. 2010). Stable isotopes are especially useful for evaluating the trophic relationships among organisms since they integrate changes in space and time (Layman 2012). To my knowledge, the use of stable isotopes and DNA metabarcoding has not been used previously to evaluate the responses of freshwater communities following rotenone treatments.

Objective and research questions

The main objective of this thesis was to investigate how freshwater ecosystems, with a focus on benthic invertebrates, respond to alien species eradication using rotenone treatments. The four papers included in the thesis aim to complement the knowledge gaps as identified in the previous paragraphs. The knowledge gaps are addressed through answering the following research questions:

1. What is the effect in terms of taxa composition of repeated rotenone treatments on riverine benthic invertebrates? (paper I).
2. What are the long-term impacts (i.e., up to four years) of rotenone treatments on riverine and lake benthic invertebrate communities? (paper II).
3. Can increased taxonomic resolution in identification by use of DNA metabarcoding give further insight into responses of benthic invertebrates to rotenone treatments?(paper III).
4. Do rotenone treatments alter the trophic resource use and trophic diversity of benthic invertebrate communities? (paper IV).

Study sites and rotenone treatments

The field experiments were conducted in three boreal study sites, two in Central Norway and one in southern part of Northern Norway (Figure 1). The purpose of the rotenone treatments was to eradicate alien species, the salmon parasite *Gyrodactylus slaris* in River Ogna (paper I) and River Fusta (paper II) and roach (*Rutilus rutilus*) in the Bymarka lakes (paper III and IV).

Data for paper I were sampled in River Ogna situated in the county of Trøndelag at 64˚ N. River Ogna drains mainly lowland areas and flows into Trondheimsfjorden. The river has a mean annual water discharge of 22 $m^3 s^{-1}$. The lower 18 km of the river was rotenone treated three times: in April and October 2001, and in August 2002. River stretches upstream of the treated area served as control areas (figure 2, upper panel). The rotenone formulation CFT-Legumin with an active ingredient of 2.5% was used in all three treatments.

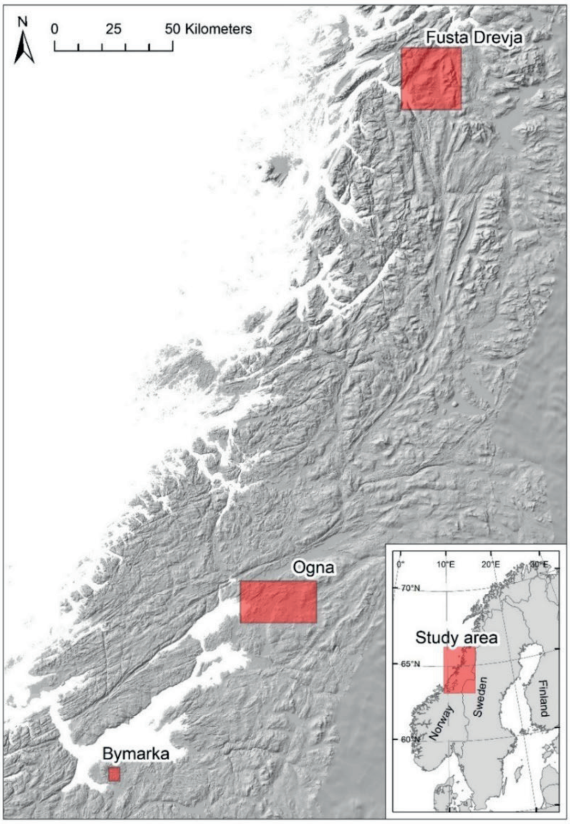

Figure 1. Overview of the location of the study sites (shown in red).

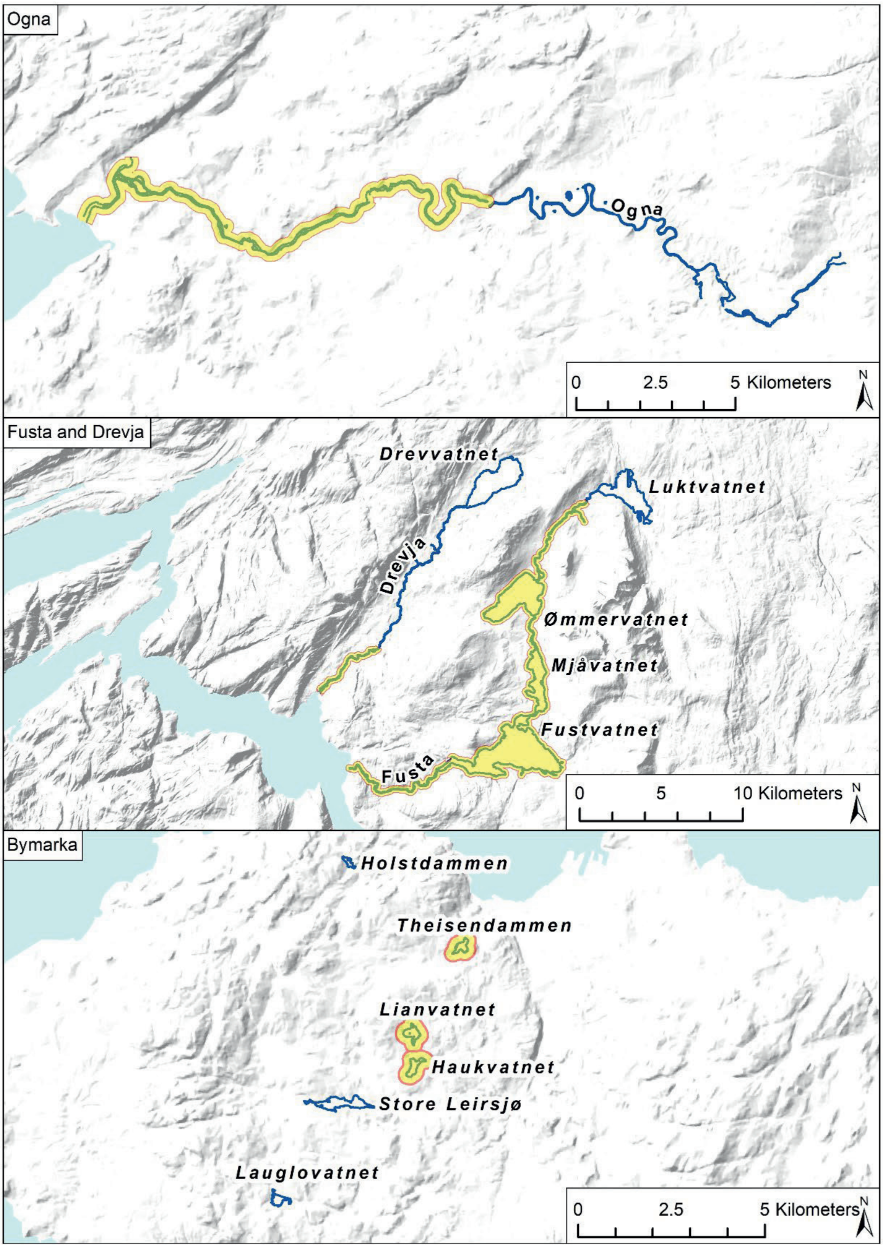

Figure 2. Maps showing the study sites; Upper panel: Ogna (paper I), middle panel: Fusta and Drevja (paper II) and lower panel: Bymarka (paper III and IV). Yellow markings indicate the rotenone treated sites and blue markings untreated control sites, except for Lake Luktvatnet, which was not part of the study.

Sampling of the Fusta watercourse in the county of Nordland at 65˚N was the basis for paper II, along with the adjacent untreated part of the Drevja watercourse, which served as a control site (Figure 2, middle panel). Both watercourses flow into Vefsnfjorden and drain sparsely vegetated mountain areas, spruce dominated forests and scattered farmlands. In the Fusta watercourse, both the oligotrophic lake Fustvatnet, which is the lowermost lake, and its outlet River Fusta, were studied. The lake has a surface area of 11 km^2 and the river has a mean annual water discharge of 34 $m^3 s^{-1}$. The control sites were the oligotrophic Lake Drevvatnet with a surface area of 5 km^2, and River Drevja with a mean annual water discharge of 12 $m^3 s^{-1}$. River Fusta was rotenone treated in August 2011 and August 2012, whereas Lake Fustvatnet was treated in October 2012. River Fusta was affected by rotenone a third time due to supply of rotenone from the upstream lake Fustvatnet during late autumn of 2012 and spring 2013. In River Fusta and in the upper surface layers of Lake Fustvatnet, CFT-Legumin with an active ingredient of 2.5% was used, whereas an active ingredient of 3.3% was used for the deeper layers.

Data from oligotrophic lakes in Bymarka, which is a popular recreational area near the city of Trondheim at 63˚N, were used in papers III and IV (Figure 2, lower panel). The six lakes are situated in coniferous dominated forests and are relatively small with a surface area of 4.4-11.1 ha. All lakes (3 rotenone treated and 3 untreated control lakes) were investigated in paper IV, while four of them (2 rotenone treated and 2 control lakes) were investigated in paper III. The treatment took place in September 2016 using CFT-Legumin with an active ingredient of 3.3%.

Methods

The methodological toolkit employed included both traditional sampling and morphological taxa identification methods, identification of specimens through DNA metabarcoding from preservative ethanol (peDNA), as well as inference of resource use and trophic diversity of the benthic invertebrate communities from stable isotope analyses (SIA). Below, I give a brief overview of the methods used. More detailed descriptions are given in the methods sections of each paper.

Benthic invertebrate sampling was always performed before and after the rotenone treatments both in treated and untreated control sites, either upstream and downstream of the rotenone release (paper I), or in treated and untreated control lakes or rivers (paper II-IV). Benthic invertebrates were sampled quantitatively by Surber sampling (paper I) and qualitatively by kick sampling (paper I and II) or Z-sweep sampling (paper III and IV). All nets had a mesh size of 250 µm. Samples for analyses of benthic invertebrate community composition collected from each sampling station were stored separately in 96% ethanol. Using a stereo microscope, benthic invertebrates were determined to the lowest possible taxonomic level (paper I-IV). Additional Z-sweeps as well as a hand sieve (1 mm mesh) were used to collect material for stable isotope analyses (SIA) from various littoral lake habitats (paper IV). All benthic invertebrates were picked and cleaned from detritus and other unwanted material prior to storage into sample tubes for subsequent analyses. After sorting and taxa identification, SIA samples were frozen at -20˚C and later freeze-dried.

In addition to traditional morphological identification of taxa of each sample, DNA from preservative ethanol (peDNA) from the same samples were used in combination with metabarcoding in paper III. The peDNA was extracted from all Z-sweep samples using Qiagen DNeasy Blood & tissue kit on filtered ethanol with a maximum volume of 300 mL per sample. Each peDNA extract was amplified and sequenced in three replicates. Quality trimming of the reads was carried out in the R package DADA2 1.14 (Callahan et al. 2016). Metazoan amplicon sequence variants (ASVs) were selected and the taxonomic affiliations were cross-checked against a reference database.

Stable isotopes can be used to track the basal sources of energy in a food web (Layman et al. 2012). In paper IV, resource use and trophic diversity of the invertebrate community was inferred using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios. In freshwater lakes, the stable isotope values of consumers reflect those of their primary carbon sources, with littoral benthic primary producers and consumers being commonly enriched in the heavier 13C isotope (thus having higher $\delta^{13}C$ values) as compared to planktonic producers and consumers in the pelagic open water areas. Stable nitrogen isotope signatures are typically used to identify the trophic position as consumers become enriched in the heavier 15N isotope with each trophic step in the food web. Samples from both the invertebrate community and their basal food resources (particulate organic matter, detritus, periphyton and leaves) were analyzed. The relative change of benthic invertebrate functional feeding groups (predators, gatherers-collectors, grazers, filter-feeders and shredders) following treatment was examined.

Findings

Paper I: Effects of three consecutive rotenone treatments on the benthic macroinvertebrate fauna of the River Ogna, Central Norway

Research question: What is the effect of repeated rotenone treatments on riverine benthic invertebrates?

Rotenone treatments may be performed as single treatments or as several consecutive treatments. However, most studies of the impacts on non-target taxa are based on single rotenone treatments. In this study, the effects in terms of taxa composition of three treatments in April and October within the same year and in August the following year on riverine benthic invertebrates were investigated.

The two first treatments showed minor negative effects on the benthic fauna with reduction in densities of only a few rotenone sensitive taxa like the mayfly *Baetis rhodani* and the caddisfly *Rhyacophila nubila*. Immediately after the third treatment, the densities of most taxa declined, and several were not recorded at all in the treated area. Despite the severe impact of the third treatment, most taxa had recovered to pre-treatment abundances within a year. Immediately after each treatment, the difference in community composition between the rotenone treated and untreated area increased, being highest after the third treatment (figure 3), indicating treatment effects. Later, the community composition of the treated and control area became more similar, probably mainly due to recolonization.

Toxicity of rotenone is known to increase with increasing water temperature (Andreasson 1963, Meadows 1973). During the third treatment, the water temperature was much higher (20˚C) compared to the two first treatments (4 and 8˚C). High water temperature enhancing the toxicity is likely the main reason for the severe effects of the third treatment, rather than the number of treatments.

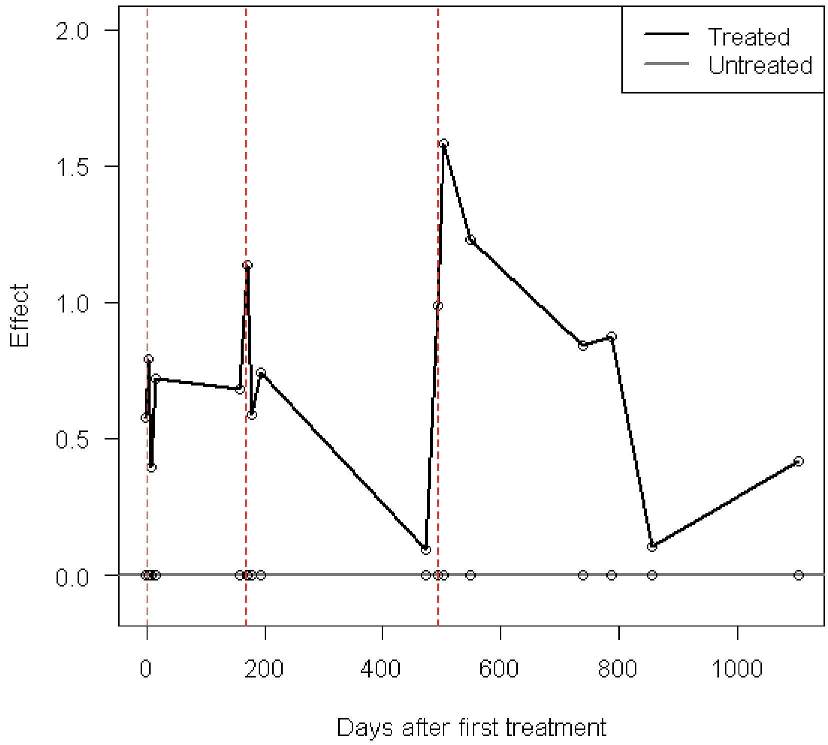

Figure 3. Overall change in community composition over time in River Ogna shown as principal response curve (Van den Brink & Ter Braak 1999) based on kick samples of benthic invertebrates (log transformed counts). The black line shows the effect of the treatment relative to the samples of the untreated stations (grey line at y = 0). Points show the dates when samples were taken. The dashed red vertical lines show the timing of the three rotenone treatment applications.

Paper II: Long-term responses of benthic invertebrates to rotenone treatment

Research question: What are the long-term impacts (i.e., up to four years) of rotenone treatments on riverine and lake benthic invertebrate communities?

Most investigations of impacts of rotenone treatments on benthic invertebrates are typically conducted for a period of up to one year after treatment and thus potential long-term effects are poorly known. Here, we investigated one treated and one control river and lake for up to four years after rotenone exposure. The river was rotenone treated in August 2011 and August 2012 and the upstream lake was treated in October 2012.

Both the immediate (i.e., < 1 month) and the long-term (4 years) effects in terms of abundance and occurrence of taxa for lotic and lentic habitats were minor. The breakdown of rotenone in the lake was slow, probably due to low water temperatures. Therefore, the outlet river received low-dose rotenone from the lake for a long period, causing the lotic fauna to be severely affected eight months after the lake treatment. The temporal beta diversity index (TBI) confirms that changes in taxa composition (based on occurrence and abundance data) of the river invertebrate community were significant and most evident in the period after the lake treatment from October 2012 to June 2013 (figure 4a and d). This change in taxa composition was mainly due to losses in terms of abundance and occurrence of taxa (figure 4b and e). The highest gains in terms of abundance and occurrence of taxa in the treated river occurred between June and August 2013, indicating rapid recovery.

The differences in TBI between the treated and untreated lakes were minor as compared to those observed in rivers. The most pronounced divergence was seen in 2015, when the losses in benthic invertebrate abundances were at the highest level in the treated lake, most likely due to heavy predation by restocked fish.

This study shows that duration of rotenone exposure is of high importance. Although the long-term effects were minor, the findings highlight the need for long-term monitoring studies as reoccurrence of some taxa lasted for several years and some were still missing after four years.

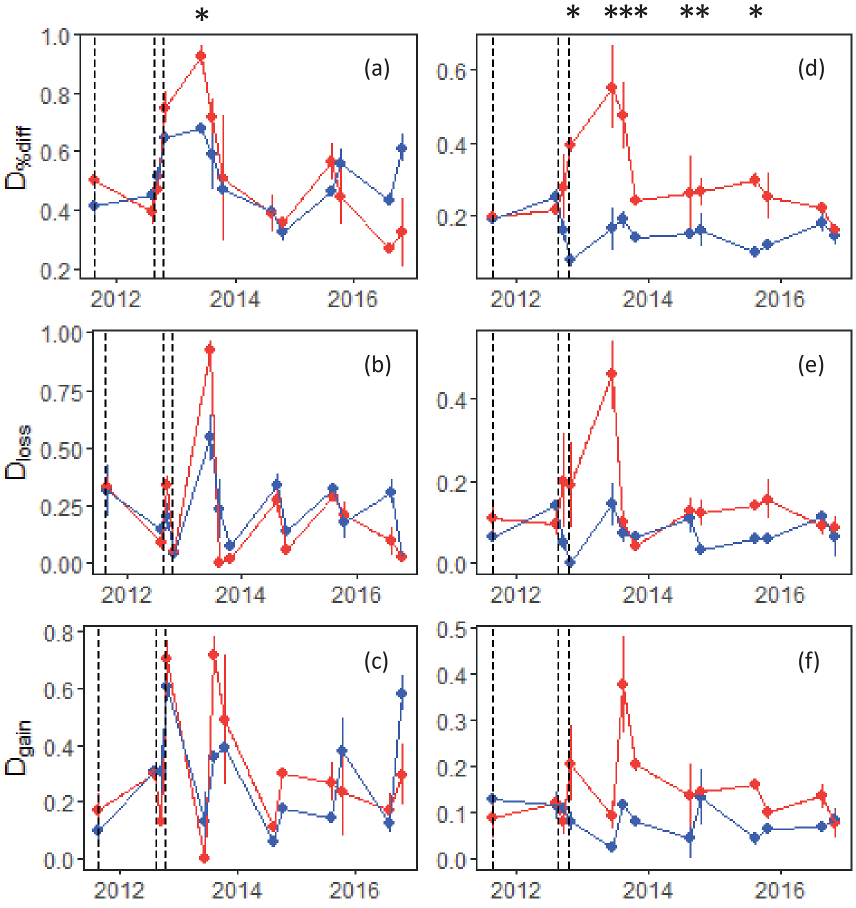

Figure 4. Mean changes in dissimilarity (% difference) for abundance data (a - c) and for occurrence data (d - f) between sampling occasions (a and d) and their components: losses (b and e) and gains (c and f). Red: River Fusta (rotenone treated), blue: River Drevja (untreated). Dashed vertical lines indicate the timing of the rotenone treatments. Error bars indicate maximum and minimum values of the temporal biodiversity indices at individual sampling stations. Significant differences in diversity indices between sampling periods ($p < 0.05$) in River Fusta are marked with an asterisk (no significant differences detected in River Drevja).

Paper III: DNA metabarcoding of preservative ethanol reveals changes in invertebrate community composition following rotenone treatment

Research question: Can increased taxonomic resolution in identification by use of DNA metabarcoding give further insight into responses of benthic invertebrates to rotenone treatments? (paper III).

DNA-based taxa identification methods have the potential to overcome some of the limitations of traditional morphological methods, which are time consuming and expensive, and often problematic regarding identification of early life stages or poorly known taxa (Chang 2020, Rimet et al. 2021). Here, we use both preservative ethanol DNA (peDNA) in combination with metabarcoding as well as morphology-based taxa identification to evaluate the effect of rotenone treatment on benthic invertebrates expressed as Bray-Curtis dissimilarity, number of species and number of individuals and DNA reads.

Both methods showed a significant impact of rotenone treatment on taxa composition, expressed as Bray-Curtis dissimilarity. The Bray-Curtis dissimilarity was higher for the treated lakes compared to the control lakes for both methods, except for samples taken within the same stations and same time (figure 5).

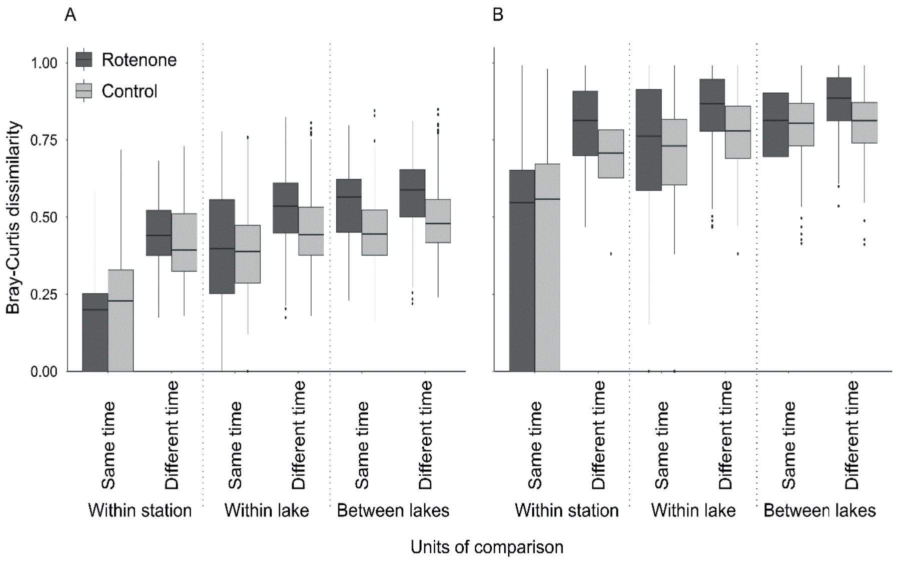

Figure 5. Pairwise Bray-Curtis dissimilarity values of rotenone treated and control lakes. (A) Data based on morphological identifications. (B) Data based on peDNA. The horizontal line in each box represents the median, the boxes depict the 1st to the 3rd quantile and the blind ends of the whiskers represent the maximum and minimum values, except outliers marked as dots. The stippled line differentiates the units of comparisons (within stations, within lakes and between lakes).

Based on the peDNA metabarcoding data, the number of taxa dropped after the treatment but increased one year after the treatment in the treated lakes, whereas no significant changes were observed in the control lakes, indicating treatment effects. Based on the morphological identification data, similar changes in the number of taxa were observed both in the treated and control lakes, indicating natural variation rather than treatment effects. The different results of the two methods were most likely due to a higher taxonomic resolution by the peDNA metabarcoding method, especially for species rich groups, which are difficult to identify to the species level by morphology. Both the number of specimens and the number of reads (DNA sequences) decreased just after the treatment and increased again one year later in the treated lakes. For the control lakes the amounts were generally more even over time, but an increase was observed for the number of specimens one year after the treatment.

Due to the ability of the DNA-based method to detect far more taxa (333) than the morphological method (90), it seems like a useful method for investigations of stressor impacts on invertebrate communities. However, since it also detected taxa not present in the samples, it needs to be further developed and to be used in combination with morphological-based identification.

Paper IV: Impacts of piscicide-induced fish removal on resource use and trophic diversity of lake invertebrates

Research questions: Do rotenone treatments alter the trophic resource use and trophic diversity of benthic invertebrate communities?

The vast majority of studies of impacts of rotenone treatment on invertebrates have focused on taxon-specific responses of rotenone and recovery time. However, little is known of the potential effect of rotenone treatments on the resource use and trophic diversity of the benthic invertebrate community. In paper IV, stable carbon and nitrogen isotope data were used to examine rotenone impacts on the trophic niche of benthic invertebrates by comparing data from one year before and one year after the treatment.

Based on pooled isotope data, the $\delta^{13}C$ and $\delta^{15}N$ values of benthic invertebrates did not differ between the before and after treatment periods in the treated or untreated lakes. (figure 6). The functional feeding groups predators (dragonflies - Odonata) and grazers (snails - Lymnaeidae) showed increased $\delta^{13}C$ values after rotenone treatment, suggesting increased feeding on littoral benthic algae, whereas gatherer-collectors (mayfly larvae - *Leptophlebia*) showed decreased $\delta^{13}C$ values following fish removal in treated lakes. Grazers (Lymnaeidae) shifted to a lower trophic position (low $\delta^{15}N$), whereas filter-feeders and shredders showed no changes in $\delta^{15}N$ following rotenone-induced fish removal.

The relative abundance of predators increased whereas that of grazers decreased after rotenone treatment in the treated lakes. No consistent changes were observed in the community-level isotopic niche areas of benthic invertebrates. However, both the sample-size corrected SEA_C and the Bayesian SEA_B estimates of standard ellipse areas slightly increased in two of the three treated lakes, apparently due to increased range in $\delta^{15}N$.

Overall, the study suggests minor changes in the resource use and trophic diversity of benthic invertebrate communities following rotenone treatments of lakes.

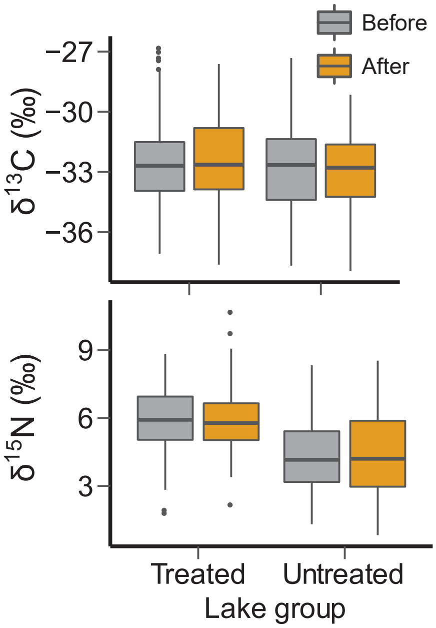

Figure 6. Boxplots showing $\delta^{13}C$ and $\delta^{15}N$ values (in ‰) of benthic invertebrates collected from treated and untreated lakes before and after rotenone treatment. The horizontal line in each box represents the median, the boxes depict the 1st to the 3rd quantile and the blind ends of the whiskers represent the maximum and minimum values, except outliers marked as dots.

Discussion

In this thesis, I have demonstrated both short- and long-term impacts of rotenone treatments on freshwater benthic invertebrate communities, as well shown the effects of rotenone treatment on the benthic food-web compartment in lake ecosystems.

In Rivers Ogna and Fusta, consecutive rotenone treatments were conducted and studied (paper I and II). In both instances, the two first treatments had relatively minor effects on benthic invertebrates. This has also been found in other projects involving two treatments (Kjærstad & Arnekleiv 2016, Bellingan et al. 2019). The most severe impact was found after the third treatment, when several taxa surviving the first two treatments went missing. However, it is not likely that this strong negative impact was a result of being number three in a row of consecutive treatments. Instead, the third treatments differed from the previous ones in terms of dose or duration. In River Ogna (Paper I), there was a short-term high-dose exposure. In case of the River Fusta (Paper II), there was a long-term low-dose exposure. The negative impacts of rotenone in River Ogna were probably further enhanced by the higher water temperature during the third treatment, since rotenone toxicity increases with increasing temperature (Andreasson 1963, Meadows 1973). The findings in paper II show that rivers situated downstream of rotenone treated lakes may be exposed to rotenone over a long period if breakdown of rotenone is slow due to e.g., low water temperature. The prolonged low-dose exposure time following the lake treatment, probably of several months, had a much larger negative effect on the benthic fauna than the short (hours) high-dose exposure of the river.

The long-term effects of piscicide treatments on invertebrates are poorly known (Vinson et al. 2010). In paper II, the effects on the benthic community after four years following rotenone treatment were found to be minor, and total invertebrate abundances reached pre-treatment levels within a year. In accordance with the findings of Mangum & Madrigal (1999), recolonization of some taxa took several years and a few taxa never reappeared. Species turnover and abundance co-varied markedly between treatment and control sites, indicating that natural variation may to a high degree override impacts of rotenone treatments. This is also recorded by Bellingan et al. (2019) who found large fluctuations in benthic invertebrate abundance both in treatment and control sites, but no apparent rotenone treatment effect. Paper II highlights the importance of having long-term monitoring with control sites.

The relatively rapid recovery seen in papers I–IV indicates high dispersal abilities of benthic invertebrates. Even if relatively large parts of the watersheds were treated (e.g., paper II), there were still numerous untreated tributaries from which drifting invertebrates could enter the treated areas. Moreover, eggs or later instars may be burrowed in the bottom substrate and thereby escape rotenone exposure (Mangum & Madrigal 1999). Another source of colonization may be flying aquatic insects that lay their eggs soon after rotenone treatment, or organisms that use birds or insects as dispersal vectors (Reynolds et al. 2015).

The response of benthic invertebrates to rotenone treatment in terms of number of individuals was studied thoroughly in papers I and II. The findings are in line with previous studies showing large variation in taxon-specific responses to rotenone, ranging from sensitive to tolerant (e.g., Eriksen et al. 2009, Kjærstad & Arnekleiv 2011, Dalu et al. 2015). However, papers I and II demonstrate that even taxa that are known to be rotenone tolerant, like the mayfly genus *Ephemerella* and elmid beetles, may be negatively affected. This is more likely to happen when the acute toxic effect of rotenone is high, as is the case during high water temperature (paper I), or if the exposure time is long due to slow breakdown of rotenone (paper II).

Paper III demonstrated that both traditional methods based upon morphological identification and methods based upon identification using DNA from preservative ethanol (peDNA) in combination with metabarcoding do detect rotenone treatment impacts on community composition. However, unlike the traditional method, the peDNA based method indicated an initial decrease in the average number of taxa shortly after the treatment and a subsequent increase one year later in the treated lakes. This indicates that the method was able to detect an initial negative effect of rotenone and a later recolonization of taxa. The major difference between the two methods is that peDNA based identification detected more taxa at the species-level than the morphological identification method. These findings suggest that the peDNA based method has a high potential for a more accurate monitoring of invertebrate communities. However, there were only 28 common taxa in the traditional and peDNA based method identification. Several taxa were identified using morphological methods that were not detected by using peDNA. Additionally, the peDNA based method detected taxa that were apparently not present in the samples, but most likely originated from DNA transported from upstream areas. Our results are in line with a recent meta-analysis were taxa identification of macroinvertebrates by DNA metabarcoding generally give complementary rather than identical results compared to traditional methods (Keck et al. 2022).

Paper IV provides novel knowledge of the effects of rotenone treatment and fish removal on the trophic niche of lake invertebrate communities. The removal of the predation pressure from fish has probably allowed large-sized predators like Odonata to increase in abundance and partly replace fish as the top predator. This argument is also supported by the observed slight community-level niche expansion in two treated lakes, associated with increased variation in $\delta^{15}N$ values of predatory invertebrates. The reduction of grazers (snails) was unexpected as this group is known to be rotenone tolerant (Holocombe et al. 1987, Kjærstad & Arnekleiv 2011). In the absence of fish predation, an increase rather than a decrease would have been expected for the snails that are often heavily predated by fish (e.g., Batzer et al. 2000). In paper IV, we cannot fully separate between the chemical effects of rotenone treatment and the biotic impacts in terms of fish removal and/or altered competitive and predatory impacts among benthic invertebrates. Moreover, in paper II, fish predation was shown to influence the benthic community. Here, an increase of the large mayfly larva *Ephemera* was recorded after fish removal. After restocking of salmonid fishes, the abundance decreased. The stable isotope data in paper IV indicated minor shifts in resource use and trophic diversity among benthic invertebrate communities. Following rotenone treatment, the community-level isotopic niche areas (SEA_C and SEA_B) slightly increased in two of the treated lakes due to increased range in $\delta^{15}N$.

Although this thesis is relatively comprehensive and uses different study approaches to elucidate rotenone treatment impacts, it has some limitations. In paper II-IV, only littoral lake habitats were investigated. The invertebrates in the pelagic and profundal habitats could potentially be more negatively affected by rotenone than invertebrates in the littoral zone, as shown for pelagic crustaceans in previous studies (e.g. Melaas et al. 2001, Beaulieu 2021). The breakdown of rotenone in deeper and colder areas may be slow and thus the toxicity of rotenone may persist longer than in the shallower areas. Our investigation of only one of the main lake habitats may therefore have underestimated the effect of rotenone treatment on the total invertebrate community. The sampling of basal feeding resources of the benthic invertebrates was limited to only a few samples collected before and after the treatment. This made the evaluation of potential changes in the resource use of benthic invertebrates uncertain. Sampling of invertebrates was mainly qualitative, except the data obtained from Surber sampling (paper I). Quantitative and more standardized sampling with e.g. an Ekman grab could have resulted in more accurate measures of density and biomass of various benthic invertebrate taxa. The relatively short study period of one year (paper III) prevented detection of potential long-term impacts, including the restocking of native brown trout (*Salmo trutta*) to treated lakes in the Bymarka area.

From a management perspective, a thorough mapping of non-target taxa should be conducted prior to treatment. This is important since the distribution of rare or vulnerable species may be limited to the treated areas. If the mapping of taxa is poor, unwanted eradication of such valuable species may occur undetected. Managers could consider rescuing rare or vulnerable species prior to rotenone treatment and restocking them after treatment. Treatment during very high or very low temperature periods is not recommended since the rotenone toxicity increases with temperature, whereas the breakdown of rotenone slows down during low temperature periods. This may induce short-term high-dose exposure in warm periods and long-term low-dose exposure during low temperature periods. In both situations, non-target taxa may be severely affected.

For management projects aiming to eradicate fish ectoparasites, new treatment methods using chemicals like aluminum sulphate and chlorine have been developed (Hindar et al. 2015, Hagen et al. 2018). The lower tolerance of the salmon parasite *Gyrodactylus salaris* than of the fish to these compounds allows for eradication of the parasite without killing the fish host. Both aluminum sulphate and chlorine have been shown to have minor impacts on benthic invertebrates compared to rotenone (Eriksen et al. 2009, Eriksen 2018). The use of such compounds, especially chlorine, is expected to increase in the future.

Future research on long-term impacts of rotenone treatment on non-target taxa is still limited, and more studies spanning over several years should be conducted. The temporal fluctuations in abundance and occurrence of invertebrate taxa are high in freshwater ecosystems and therefore control localities are needed to account for natural variation. Rotenone tolerance for many taxa is unknown, especially within species rich groups such as non-biting midges (Chironomidae), aquatic annelids (Oligochaeta) and water mites (Hydrachnidia). Consequently, the taxonomic resolution of poorly known taxa needs to be improved in future research to understand the responses of non-target taxa to rotenone. This could be facilitated by including new methods like DNA metabarcoding. The impact of rotenone treatment on food webs in freshwater ecosystems is largely unknown and further studies are needed.

Conclusion

Freshwater ecosystems are considered as one of the most vulnerable ecosystems to biodiversity loss, with invasive species being among the main drivers of homogenization of freshwater communities (Villéger et al. 2011, Reid et al. 2019). Other human stressors, such as climate change, pollution and habitat deterioration often interact with species invasions and cause major negative impacts on the recipient ecosystems (Kovach et al. 2016, Zhang et al. 2019). Chemical eradication of invasive species has proven as one of the most successful and efficient ways to control establishment and further spread of unwanted non-native species in freshwater ecosystems (Rytwinski et al. 2018). However, such major ecosystem disturbances, including rotenone treatments, should be planned and conducted with caution to avoid irreversible negative impacts on non-target and potentially endangered native species. Therefore, to supplement the findings of this thesis and previous studies, new comprehensive research of aquatic species and ecosystems are needed to understand and predict the potential positive and negative impacts of rotenone treatments on different spatial and temporal resolutions.

Co-author contributions

Paper I: GK and JVA conceived the initial ideas. JMDS led the data analyses. GK led the writing. All authors contributed to the writing and final version of the text.

Paper II: GK and JVA conceived the initial ideas. AGF, GK and GV contributed to the data analyses. GK led the writing. All authors contributed to the writing and final version of the text.

Paper III: GK, TE and MAM conceived the initial ideas. MF and LT led the lab work. MAM led the data analyses and GK the writing. All authors contributed to the writing and final version of the text as submitted here.

Paper IV: GK and APE conceived the initial ideas. APE led the data analyses and the writing. All authors contributed to the writing and final version of the text as submitted here.

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References

Adolfsen, P., Sandvik M. & Waaler, T. 2014. Rotenonanalyser og resultater. In: Bekjempelse av *Gyrodactylus salaris* i Vefsnaregionen. (Eds: J. H. Stensli & H. Bardal), pp. 119–129. Veterinærinstituttets rapportserie 2-2014.

Andreasson, S. 1963. Rotenonets inverkan på evertebrater i relation til vissa miljöfaktorer. Svensk Fiskeritidskrift 72: 90–95.

Arnekleiv, J.V., Dolmen D. & Rønning L. 2001. Effects of rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. In: Trends in Research in Ephemeroptera and Plecoptera. (Ed: E. Dominguez), pp. 77–88. Kluwer Academic/Plenum Publishers.

Bardal, H. 2019. Small- and large-scale eradication of invasive fish and fish parasites in freshwater systems in Norway. In: Island invasives: scaling up to meet the challenge. (Eds: C.R. Veitch, M.N. Clout, A.R. Martin J.C. Russell & C.J. West.), pp. 447–451. IUCN, Gland, Switzerland. https://doi.org/10.2305/IUCN.CH.2019.SSC-OP.62.en

Batzer, D.P., Pusateri, C.R. & Vetter, R. 2000. Impacts of fish predation on marsh invertebrates: Direct and indirect effects. Wetlands 20: 307–312. https://doi.org/10.1672/0277-5212(2000)020[0307:IOFPOM]2.0.CO;2

Beaulieu, J., Trépanier-Leroux, D., Fischer, J. M., Olson, M. H., Thibodeau, S., Humphries, S., Fraser, D. J. & Derry, A. M. 2021. Rotenone for exotic trout eradication: nontarget impacts on aquatic communities in a mountain lake. Lake and Reservoir Management 37: 323–338. https://doi.org/10.1080/10402381.2021.1912864

Beermann, A.J., Zizka, V.M.A., Elbrecht, V., Baranov, V. & Leese, F. 2018. DNA metabarcoding reveals the complex and hidden responses of chironomids to multiple stressors. Environmental Science Europe 30: 26. https://doi.org/10.1186/s12302-018-0157-x

Bellingan, T., Sanet, H., Woodford, D.J., Gouws, J., Villet, M.H. & Weyl, O.L.F. 2019. Rapid recovery of macroinvertebrates in a South African stream treated with rotenone. Hydrobiologia 834: 1–11. https://doi.org/10.1007/s10750-019-3885-z

Bernot, R.J. & Turner, A.M. 2001. Predator identity and trait-mediated indirect effects in a littoral food web. Oecologia 129: 139–146. https://doi.org/10.1007/s004420100705

Binns, NA. 1967. Effects of rotenone treatment on the fauna of the Green river, Wyoming. Fish Research Bulletin 1: 1–114.

Blakely, T. J., Chadderton W.L. & Harding, J. S. 2005. The effect of rotenone on orchard-pond invertebrate communities in the Motueka area, South Island, New Zealand. Doc Research & Development Series 220. 26 pp.

Blackwell, T., Ford, A. G. P., Ciezarek, A. G., Bradbeer, S. J., Gracida Juarez, C. A, Smith, A.M., Ngatunga, B.P.,… Genner, M.J. 2020. Newly discovered cichlid fish biodiversity threatened by hybridization with non-native species. Molecular Ecology 30: 895–911. https://doi.org/10.1111/mec.15638

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. 2016. dada2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13: 581–583. https://doi.org/10.1038/nmeth.3869

Chandler, J. H. & Marking, L.L. 1982. Toxicity of Rotenone to Selected Aquatic Invertebrates and Frog Larvae. The Progressive Fish-Culturist 44: 78–80. https://doi.org/10.1577/1548-8659(1982)44[78:TORTSA]2.0.CO;2

Chang, J. 2020. Monitoring Biodiversity and Water Pollution via High-Throughput eDNA Metabarcoding. Berkeley Scientific Journal 24: 50–58. http://dx.doi.org/10.5070/BS3242049349

Cobbaert, C., Bayley S. E. & Greter J.-L. 2010. Effects of a top invertebrate predator (*Dytiscus alaskanus*; Coleoptera: Dytiscidae) on fishless pond ecosystems. Hydrobiologia 644: 103–114. https://doi.org/10.1007/s10750-010-0100-7

Covi, J.A., Hutchison, E.R., Neumeyer, C.H. & Gunderson, M.D. 2016. Rotenone decreases hatching success in brine shrimp embryos by blocking development: implications for zooplankton egg banks. PLOS ONE 11: e0163231. https://doi:10.1371/journal.pone.0163231

Dalu, T., Wasserman, R. J., Jordaan, M., Froneman, W.P. & Weyl, O.L.F. 2015. An Assessment of the Effect of Rotenone on Selected Non-Target Aquatic Fauna. PLOS ONE 10: e0142140. https://doi.org/10.1371/journal.pone.0142140

Dawson, V. K., Gingerich, W. H., Davis, R. A. & Gilderhus, P. A. 1991. Rotenone Persistence in Freshwater Ponds: Effects of Temperature and Sediment Adsorption. North American Journal of Fisheries Management 11: 226–231. https://doi.org/10.1577/1548-8675(1991)011<0226:RPIFPE>2.3.CO;2

Dittel, A. & Epifanio, C. 2009. Invasion biology of the Chinese mitten crab *Eriochier sinensis*: A brief review. Journal of Experimental Marine Biology and Ecology 374: 79–92. https://doi.org/10.1016/j.jembe.2009.04.012

Dudgeon, D. 1990. Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks in two Papua Guinea streams. Archives of Hydrobiology 119: 35–53.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.-I., Knowler, D.J., Lévêque, C., Naiman, R. J.,… Sullivan, C.A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163–182. https://doi.org/10.1017/S1464793105006950

Elbrecht, V., Vamos, E.E., Meissner, K., Aroviita, J. & Leese, F. 2017. Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. Methods in Ecology and Evolution 8: 1265–1275. https://doi.org/10.1111/2041-210X.12789

Engstrom-Heg, R., Colesante, R.T. & Silco, E. 1978. Rotenone tolerances of stream-bottom insects. New York Fish Game Journal 25: 31–41.

Eriksen, T. E., Arnekleiv, J. V. & Kjærstad, G. 2009. Short-Term Effects on Riverine Ephemeroptera, Plecoptera, and Trichoptera of Rotenone and Aluminum Sulfate Treatment to Eradicate *Gyrodactylus salaris*. Journal of Freshwater Ecology 24: 597–607. https://doi.org/10.1080/02705060.2009.9664337

Eriksen, T. E. 2018. Korttidseffekter på elvelevende bunnfauna av kloraminbehandling mot parasitten *Gyrodactylus salaris* i Glitra. NIVA-rapport 7237–2018. 28 pp.

Finlayson, B. J., Schnick, R. A. Cailteux, R. L., DeMong, L., Horton, W. D. McClay, W., Thompson, C. W. & Tichacek, G. J. 2000. Rotenone use in fisheries management: administrative and technical guidelines manual. American Fisheries Society, Bethesda, Maryland.

Fukami, J. I., Shisido, T., Fukunaga, K. & Casida, J.E. 1969. Oxidative metabolism of rotenone in mammals, fish and insects, and its relation to selective toxicity. Journal of Agricultural and Food Chemistry 17: 1217–1226.

Gibson, J.F., Shokralla, S., Curry, C., Baird, D.J., Monk, W.A., King I. & Hajibabaei, M. 2015. Large-scale biomonitoring of remote and threatened ecosystems via high-throughput sequencing. PLOS ONE 10:e0138432. https://doi.org/10.1371/journ al.pone.01384 32

Gladsø, J.A. & Raddum, G.G. 2002. Rotenone treatment of a west Norwegian River: effects on drift of invertebrates. Verhandlungen—Internationale Vereinigung für Theoretische und Angewandte Limnologie 28: 764–769. https://doi.org/10.1080/03680770.2001.11901816

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F.,… Taberlet, P. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution 7: 1299–1307. https://doi.org/10.1111/2041-210X.12595

Hagen, A. G., Garmo, Ø., Hytterød, S., Olstad, K., Darrud, M., Holter, T., Svendsen, J.,… Gjessing, M. 2018. Forsøksbehandling med monokloramin mot *Gyrodactylus salaris* i elva Glitra. NIVA rapport 7328–2018. 27 pp.

Hamilton, B.T., Moore, S.E., Williams T.B., Darby, N. & Vinson, M.R. 2009. Comparative effects of rotenone and antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. North American Journal of Fisheries Management 29: 1620–1635. https://doi.org/10.1577/M08-178.1

Hindar, A., Hagen, A.G., Hytterød, S., Høgberget, R., Moen, A. & Olstad, K. 2015. Tiltak med ALS for utryddelse av lakseparasitten *Gyrodactylus salaris* i Lærdalselva i 2011 og 2012. NIVA-rapport 6701–2015. 75 pp.

Holocombe, G. W., Phipps, G. L., Sulaiman, A. H. & Hoffman, A. D. 1987. Simultaneous multiple species testing: Acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. Archives of Environmental Contamination and Toxicology 16: 697–710. https://doi.org/10.1007/BF01055420

Hulme, P.E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46: 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

IPBES. 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. (Eds: E. S. Brondizio, J. Settele, S. Díaz & H. T. Ngo). IPBES secretariat, Bonn, Germany.

Jeppesen, E., Lauridsen T.L., Christoffersen K.S., Landkildehus F., Geertz-Hansen P., Amsinck S.L., Søndergaard, M.,…Rigét F. 2017. The structuring role of fish in Greenland lakes: an overview based on contemporary and paleoecological studies of 87 lakes from the low and the high Arctic. Hydrobiologia 800: 99–113. https://doi.org/10.1007/s10750-017-3279-z

Johnsen, B.O., Møkkelgjerd, P.I. & Jensen, A.J. 1999. Parasitten *Gyrodactylus salaris* på laks i Norske vassdrag, statusrapport ved inngangen til år 2000. Norsk Institutt for Naturforskning. NINA Oppdragsmelding 617. 129 pp.

Keck, F., Blackman, R.C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D.,…Altermatt, F. 2022. Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. Molecular Ecology. https://doi.org/10.1111/mec.16364

Kjærstad, G. & Arnekleiv, J.V. 2011. Effects of Rotenone Treatment on Lotic Invertebrates. International Review of Hydrobiology 96: 58–71. https://doi.org/10.1002/iroh.201111261

Kjærstad, G. & Arnekleiv, J.V. 2016. Bunndyrundersøkelser i Rauma i 2013-2015 i forbindelse med rotenonbehandling. NTNU Vitenskapsmuseet naturhistorisk notat 2016-11. 25 pp.

Koksvik, J.I. & Aagaard, K. 1984. Effects of rotenone on the benthic fauna of a small eutrophic lake. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen 22: 658–665. https://doi.org/10.1080/03680770.1983.11897361

Kovach, R. P., Al-Chokhachy, R., Whited, D. C., Schmetterling, D. A., Dux, A. M. & Muhlfeld, C.C. 2016. Climate, invasive species and land use drive population dynamics of a cold-water specialist. Journal of Applied Ecology 54: 638–647. https://doi.org/10.1111/1365-2664.12766

Kumschick, S., Gaertner M., Vilà M., Essl, F., Jeschke, J.M., Pysek, P., Ricciardi, A.,…Winter, M. 2015. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. BioScience 65: 55–63. https://doi.org/10.1093/biosci/biu193

Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P.,… Bearhop, S. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews 87: 545–562. https://doi.org/10.1111/j.1469-185X.2011.00208.x

Ling, N. 2011. Rotenone - a review of its toxicity and use for fisheries management. Science for conservation 211. DOC science investigation no. 3414. 39 pp.

Mangum, M.A & Madrigal, J.L. 1999. Rotenone Effects on Aquatic Macroinvertebrates of the Strawberry River, Utah: A Five-Year Summary. Journal of Freshwater Ecology 14: 125–135. https://doi.org/10.1080/02705060.1999.9663661

McClay, W. 2000. Rotenone use in North America (1988-1997). Fisheries 20: 15–21. https://doi.org/10.1577/1548-8446(2000)025%3C0015:RUINA%3E2.0.CO;2

McClay, W. 2005. Rotenone use in North America (1998-2002). Fisheries 30: 29–31. https://doi.org/10.1577/1548-8446(2005)30[29:RUINA]2.0.CO;2

Meadows, B.S. 1973. Toxicity of rotenone to some species of coarse fish and invertebrates. Journal of Fish Biology 5: 155–163. https://doi.org/10.1111/j.1095-8649.1973.tb04444.x

Melaas, C.L., Zimmer, K.D., Butler, M.G., & Hanson, M.A. 2001. Effects of rotenone on aquatic invertebrate communities in prairie wetlands. Hydrobiologia 459: 177–186. https://doi.org/10.1023/A:1012514124430

Oliver, T. H., Heard, M. S., Isaac, N. J. B., Roy, D. B., Procter, D., Eigenbrod, F., Freckleton, R.,… Bullock, J. M. 2015. Biodiversity and resilience of ecosystem functions. Trends in Ecology & Evolution 30: 673–684. https://doi.org/10.1016/j.tree.2015.08.009

Pham, L., Jarvis, M. G., West, D & Closs, G. P. 2018. Rotenone treatment has a short-term effect on New Zealand stream macroinvertebrate communities. New Zealand Journal of Marine and Freshwater Research 52: 42–54. https://doi.org/10.1080/00288330.2017.1330273

Rahel, F. J & Olden, J. D. 2008. Assessing the Effects of Climate Change on Aquatic Invasive Species. Conservation Biology 22: 521–533. https://doi.org/10.1111/j.1523-1739.2008.00950.x

Reed, K.A., Park, H., Lee, S.G., Lee, W., Lee, S.-H., Bleau, J.M., Munden, T.N.M &, Covi J.A. 2018. Embryos of an Antarctic zooplankton require anoxia for dormancy, are permeable to lipophilic chemicals, and reside in sediments containing PCBs. Scientific Reports 8: 1–13. https://doi.org/10.1038/s41598-018-34689-w

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A.,… Cooke, S.J. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94: 849–873. https://doi.org/10.1111/brv.12480

Reise, K., Olenin, S. & Thieltges, D.W. 2006. Are aliens threatening European aquatic coastal ecosystems? Helgoland Marine Research 60: 70–83. https://doi.org/10.1007/s10152-006-0024-9

Reynolds, C., Miranda, N.A.F. & Cumming, G.S. 2015. The role of waterbirds in the dispersal of aquatic alien and invasive species. Diversity and Distributions 21: 744–754. https://doi.org/10.1111/ddi.12334

Rimet, F., Alagas, E., Borja, A., Bouchez, A., Chauvin., Chonova, T., Ciampor, F.,… Ekrem, T. 2021. Metadata standards and practical guidelines for specimen and DNA curation when building barcode reference libraries for aquatic life. Metabarcoding and Metagenomics 5: 17–33. https://doi.org/10.3897/mbmg.5.58056

Russell, J. C. & Blackburn, T.M. 2017. Invasive Alien Species: Denialism, Disagreement, Definitions, and Dialogue. Trends in Ecology & Evolution 32: 312–314. https://doi.org/10.1016/j.tree.2017.02.

Rytwinski, T., Taylor, J.J., Donaldson, L.A., Britton, J. R., Browne, D.R., Gresswell, R.E.,… Coock, S. J. 2018. The effectiveness of non-native fish removal techniques in freshwater ecosystems: a systematic review. Environmental Reviews 27: 71–94. https://doi.org/10.1139/er-2018-0049

Sandodden, R., Brazier, M. Sandvik, M., Moen, A., Wist, A. N. & Adolfsen, P. 2018. Eradication of *Gyrodactylus salaris* infested Atlantic salmon (*Salmo salar*) in the Rauma River, Norway, using rotenone. Management of Biological Invasions 9: 67–77. https://doi.org/10.3391/mbi.2018.9.1.07

Schlaepfer, M.A. 2018. Do non-native species contribute to biodiversity? PLOS BIOLOGY 16: e2005568. https://doi.org/10.1371/journal.pbio.2005568

Schlaepfer, M.A., Sax, D. F. & Olden, J. D. 2011. The Potential Conservation Value of Non-Native Species. 2011. Conservation Biology 25: 428–437. https://doi.org/10.1111/j.1523-1739.2010.01646.x

Schnick, R. 1974. A Review of the Literature on the Use of Rotenone in Fisheries. U.S. Department of the Interior Fish and wildlife service. 133 pp.

Singer, T.P. & Ramsey, R.R. 1994. The reaction site of rotenone and ubiquinone with mitochondrial NADH dehydrogenase. Biochimica et Biophysica Acta 1187: 198–202. https://doi.org/10.1016/0005-2728(94)90110-4

USEPA (US Environmental Protection Agency). 2007. Reregistration eligibility decision for Rotenone EPA 738-R-07-005. Washington, DC: USEPA. Prevention, Pesticides, and Toxic Substances. Special Review and Reregistration Division. 44 pp. https://pdfs.semanticscholar.org/2260/bc61d208e42bbb7a23fcd8d89bdd1d78f1ac.pdf

Villéger, S., Blancheta, S., Beauchard, O., Oberdorff, T. & Brosse, S. 2011. Homogenization patterns of the world's freshwater fish faunas. PNAS 108: 18003-18008. https://doi.org/10.1073/pnas.1107614108

Vinson, M.R., Dinger, E.C. & Vinson, D.K. 2010. Piscicides and invertebrates: after 70 years, does anyone really know? Fisheries 35: 61–71. https://doi.org/10.1577/1548-8446-35.2.61

Van den Brink, P. J. & Ter Braak, C. J. F. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18: 138–148. https://doi.org/10.1002/etc.5620180207

Warren, C. R. 2021. Beyond ‘Native V. Alien’: Critiques of the Native/alien Paradigm in the Anthropocene, and Their Implications. Ethics, Policy & Environment 2021, 1–31. https://doi.org/10.1080/21550085.2021.1961200

Williamson, M. 1996. Biological invasions. London: Chapman & Hall.

Weyl, P.S.R., de Moor, F. C., Hill, M.P. & Weyl, O.L.F. 2010. The effect of largemouth bass *Micropterus salmoides* on aquatic macroinvertebrate communities in the Wit River, Eastern Cape, South Africa. African Journal of Aquatic Science 35: 273–281. https://doi.org/10.2989/16085914.2010.540776

Zhang, Z., Capnha, C., Usio, N., Weterings, R., Liu, X., Li, Y., Landeira, J.M,…Yokota, M. 2019. Impacts of climate change on the global potential distribution of two notorious invasive crayfishes. Freshwater Biology 65:353–365. https://doi.org/10.1111/fwb.13429

Paper I

RIVER RESEARCH AND APPLICATIONS

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EFFECTS OF THREE CONSECUTIVE ROTENONE TREATMENTS ON THE BENTHIC MACROINVERTEBRATE FAUNA OF THE RIVER OGNA, CENTRAL NORWAY

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ABSTRACT

The effects of piscicides on aquatic invertebrates are often studied after one treatment, even though piscicides may be repeatedly applied within river management. Here we investigate the impacts of repeated piscidie treatment on riverine benthic invertebrates. The River Ogna, Norway, was treated with rotenone three times over a 16-month period. The two first treatments caused temporary density reduction of a few rotenone sensitive benthic invertebrate taxa. Effects of the third treatment were variable with some taxa unaffected while all Plecoptera, were locally extinct. The toxic effect of rotenone increases with water temperature and high water temperature (20 °C) combined with high rotenone concentration was probably the main reason why the benthic community in the third treatment was more negatively affected than during the two previous treatments (4 and 8 °C). Eight months after the treatment benthic densities had not reached pre-treatment levels, but most taxa had recolonized the treated area within a year. Our data suggest that the severe effects of the third treatment were not influenced by the two former ones. This implies that the timing of piscicide treatment has a greater impact on the benthic invertebrate community than the number of treatments. Copyright © 2015 John Wiley & Sons, Ltd.

KEY WORDS: rotenone treatment; benthic invertebrates; EPT; recovery; river

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INTRODUCTION

The piscicide rotenone has been an important tool for fisheries management and research in the U.S. since the 1930s. Uses include manipulation of fish communities to maintain sport fisheries, quantification of fish populations (sampling), treatment of rearing facilities and eradication of exotic fish (Finnlayson *et al*., 2000). During the two last decades rotenone has been widely used in Norway to eradicate the salmon parasite *Gyrodactylus salaris* Malmberg, which causes high mortality in Atlantic salmon (*Salmo salar* L.) juveniles. The aim of the treatments is to eradicate the parasite by killing all salmon present in a watershed, since the parasite can only survive for a short time period without a host.

Rotenone affects cellular aerobic respiration, blocking mitochondrial electron transport by inhibiting NADH-ubiquinone reductase (Singer and Ramsey, 1994). The toxicity of rotenone to fish is very high (Ling, 2003), although non-target organisms including benthic invertebrates may also be affected during rotenone treatments (Morrison, 1977; Mangum and Madrigal, 1999; Gladsø and Raddum, 2002; Eriksen *et al*., 2009). A number of studies investigating the effects of rotenone on invertebrates have been conducted and many report severe impacts (Binns, 1967; Arnekleiv *et al*., 1997; Hamilton *et al*., 2009). On the other hand, minor effects have also been registered (e.g. Coock and Moore, 1969; Koksvik and Aagaard, 1984; Dudgeon, 1990). Generally lotic invertebrates seem to be more sensitive to rotenone compared to lentic taxa. Taxa from the orders Ephemeroptera, Plecoptera and Trichoptera (EPT) are particularly sensitive, although some species within these orders are tolerant (Mangum and Madrigal, 1999; Arnekleiv *et al*., 2001). Some studies report tolerance differences in larval stages, with early stages more sensitive than later stages (Gladsø and Raddum, 2002; Kjærstad and Arnekleiv, 2011). Recovery time of invertebrate densities after rotenone treatments varies, but has been registered as fast as within a year (Binns, 1967; Kjærstad and Arnekleiv, 2004). However, recovery of single taxa may take several years (Arnekleiv *et al*., 1997; Mangum and Madrigal, 1999). Despite the relative high number of investigations of rotenone effects on invertebrates, the results are somewhat contradictory. This led Vinson *et al*. (2010) to suggest that the true impacts of rotenone on invertebrate assemblages are not well known.

Normally, investigations only report effects of rotenone on invertebrates from a single treatment. In this paper we document the effect of three subsequent treatments over a 16-month period. Other objectives were to assess the effects

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of rotenone on different taxa and the recovery time of the benthic community. We hypothesized that three consecutive rotenone treatments would result in higher short-term toxic effects on the macroinverte community than each of them separately.

METHODS

Study area

The River Ogna is situated in Central Norway (Figure 1), draining mainly lowland areas and flowing into Trondheimsfjorden. It has a catchment area of 578 km^2 and a mean annual discharge of 22 m^3 s^{-1}.

In 1980 *G. salaris* was found on juvenile salmon in the River Ogna. The river was treated with PW-rotenone in 1993, but for unknown reasons the parasite reoccurred in 1997. This study deals with the rotenone treatments where CFT-Legumin (liquid formulation with a 2.5% by volume active gradient) was applied three times over a two-year period, April and October 2001, as well as in August 2002. The environmental authorities aimed to keep a minimum concentration of 0.5-ppm rotenone solution during the treatments. Rotenone was applied at the upper end of the anadromous river reach at Støafoss, 18 km from the river mouth (Figure 1), as well as other points downstream the river to maintain the desired rotenone concentration. Boats equipped with pumps were used to spray rotenone on gravel banks and river margins parallel to the rotenone application. Drip barrels were set up in anadromous parts of all tributaries, and backpack sprayers and watering cans were used to treat various types of wetlands adjacent to the rivers. Water temperatures during the treatments in April and October 2001 were approximately 4 °C and 8 °C, respectively. During the treatment in August 2002 the water temperature was approximately 20 °C.

Sampling

To register benthic invertebrate densities, Surber samples were carried out at two stations, one rotenone treated and one untreated. Six replicates were taken on each station on each sampling occasion. The Surber sampler had an area of 900 cm^{-2} and a mesh size of 250 μm. In order to enhance chances of detecting invertebrate taxa, three rotenone treated and three untreated stations were also sampled by a kick net. For each sampling occasion 1–2 kick samples, each of 1-min duration, were taken. The kick net had a square-shaped frame with an opening of 25 × 25 cm and a mesh size of 250 μm. Benthic samples were taken a few days before and a few days after each rotenone treatment, as well as on other occasions (see Table I for details).

Statistical analyses

To assess the temporal response of the riverine invertebrate fauna to repeated rotenone treatment we used principal response curves. Principal response curves (PRC) are based upon redundancy analysis (RDA), and developed to

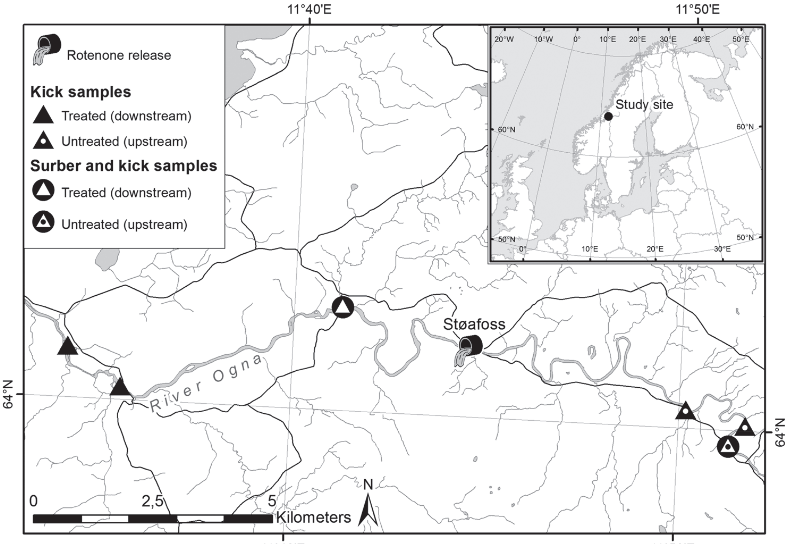

Figure 1. Study area and sampling stations

Table I. Dates of benthic sampling and rotenone treatments and number of days since the first, second and third treatments

Date	Surber sampling	Kick sampling	Rotenone treatment	Days since first treatment	Days since second treatment	Days since third treatment
18.04. 2001	x	x				
21.04. 2001			x			
24.04. 2001	x	x		3		
30.04. 2001	x	x		9		
07.05. 2001	x	x		16		
25.09. 2001	x	x		157		
04.10. 2001			x			
08.10. 2001	x	x		170	4	
15.10. 2001		x		177	8	
31.10. 2001		x		193	24	
07.08. 2002	x	x		473	304	
26.08. 2002			x			
28.08. 2002	x	x		494	325	2
05.09. 2002	x	x		502	333	8
22.10. 2002	x	x		549	280	55
29.04. 2003	x	x		738	569	244
17.06. 2003		x		787	618	293
25.08. 2003	x	x		856	687	362
29.04. 2004		x		1104	935	510

facilitate interpretation of multivariate response to an intervention within a repeated measures design (Van den Brink and Braak, 1999). Principal response curve analysis was carried out using the kick sample data. Counts were log + 1 transformed prior to analysis.

Invertebrate densities (Surber sampled data) were analysed for the total invertebrate fauna and for each of the EPT orders. Since this data was collected through overdispersed counts, it was analysed using quasi-Poisson family generalized linear modelling. Separate models were run for each sampling date, testing for an effect of treatment on the density of each group. The sample was taken as the unit. The differences in density of individual taxa were estimated using standardized mean differences to allow for comparison of rotenone treatment effect between taxa differing in background abundance. We estimated the standardized mean difference as the difference in mean density (treated – untreated) divided by the pooled standard deviation. Only the most abundant and consistently present taxa were analysed. Data were analysed in the R statistical environment version 3.0.2 (R Core Team, 2013). PRC analysis was carried out using the vegan package (Oksanen *et al.*, 2013)

RESULTS

The differences in the invertebrate community composition between the treated and untreated stations rapidly increased after each of the three rotenone treatments (Figure 2). However, a few days after the treatments the difference between the treated and untreated stations was reduced, indicating that the community composition between the sites became again more similar. On day 856, one year after the third and last treatment, the community composition between the two stations was as similar as in the baseline data.

The combined densities of all invertebrates showed only minor differences between the treated and untreated site immediately after the first treatment, but significantly higher densities in the untreated area on the first sampling occasions after the two last treatments (Figure 3). Significant differences in densities between the two sites were also registered 9, 16 and 549 days after the first treatment, but with the greatest densities in the treated area.

Densities within the EPT groups (**E**phemeroptera, **P**lecoptera, **T**richoptera) showed that Ephemeroptera had significant differences between the treated and untreated sites on all sampling occasions, except on the first sampling occasion three days before the first treatment, as well as one year after the third treatment (Figure 4a). Apart from the sampling three days prior to and nine days after the first treatment densities were highest at the untreated site. Plecoptera had significant higher densities at the untreated site on day 170 (one day after the second treatment) and on day 738 after the first treatment (8 months after the third treatment) (Figure 4b). However, on day 473 (16 days before the third treatment) densities were significantly higher at the treated site. Due to zero Plecoptera specimens at the treated site on the two first sampling occasions after the third treatment, no calculations of differences between sites were made.

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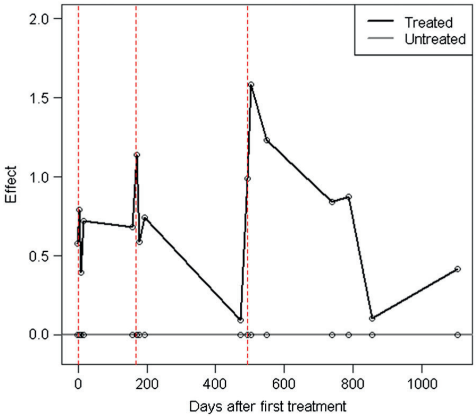

Figure 2. Principle response curve based on kick samples (total material) in the river Ogna (log transformed counts). The dashed vertical lines show the timings of the three treatment applications. The black line shows the effect of the treatment against the untreated samples (grey line at y = 0). Points show the dates when samples were taken. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

However, at the untreated site densities on the same sampling dates yielded 321 and 45 individuals m^{-2}, respectively. Significant higher Trichoptera densities were found at the treated site immediately before and after the first treatment, 16 days after the first treatment and 157 days after the first treatment (12 days before the second treatment) (Figure 4c). On the three first sampling occasions after the third treatment; on day 494, 502 and 549 after the first treatment, respectively, the densities were significantly higher at the untreated site.

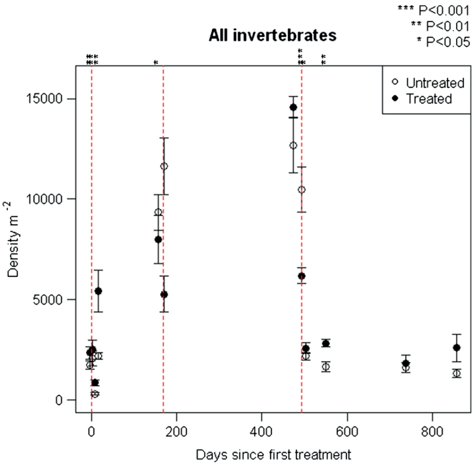

Figure 3. Density (mean ± standard error) of total material sampled at stations with and without rotenone treatment. The vertical lines indicate the dates of treatment application. Invertebrates are sampled using Surber. The stars at the top of the figure denote the significance levels from quasi-Poisson GLMs based on the counts rather than densities. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

Figure 5 shows the standardized mean differences of selected taxa densities between treated and untreated stations three days after the first, one day after the second and one day after the third rotenone treatment. The density differences between the two stations were smallest in the first treatment and largest in the third treatment. The plots are skewed to the negative side after the third treatment, less after the second treatment and least after the first treatment. This indicates that densities of the treated station has a higher drop than the untreated station, especially after the third treatment, but also after the second treatment, but to a smaller extent after the first treatment.

Out of a total of 28 selected taxa Oligochaeta, Nematoda, Ceratopogonidae and the mayfly *Caenis* sp. densities increased more at the treated than the untreated station after the third treatment (Figure 5). The same situation was true for seven taxa after the first and second treatment. The mayflies *Baetis rhodani*, *Heptagenia dalecarlica* and *Ephemerella aurivillii*, the stoneflies *Diura nanseni* and *Amhinemura* sp., the caddisfly *Rhyacophila nubila* and the beetles *Elmis aenea* and Elmidae (= elmids except *E. aenea*) had decreased densities at the treated station compared to the untreated stations after all three treatments. No taxa had increased densities on the treated station after all three treatments, but Oligochaeta, the stonefly Chloroperlidae (*Siphonoperla burmeisteri* or/and *Xanthoperla apicalis*) and the caddisfly *Agapetus* sp. and Leptoceridae did so after two of the treatments. A detailed taxa list of Surber densities from all sampling occasions is given in Appendix A.

The number of EPT taxa present in the treated and untreated river section throughout the project period is given in Figure 6. Number of Ephemeroptera, Plecoptera and Trichoptera taxa varied from 11 to 15, 7 to 10 and 6 to 16 in the untreated section, respectively, and from 5 to 16, 0 to 11 and 4 to 12 in the treated section, respectively (Figure 6a). In the treated section all three taxa groups decreased immediately after the third treatment, and the lowest taxa number was registered after the third treatment (Figure 6b). This was, however, not the case in the untreated section.

DISCUSSION

The River Ogna was treated with rotenone three times in different seasons; spring, summer and fall. Different life stages and different species with different sensitivity will

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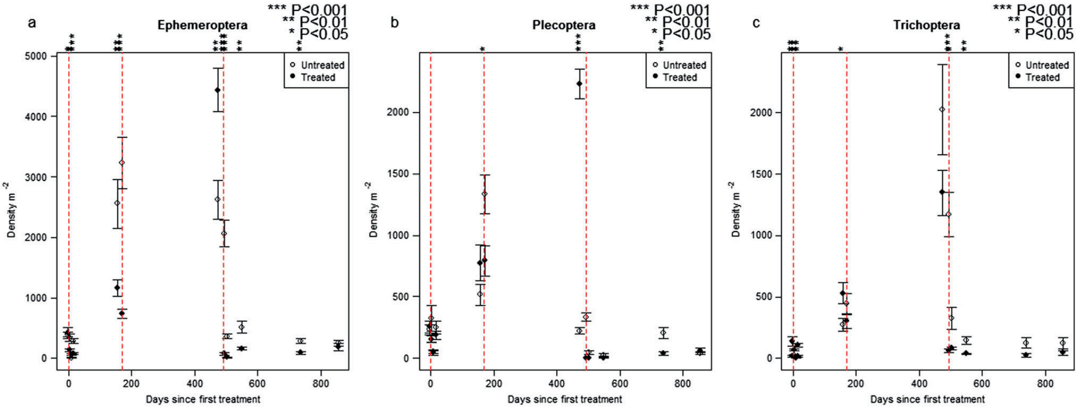

Figure 4. Densities (mean ± standard error) of EPT at the treated and untreated station of a) Ephemeroptera, b) Plecoptera and c) Trichoptera. The vertical lines indicate the dates of treatment application. The stars at the top of the figure show the *P* values from quasi-Poisson GLMs based on the count data rather than densities. This figure is available in colour online at wileyonlinelibrary.com/journal/rra

be present during the different treatments. It is consequently not straightforward to compare rotenone effects between treatments or the effect of three consecutive treatments. Nevertheless many of the same species including many which are rotenone sensitive were present during all three treatments. The first and second treatments were carried out in April and October, respectively, and densities of a few sensitive taxa decreased, but the species were not locally extinct after the treatments. After the third treatment, however, many taxa remained absent until several months after the treatment. Our data do not suggest that the severe effects of the third treatment were influenced by the two first ones, but rather other factors, including high water temperature during the third treatment. The toxicity of rotenone

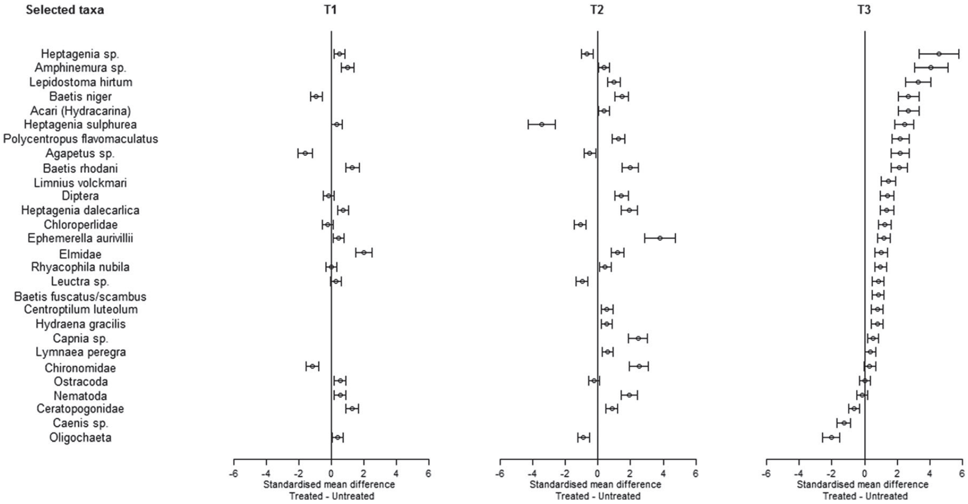

Figure 5. Standardized mean differences (mean difference divided by pooled standard deviation) between taxa densities in treated and untreated stations immediately after the three treatment applications (T1 = 1st treatment, T2 = 2nd treatment and T3 = 3rd treatment). Mean and standard errors presented (estimated across 6 Surber samples per treatment). A positive standardized mean difference indicates that the taxa had a higher density in the treated than untreated samples. Taxa selected are those that are most abundant and consistently present across time periods. Taxa are ordered according to the mean difference after the third treatment application

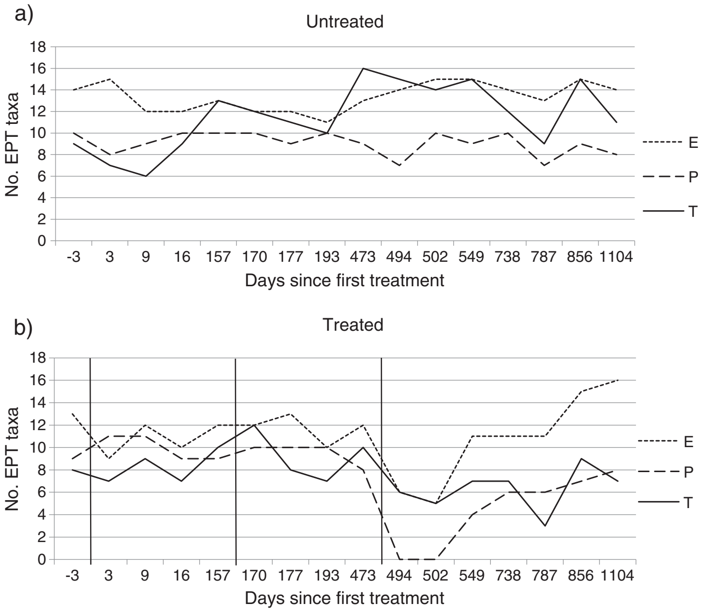

Figure 6. Number of recorded EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa in the a) untreated and b) treated area of the River Ogna based on Surber and kick samples. The black vertical lines show the timings of the three treatment applications

increases with temperature (Andreasson, 1963; Meadows, 1973). Water temperature in the first, second and third treatments was 4, 8 and 20 °C, respectively.

The recovery time of lotic invertebrates after rotenone treatment has been shown to vary. Were rotenone treatments include entire watersheds, recovery time is slow and may take several years (Mangum and Madrigal, 1999). However, in many rotenone treatments only a small section of the watershed is treated, and large untreated areas can serve as refuges for dispersal to treated areas. In such cases benthic densities/abundances will often reach pre-treatment levels within a year (Binns, 1967; Gladsø and Raddum, 2000; Arnekleiv *et al*., 2001; Kjærstad and Arnekleiv, 2004). In the River Ogna several taxa disappeared after the third rotenone treatment, but most of them reoccurred within a year. Only the lowermost 18 km of the river, which constitutes a minor part of the watershed, was treated. Large untreated upstream areas, both of the main river and tributaries, will allow rapid re-colonization through drift. Invertebrate drift is important for downward movement of lotic invertebrates (Maurer and Brusven, 1983; Fenoglio *et al*., 2002), but also other dispersal mechanisms like ovipositing adults may contribute to recolonization. In addition invertebrates which inhabit the hyphoreic zone may have avoided direct contact with rotenone. Invertebrate eggs, which are probably less sensitive to rotenone than later life stages as demonstrated for fish (Marking and Bills, 1976), may have survived the treatments, but due to the vast untreated upstream areas we believe drift to be the most important factor.

The maximum rotenone concentration was 59 $\mu g\ l^{-1}$ in the first treatment and 32 $\mu g\ l^{-1}$ in the third treatment (no measurements were taken during the 2nd treatment) (Guttvik *et al*., 2008). However, only occasional measurements were taken, so the true rotenone concentration in terms of ppm-hours cannot be calculated. The total use of CFT-Legumin was much higher in the third treatment with 3300 l, compared to 1145 l in the first and 1675 l in the second treatment. Since the water discharge was much lower during the third treatment compared to the two previous ones, the rotenone concentration was probably highest during the third treatment. Additionally low water discharge during the third treatment resulted in extensive dewatered river banks being sprayed with rotenone. Rotenone leaking from the banks probably gave an additional and prolonged effect, compared to the two other treatments where exposed areas of dried banks were smaller because of higher water discharge. On the other hand, due to high water temperatures the breakdown of rotenone would have been faster in the third treatment. It is therefore difficult to predict the effects of different amounts of rotenone in the three treatments on the benthic fauna. However, during the third treatment both high water temperature and a high rotenone concentration resulted in a more toxic effect on the macroinvertebrates than the two former treatments.

The sensitivity to rotenone is highly variable between invertebrate taxa (e.g. Engstrom-Heg *et al*., 1978; Chandler and Marking, 1982; Arnekleiv *et al*., 2001; Eriksen *et al*., 2009). Although some species within the EPT groups are relatively rotenone tolerant, these orders are often reported to be among the most sensitive invertebrate groups (Binns, 1967; Arnekleiv *et al*., 1997; Mangum and Madrigal, 1999). All EPT groups had a marked drop in densities immediately after all treatments, except Plecoptera which had a slight increase after the second treatment. The decrease in densities was most pronounced after the third treatment, especially for Ephemeroptera and Plecoptera. Epemeroptera densities in the treated area dropped from 4200 individuals per m^{-2} before the third treatment to 80 individuals after the treatment. Plecoptera, which had a density of 2100 individuals per m^{-2} immediately before the third treatment, was not registered immediately after the treatment. At the reference station the densities were lower, but relatively stable throughout the treatment. For Trichoptera there was also a high density decline, but this was the case at both the treated and untreated stations. The mayfly *Baetis rhodani* had a marked decrease in densities at the treated station immediately after all three treatments, but densities remained stable or increased at the untreated site during the same time period. In fact no specimen of *B. rhodani* was registered in the Surber samples immediately after the third treatment (337 individuals m^{-2} before treatment). *Baetis* is known to be relatively rotenone sensitive (Engstrom-Heg *et al*.,1978; Dudgeon, 1990; Eriksen *et al*., 2009). The difference in tolerance between *B. rhodani* and *B. muticus* has been documented, the former being the most sensitive (Kjærstad and Arnekleiv, 2011). Other mayfly species which seemed to be negatively affected by the treatments included *Ameletus inopinatus*, *Heptagenia dalecarlica* and *H. suphurea*. *Ameletus* has shown to be rotenone sensitive (Gladsø and Raddum, 2000) and Heptageniidae to have an intermediate tolerance (Engstrom-Heg *et al*., 1978; Kjærstad and Arnekleiv, 2011). Even the relative rotenone tolerant *Ephemerella* (Engstrom-Heg *et al*., 1978) had a marked density decrease after the third treatment.

Among stoneflies *Isoperla* sp. and *Leuctra* sp. showed decreased densities during the first treatment. According to Kjærstad and Arnekleiv (2011) these taxa have low rotenone tolerance. No Plecoptera taxa appeared to be negatively affected by the second treatment. However, immediately after the third treatment no Plecoptera specimen was recorded. Plecoptera taxa present in Surber samples immediately before the third treatment included *Amphinemura* sp., *Isoperla* sp., Chloroperlidae, *Capnia* sp, *Diura nanseni* and *Leuctra* sp. The two first of these taxa occurred in high densities at the treated station immediately before the third treatment; 1700 and 287 ind. m^{-2} respectively.

For Trichoptera, the two taxa *Rhyacophila nubila* and *Agapetus* sp. had reduced densities at the treated station after all three treatments, while the densities at the untreated station had a slight increase during the same time period. *Rhyacophila* is known to be rotenone sensitive (Engstrom-Heg *et al*., 1978; Arnekleiv *et al*., 1997; Gladsø and Raddum, 2002), and *Agapetus ochripes* has been cited with an intermediate rotenone tolerance (Kjærstad and Arnekleiv, 2011). During the third treatment all Trichoptera taxa showed decreased densities at the treated station, but all were present immediately after the treatment, but in low densities. The third treatment seemed to have the most severe effect on the benthic community, and most taxa in the treated area showed a decline in densities. Only four taxa, Chironomidae, Ceratopogonidae, the snail *Lymnaea peregra* and the mayfly *Caenis* sp. had higher densities at the treated station immediately after the third treatment, compared to immediately before the treatment. This indicates a high tolerance to rotenone for these taxa. However, within Chironomidae certain genera, especially *Chironomus*, are known to be sensitive to rotenone (Koksvik and Aagaard, 1984; Melaas *et al*., 2001). Engstrom-Heg *et al*. (1978) reported an intermediate rotenone tolerance of Chironomidae. There are obviously substantial differences in the sensitivity within this species rich group, and this may also be the case for Ceratopogonidae. High rotenone tolerance in *Lymnaea peregra* is in accordance with other investigations involving freshwater snails, e.g. Arnekleiv *et al*. (1997), Chandler and Marking (1982), Holocombe *et al*. (1987) and Kjærstad and Arnekleiv (2011).

Overall benthic densities at the treated and untreated sites were fairly stable between samples taken immediately before and immediately after the first treatment. After the second treatment densities dropped at the treated station and increased at the untreated station. Densities at both stations decreased after the third treatment, but most at the treated station. The reduction in overall benthic densities seemed to be highest immediately after the third treatment and lowest after the first treatment.

Overall benthic densities before the third treatment were relatively high with 14 000 individuals per m^{-2} at the treated site and 12 000 individuals per m^{-2} at the untreated site. The high density is probably partly due to the very low water discharge, only 1–2 m^{3} s^{-1}, which was lower than on the other sampling occasions (4–58 m^{3} s^{-1}). As water discharge decreases the submerged area will decrease, resulting in higher benthic densities. The highest water discharge was registered nine days after the first treatment, and densities both at the treated and untreated stations were by far the lowest registered, and much lower than on the previous sampling occasion, three days after the first treatment.

The overall densities of the benthic fauna in the treated river section in April 2003 (1700 ind. m^{-2}), eight months after the third treatment, did not reach pre-treatment densities (2200 ind. m^{-2}). This was also the case in the untreated section. However, the water discharge was much higher with 28 m^3 s^{-1} in April 2003, compared to 8 m^3 s^{-1} before the first treatment in April 2001. If the water discharge had been equal on both sampling occasions, densities in the treated section may have been more close to, or perhaps exceeding, the pre-treatment densities. However, seasonal changes in the abundance of various taxa may also be considered as an explanation for differences in densities between sampling occasions.

EPT taxa richness was relatively stable for the whole period in the untreated section, and also in the treated section before the third treatment. However, after the third treatment taxa numbers of all three groups decreased in the treated section. Plecoptera had the highest drop from eight taxa prior to the treatment to zero on the first sampling occasion, two days after the treatment. On the second sampling occasion, eight days after the third treatment, we again did not find any Plecoptera specimens in the treated river section. On the third sampling occasion, 55 days after the third treatment, four Plecoptera taxa were found in the treated area (Surber and kick samples combined). One year after the treatment seven taxa were present, which is close to the number immediately before the third treatment. For Ephemeroptera and Trichoptera taxa numbers reached pre-treatment levels on the third sampling occasion, 55 days after the third treatment. Six EPT taxa in the treated area and two in the untreated area detected before treatment were absent after the third treatment. Moreover, two EPT taxa in the treated area and three in the untreated area were only registered after the third treatment. However, densities of these taxa were low, and their absence may as well be due to chance than other factors, including treatment effects. Missing taxa after rotenone treatments have been reported earlier (Mangum and Madrigal, 1999; Kjærstad and Arnekleiv, 2004; Hamilton *et al*., 2009).

In conclusion the two first treatments had only minor effects on the benthic invertebrate fauna, but the third treatment caused severe effects. Much higher water temperature combined with high rotenone concentration during the third treatments compared to the two former ones is the most likely explanation for the observed differences. Despite the severe effects during the third treatment, with parts of the benthic fauna being locally extinct, most taxa registered before the treatment were present in the treated river section within a year of the final treatment. Since only a minor part of this watercourse was treated large untreated upstream areas allowed rapid re-colonization through drift, with the exception of those species restricted to the lower reaches. The three consecutive treatments do not appear to have had a greater influence on the invertebrate fauna community than one single treatment.

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REFERENCES

Andreasson S. 1963. The effect of rotenone on invertebrates in relation to certain environmental factors. Svensk Fiskeritidskrift**72**: 90–95. (in Swedish).

Arnekleiv JV, Dolmen D, Aagaard K, Bongard T, Hanssen O. 1997. Effects of rotenone treatment on the bottom-fauna of the Rauma and Henselva watercourses, Møre og Romsdal County. Part 1: qualitative investigations. Vitenskapsmuseet Rapport Zoologisk Serie **1997–8**. (in Norwegian).

Arnekleiv JV, Dolmen D, Rønning L. 2001. Effects of rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. – In: E. Dominguez(ed). *Trends in Research in Ephemeroptera and Plecoptera*. Kluwer Academic/Plenum Publishers; 77–88.

Binns NA. 1967. Effects of rotenone treatment on the fauna of the Green river, Wyoming. *Fish Research Bulletin*. **1**: 1–114.

van den Brink PJ, Braak CJT. 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry* **18**: 138–148.

Chandler, JH Jr, Marking LL. 1982. Toxicity of rotenone to selected aquatic invertebrates and frog larvae. *Progressive Fish Culturist* **44**: 78–80.

Coock SF Jr, Moore RL. 1969. The effects of rotenone treatment on the insect fauna of a California stream. *Transactions of the American Fisheries Society* **98**: 539–544.

R Core Team, 2013: *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria.

Dudgeon D. 1990. Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks in two Papua New Guinea streams. *Archives of Hydrobiology* **119**: 35–53.

Engstrom-Heg R, Colesante RT, Silco E. 1978. Rotenone tolerances of stream-bottom insects. *New York Fish Game Journal* **25**: 31–41.

Eriksen TE, Arnekleiv JV, Kjærstad G. 2009 Short-term effects on riverine Ephemeroptera, Plecoptera and Trichoptera of rotenone and aluminum sulfate to eradicate *Gyrodactylus salaris*. *Journal of Freshwater Ecology* **24**: 597–607.

Fenoglio P, Agosta P, Bo T, Cucco M. 2002. Field experiments on colonization and movements of stream invertebrates in an Apennine river (Visone, Italy). *Hydrobiologia* **474**: 125–130.

Finnlayson BJ, Scnick RA, Cailteux RL, Demong L, Horton WD, McClay W, Thompson CW, Tichacek GJ. 2000. *Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual*. American Fisheries Society, Bethesda, Maryland.

Gladsø JA, Raddum GG. 2000. Rotenone treatment and effects on the bottom-fauna in River Lærdalselva. Laboratory of Freshwater Ecology and Inland Fisheries, Univ. Bergen, Rapport **113**: 1–53. (in Norwegian).

Gladsø JA, Raddum GG. 2002. Rotenone treatment of a west Norwegian River: effects on drift of invertebrates. *Verhandlungen—Internationale*

Vereinigung fur Theoretische und Angewandte Limnologie **28**: 764–769.

Guttvik KT, Stensli JH, Sandodden, R. 2008. Rotenone treatment of the Steinkjer water courses 2001 and 2002. National Veterinary Institute's Series. Report **11**. (in Norwegian).

Hamilton BT, Moore SE, Williams TB, Darby N, Vinson MR. 2009: Comparative effects of Rotenone and Antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. *North American Journal of Fisheries and Management* **29**: 1620–1635.

Holocombe GW, Phipps GL, Sulaiman AH, Hoffman AD. 1987. Simultaneous multiple species testing: acute toxicity of 13 chemical to 12 diverse freshwater amphibian, fish, and invertebrate families. *Archives of Environmental Contamination and Toxicology* **16**: 697–710.

Kjærstad G, Arnekleiv JV. 2004. Rotenone treatment of rivers in the Rana region 2003 and 2004: Effects on benthic invertebrates. Vitenskapsmuseet Rapport Zoologisk Serie 2004–4. (in Norwegian).

Kjærstad G, Arnekleiv JV. 2011. Effects of rotenone treatment on lotic invertebrates. *International Review of Hydrobiology* **96**: 58–71. DOI: 10.1002/iroh.201111261.

Koksvik JI, Aagaard K. 1984. Effects of rotenone on the benthic fauna of a small eutrophic lake. *Verhandlungen—Internationale Vereinigung fur Theoretische und Angewandte Limnologie* **22**: 658–665.

Ling N. 2003. Rotenone—a review of its toxicity for fisheries management. New Zealand Department of Conservation, Science for Conservation 211: Wellington.

Mangum FA, Madrigal JL. 1999. Rotenone effects on aquatic macroinvertebrates of the Strawberry River, Utah: a five-year summary. *Journal of Freshwater Ecology* **14**: 125–135.

Marking LL, Bills TD. 1976. Toxicity of rotenone to fish in standardised laboratory tests. *U.S. Fish and Wildlife Service Investigations in Fish Control* **72**: 1–11.

Maurer MA, Brusven MA. 1983. Insect abundance and colonization rate in *Fontinalis neo-mexicana* (Bryophyta) in an Idaho Batholit steram, U.S. A. *Hydrobiologia* **98**: 9–15.

Meadows BS. 1973. Toxicity of rotenone to some species of coarse fish and invertebrates. *Journal of Fish Biology* **5**: 155–163.

Melaas CL, Zimmer KD, Butler MG, Hanson MA. 2001. Effects of rotenone on aquatic communities in prairie wetlands. *Hydrobiologia* **459**: 177–186.

Morrison BRS. 1977. The effects of rotenone on the invertebrate fauna of three hill streams in Scotland. *Fish Management* **8**: 128–139.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. Vegan: community ecology package. http://CRAN.R-project.org/package=vegan

Singer TP, Ramsey RR. 1994. The reaction site of rotenone and ubiquinone with mitochondrial NADH dehydrogenase. *Biochimica et Biophysica Acta* **1187**: 198–202.

Vinson MR, Dinger EC, Vinson DK. 2010. Piscicides and invertebrates: after 70 years, does anyone really know? *Fisheries* **35**(2): 61–71. DOI: 10.1577/1548-8446-35.2.61.

APPENDIX A

						Untreated						
Days since first treatment	**–3**	**3**	**9**	**16**	**157**	**170**	**473**	**494**	**502**	**549**	**738**	**856**
Nematoda		29±29		75±28	246±68	116±22	287±37	385±191	20±7	12±6		4±2
Oligochaeta	160±31	208±36	62±8	178±27	834±109	967±135	510±77	290±49	371±55	203±30	337±48	235±57
Acari (Hydracarina)	2±2			2±2	78±12	107±41	367±56	894±145	7±4	5±2	2±2	7±2
Ostracoda		7±7		2±2	53±11	118±23	25±10	2±2				
Ameletus inopinatus	25±12	20±8		32±16	219±32	251±39	271±96	118±40	25±8	14±8	12±7	5±2
Centroptilum luteolum					23±19	46±44	1302±425	69±45	29±24			4±4
Baetis niger	2±2			2±2	102±28	125±45	141±41	201±40	53±22	45±34	4±2	7±2
Baetis rhodani	150±28	150±45		77±9	105±47	121±32	5±4	96±24	5±2	93±30	71±10	27±13
Heptagenia sp.	36±6	21±10		14±6	301±93	264±101	273±66	691±80				
Heptagenia dalecarlica	21±9	7±4		14±4	32±10	36±9	39±8	59±23	45±13	59±13	9±5	59±18
Heptagenia sulphurea	9±6	4±2	2±2		9±4	2±2	78±5	116±25	110±31	169±29	43±15	80±15
Ephemerella aurivillii	115±5	11±4	4±2	11±3	32±8	125±16	45±11	30±13	25±13	5±4	11±3	18±8
Ephemerella mucronata	46±8	45±12	4±2	6427	1589±366	2102±373	337±101	533±157	18±6	80±32	103±26	
Caenis sp.												
Diura nanseni	2±2	5±5	9±7	20±3	11±6	27±7	18±4	9±5	12±5		9±4	36±13
Isoperla sp.	11±4	32±13	4±2	16±2	23±17	34±16	14±5	71±13	11±5	9±6	20±9	
Chloroperlidae	5±2	2±2			11±4	4±2	18±4	53±23				
Siphonoperla burmeisteri	4±2	5±4							11±6	5±4	27±8	2±2
Taeniopteryx nebulosa					7±5	5±4	11±4	16±5		2±2		
Amphinemura sp.	116±16	166±43	18±9	139±44	226±60	821±166	114±25	166±22		5±2	132±40	
Capnia sp.					200±49	369±70	27±8	2±2		4±4		2±2
Capnia pygmaea	4±2			2±2								
Leuctra sp.	80±20	86±54		61±7	9±3	4±2	2±2	4±2	5±4		7±7	
Elmidae	23±5	107±28		16±9	586±130	360±123	992±151	962±181	349±64	228±34	61±42	333±66
Elmis aenea		7±4		5±4	176±36	148±45	351±53	269±47	78±20	30±8	27±19	46±8
Limnius volckmari					5±4		11±4	11±4	4±2	5±2	4±2	4±2
Oulimnius tuberculatus					2±2	2±2	2±2	2±2	2±2	2±2		4±2
Rhyacophila nubila	2±2	11±7		2±2	9±5	16±9	2±2	36±16		5±4	5±4	5±4
Agapetus sp.	5±4	9±3		5±4	102±33	130±38	447±184	463±111	123±47	66±20	69±28	39±14
Hydroptila sp.					87±32	144±25	1144±180	280±87	98±29	16±9	12±10	5±4
Oxyethira sp.				2±2	11±6	30±8	73±23	48±29	20±9	5±2		2±2
Polycentropus flavomaculatus	2±2			2±2	20±5	23±10	52±12	53±13	14±5	9±4		
Hydropsyche contubernalis					4±2	12±7	4±2	25±5	2±2	9±6	12±6	9±7
Hydropsyche nevae					7±4	4±2	23±15	45±14			5±2	5±5
Lepidostoma hirtum					2±2	7±2	41±11	46±7	7±5	7±4	2±2	5±2
Leptoceridae					11±7		41±15	21±7	5±4	7±5		9±4
Diptera		16±8	2±2	14±5	98±28	126±27	50±9	77±29	39±13	21±5	11±7	34±10
Chironomidae	786±129	894±193	157±38	1272±123	3560±472	4296±668	4659±742	3497±641	349±31	362±73	451±86	139±20
Simuliidae	9±7	14±6	4±2	4±2	2±2	4±2	5±2			5±5		2±2
Ceratopogonidae	37±10	50±15		12±4	102±18	93±34	128±30	205±38	69±18	41±15	25±9	25±5
Lymnaea peregra					12±5	21±11	105±49	36±13	110±33	21±6	37±12	39±10

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					Treated						
−3	**3**	**9**	**16**	**157**	**170**	**473**	**494**	**502**	**549**	**738**	**856**
2±2		4±4		135±57	29±11	700±161	444±77	23±6	2±2		4±2
353±94	151±71	162±33	862±204	2020±428	1848±506	1881±54	1249±245	1163±157	625±71	387±179	1481±455
5±2			4±2	173±33	75±16	494±132	134±40	5±2	2±2		11±5
			16±16	410±169	167±110	5±2	2±2				
	11±7	12±6	4±4	9±6	4±2	52±34					
						2±2	±2		2±2	4±2	
	9±5				2±2			2±2	2±2		25±17
237±31	30±12		7±4	306±49	2±2	337±61			123±21	84±23	30±10
37±17	11±6	4±2	29±8	483±56	401±46	290±322	4±2				
2±2	2±2			29±12	4±2	27±5					43±9
7±5	2±2	2±2	5±2	87±16	53±8	151±23					75±30
	5±5	4±2		2±2	4±4						
87±45	57±10	34±6	29±7	132±36	210±32	353±67	18±7		7±4		
11±11						11±9	52±22				
		2±2	2±2	5±2	2±2	18±7					14±5
21±6	4±4	2±2	9±5	4±2	2±2	287±36				5±2	2±2
9±4	4±4		4±2	21±13	18±7	78±17					
		2±2									
				2±2	2±2						2±2
93±37	80±17	29±10	87±22	673±132	677±111	1698±145				25±11	
				34±9	43±11	50±13					
2±2	2±2	2±2	2±2								
105±27	55±9	9±6	59±10		12±4	4±4				4±2	2±2
4±4			36±20	278±63	84±11	693±55	538±123	273±57	41±12	41±11	189±39
18±12				4±2	9±4	9±9	20±8	25±11	11±4	5±4	9±4
				4±2		12±6		11±7	4±2		7±4
18±8	11±6	2±2	5±2	20±8	7±5	89±13	5±4	2±2	12±5	5±4	14±7
66±33	34±8	5±4	78±18	401±84	192±56	1049±167	5±4	2±2	7±2	12±12	14±8
				25±8	16±10	23±4	11±5	43±9	4±2	2±2	2±2
									2±2		
	2±2										5±2
32±22	7±4	2±2	9±3	34±11	41±12	50±12	18±7	16±5	9±3	4±4	5±2
2±2					2±2	12±4	4±2				
2±2	4±2		2±2	2±2	2±2	30±8	20±12	16±6	2±2	2±2	
18±8	20±6	7±5	41±15	121±30	43±17	109±20		5±4	9±3	23±10	5±4
983±215	1853±399	535±98	3822±841	2083±343	1005±207	2152±180	3091±172	780±73	1714±217	645±102	387±96
43±25	20±9		32±9	12±7	11±3	9±3			34±8	346±142	4±2
16±5	12±5		39±17	39±17	36±12	185±17	303 ±71	46±11	45±15	93±16	53±20
11±7	2±2			27±11	9±3	18±7	25 ±7	55±26	45±21	41±15	25±10

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SPECIAL ISSUE PAPER

Long-term responses of benthic invertebrates to rotenone treatment

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Abstract

Biological invasions are regarded as one of the largest threats to native biodiversity. The eradication of alien invasive fish or parasites by culling of hosts is a controversial conservation strategy, particularly when using indiscriminate methods involving whole ecosystem collateral damage. While short-term effects are abundantly documented, long-term surveys needed to detect potential wider ecosystem effects are scarce. Here, we report a five-year study on effects of the piscicide rotenone on invertebrate communities from a Norwegian watercourse using a Before-After-Control-Impact design. Kick-net samples of benthic invertebrates were collected from three lentic sampling stations and two lotic stations two to four times per year in both a treated and nearby control catchment. In general, only relatively minor short-term effects, measured as temporal beta-diversity of benthic invertebrates, were observed in lentic and lotic locations following rotenone treatment. However, the lotic fauna was severely negatively affected following a period of long-term rotenone exposure from a late autumn treated upstream lake where rotenone degradation was slow, likely due to low temperatures. Species turnover co-varied markedly between control and treatment locations, indicating that natural environmental variation override effects of rotenone treatment. Likewise, the abundance of invertebrate taxa varied considerably both over time and between control and treatment locations. Our study indicates minor short-term (i.e., <1 month) or long-term (i.e., 4 years) effects of rotenone treatment on benthic invertebrates, but severe effects on the lotic fauna 8 months after treatment following long-term low-dose exposure. This suggests that exposure time is of high importance. The findings from the current study therefore highlight both the importance of long-term monitoring including control sites for the study of rotenone treatment effects on benthic invertebrates as well as the importance of minimizing exposure time.

KEYWORDS
alien invasive species, benthic invertebrates, beta diversity, piscicide, recolonization

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1 | INTRODUCTION

Biological invasions are regarded as one of the largest threats to native biodiversity (Mack et al., 2000), also in freshwater (Caffrey et al., 2014; Sala et al., 2000). Alien species may display invasive properties inducing serious ecological consequences through both direct and indirect ecological interactions (Kumschick et al., 2015), as well as major economic impacts (Dittel & Epifanio, 2009). Such alien invasive species may spread rapidly and cause problems that escalate from local to regional scale following establishment. Early targeted eradication actions are often therefore the only effective management method. This approach may be particularly effective when species are required to move through environmental corridors, such as in freshwater ecosystems (Hulme, 2009). However, options for cost-effective and reliable measures are often limited since it may become virtually impossible to eradicate invasive aquatic species in anything but small, enclosed waterbodies (Holdich, Reynolds, Souty-Grosset, & Sibley, 2009).

In aquatic ecosystems, one of the most widely used measures for eradication of invasive species is rotenone. This is a piscicide which is highly toxic to fish and to certain invertebrates but has low toxicity to birds and mammals (Ling, 2003). Rotenone is a compound originating from plant species in the Leguminosae family and has been used for centuries as a mean of capturing fish in areas where these plants are naturally occurring (Brooks & Price, 1961; Meadows, 1973). It has also been used as a fish management tool for almost a century (Finlayson et al., 2010a), either with the purpose of quantifying fish abundances, manipulating fish populations to maintain sport fisheries, or treatment of rearing ponds (McClay, 2000, 2005). It is one of the most successful measures for eradicating populations of invasive fish (Rytwinski et al., 2018). In addition, rotenone has been widely used for eradication of fish parasites through culling of host populations.

Use of rotenone is controversial largely due to its indiscriminate impacts on the wider aquatic fauna. Short-term impacts on nontarget organisms like benthic invertebrates is well documented (e.g., Dalu, Wasserman, Jordaan, Froneman, & Weyl, 2015; Koksvik & Aagaard, 1984; Melaas, Zimmer, Butler, & Hanson, 2001). The observed effects often include reduction in the total abundance of benthic invertebrates comparted to pretreatment levels. However, there are large variations ranging from little or no reduction (Bellingan et al., 2019; Cook, & jr., & Moore, R.L., 1969; Dudgeon, 1990) to up to 95% reduction (Hamilton, Moore, Williams, Darby, & Vinson, 2009). Part of the variation may be explained by taxa-specific tolerance to rotenone. Ephemeroptera, Plecoptera, and Trichoptera, which are abundant in oxygen rich lotic habitats, are generally reported to be rotenone sensitive (Arnekleiv, Dolmen, & Rønning, 2001; Eriksen, Arnekleiv, & Kjærstad, 2009; Mangum & Madrigal, 1999), whereas Coleoptera, Odonata, and Gastropoda, which are often abundant in lentic habitats, are considered rotenone tolerant (Arnekleiv, Dolmen, Aagaard, Bongard, & Hanssen, 1997; Chandler & Marking, 1982; Holocombe, Phipps, Sulaiman, & Hoffman, 1987; Kjærstad & Arnekleiv, 2011). There are also species-specific differences in tolerance among closely related taxa. For example, species of gill breathing Ephemeroptera are particularly sensitive to rotenone (Arnekleiv et al., 2001). Recovery of the total benthic invertebrate density can occur within a year (Kjærstad & Arnekleiv, 2004; Pham, Jarvis, West, & Closs, 2018). However, recovery of single taxa may potentially take several years (Arnekleiv et al., 1997; Mangum & Madrigal, 1999).

Long-term consequences of failing to act toward continuous spread of invasive fish or parasites may be severe to the ecosystem and ecosystem services (Crowl, Crist, Parmenter, Belovsky, & Lugo, 2008; Pyšek et al., 2020). Short-term disturbances to the ecosystem are expected and, to certain degree accepted, as appropriate collateral damage. However, long-term consequences of rotenone treatment may be less accepted. There is currently a lack of studies assessing long-term ecosystem effects caused by rotenone treatments (Vinson, Dinger, & Vinson, 2010). Therefore, this study focused on the long-term consequences of an intensive rotenone treatment on lotic and lentic benthic invertebrate communities. Specifically, we tested for the impact of rotenone treatment on the long-term abundance and temporal turnover of key taxa by comparing time series from a rotenone-treated watercourse and a nearby control watercourse.

2 | METHODS

2.1 | Study sites

The Fusta (rotenone treated) and Drevja (control) water courses are situated in Northern Norway (Figure 1). The Fusta watercourse has a catchment area of 538 km^2. The largest and lowermost lake of the catchment, Lake Fustvatnet (38 m a.s.l.), has a surface area of 11 km^2 and a maximum depth of 68 m. Concentrations of total nitrogen and total phosphorus from water samples taken in August 2014 of 70 and <2 μg/L, respectively, indicate oligotrophic conditions. River Fusta, which is the outlet of Lake Fustvatnet, is 8.5 km long and drains to the sea. The river has a mean annual discharge of 34 $m^3 s^{-1}$.

The Drevja watercourse (untreated), which is bordering the Fusta watercourse, has a catchment area of 178 km^2. Lake Drevvatn (47 m a.s.l.), the largest and lowermost lake in the catchment, has a surface area of 5 km^2 and a maximum depth of 40 m. Concentrations of total nitrogen and phosphorus from water samples taken in August 2014 of 55 and <2 μg/L, respectively, indicate oligotrophic conditions. River Drevjaelva, which is the outlet of Lake Drevvatn, is 17.7 km long and drains to the sea. The river has a mean annual discharge of 12 $m^3 s^{-1}$.

Both watercourses are surrounded by sparsely vegetated mountain areas, spruce dominated forests and scattered farmlands.

2.2 | Study design

We performed the study using a Before-After-Control-Impact design (Josefsson et al., 2020). Rotenone was used in the Fusta watercourse to eradicate the invasive parasite *Gyrodactylus salaris* through exterminating its host populations of salmonid fish. Three rotenone treatments were conducted in the watercourse, with two treatments of the River Fusta (August 2011 and 2012) and one subsequent treatment of the upstream Lake Fustvatnet (October 2012), causing the downstream River Fusta to be treated a third time. The neighboring

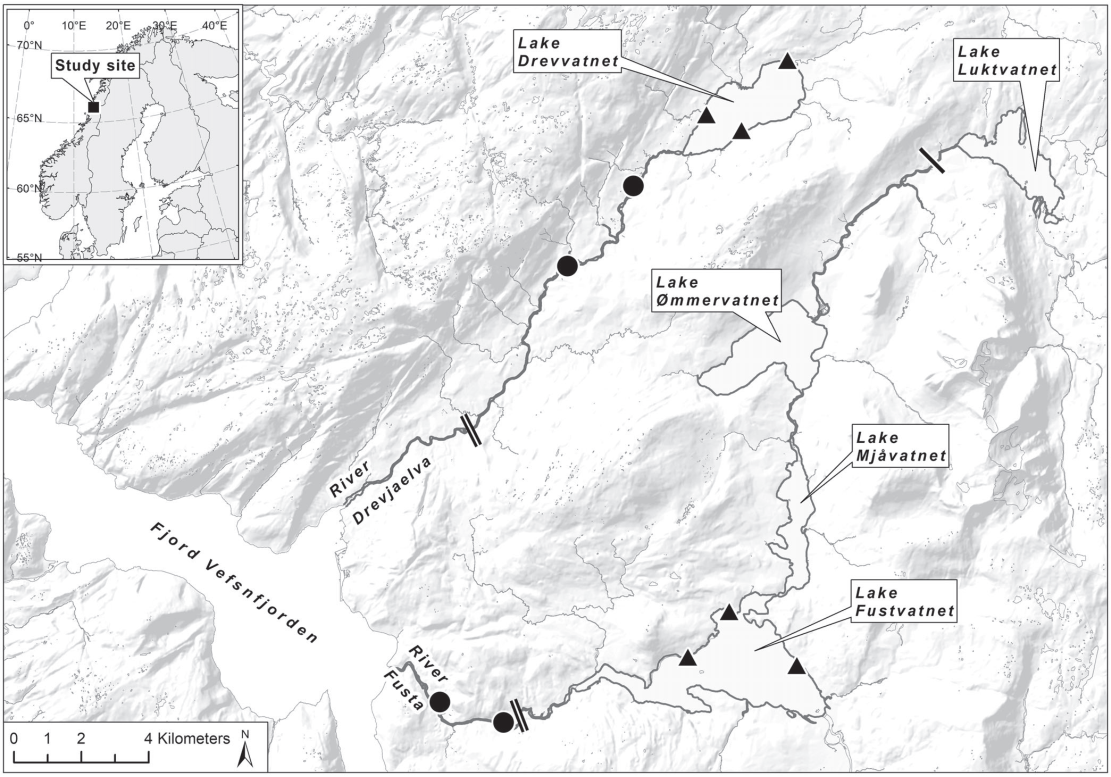


FIGURE 1 Overview of the two studied watercourses Fusta and Drevja. Triangles indicate the position of the lentic sampling stations and dots of the lotic sampling stations. A double black bar indicates the upstream limit of the treatment of River Fusta and River Drevjaelva in August 2011 and August 2012, and a single black bar indicates the upstream limit of the treatment of the Fusta watercourse in October 2012

Drevja watercourse was used as a control site (Figure 1). Sampling was conducted at two lotic and three lentic sampling stations in each watercourse during the autumn from 2011 to 2016, as well as one spring sampling in June 2013, 8 months after the lake treatment. Sampling started in August 2011 before the first rotenone treatment and ended in Autumn 2016. An overview of times of sampling and rotenone treatments is given in Table 1.

Riverine stations were situated in riffle areas dominated by cobbles (particle size of 64–256 mm) and pebbles (particle size 4–64 mm) and with scattered patches of river moss and alga. Two of the stations in each lake were situated in exposed areas where pebbles (particle size of 4–64 mm) dominated the substrate. One of the stations in each lake was situated in sheltered areas with a substrate dominated by finer inorganic and organic substrate (particle size <2 mm) and with little or no aquatic vegetation.

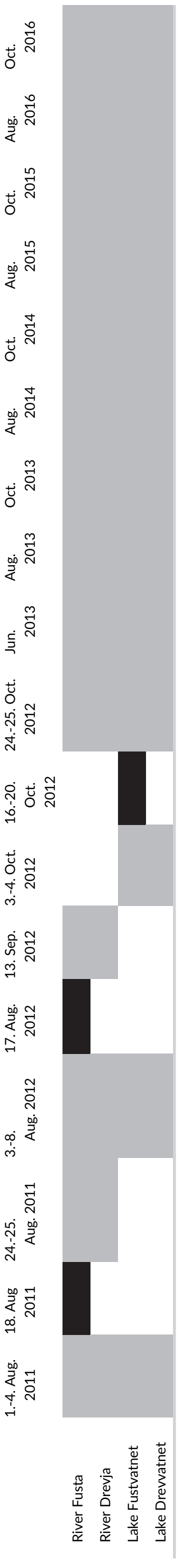
2.3 | Sampling and sample processing

Benthic invertebrates were collected using a kick net with a 25 × 25 cm frame and a mesh size of 0.25 mm. For each sample, an area of approximately 0.5 m^2 was covered with a sampling duration of 1 min. At each combination of sampling occasion and sampling station, five replicate samples were taken. The samples were preserved in 96% ethanol before subsequent subsampling of each sample (1/10) in the laboratory. All invertebrates in the subsample were sorted and identified to the lowest possible taxonomic unit using a stereo microscope and following Engblom (1996) for Ephemeroptera, Lillehammer (1988) for Plecoptera, Rinne and Wiberg-Larsen (2017) for Trichoptera, Holmen (1987) and Nilsson and Holmen (1995) for Coleoptera and Glöer (2002) for Gastropoda. The number of specimens from the sub sample was then multiplied by 10. All specimens of the remaining sample that differed morphologically from the specimens in the sub sample were picked out, identified to lowest possible taxon and counted.

2.4 | Rotenone treatment and fish re-establishment

The rotenone treatments of River Fusta were performed on August 18th 2011 and August 17th 2012 using a rotenone formulation CFT-Legumine with a 2.5% active gradient. In both treatments, the release of rotenone took place over a 7-hr period from two stations in the

TABLE 1 Time of sampling (grey) and rotenone treatments (black) in River Fusta, River Drevjaelva, Lake Fustvatnet, and Lake Drevvatnet



	1.-4. Aug. 2011	18. Aug 2011	24.-25. Aug. 2011	3.-8. Aug. 2012	17. Aug. 2012	13. Sep. 2012	3.-4. Oct. 2012	16.-20. Oct. 2012	24.-25. Oct. 2012	Jun. 2013	Aug. 2013	Oct. 2013	Aug. 2014	Oct. 2014	Aug. 2015	Oct. 2015	Aug. 2016	Oct. 2016
River Fusta	▨	■	▨	▨	■	▨			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
River Drevja	▨		▨	▨		▨			▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Lake Fustvatnet	▨			▨			▨	■	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Lake Drevvatnet	▨			▨			▨		▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

river, in the upper and in the middle part (Stensli & Wist, 2014). The upper release was situated approximately 1 km upstream of our uppermost sampling station, and the release in the middle part was just downstream of our lowermost sampling station. In addition, riverbanks of slow flowing areas, adjacent ponds, oxbow lakes, and streams were rotenone treated with local point releases. The total use of CFT-Legumine in River Fusta was 754 and 573 L in 2011 and 2012, respectively. The water temperature was approximately 15°C during both treatments and the water flow was 12 $m^3\ s^{-1}$ in 2011 and 11 $m^3\ s^{-1}$ in 2012. In the lower part of River Fusta, treatment concentration of CFT-Legumine was higher than 0.5 ppm over a period of several hours, which was the desired minimum concentration, periodically reaching up to 7 ppm (Adolfsen, Sandvik, & Waaler, 2014).

The rotenone treatment of Lake Fustvatnet took place between October 16th and October 20th 2012. The total use of CFT-Legumine in Lake Fustvatnet was 139,200 L, of which 96,600 L were used for the surface waters and 42,600 L were used for deeper areas. A CFT-Legumine formula of 3.3% rotenone was used, except for the surface layer where the treatment included 25,800 L with 2.5% rotenone (Moen & Bardal, 2014). The 3.3% formula did not contain the synergist piperonylbutoxid and the solvent N-metylpyrrolidone since the former increases the toxicity to invertebrates and not to fish (Finlayson et al., 2010b). Measurements of rotenone concentration were performed over scattered sites in the surface water and at different depths during and after the treatment. On October 21, 2012, no measurements were below 0.5 ppm CFT-Legumine, and the average concentration was 0.68 ppm. Due to low water temperatures, the breakdown of rotenone was slow. In April 2013, the average concentration of CFT-Legumine was 0.1 and 0.3–0.4 ppm in the surface layers and in the deeper parts (>10 m), respectively. In June 2013, the concentration was below 0.1 ppm at all depths. No rotenone was detected in mid-October 2013 (Adolfsen et al., 2014).

Fish was reestablished by stocking of Atlantic salmon (*Salmo salar*) in River Fusta and Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in Lake Fustvatnet. Three thousand Atlantic salmon smolts were released in the river in 2013 (Lo & Holthe, 2014) followed by planting of 187,000 Atlantic salmon eggs in 2014. In both 2015 and 2016, 12,000 Atlantic salmon smolts were released, as well as 384,000 young of the year (YOY) Atlantic salmon in 2015 and 120,000 YOY in 2016 (Holthe, Bjørnå, & Lo, 2018). In Lake Fustvatnet 35,000 Arctic charr, mainly 2-year-old, were released during the spring of 2014, as well as 93,000 brown trout YOY (Holthe, Bjørnå, & Lo, 2015; Lo & Holthe, 2014).

The two lakes upstream of Lake Fustvatnet, Lake Ømmervatnet and Lake Mjåvatnet, were rotenone treated on October 14th–16th and October 19, 2012, respectively.

The lowest 5 km of River Drevjaelva, from a fish upstream migration barrier and downstream to the fjord-outlet of the river, was rotenone treated in August 2011 and August 2012. Our control area was situated 6–10 km upstream of this fish barrier and as such without influence of this treatment. Fish were also stocked in River

Drevjaelva after the treatment but only in the lower treated part below the barrier. We lack information of the abundance or natural variation in fish populations size in the untreated part of River Drevjaelva (brown trout) or in Lake Drevvatnet (brown trout and Arctic charr).

2.5 | Data analyses

Changes in taxa composition between each subsequent sampling period was measured as temporal beta-diversity index (TBI). We calculated two different TBI indices, using the percentage difference from abundance data and the Sorensen index based on presence–absence data. The percentage difference ($D_{\%\mathrm{diff}}$) between two sampling periods (t_i and t_{i+1}) was defined as $D_{\%\mathrm{diff}} = (\mathrm{B} + \mathrm{C})/(2\mathrm{A} + \mathrm{B} + \mathrm{C})$, where A is the sum of the abundance of all taxa found in both sampling period t_i and t_{i+1}. This represents the unscaled similarity between the two sampling periods. B is the part of abundance of the common taxa that is higher in samples at t_i than at t_{i+1} and is the unscaled sum of taxa *losses* between sampling periods. C is the part of the abundance that is higher t_{i+1} than at t_i and is the unscaled sum of taxa *gains* between the two sampling periods. The equation for the Sorensen index, using presence–absence data, is the same as for the $D\%_{\mathrm{diff}}$ outlined above (see Legendre, 2019 for more details). Differences in abundances and presence–absence data among subsequent sampling occasions were tested for significance with a paired *t* test. The *p*-values (based upon 9,999 random permutations) were controlled for the family-wise error rate using Holm correction. The calculations of the TBI indices and significance tests were done in the R package "adespatial" (Dray et al., 2019).

We analyzed data from riverine and lake habitat separately. Responses to rotenone treatment is expected to vary among habitats. The abundance of each invertebrate taxon is expressed as average number of specimens per kick-sample for each sampling period at each site.

A permutational multivariate analysis of variance (PERMANOVA) with 9,999 permutations was performed (see Anderson, 2001) with the "adonis" function of the R package "vegan" (Oksanen et al., 2020). We tested for differences in community composition (Bray–Curtis dissimilarity) over time, between treated and untreated localities and the interaction between time and treatment.

3 | RESULTS

3.1 | Rivers

The temporal species turnover appeared to co-vary in the treated and untreated river, especially for abundance data (Figure 2). The exception was the period after the lake rotenone treatment in October 2012 to summer 2013 where benthic invertebrate abundances of the rotenone treated River Fusta changed significantly (Figure 2a, Appendix S1), mainly due to losses (Figure 2b). For presence–absence data, there was a significant dissimilarity of species occurrences in the treated river from immediately after the lake treatment in October 2012 until August 2015 (Figure 2d, Appendix S2). The species loss-rate of the treated river was at its highest from October 2012 to June 2013 (Figure 2e) and gain-rate at its highest from June 2013 to August 2013 (Figure 2f, Appendix S2). No significant changes in abundance or species occurrence were found in the untreated river (Figure 2a,b, Appendices S1 and S2).

The total abundance of benthic invertebrates was generally higher in the untreated river compared to the treated river both before and after the treatments (Figure 3a), and this was especially prominent for Ephemeroptera and Oligochaeta (Figure 3b,f, respectively).

The total abundance of benthic invertebrates reflects the temporal turnover indices (Figure 3a). There were no clear short-term effects on the total abundance or the abundance on most taxa following the two rotenone treatments of River Fusta (Figure 3). As an exception, the abundance of Plecoptera decreased strongly in the treated river immediately after the first and second river treatment (89% and 92%, respectively), whereas the changes were smaller in the untreated river (+17% and −37%, respectively) (Figure 3c).

In contrast, there was a marked increase in the abundance of taxa observed in samples from both the treated and the untreated river in October 2012, followed by a pronounced decline in the treated river in June 2013 (Figure 3a). However, the observed response in total abundance masks distinct differences in response to the lake rotenone treatment of riverine benthic invertebrate taxa.

The abundance of Ephemeroptera in the treated river decreased shortly after the two river treatments (Figure 3b). This was also found in the untreated river after the first river treatment. The Ephemeroptera *Baetis rhodani and Baetis* sp. decreased in the treated river after both treatments and in the untreated river after the first treatment. Abundances of all taxa from the rivers not presented in the figures are listed in Appendix S3. For other Ephemeroptera, like *Heptagenia dalecarlica* and *Ephemerella* sp., the abundance in both rivers was relatively high shortly after the river treatments. Immediately after the lake treatment in October 2012, *B. rhodani* was the only Ephemeroptera with reduced abundance and was not recorded in the treated river. In June 2013, 8 months after the lake treatment, there was a dramatic decline in the Ephemeroptera abundance in the treated river (Figure 3b) with only a few specimens present of *Siphlonurus* sp., *B. rhodani*, and *H. dalecarlica*. However, a decline was also found in the untreated river, but here most taxa were present in June 2013. In the subsequent years, the Ephemeroptera abundances in the treated river increased, mainly *B. rhodani* and *H. dalecarlica*. A similar increase occurred in untreated river, in addition to an increase in *B. muticus*.

There was a strong decrease in the abundance of Plecoptera in the treated river after both river treatments, whereas there was an increase in the untreated river. Taxa with reduced abundances included *Diura* sp., *Isoperla* sp. and *Leuctra* sp. Shortly after the lake treatment in October 2012, the abundance of Plecoptera in the treated river decreased with 95% compared to September 2012

(Figure 3c), and only three specimens of the genus *Nemoura* were recorded. Most taxa were present in the untreated river and with a relatively high abundance of *Amphinemura borealis*, *Diura* sp., and *Isoperla* sp. In June 2013, 8 months after the lake treatment, no Plecoptera were detected in the treated river, while at the same time, there was an average of more than 200 specimens of Plecoptera in the untreated river (Figure 3c).

Contrary to the river treatments, where the negative effect was minor, Trichoptera abundances decreased in the treated river immediately after the lake treatment but did not change in the untreated river (Figure 3d). Eight months after the lake treatment in June 2013, there was a dramatic decrease (99%) in the abundance of Trichoptera in the treated river, and only a few specimens of *Rhyacophila nubila*, *Hydropsychidae* sp., and *Micrasema* sp. were present. A corresponding but smaller decline (41%) was observed in the untreated river.

Both rivers had high abundance of Chironomidae shortly after the lake treatment (Figure 3e). The abundance of Chironomidae co-varied considerably among rivers. The abundance of Oligochaeta in the treated river decreased shortly after the first river treatment and increased subsequent to the second river treatment and the lake treatment (Figure 3f). In June 2013, 8 months after the lake treatment, the abundance of Oligochaeta was at its minimum in the treated river.

There was a low abundance of Hydrachnidia in the treated river, and the taxon was absent in 2013 (Appendix S3). In the untreated river, Hydrachnidia was recorded in all sampling occasions and with the highest abundances after the treatments. The abundance of the riffle beetles (Elmidae) decreased in the treated river following the treatment. In the untreated River Drevjaelva, the taxon also decreased in abundance, albeit at a much lower rate than in the treated River Fusta.

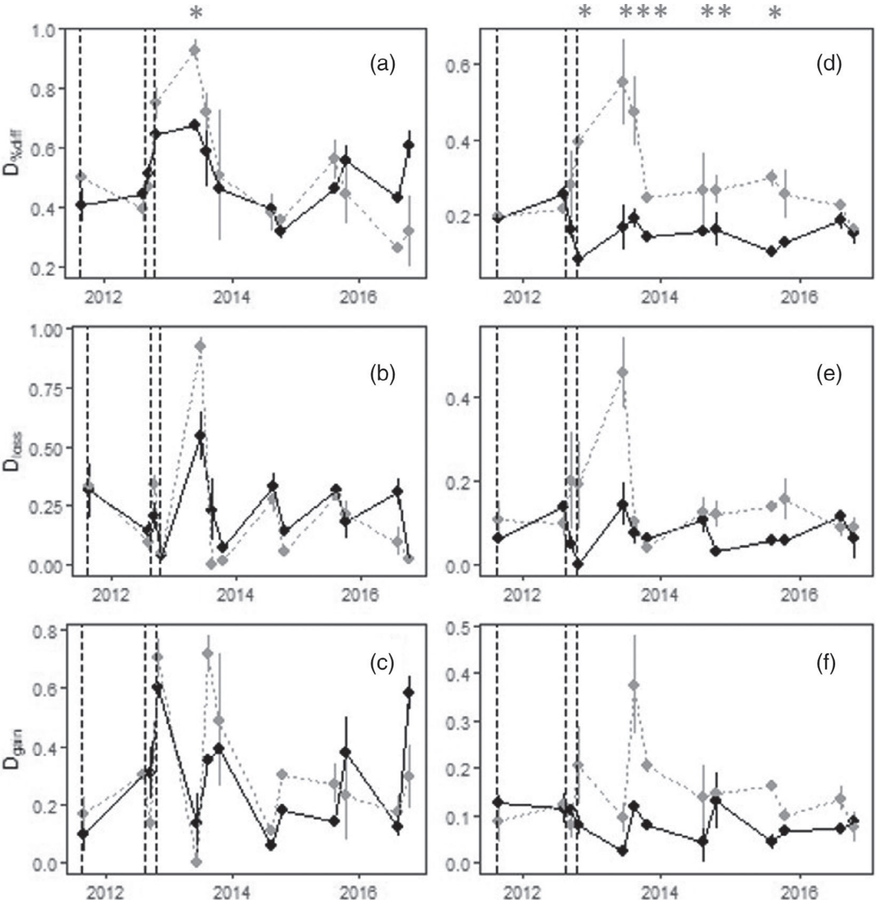


FIGURE 2 Rivers: Mean changes in dissimilarity (% difference) for abundance data (a–c) and for presence–absence data (d–f) between sampling occasions (a and d) and its components; losses (b and e) and gains (c and f). Grey: River Fusta (rotenone treated), black: River Drevjaelva (untreated). Dashed vertical lines indicate the timing of the rotenone treatments. Error bars indicate maximum and minimum values of the temporal biodiversity indices at individual sampling stations. Significant differences in diversity indices between sampling periods ($p < 0.05$) in River Fusta are marked with grey asterisk (no significant differences detected in River Drevjaelva)

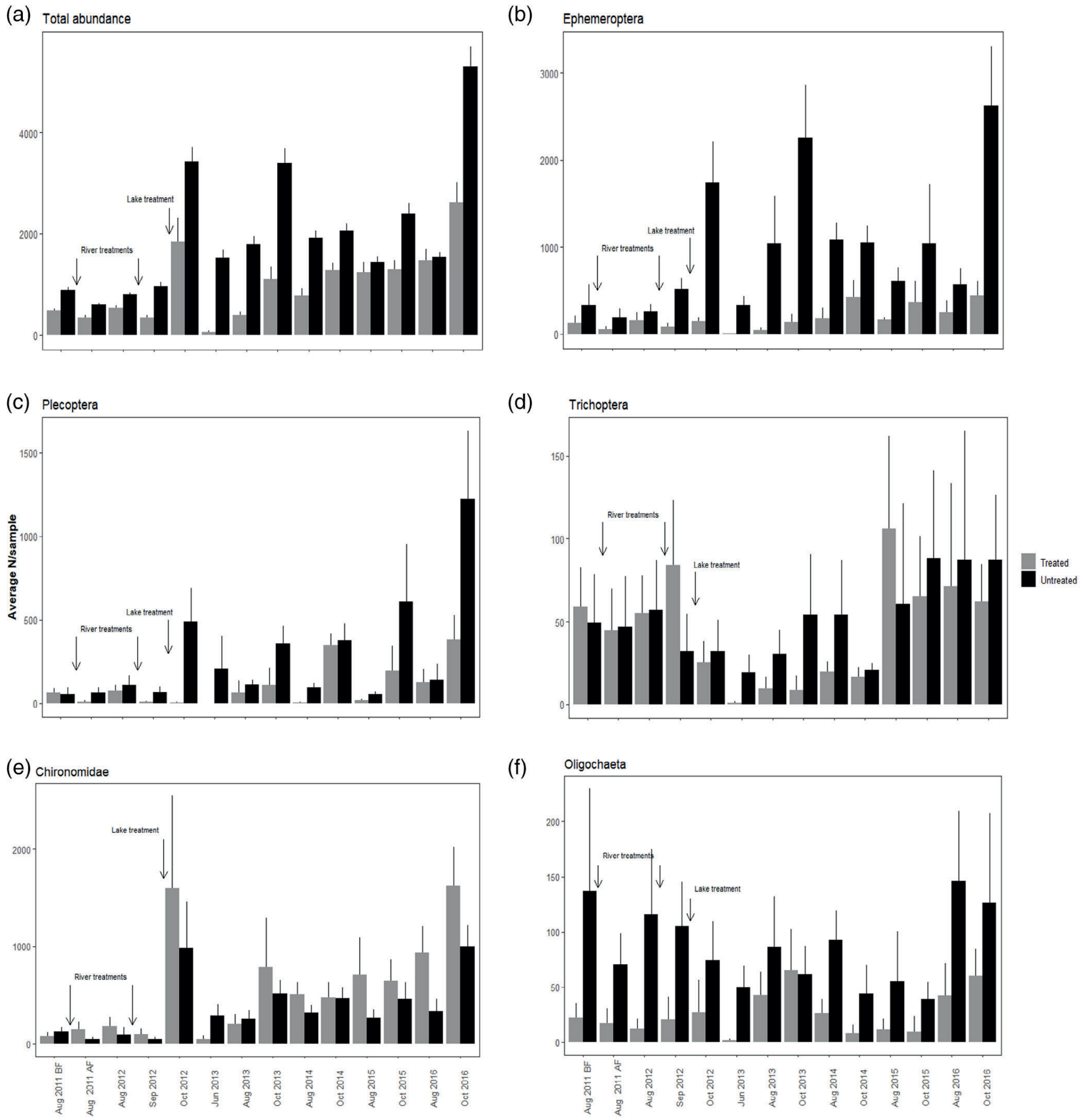


FIGURE 3 Rivers: Average abundance (with standard deviation) of benthic invertebrates of River Fusta (rotenone treated) and River Drevjaelva (untreated) in the period 2011–2016. (a) Total abundance, (b) Epemeroptera, (c) Plecoptera, (d) Trichoptera, (e) Chironomidae, and (f) Oligochaeta. Timing of rotenone treatments are marked with arrows

Permutation tests showed that the taxa composition expressed as Bray–Curtis dissimilarity, differed significantly over time irrespective of rotenone treatment status ($p < 0.001$) and between the treated and untreated river ($p < 0.001$). However, the difference was nonsignificant between the two rivers before the rotenone treatments ($p = 0.333$). The interaction between treatment (treated and untreated) and time (before and after the first river treatment) were nonsignificant ($p = 0.125$). However, when testing for effects of the lake treatment, this interaction was significant ($p < 0.001$). Overview of the full test results are given in Appendices S7–S8 and S10.

3.2 | Lakes

Temporal species turnover appeared to co-vary in the treated and untreated lakes (Figure 4). The untreated lake tended to have a

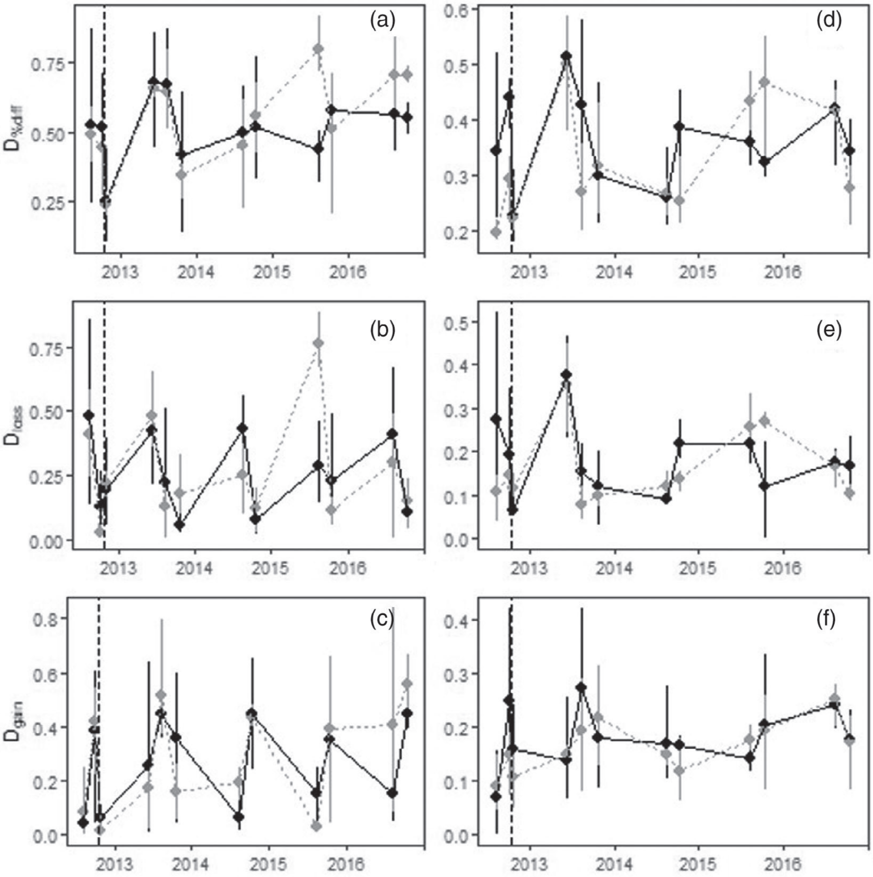


FIGURE 4 Lakes: Mean changes in dissimilarity (% difference) for abundance data (a–c) and for presence–absence data (d–f) between sampling occasions (a and d) and its components; losses (b and e) and gains (c and f). Grey: Lake Fustvatnet (rotenone treated), black: Lake Drevvatnet (untreated). Dashed vertical lines indicate the timing of the rotenone treatment of the lake. Error bars indicate maximum and minimum values of the temporal biodiversity indices at individual sampling stations

greater temporal turnover, both measured as dissimilarity in taxa abundances and species occurrences throughout most of the study period (Figure 4a,d). The highest losses in terms of species abundance occurred in the untreated lake in August 2012 and for the treated lake in August 2015 (Figure 4b). Gains were generally highest for the treated lake (Figure 4c,f), especially for abundance data. No significant changes in abundance or species occurrence indices between sampling periods were found in the treated or untreated lake (Appendices S4 and S5).

The total abundance of benthic invertebrates in the treated and untreated lake was similar or higher in the treated lake on most sampling occasions, both before and after the rotenone treatment (Figure 5a). However, abundances of invertebrate groups varied considerably between the two lakes, for example, with dominance of Plecoptera in the untreated lake and Chironomidae in the treated lake (Figures 7c and 5e, respectively).

The total abundances of benthic invertebrates in the lakes showed major variations even before the rotenone treatment, that is, the abundances decreased by about 50% from August 2011 to August 2012 in both lakes (Figure 5a). In October 2012, shortly after the treatment, the total abundance in both lakes decreased but stronger in the rotenone treated Lake Fustvatnet. In June 2013, 8 months after the treatment, a minor decrease occurred in both lakes. From August 2013 and onward the variations in abundance differed between the lakes, with higher values in the treated lake in August 2013, August and October 2014, October 2015, and August and October 2016.

The Ephemeroptera abundance varied over time both within and between lakes (Figure 5b). There was a high abundance in both lakes in June 2013, compared to pretreatment abundances mainly caused by the Ephemeroptera *Siphlonurus* sp. Abundances of all taxa from the lakes not presented in the figures are listed in Appendix S6. High abundances were also registered in the treated lake in August 2014

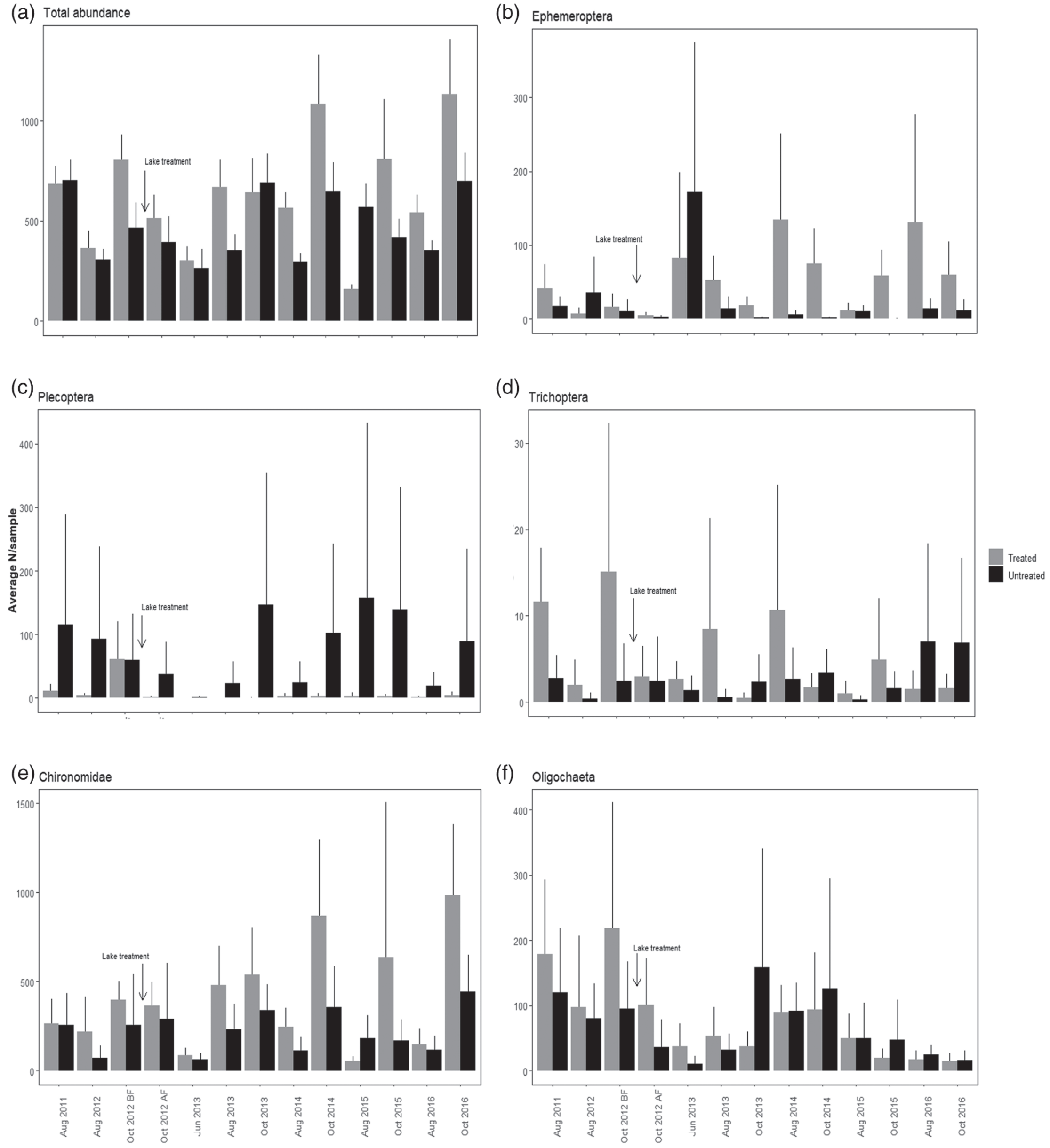


FIGURE 5 Lakes: Average abundance (with *SD*) of benthic invertebrates of Lake Fustvatnet (rotenone treated) and Lake Drevvatnet (untreated) in the period 2011–2016. (a) Total abundance, (b) Ephemeroptera, (c) Plecoptera, (d) Trichoptera, (e) Chironomidae, and (f) Oligochaeta. Timing of the rotenone treatment is marked with an arrow

and August 2016. In October 2012, shortly after the lake treatment the abundances was lower in both lakes, compared to samples taken just before the treatment. The abundance of Ephemeroptera taxa decreased immediately after the lake treatment, especially *Centroptilum luteolum* in the treated lake and Leptophlebiidae in both the treated and untreated lakes. Species with increased abundances in the treated lake after the treatment included *Centroptilum luteolum*, *Caenis horaria*, and *Ephemera vulgata*. However, the abundance of *E. vulgata* decreased strongly in August and October 2015 (73% and 71%, respectively), compared to 2014. Except from *Siphlonurus* sp., there was no marked temporal change in the abundances of Ephemeroptera in the untreated lake.

The abundance of Plecoptera in the treated lake was generally low except in October 2012 just before the rotenone treatment (Figure 5c). In the untreated lake, abundances varied but were generally much higher than in the treated lake. In the treated lake shortly after the treatment in October 2012, there was a strong decrease in Plecoptera abundances (Figure 5c), with only a few specimens of the genus *Nemoura* present. The decline was much smaller in the untreated lake. In June 2013, 8 months after the treatment, no Plecoptera were recorded in the treated lake, and only a few specimens were observed in the untreated lake. The genus *Capnia* had the most marked decrease in abundance in the treated lake following the treatment, whereas the abundance of *Capnia* peaked in some of the years after the treatment in the untreated lake.

For Trichoptera, the abundances in both lakes varied across sampling occasions (Figure 5d). In October 2012, shortly after the treatment, abundances decreased markedly in the treated lake but did not change in the untreated lake. However, a decreased abundance also occurred before the treatment in both lakes between August 2011 and August 2012. The highest abundances in the treated lake after the treatment were recorded in August 2013 and in August 2014. In the untreated lake, the abundances peaked in 2016. Polycentropodidae had the most pronounced decrease among Trichoptera after the treatment in the treated lake, whereas the family increased slightly during the same period in the untreated lake. A few Trichoptera taxa in the treated lake, all found at low abundances, were only recorded before or after the treatment.

The abundance of Chironomidae showed only minor changes after the treatment in both lakes (Figure 5e). In subsequent years, the abundances of Chironomidae in the treated lake varied compared to pretreatment levels, whereas it did not change or were higher in the untreated lake.

The abundance of Oligochaeta (Figure 5f) and Hydrachnidia generally varied to a similar extent in both lakes. For Corixidae and Coleoptera, and especially of the genus *Callicorixa* and dytiscid beetles, the abundances increased in the treated Lake Fustvatnet after the treatment. No Corixidae was recorded in untreated Lake Drevvatnet, while Dytiscidae occurred sporadically.

Permutation tests showed that the taxa composition expressed as Bray–Curtis dissimilarity, differed significantly over time irrespective of rotenone treatment status ($p < 0.001$) and between the treated and untreated lake ($p < 0.001$). However, the difference was nonsignificant between the two lakes before the rotenone treatment ($p = 0.255$) or over time before the treatment ($p = 0.120$). Additionally, there was no significant difference in taxa composition when testing the interaction between treatment and time (before and after the lake treatment) ($p = 0.371$). Overview of the full test results are given in Appendices S9 and S11.

4 | DISCUSSION

The immediate impact of rotenone treatments in August 2011 and 2012 was minor for benthic invertebrates in the rotenone-treated River Fusta. Only a few taxa showed a marked decrease in abundance in the treated river compared to the untreated River Drevjaelva, including the Ephemeroptera *Baetis rhodani* and the Plecoptera genera *Diura* and *Isoperla*. Because the upstream Lake Fustvatnet was treated 2 months after the last river treatment, River Fusta also received rotenone from the lake. Still, the immediate effect of the lake rotenone treatment on benthic invertebrate community in the downstream river was minor. One exception was the Trichoptera *Rhyacophila nubila*, which occurred at high abundances in both rivers, but which disappeared from the River Fusta subsequently to the treatment of the upstream lake. All the above-mentioned taxa are known to be rotenone sensitive (Arnekleiv et al., 2001; Engstrom-Heg, Colesante, & Silco, 1978; Gladsø & Raddum, 2002), and negative impacts were expected.

The rotenone treatment of Lake Fustvatnet took place in October, just before the lake ice formed. Breakdown of rotenone was thus slowed down due to low temperatures and limited light. Consequently, the treated river was exposed to rotenone from the lake for several months. Even though the toxic effect of rotenone decreases with decreasing water temperatures (Kjærstad, & Arnekleiv, & Speed, J.D.M., 2015; Meadows, 1973), the long exposure apparently had a pronounced negative effect. In June 2013, 8 months after the lake treatment, we recorded the lowest abundance of benthic invertebrates in the treated river. A similar decrease did not occur in the untreated river. The benthic invertebrate community in the treated river at this time was dominated by Chironomidae, Oligochaeta, Nematoda, and elmid beetles, the latter group adversely affected known to be rotenone tolerant (Kjærstad & Arnekleiv, 2011). Oligochaeta are reported to be sensitive (Arnekleiv et al., 1997; Mangum & Madrigal, 1999) or tolerant to rotenone (Fjellheim 2004, Kjærstad, & Arnekleiv, & Speed, J.D.M., 2015, Pham et al., 2018), suggesting that tolerance to rotenone is taxa specific within oligochaetes. Other taxa occurred at very low abundances, and several taxa were missing, including rotenone-tolerant species like the Ephemeroptera *Ephemerella* (Engstrom-Heg et al., 1978). Moreover, despite that the freshwater pearl mussel (*Margaritifera margaritifera*) is known to be rotenone tolerant (Dolmen, Arnekleiv, & Haukebø, 1995), all known specimens in the treated river died during the spring of 2013 (Larsen, 2015). Permutations tests showed a significant interaction effect of taxa composition (Bray–Curtis dissimilarity) between treatment and time (before versus after the lake treatment) for the rivers. The temporal beta diversity confirms that the changes in taxa composition were by far highest, and significant, in the treated river from October 2012 to June 2013. This decline in benthic invertebrates was mainly due to losses both in terms of taxa abundances and presence–absence. This indicates that rivers may receive rotenone for several months following treatment of upstream lakes, which can have major impacts. Most taxa in the treated river, including rotenone-tolerant taxa, decreased to very low abundances and some disappeared, like the freshwater pearl mussel.

Despite the strong negative impacts recorded in the treated river 8 months after the lake rotenone treatment, recolonization was relatively fast. The highest gains in terms of species occurrence in the treated river occurred in August 2013. Already in August 2014, the total abundances exceeded pretreatment levels. In August and October 2016, the change in taxa composition, in terms of abundance and presence–absence of taxa between sampling occasions, were rather similar for the treated and the untreated river. This indicates that the benthic fauna of the treated river had recovered to a great extent. Several taxa, like the Ephemeroptera *Heptagenia dalecarlica* and *Ephemerella*, the Plecoptera *Isoperla* and *Amphinemura borealis* and Oligochaeta reached much higher abundances in 2013, as compared to pretreatment levels. Ephemeroptera and Plecoptera are known to be among the most abundant groups in the drift of swift-flowing temperate streams (Brittain & Eikeland, 1988). These groups likely colonized rapidly from drift in untreated tributary streams, although some may have survived as eggs. Oligochaeta may have avoided rotenone exposure by digging deeper into the substratum. However, it took almost 4 years after the last rotenone treatment before most of the taxa had recolonized. The Plecoptera *Taeniopteryx nebulosa* were among the slowest to recolonize and appeared in 2016. Also, two rotenone tolerant taxa, the Ephemeroptera *Ephemerella* and elmid beetles, did not reached pretreatment abundances in the treated river 4 years after the treatments.

According to Vinson et al. (2010), the overall invertebrate abundances generally return to pretreatment levels quicker than the biodiversity and taxonomic composition measures. This is in accordance with our findings. However, the recolonization will greatly depend on the taxonomic resolution of the data. Biodiversity measured at family and genera level will recolonize fast, while it may take longer for all species to recolonize. We have identified some taxa to species level, such as Ephemeroptera, Plecoptera, and Trichoptera, and some taxa to a higher taxonomic level, such as the specious Chironomidae and Hydrachnidia. Thus, changes in abundance and recolonization patterns of many species in this study remain unknown.

A reduction of total benthic invertebrate abundance was observed both in the treated lake and untreated lake immediately after the lake treatment, but to a higher extent in the treated lake. This indicates both natural changes and a negative effect of rotenone. Taxa with clearly decreasing abundances shortly after the treatment in Lake Fustvatnet, but not in the untreated Lake Drevvatnet, included the Ephemeroptera *Centroptilum luteolum* and the Plecoptera *Capnia* sp. While *Capnia* is known to be rotenone sensitive, *C. luteolum* is quite tolerant (Kjærstad & Arnekleiv, 2011). The reason for the seemingly high sensitivity of *C. luteolum* to rotenone in the treated lake is unknown.

The recolonization in terms of abundance of benthic invertebrates in the treated lake was fast, reaching pretreatment levels 8 months after the treatment. A few increased in abundance after the treatment in the treated lake but not in the untreated lake. This was particularly evident for the diving beetles (Dytiscidae). Beetles are generally known to be very rotenone tolerant (Engstrom-Heg et al., 1978). Some will also benefit from increased prey availability because of the general increase in invertebrate abundance after the recolonization. Even though most taxa increased in abundance during the years following the treatments, the Plecoptera *Capnia*, the Trichoptera Polycentropodidae, and the Gastropoda *Gyraulus acronicus* decreased and had not reached their pretreatment levels in the treated lake 4 years after the treatment. Considering that Gastropoda are rotenone tolerant (Arnekleiv et al., 1997; Holocombe et al., 1987), the decline of *G. acronicus* in the treated lake was surprising. Mangun & Madrigal (1999) found that of several taxa missing 5 years after rotenone treatments, most belonged to rotenone sensitive groups, like Ephemeroptera, Plecoptera, and Trichoptera. Taxa that were tolerant to rotenone were identified as “nonmissing” or “briefly missing” (Mangum & Madrigal, 1999).

Fish may play an important role in structuring benthic invertebrate communities (e.g., Alexiades & Kraft, 2017; Weyl, de Moor, Hill, & Weyl, 2010). Stocking of Atlantic salmon (*S. salar*) took place in the treated river between 2013 and 2016, whereas no fish was stocked in the untreated river. Despite fish removal, the abundance of most benthic taxa in the treated river did not increase after the river treatments compared to pretreatment abundances, indicating that the benthic community not yet was re-established. Total abundances strongly increased immediately after the lake treatment, mainly due to an increase in Chironomidae. This occurred in both rivers, suggesting natural variation rather than absence of fish. After fish stocking from 2013 and onward, abundances generally increased in the treated and untreated rivers, and consequently fish stocking had a limited effect on the abundances of benthic invertebrates. However, for Trichoptera, which mostly constituted of large-bodied taxa like *Arctopsyche ladogensis*, Hydropsychidae, and *Rhyacophila nubila*, the abundances of the treated river did not reach pretreatment levels until 2015, probably due to fish predation.

The total invertebrate abundances of the treated and untreated lakes covaried, except for August 2015 when the total abundances decreased markedly in the treated lake. This was most likely due to predation from fish stocked during the previous year. The decrease was particularly high for Chironomidae and Ephemeroptera, including the large-sized and important fish prey, *Ephemera vulgata*. Contrary to the untreated lake, the abundances during some years after the treatment in the treated lake were higher than pretreatment levels, indicating that predation pressure from fish was lower after than before the treatment. The change in invertebrate abundances was more prominent in the impacted treated localities than in the untreated localities. This illustrates the importance of using control sites when performing time-series experiments.

It is striking to observe the large fluctuations in the abundance and in the presence–absence of benthic invertebrates over time of both treated and untreated localities. Even before the lake treatment, the invertebrate abundances in both lakes were reduced by approximately 50% from August 2011 to August 2012. According to Vinson et al. (2010), the accumulation curve for benthic invertebrate genera showed little inclination for flattening out during a 10-year monthly sampling of a Utah river. We did not see the same pattern in our data, perhaps due to fewer taxa present and low taxonomic resolution for several species-rich groups. Even though it will vary greatly with habitat type, latitude, etc., a rising taxa accumulating curve would certainly be found elsewhere and shows how random the community composition may be registered at any given time.

Our study found minor short-term (i.e., <1 month) effects of the river rotenone treatments, whereas the lake treatment caused major temporary negative effects on the lotic fauna 8 months after the treatment. This is likely caused by leaking of rotenone to the lotic system during the subsequent winter and spring. Accordingly, long-term low-dose exposure seem to have higher negative effects than high-dose short-term exposure, suggesting that it is important to minimize exposure time. For example, this could likely be accommodated by choosing an earlier date for the lake treatment in the River Fusta. No major short- or long-term (i.e., 4 years) effects on lake benthic invertebrate communities where detected. Recovery time of invertebrate abundance after the lake treatment was fast whereas the recovery in terms of presence–absence of species were comparably much longer, lasting for several years. The conclusion of this study would not have been possible with short-term monitoring without controls. The current study underpins the crucial importance of performing long-terms studies with before-after-control-impact during investigation of the effects of rotenone on benthic invertebrates.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.16791889.v1.

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REFERENCES

Adolfsen, P., Sandvik, M., & Waaler, T. (2014). Rotenonanalyser og resultater. In J. H. Stensli & H. Bardal (Eds.), *Bekjempelse av Gyrodactylus salaris i Vefsnaregionen* (Vol. 2, pp. 119–129). Veterinærinstituttets rapportserie, Oslo: Veterinærinstituttet.

Alexiades, A. V., & Kraft, C. E. (2017). Effects of stocked trout on stream invertebrate communities. *Journal of Freshwater Ecology*, *32*, 95–102. https://doi.org/10.1080/02705060.2016.1248502

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*, 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Arnekleiv, J. V., Dolmen, D., Aagaard, K., Bongard, T. & Hanssen, O. (1997). Rotenonbehandlingens effekt på bunndyr i Rauma og Hensvassdraget, Møre & Romsdal. Del I: Kvalitative undersøkelser. Vitenskapsmuseet Rapport Zoologisk Serie (pp. 1997–1998); Trondheim: NTNU Vtenskapsmuseet.

Arnekleiv, J. V., Dolmen, D., & Rønning, L. (2001). Effects of rotenone treatment on mayfly drift and standing stocks in two Norwegian rivers. In E. Dominguez (Ed.), *Trends in research in Ephemeroptera and Plecoptera*. New York: Kluwer Academic/Plenum Publishers.

Bellingan, T. A., Hugo, S., Woodford, D. J., Gouws, J., Villet, M. H., & Weyl, O. L. F. (2019). Rapid recovery of macroinvertebrates in a south African stream treated with rotenone. *Hydrobiologia*, *834*, 1–11. https://doi.org/10.1007/s10750-019-3885-z

Brittain, J. E., & Eikeland, T. J. (1988). Invertebrate drift—A review. *Hydrobiologia*, *166*, 77–93. https://doi.org/10.1007/BF00017485

Brooks, I. C., & Price, R. W. (1961). Studies on the chronic toxicity of Pro-Noxfish®, a proprietary synergized rotenone fish-toxicant. *Toxicology and Applied Pharmacology*, *3*, 49–56. https://doi.org/10.1016/0041-008X(61)90007-2

Caffrey, J. M., Baars, J. R., Barbour, J. H., Boets, P., Boon, P., & Davenport, K. (2014). Tackling invasive alien species in Europe: The top 20 issues. *Management of Biological Invasions*, *5*, 1–20. https://doi.org/10.3391/mbi.2014.5.1.01

Chandler, J. H., & Marking, L. L. (1982). Toxicity of rotenone to selected aquatic invertebrates and frog larvae. *The Progressive Fish-Culturist*, *44*, 78–80. https://doi.org/10.1577/1548-8659(1982)44[78:TORTSA]2.0.CO;2

Cook, S. F., & Moore R. L. (1969). The effects of rotenone treatment on the insect fauna of a California stream. *Transactions of the American Fisheries Society*, *98*, 539–544. https://doi.org/10.1577/1548-8659(1969)98[539:TEOART]2.0.CO;2

Crowl, T. A., Crist, T. A., Parmenter, R. R., Belovsky, G., & Lugo, A. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers of Ecology and the Environment*, *6*(5), 238–246. https://doi.org/10.1890/070151

Dalu, T., Wasserman, R. J., Jordaan, M., Froneman, W. P., & Weyl, O. L. F. (2015). An assessment of the effect of rotenone on selected non-target aquatic Fauna. *PLoS One*, *23*, e0142140. https://doi.org/10.1371/journal.pone.0142140

Dittel, A., & Epifanio, C. (2009). Invasion biology of the Chinese mitten crab *Eriochier sinensis*: A brief review. *Journal of Experimental Marine Biology and Ecology*, *374*, 79–92. https://doi.org/10.1016/j.jembe.2009.04.012

Dolmen, D., Arnekleiv, J. V., & Haukebø, T. (1995). Rotenone tolerance in the freshwater pearl mussel *Margaritifera margaritifera*. *Nordic Journal of Freshwater Research*, *70*, 1–30.

Dray, S., Bauman, D., Blanchet, G., Borchard, D., Clappe, S., Guénard, G. & Wagner, H.H. … (2019). Adespatial: Multivariate multiscale spatial analsysis. R package version 0.3-3. Retrieved from https://cran.r-project.org/package=adespatial

Dudgeon, D. (1990). Benthic community structure and the effect of rotenone piscicide on invertebrate drift and standing stocks in two Papua New Guinea streams. *Archives of Hydrobiology*, *119*, 35–53.

Engblom, E. (1996). Ephemeroptera, mayflies. In A. Nilsson (Ed.), *Aquatic insects of North Europe. A taxonomic handbook. Vol 1*. Stenstrup: Apollo Books.

Engstrom-Heg, R., Colesante, R. T., & Silco, E. (1978). Rotenone tolerances of stream-bottom insects. *New York Fish Game Journal*, *25*, 31–41.

Eriksen, T. E., Arnekleiv, J. V., & Kjærstad, G. (2009). Short-term effects on riverine Ephemeroptera, Plecoptera, and Trichoptera of rotenone and aluminum sulfate treatment to eradicate *Gyrodactylus salaris*. *Journal of Freshwater Ecology*, *24*, 507–607. https://doi.org/10.1080/02705060.2009.9664337

Finlayson, B., Schnick, R., Skaar, D., Anderson, J., Demong, L., Duffield, W, D., … Steinkjer, J. (2010a). *Planning and standard operating procedures for the use of rotenone in fish management—Rotenone SOP manual*. Bethesda, MD: American Fisheries Society.

Finlayson, B., Somer, W. L., & Vinson, M. R. (2010b). Rotenone toxicity to rainbow trout and several mountain stream insects. *North American Journal of Fisheries Management*, *30*, 102–111. https://doi.org/10.1577/M09-078.1

Gladsø, J., & Raddum, G. G. (2002). Rotenone treatment of a west Norwegian river: Effects on drift of invertebrates. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen*, *28*, 764–769. https://doi.org/10.1080/03680770.2001.11901816

Glöer, P. (2002). *Süsswassergastropoden Nord- und Mitteleuropas*. Bestimmungsschlussel, Lebenweise, Verbreitung: ConchBooks.

Hamilton, B. T., Moore, S. E., Williams, T. B., Darby, N., & Vinson, M. R. (2009). Comparative effects of rotenone and antimycin on macroinvertebrate diversity in two streams in Great Basin National Park, Nevada. *North American Journal of Fisheries of Management*, *29*, 1620–1635. https://doi.org/10.1577/M08-178.1

Holdich, D., Reynolds, J., Souty-Grosset, C., & Sibley, P. (2009). A review of the ever-increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems*, *394-395*, 11. https://doi.org/10.1051/kmae/2009025

Holmen, M. (1987). In *The aquatic Adephaga (Coleoptera) of Fennoscanida and Denmark, volume 1. Gyrinidae, Haliplidae, Hygrobiidae and Noteridae* (Vol. 20, p. 168). Fauna Entomologica Scandinavica, Leiden, Copenhagen: E.J. Brill/Scandinavian Science Press Ltd.

Holocombe, G. W., Phipps, G. L., Sulaiman, A. H., & Hoffman, A. D. (1987). Simultaneous multiple species testing: Acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. *Archives of Environmental Contamination and Toxicology*, *16*, 697–710. https://doi.org/10.1007/BF01055420

Holthe, E., Bjørnå, T. & Lo, H. (2015). Reetableringsprosjektet i Vefsnaregionen—Årsrapport for aktiviteten i 2014. Veterinærinstituttets Rapportserie, 14, 1-29.

Holthe, E., Bjørnå, T. & Lo, H. (2018). Reetableringsprosjektet i Vefsnaregionen. Årsrapport 2017. Veterinærinstituttets Rapportserie 11, 1-29.

Hulme, P. E. (2009). Trade, transport and trouble: Managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, *46*, 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x

Josefsson, J., Hiron, M., Arlt, D., Auffret, A. G., Berg, Å., Chevalier, M., …, Pärt, T. (2020). Improving scientific rigor in conservation evaluations and a plea deal for transparency on potential biases. *Conservation Letters*, *13*, e12726. https://doi.org/10.1111/conl.12726

Kjærstad, G., Arnekleiv J. V., & Speed, J. D. M., & (2015). *Effects of three consecutive rotenone treatments on the benthic macroinvertebrate fauna of the river Ogna*. Central Norway: Rivers Research and Applications. https://doi.org/10.1002/rra.2873

Kjærstad, G. & Arnekleiv, J. V. (2004). Rotenonbehandling av elver i Ranaregionen i 2003 og 2004: effekter på bunndyr. Vitenskapsmuseet Rapport Zoologisk Serie, 4, 1-23; Trondheim: NTNU Vitenskapsmuseet.

Kjærstad, G., & Arnekleiv, J. V. (2011). Effects of rotenone treatment on lotic invertebrates. *International Review of Hydrobiology*, *96*, 58–71. https://doi.org/10.1002/iroh.201111261

Koksvik, J. I., & Aagaard, K. (1984). Effects of rotenone on the benthic fauna of a small eutrophic lake. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen*, *22*, 658–665. https://doi.org/10.1080/03680770.1983.11897361

Kumschick, S., Gaertner, M., Vilà, M., Essl, F., Jeschke, J. M., Pysek, P., … Winter, M. (2015). Ecological impacts of alien species: Quantification, scope, caveats, and recommendations. *Bioscience*, *65*, 55–63. https://doi.org/10.1093/biosci/biu193

Larsen, B.M. (2015). Elvemusling i Fusta, Nordland—konsekvenser av rotenonbehandling i vassdraget og tiltak for å sikre bestanden av muslinger, NINA Rapport 1189.

Legendre, P. (2019). A temporal beta-diversity index to identify sites that have changed in exceptional ways in space-times surveys. *Ecology and Evolution*, *9*, 3500–3514. https://doi.org/10.1002/ece3.4984

Lillehammer, A. (1988). *Stoneflies (Plecoptera) of Fennoscandia and Denmark, Vol. 21*. Leiden, The Netherlands: E. J. Brill/Scandinavian Science Press Ltd.

Ling, N. & (2003). Rotenone—Review of its toxicity and use for fisheries management. Science for Conservation, 211, 39; Wellington.

Lo, H., & Holthe, E. (2014). Bevaring av fiskebestander. In J. H. Stensli & H. Bardal (Eds.), *Bekjempelse av Gyrodactylus salaris i Vefsnaregionen* (p. 146–158). Oslo: Veterinærinstituttet.

Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, *10*, 689–710. https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Mangum, M. A., & Madrigal, J. L. (1999). Rotenone effects on aquatic macroinvertebrates of the Strawberry River, Utah: A five-year summary. *Journal of Freshwater Ecology*, *14*, 125–135. https://doi.org/10.1080/02705060.1999.9663661

McClay, W. (2000). Rotenone use in North America (1988-1997). *Fisheries*, *20*, 15–21. https://doi.org/10.1577/1548-8446(2000)025%3C0015:RUINA%3E2.0.CO;2

McClay, W. (2005). Rotenone use in North America (1998-2002). *Fisheries*, *30*, 29–31. https://doi.org/10.1577/1548-8446(2005)30[29:RUINA]2.0.CO;2

Meadows, B. S. (1973). Toxicity of rotenone to some species of coarse fish and invertebrates. *Journal of Fish Biology*, *5*, 155–163. https://doi.org/10.1111/j.1095-8649.1973.tb04444.x

Melaas, C. L., Zimmer, K. D., Butler, M. G., & Hanson, M. A. (2001). Effects of rotenone on aquatic invertebrate communities in prairie wetlands. *Hydrobiologia*, *459*, 177–186. https://doi.org/10.1023/A:1012514124430

Moen, A., & Bardal, H. (2014). Innsjøbehandlingen. In J. H. Stensli & H. Bardal (Eds.), *Bekjempelse av Gyrodactylus salaris i Vefsnaregionen* (pp. 92–101). Veterninærinstituttets rapportserie, Oslo: Veterinærinstituttet.

Nilsson, A. N., & Holmen, M. (1995). *The aquatic Adephaga (Coleoptera) of Fennoscandia and Denmark, Vol. II. Dytiscidae*. (p. 32). Fauna Entomologica Scandinavica, Leiden, New York: E.J. Brill.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2020). Vegan: Community ecology package (version 2.5-5). Retrieved from https://cran.r-project.org/web/packages/vegan/index.html

Pham, L., Jarvis, M. G., West, D., & Closs, G. P. (2018). Rotenone treatment has a short-term effect on New Zealand stream macroinvertebrate communities. *New Zealand Journal of Marine and Freshwater Research*, *52*, 42–54. https://doi.org/10.1080/00288330.2017.1330273

Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., … Richardson, D. M. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, *95*, 1511–1534. https://doi.org/10.1111/brv.12627

Rinne, A., & Wiberg-Larsen, P. (2017). *Trichoptera larvae of Finland: Identification key to the caddis larvae of Finland and nearby countries*. Helsinki: Trificon Books.

Rytwinski, T., Taylor, J. J., Donaldson, L. A., Britton, J. R., Browne, D. R., Gresswell, R. E., … Cooke, S. J. (2018). The effectiveness of non-native fish removal techniques in freshwater ecosystems: A systematic review. *Environmental Reviews*, *00*, 1–24. https://doi.org/10.1139/er-2018-0049

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., … Wall, D. H. (2000). Biodiversity: Global biodiversity scenarios for the year 2100. *Science*, *287*, 1770–1774. https://doi.org/10.1126/science.287.5459.1770

Stensli, J.H. & Wist, A.N. (2014). Behandlingene i sone Indre Vefsnfjorden. In: Stensli, J.H. & Bardal, H. (eds.). *Bekjempelse av Gyrodactylus salaris i Vefsnaregionen*. (Eds: J. H. Stensli & H. Bardal). Oslo: Veterinærinstituttet Veterninærsinstituttets rapportserie, 2, 54-74.

Vinson, M. R., Dinger, E. C., & Vinson, D. K. (2010). Piscicides and invertebrates: After 70 years, does anyone really know? *Fisheries*, *35*, 61–71. https://doi.org/10.1577/1548-8446-35.2.61

Weyl, P. S. R., de Moor, F. C., Hill, M. P., & Weyl, O. L. F. (2010). The effect of largemouth bass *Micropterus salmoides* on aquatic macroinvertebrate communities in the Wit River, Eastern Cape, South Africa. *African Journal of Aquatic Science*, *2010*(35), 273–281. https://doi.org/10.2989/16085914.2010.540776

SUPPORTING INFORMATION

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Paper III

Kjærstad, Gaute; Majaneva, Markus; Falahati-Anbaran, Mohsen; Topstad, Lasse; Finstad Anders Gravbrøt; Arnekleiv, Jo Vegar; Ekrem, Torbjørn. DNA metabarcoding of preservative ethanol reveals changes in invertebrate community composition following rotenone treatment.

This paper is submitted for publication and is therefore not included.

Paper IV

Paper 4: Eloranta, Antti P.; Kjærstad, Gaute; Power, Michael; Lakka, Hanna-Kaisa; Arnekleiv, Jo Vegar; Finstad, Anders G. Impacts of piscicide-induced fish removal on resource use and trophic diversity of lake invertebrates.

This paper is submitted for publication and is therefore not included.

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	**Name**	**Degree**	**Title**
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr. philos Botany	The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exposed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflectometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos Zoology	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels and some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Botany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Botany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses

1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*
1994	Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994	Solveig Bakken	Dr. scient Botany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994	Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995	Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995	Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995	Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995	Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995	Hans Haavardsholm Blom	Dr. philos Botany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996	Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996	Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996	Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996	Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997	Gunvor Øie	Dr. scient Botany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spruce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997	Ole Reitan	Dr. scient Zoology	Responses of birds to habitat disturbance due to damming
1997	Jon Arne Grøttum	Dr. scient Zoology	Physiological effects of reduced water quality on fish in aquaculture

1997	Per Gustav Thingstad	Dr. scient Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997	Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as
1997	Signe Nybø	Dr. scient Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997	Atle Wibe	Dr. scient Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997	Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997	Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997	Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos Zoology	Population responses of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998	Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservation biological approach
1998	Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999	Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Botany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway

1999	Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis
1999	Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gadus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999	Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer’s disease
1999	Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families:
1999	Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient Zoology	Host specificity as a parameter in estimates of arthropod species richness
1999	Sonja Andersen	Dr. scient Zoology	Expressional and functional analyses of human, secretory phospholipase A2
2000	Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial control of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species

2001	Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forest systems
2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient Zoology	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequences of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr. scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr. scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr. scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr. scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr. scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo salar* L.) parr and smolt
2004	Torkild Bakken	Dr. scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)

2004	Ingar Pareliussen	Dr. scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr. scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr. scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr. scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr. scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr. scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr. scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr. scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr. scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr. scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr. scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	PhD Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr. scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr. scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyroid hormone and vitamin A concentrations
2005	Christian Westad	Dr. scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	PhD Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	PhD Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	PhD Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	PhD Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	PhD Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr. scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	PhD Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds, Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr. scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	PhD Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr. philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr. scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	PhD Biology	*Acesta oophaga* and *Acesta excavata* – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	PhD Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	PhD Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	PhD Biology	Wildlife conservation and local land use conflicts in Western Serengeti Corridor, Tanzania
2006	Anna Maria Billing	PhD Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	PhD Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	PhD Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	PhD Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	PhD Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	PhD Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	PhD Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	PhD Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	PhD Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	PhD Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	PhD Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	PhD Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania
2007	Julius William Nyahongo	PhD Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania

2007	Shombe Ntaraluka Hassan	PhD Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	PhD Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	PhD Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	PhD Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	PhD Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	PhD Biology	The ecology and behaviour of the Masai Ostrich (*Struthio camelus massaicus*) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	PhD Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, *Taeniopygia guttata*
2008	Sølvi Wehn	PhD Biology	Biodiversity dynamics in semi-natural mountain landscapes - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	PhD Biology	The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations
2008	Katarina Mariann Jørgensen	Dr. scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	PhD Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	PhD Biology	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	PhD Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	PhD Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	PhD Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	PhD Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	PhD Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	PhD Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease
2009	Pål Kvello	PhD Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	PhD Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	PhD Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches

2009	Zsolt Volent	PhD Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	PhD Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	PhD Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	PhD Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments - Focus on formulated diets
2010	Eli Kvingedal	PhD Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	PhD Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	PhD Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. The Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	PhD Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	PhD Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	PhD Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	PhD Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	PhD Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	PhD Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	PhD Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	PhD Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	PhD Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS: The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	PhD Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	PhD Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in Arabidopsis thaliana
2011	Grethe Robertsen	PhD Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	PhD Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	PhD Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	PhD Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology

2011	Sajeda Begum	PhD Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	PhD Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	PhD Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	PhD Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	PhD Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	PhD Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	PhD Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	PhD Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	PhD Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	PhD Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	PhD Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	PhD Biology	Foraging in a variable world: adaptations to stochasticity
2012	Aleksander Handå	PhD Biology	Cultivation of mussels (*Mytilus edulis):* Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	PhD Biology	Reproductive and migratory challenges inflicted on migrant brown trout (*Salmo trutta* L.) in a heavily modified river
2012	Jisca Huisman	PhD Biology	Gene flow and natural selection in Atlantic salmon
2012	Maria Bergvik	PhD Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	PhD Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	PhD Biology	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	PhD Biology	Optimal performance in the cold
2012	Robert Dominikus Fyumagwa	Dr. Philos Biology	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	PhD Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway. Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	PhD Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	PhD Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters

2012	Lise Cats Myhre	PhD Biology	Effects of the social and physical environment on mating behaviour in a marine fish
2012	Tonje Aronsen	PhD Biology	Demographic, environmental and evolutionary aspects of sexual selection
2012	Bin Liu	PhD Biology	Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana*
2013	Jørgen Rosvold	PhD Biology	Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective
2013	Pankaj Barah	PhD Biology	Integrated Systems Approaches to Study Plant Stress Responses
2013	Marit Linnerud	PhD Biology	Patterns in spatial and temporal variation in population abundances of vertebrates
2013	Xinxin Wang	PhD Biology	Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming
2013	Ingrid Ertshus Mathisen	PhD Biology	Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia
2013	Anders Foldvik	PhD Biology	Spatial distributions and productivity in salmonid populations
2013	Anna Marie Holand	PhD Biology	Statistical methods for estimating intra- and inter-population variation in genetic diversity
2013	Anna Solvang Båtnes	PhD Biology	Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night
2013	Sebastian Wacker	PhD Biology	The dynamics of sexual selection: effects of OSR, density and resource competition in a fish
2013	Cecilie Miljeteig	PhD Biology	Phototaxis in *Calanus finmarchicus* – light sensitivity and the influence of energy reserves and oil exposure
2013	Ane Kjersti Vie	PhD Biology	Molecular and functional characterisation of the IDA family of signalling peptides in *Arabidopsis thaliana*
2013	Marianne Nymark	PhD Biology	Light responses in the marine diatom *Phaeodactylum tricornutum*
2014	Jannik Schultner	PhD Biology	Resource Allocation under Stress - Mechanisms and Strategies in a Long-Lived Bird
2014	Craig Ryan Jackson	PhD Biology	Factors influencing African wild dog *(Lycaon pictus*) habitat selection and ranging behaviour: conservation and management implications
2014	Aravind Venkatesan	PhD Biology	Application of Semantic Web Technology to establish knowledge management and discovery in the Life Sciences
2014	Kristin Collier Valle	PhD Biology	Photoacclimation mechanisms and light responses in marine micro- and macroalgae
2014	Michael Puffer	PhD Biology	Effects of rapidly fluctuating water levels on juvenile Atlantic salmon (*Salmo salar* L.)
2014	Gundula S. Bartzke	PhD Biology	Effects of power lines on moose *(Alces alces)* habitat selection, movements and feeding activity
2014	Eirin Marie Bjørkvoll	PhD Biology	Life-history variation and stochastic population dynamics in vertebrates
2014	Håkon Holand	PhD Biology	The parasite *Syngamus trachea* in a metapopulation of house sparrows
2014	Randi Magnus Sommerfelt	PhD Biology	Molecular mechanisms of inflammation – a central role for cytosolic phospholiphase A2
2014	Espen Lie Dahl	PhD Biology	Population demographics in white-tailed eagle at an on-shore wind farm area in coastal Norway

2014	Anders Øverby	PhD Biology	Functional analysis of the action of plant isothiocyanates: cellular mechanisms and in vivo role in plants, and anticancer activity
2014	Kamal Prasad Acharya	PhD Biology	Invasive species: Genetics, characteristics and trait variation along a latitudinal gradient.
2014	Ida Beathe Øverjordet	PhD Biology	Element accumulation and oxidative stress variables in Arctic pelagic food chains: *Calanus*, little auks (*Alle alle*) and black-legged kittiwakes (*Rissa tridactyla*)
2014	Kristin Møller Gabrielsen	PhD Biology	Target tissue toxicity of the thyroid hormone system in two species of arctic mammals carrying high loads of organohalogen contaminants
2015	Gine Roll Skjervø	Dr. philos Biology	Testing behavioral ecology models with historical individual-based human demographic data from Norway
2015	Nils Erik Gustaf Forsberg	PhD Biology	Spatial and Temporal Genetic Structure in Landrace Cereals
2015	Leila Alipanah	PhD Biology	Integrated analyses of nitrogen and phosphorus deprivation in the diatoms *Phaeodactylum tricornutum* and *Seminavis robusta*
2015	Javad Najafi	PhD Biology	Molecular investigation of signaling components in sugar sensing and defense in *Arabidopsis thaliana*
2015	Bjørnar Sporsheim	PhD Biology	Quantitative confocal laser scanning microscopy: optimization of in vivo and in vitro analysis of intracellular transport
2015	Magni Olsen Kyrkjeeide	PhD Biology	Genetic variation and structure in peatmosses (*Sphagnum*)
2015	Keshuai Li	PhD Biology	Phospholipids in Atlantic cod (*Gadus morhua* L.) larvae rearing: Incorporation of DHA in live feed and larval phospholipids and the metabolic capabilities of larvae for the de novo synthesis
2015	Ingvild Fladvad Størdal	PhD Biology	The role of the copepod *Calanus finmarchicus* in affecting the fate of marine oil spills
2016	Thomas Kvalnes	PhD Biology	Evolution by natural selection in age-structured populations in fluctuating environments
2016	Øystein Leiknes	PhD Biology	The effect of nutrition on important life-history traits in the marine copepod *Calanus finmarchicus*
2016	Johan Henrik Hårdensson Berntsen	PhD Biology	Individual variation in survival: The effect of incubation temperature on the rate of physiological ageing in a small passerine bird
2016	Marianne Opsahl Olufsen	PhD Biology	Multiple environmental stressors: Biological interactions between parameters of climate change and perfluorinated alkyl substances in fish
2016	Rebekka Varne	PhD Biology	Tracing the fate of escaped cod (*Gadus morhua* L.) in a Norwegian fjord system
2016	Anette Antonsen Fenstad	PhD Biology	Pollutant Levels, Antioxidants and Potential Genotoxic Effects in Incubating Female Common Eiders (*Somateria mollissima*)
2016	Wilfred Njama Marealle	PhD Biology	Ecology, Behaviour and Conservation Status of Masai Giraffe (*Giraffa camelopardalis tippelskirchi*) in Tanzania
2016	Ingunn Nilssen	PhD Biology	Integrated Enviromental Mapping and Monitoring: A Methodological approach for end users.
2017	Konika Chawla	PhD Biology	Discovering, analysing and taking care of knowledge.
2017	Øystein Hjorthol Opedal	PhD Biology	The Evolution of Herkogamy: Pollinator Reliability, Natural Selection, and Trait Evolvability.

2017	Ane Marlene Myhre	PhD Biology	Effective size of density dependent populations in fluctuating environments
2017	Emmanuel Hosiana Masenga	PhD Biology	Behavioural Ecology of Free-ranging and Reintroduced African Wild Dog (*Lycaon pictus*) Packs in the Serengeti Ecosystem, Tanzania
2017	Xiaolong Lin	PhD Biology	Systematics and evolutionary history of *Tanytarsus* van der Wulp, 1874 (Diptera: Chironomidae)
2017	Emmanuel Clamsen Mmassy	PhD Biology	Ecology and Conservation Challenges of the Kori bustard in the Serengeti National Park
2017	Richard Daniel Lyamuya	PhD Biology	Depredation of Livestock by Wild Carnivores in the Eastern Serengeti Ecosystem, Tanzania
2017	Katrin Hoydal	PhD Biology	Levels and endocrine disruptive effects of legacy POPs and their metabolites in long-finned pilot whales of the Faroe Islands
2017	Berit Glomstad	PhD Biology	Adsorption of phenanthrene to carbon nanotubes and its influence on phenanthrene bioavailability/toxicity in aquatic organism
2017	Øystein Nordeide Kielland	PhD Biology	Sources of variation in metabolism of an aquatic ectotherm
2017	Narjes Yousefi	PhD Biology	Genetic divergence and speciation in northern peatmosses (*Sphagnum*)
2018	Signe Christensen-Dalgaard	PhD Biology	Drivers of seabird spatial ecology - implications for development of offshore wind-power in Norway
2018	Janos Urbancsok	PhD Biology	Endogenous biological effects induced by externally supplemented glucosinolate hydrolysis products (GHPs) on *Arabidopsis thaliana*
2018	Alice Mühlroth	PhD Biology	The influence of phosphate depletion on lipid metabolism of microalgae
2018	Franco Peniel Mbise	PhD Biology	Human-Carnivore Coexistence and Conflict in the Eastern Serengeti, Tanzania
2018	Stine Svalheim Markussen	PhD Biology	Causes and consequences of intersexual life history variation in a harvested herbivore population
2018	Mia Vedel Sørensen	PhD Biology	Carbon budget consequences of deciduous shrub expansion in alpine tundra ecosystems
2018	Hanna Maria Kauko	PhD Biology	Light response and acclimation of microalgae in a changing Arctic
2018	Erlend I. F. Fossen	PhD Biology	Trait evolvability: effects of thermal plasticity and genetic correlations among traits
2019	Peter Sjolte Ranke	PhD Biology	Demographic and genetic and consequences of dispersal in house sparrows
2019	Mathilde Le Moullec	PhD Biology	Spatiotemporal variation in abundance of key tundra species: from local heterogeneity to large-scale synchrony
2019	Endre Grüner Ofstad	PhD Biology	Causes and consequences of variation in resource use and social structure in ungulates
2019	Yang Jin	PhD Biology	Development of lipid metabolism in early life stage of Atlantic salmon (*Salmo salar*)
2019	Elena Albertsen	PhD Biology	Evolution of floral traits: from ecological contex to functional integration
2019	Mominul Islam Nahid	PhD Biology	Interaction between two Asian cuckoos and their hosts in Bangladesh
2019	Knut Jørgen Egelie	PhD Biology	Management of intellectual property in university-industry collaborations – public access to and control of knowledge
2019	Thomas Ray Haaland	PhD Biology	Adaptive responses to enviromental stochasticity on different evolutionary time-scales

2019	Kwaslema Malle Hariohay	PhD Biology	Human wildlife interactions in the Ruaha-Rungwa Ecosystem, Central Tanzania
2019	Mari Engvig Løseth	PhD Biology	Exposure and effects of emerging and legacy organic pollutants in white-tailed eagle (*Haliaeetis albicilla*) nestlings
2019	Joseph Mbyati Mukeka	PhD Biology	Human-Wildlife Conflicts and Compensation for Losses in Kenya: Dynamics, Characteristics and Correlates
2019	Helene Løvstrand Svarva	PhD Biology	Dendroclimatology in southern Norway: tree rings, demography and climate
2019	Nathalie Briels	PhD Biology	Exposure and effects of legacy and emerging organic pollutants in developing birds – Laboratory and field studies
2019	Anders L.Kolstad	PhD Biology	Moose browsing effects on boreal production forests – implications for ecosystems and human society
2019	Bart Peeters	PhD Biology	Population dynamics under climate change ad harvesting: Results from the high Arctic Svalbard reindeer
2019	Alex Kojo Datsomor	PhD Biology	The molecular basis of long chain polyunsaturated fatty acid (LC-PUFA) biosynthesis in Atlantic salmon (*Salmo salar L*): In vivo functions, functional redundancy and transcriptional regulation of LC-PUFA biosynthetic enzymes
2020	Ingun Næve	PhD Biology	Development of non-invasive methods using ultrasound technology in monitoring of Atlantic salmon (*Salmo salar*) production and reproduction
2020	Rachael Morgan	PhD Biology	Physiological plasticity and evolution of thermal performance in zebrafish
2020	Mahsa Jalili	PhD Biology	Effects of different dietary ingredients on the immune responses and antioxidant status in Atlantic salmon (*Salmo salar L.):* possible nutriomics approaches
2020	Haiqing Wang	PhD Biology	Utilization of the polychaete *Hediste diversicolor* (O.F. Millier, 1776) in recycling waste nutrients from land-based fish farms for value adding applications'
2020	Louis Hunninck	PhD Biology	Physiological and behavioral adaptations of impala to anthropogenic disturbances in the Serengeti ecosystems
2020	Kate Layton-Matthews	PhD Biology	Demographic consequences of rapid climate change and density dependence in migratory Arctic geese
2020	Amit Kumar Sharma	PhD Biology	Genome editing of marine algae: Technology development and use of the CRISPR/Cas9 system for studies of light harvesting complexes and regulation of phosphate homeostasis
2020	Lars Rød-Eriksen	PhD Biology	Drivers of change in meso-carnivore distributions in a northern ecosystem
2020	Lone Sunniva Jevne	PhD Biology	Development and dispersal of salmon lice (*Lepeophtheirus salmonis Krøyer*, 1837) in commercial salmon farming localities
2020	Sindre Håvarstein Eldøy	PhD Biology	The influence of physiology, life history and environmental conditions on the marine migration patters of sea trout
2020	Vasundra Touré	PhD Biology	Improving the FAIRness of causal interactions in systems biology: data curation and standardisation to support systems modelling applications

2020	Silje Forbord	PhD Biology	Cultivation of *Saccharina latissima* (Phaeophyceae) in temperate marine waters; nitrogen uptake kinetics, growth characteristics and chemical composition
2020	Jørn Olav Løkken	PhD Biology	Change in vegetation composition and growth in the forest-tundra ecotone – effects of climate warming and herbivory
2020	Kristin Odden Nystuen	PhD Biology	Drivers of plant recruitment in alpine vegetation
2021	Sam Perrin	PhD Biology	Freshwater Fish Community Responses to Climate Change and Invasive Species
2021	Lara Veylit	PhD Biology	Causes and consequences of body growth variation in hunted wild boar populations
2021	Semona Issa	PhD Biology	Combined effects of environmental variation and pollution on zooplankton life history and population dynamics
2021	Monica Shilereyo	PhD Biology	Small Mammal Population Ecology and Ectoparasite Load: Assessing Impacts of Land Use and Rainfall Seasonality in the Serengeti Ecosystem, Tanzania
2021	Vanessa Bieker	PhD Biology	Using historical herbarium specimens to elucidate the evolutionary genomics of plant invasion
2021	Håkon Austad Langberg	PhD Biology	Fate and transport of forever chemicals in the aquatic environment: Partitioning and biotransformation of mixtures of Per- and Polyfluoroalkyl Substances (PFAS) from different point sources and resulting concentrations in biota
2021	Julie Renberg	PhD Biology	Muscular and metabolic load and manual function when working in the cold
2021	Olena Meleshko	PhD Biology	Gene flow and genome evolution on peatmosses (S*phagnum*)
2021	Essa Ahsan Khan	PhD Biology	Systems toxicology approach for evaluating the effects of contaminants on fish ovarian development and reproductive endocrine physiology:A combination of field-, in vivo and ex vivo studies using Atlantic cod (*Gadus morhua*)
2021	Tanja Kofod Petersen	PhD Biology	Biodiversity dynamics in urban areas under changing land-uses
2021	Katariina Vuorinen	PhD Biology	When do ungulates override the climate? Defining the interplay of two key drivers of northern vegetation dynamics
2021	Archana Golla	PhD Biology	Impact of early life stress on behaviour and dorsal raphe serotonergic activity in zebrafish (*Danio rerio*)
2021	Aksel Alstad Mogstad	PhD Biology	Underwater Hyperspectral Imaging as a Tool for Benthic Habitat Mapping
2021	Randi Grønnstad	PhD Biology	Per- and polyfluoroalkyl substances (PFAS) in ski products: Environmental contamination, bioaccumulation and effects in rodents

2021	Gaspard Philis	PhD Biology	Life cycle assessment of sea lice treatments in Norwegian net pens with emphasis on the environmental tradeoffs of salmon aquaculture production systems
2021	Christoffer Høyvik Hilde	PhD Biology	Demographic buffering of vital rates in age-structured populations”
2021	Halldis Ringvold	Dr.Philos	Studies on Echinodermata from the NE Atlantic Ocean - Spatial distribution and abundance of Asteroidea, including taxonomic and molecular studies on Crossaster and Henricia genera- Value-chain results, including test fishery, biology, market and nutritional analysis, on Parastichopus tremulus (Holothuroidea) from the Norwegian coast
2021	Elise Skottene	PhD Biology	Lipid metabolism and diapause timing in Calanus copepods. The impact of predation risk, food availability and oil exposure
2021	Michael Le Pepke	PhD Biology	The ecological and evolutionary role of telomere length in house sparrows
2022	Niklas Erik Johansson	Dr. Philos	On the taxonomy of Northern European Darwin wasps (Hymenoptera: Ichneumonidae).
2022	Jonatan Fredricson Marquez	PhD Biology	Understanding spatial and interspecific processes affecting population dynamics in a marine ecosystem.
2022	Anne Mehlhoop	PhD Biology	Evaluating mitigation measures to reduce negative impacts of infrastructure construction on vegetation and wildlife.
2022	Malene Østreng Nygård	PhD Biology	Integrative biosystematics and conservation genomics – holistic studies of two red-listed plants in Norway
2022	Martin René Ellegaard	PhD Biology	Human Population Genomics in Northern Europe in the Past 2000 years

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