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# Oikuben Student Journal

The aim of this student-driven journal is to provide an outlet for peer-reviewed, quality work made by students at the Department of Biological Sciences (BIO) – as part of courses, theses, or projects. Students develop a range of interesting scientific reports, texts and products during their bachelor and master studies, and most of it is never accessible to others than the teacher and the student. Here we want to make the best of these works available to a broad audience.

A publication in Bikuben student journal allows you to showcase your best work for potential employers, while exercising academic writing and the process of publishing in scientific, peer-reviewed journals. Any publication here is a valuable bullet point for your CV. This is an opportunity to show something concrete to your future employers and help you to stand out as a strong applicant.

Here is a good place to get feedback, polish and publish assignments you have already invested much time into. You can also write about something you care about, independent from course-assignments at BIO.

The journal also aims to be a source of learning for the readers. If you are unsure how to approach an upcoming assignment, some of the contributions here can serve as an inspiration for your work and guideline for how to write a given genre.

Bikuben student-journal publishes a wide array of texts and products by students at BIO – see ideas for genres and criteria on our webpage. The theme should be relevant to science or education taking place at BIO. All contributions are evaluated by the editors. Suitable contributions will be peer-reviewed by other students and the editorial board before publication.

## Table of contents

Table of contents	4
The editors' corner	5
Review	6
Let's talk about sex: Exploring evolutionary explanations for the	7
persistence of sexual reproduction in eukaryotes	
Essay	14
The Insect Apocalypse: a cause for concern or simply an exaggeration?	15
Reports	19
Particulate element composition and phytoplankton biomass in	21
Puddefjorden and Store Lungegårdsvannet during spring 2021	
Can we use fish scales to identify spring and autumn spawning	31
herring (Clupea harengus)?	
The habitat uses and behaviour of Black Grouse (Lyrurus tetrix)	39
in western Norwegian heathlands, in winter	
Naturen i Bergen lagrer mer karbon enn det nasjonale gjennomsnittet	53
Opinion piece	62
Decoupling Our Future from Our Past: Reflections on Sustainable	63
Development	
About the authors	66
Contribute to Bikuben	70
Guidelines for authors	71

## The editors' corner

Dear Reader,

We are beyond proud to present the very first volume of Bikuben Student Journal. Bikuben is the newly developed scientific journal for all students at the Department of Biological Sciences, University of Bergen. The goal of this journal is to provide students with an outlet for their research and the work they create during their studies. Students produce work with great academic potential, but most of it never make it to an audience beyond their professors. Therefore, this journal gives students the opportunity to share their work to a broader audience, while gaining valuable experience from academic publishing at the same time.

The creation of this journal has been a work in progress for over a year, and an extensive amount of work has gone into its production. As a newly established journal, we have encountered many challenges along the way. The recruitment of authors and peer-reviewers was more demanding than what we anticipated, and we were faced with making difficult editorial decisions. However, with the great guidance and support of the bioCEED team we were able to overcome all our challenges and bring our visions to life.

Bikuben Student Journal would not have been possible without the combined effort of the editorial team, authors, peer reviewers, faculty advisors, designers and photographers. We would like to express our deepest gratitude to all students that have contributed to the journal, despite having their own exams and deadlines to keep.

As this is only the first edition, we aim to make this journal a valuable part of the institute. We want to continue publishing through our web page as new texts are ready, and to have summary editions, like the one you are currently reading, published on an annual basis. BioCEED always aims to spread their ideas to other institutes and faculties, and hopefully Bikuben can include other fields at UIB in the future, as we have already received some interest from other institutes.

We, the current editorial board, are now handing the journal over to a new group of editors. We are excited to follow the development of Bikuben in the years to come.



Jørund Johansen MSc. Alumni

> Lars Martin Myhre MSc. Student

> > Tonje Olsen Totland MSc. Alumni



Honeybee (Apis mellifera) in plum flower (Prunus domestica) Photo: Silje Maria Midthjell Høydal

## Let's talk about sex: Exploring evolutionary explanations for the persistence of sexual reproduction in eukaryotes

Mads S. Michalsen

#### 1. Introduction

Why sex? Perhaps the simplest, yet deepest, question about sexual reproduction is why it exists at all. The answer seems obvious at first, to produce offspring. On second thought however, the question becomes far more intriguing. Because, as August Weismann said all the way back in 1889: "The significance of amphymixis (i.e. sex) cannot be that of making multiplication possible, for multiplication may be effected without amphimixis in the most diverse ways" (Weismann, 1889). In other words, sex is not the only option for reproduction. In eukaryotes, asexual modes of reproduction include budding, vegetative reproduction and parthenogenesis (Futuyma and Kirkpatrick, 2017). Furthermore, asexual modes of reproduction have advantages that should make them far more favorable than sex.

For one, asexual reproduction avoids the costs that come with finding a mate, including costs associated with sexual selection dynamics. The dangers of contracting sexually transmitted diseases and other risks connected to the act of mating are other costs not incurred. Additionally, sexual reproduction systematically breaks down successful genetic combinations through recombination and outcrossing. By far the most significant cost to sex is however what John Maynard-Smith coined the two-fold cost of males. Maynard-Smith (1971) argued that mutations that cause an organism to reproduce asexually should rapidly dominate if it arrived in a (dioecious) sexual population. Because asexual females do not invest in sons, the reasoning is that their birth rate should far exceed that of their sexual counterparts. Holding all else equal between sexual and asexual females, the cost of sex is expected to be twofold in outcrossing populations with separate sexes and equal sex ratios (Gibson et al., 2017). The two-fold cost also manifests itself in gene numbers. Each gamete only passes on 50 percent of its parent's genes<sup>1</sup>. If an individual were to attain a way to pass 100 percent of its genes to the next generation, e.g. by cloning, it would gain a two-fold advantage over the other individuals in the population. Its genes should soon dominate the population.

Considering all these costs, sex as a successful mode of reproduction seems paradoxical. It suffers a two-fold cost compared to asexual reproduction, it comes with the burden of finding and attracting a mate, it can transmit venereal diseases and it systematically breaks down successful genetic combinations.

Despite all of this, sex is ubiquitous among complex life forms. Almost all eukaryotes reproduce sexually at some stage of their life cycle, and many are obligate sexual reproducers. Only about 1 percent of plants and 0.1 percent of animal species reproduce asexually, i.e. by making genetic clones of themselves (Otto, 2009). Furthermore, most species with obligate asexual reproduction are recently formed, implying that fully asexually reproducing organisms generally have a short species-level life span in evolutionary time<sup>2</sup>. The fact that most eukaryotes reproduce sexually is evidence of its evolutionary success, which entails that there must be major advantages to this form of reproduction. But what are they? Essentially, why does sex exist?

The question of why sex exists comprises two related, yet separate, topics: the origin of sex and its persistence through evolutionary time<sup>3</sup>. This paper limits itself to the latter, reviewing some of the different hypotheses put forth for the evolutionary advan-

3 For a discussion on the origin of sex, see e.g. Cavalier-Smith (2002).

<sup>1</sup> Interestingly, this opens the door for yet another cost to sex; the propagation of selfish genetic parasites (see e.g. Lane (2010)).

<sup>2</sup> A notable exemption from this are bdelloid rotifers, as this group of planctonic organisms have maintained an asexual reproductive mode for more than 322 million years (Fontaneto et al., 2007).

tages of sexual reproduction, in an attempt to make sense of its enduring prevalence despite its great costs.

netics by Fisher (1930) and Muller (1932), among other things incorporating the role of mutations in a clearer way.

#### 2. Discussion

Initially, some quick definitions are in order. Reproduction is the process by which organisms give rise to offspring. Sexual reproduction can be defined as when a new organism is produced by the mixing of the genomes of two organisms of different types (parents) (Reece et al., 2014). Both parents produce gametes through meiosis, during which the chromosomes undergo recombination where genetic information is exchanged between the homologous chromosomes. Through this process every individual gamete produced by an organism are genetically unique to each other. Through outcrossing and the fusion of sex cells, 50 percent of the genetic material from each parent is combined in a zygote. This results in offspring that are genetically different from each other and from their parents. In short, sex is recombination and outcrossing, and the mixture of genes can be considered its primary feature. It does so systematically across the entire genome, breaking down previous combinations and generating new ones. But why?

#### 2.1 The Vicar of Bray and Selective Interference

An early answer to the question of why sex persists dates back to the 19th century and the previously mentioned August Weismann. He suggested that the key benefit of sex is that it generates greater variation for natural selection to act upon, claiming it as "the source of individual variability, furnishing material for the operation of natural selection" (Weismann, 1889). Weismann further argued that sex was just as likely to generate detrimental as beneficial combination of genes, meaning that there is no net advantage to sex for the individuals in any given generation. The population as a whole, however, does benefit, as detrimental combinations are weeded out of the population by means of natural selection, leaving the favorable combinations. Bell (1982) later dubbed Weismann's view on the evolutionary purpose of sex the Vicar of Bray hypothesis. Closely linked to this is the idea that sex reduces selective interference, separating alleles from their genomic backgrounds allowing for more efficient adaptive selection processes (Futuyma and Kirkpatrick, 2017).

It might be important to emphasize that sex does not itself introduce new variation to a population in terms of new genes or alleles. Mutation (or gene flow) is needed for this. Without this, sex only generates new combinations of existing genes, eliminating disadvantageous variants along the way. Without the introduction of new variants, sex actually restricts variation.

After Weismann, a continuation of the Vicar of Bray hypothesis was formulated in terms of population geFigure 1 illustrates the resulting Fischer-Muller model, which demonstrates an advantage to sexual reproduction through the avoidance of clonal interference. Consider the outcome in an asexually reproducing population if advantageous mutations A and B appear (at different loci) in different individuals. Due to the mutations being beneficial, the clones of these individuals proliferate. Becoming common in the population, the two clonal lines start to compete, potentially driving one of the lin-



**Figure 1.** The spread of advantageous mutations in a sexual (top) and an asexual population (bottom). In the sexually reproducing population, the advantageous mutations (A and B) are quickly combined and the AB genotype brought to fixation due to recombination and selection. Without sex however, A can only spread at the expense of B and vice versa, unless both mutations arise in the same clonal lineage.

eages to extinction (in this case aB). The only way for an AB genotype to get fixed in the population, is for both mutations to independently arise in the same clonal lineage, an unlikely event. In short, clonal interference is a hindrance to adaptive evolution in the population.

For a sexually reproducing population on the other hand, novel advantageous genotypes can be created more rapidly. If allele A and B arise independently in different individuals, the two alleles can rapidly be recombined in the same organism. Sexual reproduction allows the beneficial AB genotype to quickly spread to fixation.

Crow and Kimura (1965) later provided a modern demonstration of the Fischer-Muller model. Through mathematical formulations they demonstrated the relative rates of incorporation of the new mutations with and without recombination. They found that recombination is of the greatest advantage when the double mutant is more advantageous than either single mutant, when the mutant effects are small, when mutations occur with high frequency, and when the population is large. The Fisher-Muller model presents an argument for how sex can be beneficial through eliminating competition among favorable mutations that have arisen in different genetic backgrounds, instead bringing these together through recombination, speeding up adaptation. Later, Muller shifted focus from favorable mutations to deleterious ones, perhaps inspired by the fact that most non-neutral mutations are in fact deleterious (Loewe and Hill, 2010).

Muller (1964) introduced the concept later dubbed Muller's ratchet, a process in which, in the absence of recombination, deleterious mutations accumulate in a population in an irreversible manner. Without recombination, assuming that back-mutations are rare, Muller argues that offspring will at minimum carry the same mutational load as their progenitors. He proposed this mechanism as a further reason for why sex might win out over asexual reproduction, as sexually reproducing organisms avoid the ratchet due to sex's ability to recreate high-fitness individuals by bringing together unmutated alleles in the same individual. Experiments have shown that ratchet is fastest, and thus the advantage of sex the largest, when mutation rates are high, selection is weak, the organisms (genomes) are large and the size of the population is small (Bell and Graham, 1988).

Kondrashov (1988) complements the work of Muller by introducing the role of synergistic epistasis. This is the idea that most mutations are only slightly deleterious individually but that the cumulative effect of mutations has an increasingly large effect on fitness as the total number of mutations increases (see Figure 2). In a sexual population, some of the individuals born will have many mutations, while some will have few. Since there is a major selective disadvantage to individuals with more mutations, these individuals die out. Sex compartmentalizes the deleterious mutations, and rids the population of them.

The ideas presented so far seem to argue that sex persists because it benefits populations by bringing advantageous combinations of genes together, and by eliminating deleterious ones. Mystery solved? Many would argue no. One reason for this is that major parts of the Vicar of Bray thinking can essentially be deemed group selectionist, due to many of the benefits put forth mostly accruing to the group, and not the individual (at least not in any immediate sense). Given that there is a strong consensus that natural selection works on the level of the individual (or even the gene), and not on the species or group level, this poses a problem. Natural selection is not going to promote genes that benefit the species but hurt the individual, because such genes will be outcompeted by non-self-sacrificial variants (Maynard-Smith, 1978). Furthermore, Muller's ratchet, the Fischer-Muller model, and other forms of reducing selective interference are slow processes. Their benefits do not seem to counter the large costs of



**Figure 2.** Illustration of different relationships between the number of mutations and fitness. The red line represents synergistic epistasis, where each subsequent mutation has a disproportionately large effect on the organism's fitness. This is a requirement for Kondrashov's model. The other two lines illustrate additive (black) and antagonistic (blue) effects of the number of mutations on fitness (Kondrashov, 1988). Original illustration by MyvReeve, licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

sexual reproduction in the short term (Ridley, 1994). Convincing arguments for how sexual individuals can be better off than non-sexual individuals are therefore needed.

#### 2.2 The Ecological Perspective

In the attempt to answer how sex sufficiently benefits the individual despite its two-fold cost, many biologists shifted focus towards ecological explanations. Williams (1975) argued that the genetic mixing inherent to sex, might prove beneficial when the environment is changing rapidly. If the environment changes from generation to generation, the genotype advantageous in one generation might not be advantageous in the next. The much greater diversity of sexually produced offspring compared to asexual ones, could therefore be a good strategy. However, if sex' purpose mainly were as an adaptation to highly variable environments, one would predict sex to be more prevalent in highly fluctuating environments such as high latitudes and altitudes. Generally speaking, this is not what is found. In fact, data suggest sex is actually more common in stable environments such as lakes and tropical rainforests (Bell, 1982). Williams' theory is not without value, but it seems to fall short as a single explanation for the persistence of sex.

Another ecological argument for sex is the Tangled Bank hypothesis. The Tangled Bank holds that since the state of the environment varies spatially even at fairly local levels, different genotypes will be optimal at different locations. Given that each location will only support a limited number of individuals, clonal offspring from an asexual parent will compete intensely with one another for the same set of resources. The offspring of a sexual female, however, by virtue of their greater diversity, will be able to exploit a much wider range of sites as well as different niches in the same location. This might lead to sexual lineages competing less amongst themselves and thus to greater overall success compared to asexual lineages despite its lower reproductive efficiency (Bell, 1982). An elegant idea, but empirical support for the Tangled Bank hypothesis as the driving cause for the persistence of sex is not very strong (Lane, 2010).

Lastly, a promising explanation of how sex can be favored by natural selection is referred to as the Red Queen hypothesis. This ecological hypothesis is named after a character in Lewis Carroll's Through the Looking Glass, who must "do all the running [she can do] in order to stay in the same place". Whilst the Red Queen term is used by biologist as a general reference to perpetual arms races between different species, it is perhaps most strongly associated with the evolution of sex<sup>4</sup>.

The hypothesis states that species are in evolutionary arms races with other species, and particularly parasites rapidly evolving to circumvent its hosts' defenses. This produces a continually shifting adaptive landscape for the host organism, and in this context, sex can be a winning strategy. Recombination allows for the continual arrival of new combinations of alleles to which the parasite is not well-adapted. In the context of arms races, sexual reproduction and its mixing of genes can prove more advantageous than asexual reproduction and its slower production of novelty. Support for this can be found from the observation of parasite co-evolution in populations of mud snails (Potamopyrgus antipodarum), which have both sexual and asexual genotypes. Studying these populations, it was found that the populations with higher exposure to parasites shifted towards higher frequencies of the sexual genotype (Vergara et al., 2014). These studies, and others, suggest that the evolutionary benefits of the genetic mixing produced by sex can compensate for its costs.

The Red Queen hypothesis provides a strong argument for the primary purpose of sex being that of keeping parasites at bay. There are however doubts as to whether the threat of parasites is a strong enough force to explain the powerful pervasiveness of sex. For example, Howard and Lively (1994) used computer simulations to (among other things) study the role of parasites in explaining the rarity of obligate parthenogenesis in natural populations. They found that their models required that parasite transmission rates were very high, and that parasites had severe fitness effects on their hosts (>80% loss of fitness),

#### 3. Synthesis

Many promising theories have been put forth in the quest to resolve the enigma of sex. It seems, however, that no single theory can provide strong enough explanatory power to alone account for the incredible persistence of sex. That being said, there is actually no reason why one theory alone should have to explain the evolutionary success of sexual reproduction. The theories reviewed in this paper are not mutually exclusive, so perhaps the explanation for sex lies in some combination of these ideas<sup>5</sup>.

Considering Muller's ratchet and the Red Queen together, for example, does in fact provide a far stronger case for the advantage of sex over asex than if each factor is considered individually (Howard and Lively, 1994). Furthermore, more recent mathematical modelling work, such as that of Otto and Barton (1997) and Keightley and Otto (2006), have provided highly promising results through combining even more of the ideas discussed in this review. Through incorporating different mechanisms of selective interference in finite populations with the presence of strong selective forces such as parasites or rapidly changing environments into the same model, they are able to demonstrate that sex can immediately benefit the individual and perform better than cloning in a wide range of circumstances.

To conclude, sex reduces selective interference, bringing together favorable genetic combinations in the same individual and getting rid of deleterious mutations, and it arms the organism with the weapon of variability in the face of dynamic, adaptive landscapes. Sexual reproduction undeniably comes with great costs, but through a mixture of factors reminiscent of the process of sex itself, it prevails.

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in order for sex to gain a decisive advantage over parthenogenesis. Conditions like these are unquestionably present in a number of natural systems, but in no way all.

<sup>5</sup> Along with others not covered. See e.g. Bernstein et al. (1987) for an interesting argument for sex' primary value as a tool for DNA repair.

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Trout (S*almo trutta*) Photo: Vebjørn Opsanger







## Madagskaruranide Chrysiridia rhipheus

## The Insect Apocalypse: a cause for concern or simply an exaggeration?

Susanne Zazzera

Insects, being the largest and most diverse group of organisms, play a vital role in ecosystem functioning. They are key components in pollination, decomposition of organic matter, nutrient cycling as well as suppression of pests and diseases, making them of high economic value (Noriega et al., 2018). This illustrates just how critical insects are for human welfare and a decrease in insect populations is expected to have great consequences. A significant loss of insect abundance and diversity would also have cascading effects across trophic levels and influence surrounding ecosystems (Forister et al., 2019). However, understanding exactly in which ways insect populations have changed recently may be more complicated than previously assumed.

Hallmann et al. (2017) found a steep decline of flying insect biomass in Germany. They suggested an estimated 75% decline over a 27-year period. The study pointed to climate change, habitat loss and fragmentation as well as the use of agricultural insecticides as potential drivers (Hallmann et al., 2017). This has since become a high-profile case study and is often used as a reference in news articles concerning changes in insect populations. Studies focused on individual species have also shown similar trends, with one reporting a sharp decline of the monarch butterfly (Danaus plexippus) in North America (Thogmartin et al., 2017). In the UK, 52% of butterfly species have declined in abundance since 1976 and in western US, the predicted mean occupancy of one bumble bee species (Bombus occidentalis) declined by 93% over 21 years (Fox et al., 2015; Graves et al., 2020).

These trends are alarming, and the media started sharing the news of an "insect apocalypse". In one of their articles, The Guardian announced that "Insects could vanish within a century at current rates of decline" and more recently stated "Our world will grind to a halt without them" (Carrington, 2019; Goulson, 2021). The New York Times Magazine also reported that "The Insects Apocalypse Is Here", further strengthening their message with an illustration of an exploding wasp (New York Times, 2018). ABC news used a slightly different term, calling it "Insect Armageddon" and declared that "If they go, we go" (Campbell, 2019). However, the notion of an insect apocalypse has met some resistance.

#### Populations appear stable in the US

Most of the evidence for a steep decline in insect abundance come from Europe, where humans have managed landscapes for centuries and human population densities are high. This type of research has been scarce in the US, which is why Crossley et al. (2020) researched how insect populations had changed for sites with varying degrees of human disturbance in the US. They conducted a meta-analysis consisting of 82,777 arthropod observations from 68 datasets spanning up to 36 years (Crossley et al. 2020). Here, they utilized these long-term datasets to search for evidence of insect declines. The data was collected using a variety of methods, but these methods were consistent over time based on taxa within each dataset. Types of data include grasshopper per sweep in Kansas, ground arthropods per pitfall trap in New Mexico and aphids per suction trap in Midwestern US (Crossley et al. (2020)). The research areas used in the study was Long-Term Ecological Research sites (LTERs), initiated by the US National Science Foundation. Of the 25 existing LTERs, 12 were used in their analysis (Fig. 1). The study sites were chosen to cover different habitats and with a wide range of human influence from urban or farmed regions to relatively undisturbed areas (Crossley et al. 2020).

A rather surprising trend of stable insect population size was found, challenging the current view of an ongoing insect apocalypse. Crossley et al. (2020) found that some arthropod taxa at some sites had a decline in abundance, while in other sites most taxa were stable or increased in abundance. In most of the datasets, the median abundance change was modest, lying within 1.6 standard deviation of zero net difference (Crossley et al., 2020). No specific cause could be linked to any of the declines or increases detected. The results were similar when separating taxa into aquatic vs. terrestrial or when grouped based on feeding guilds. No trend was found even when comparing sites heavily altered by humans with more natural sites (Crossley et al. 2020). The seemingly robustness of insects and other arthropods in the US is comforting. Still, it begs the question of how previous research can differ so much from these findings. Despite the US study finding no net abundance and diversity declines, the stories of an insect apocalypse continue to develop. Publication bias suggests that studies with more significant findings are more likely to be published (Dickersin, 1990). Readers are more likely to be interested in papers with dramatic results, like populations declining rapidly, rather than results showing no changes at all. Therefore, scientists may execute studies that have the desire to find evidence supporting novel results. Chances are, if you go looking for species in decline, you will find them.



**Figure 1 – Map of LTERs.** Larger black circles show the Long-Term-Ecological Research sites (LTERs) included in the meta-analysis (Crossley et al. 2020). Colors indicate different ecoregions defined by climate, precipitation, temperature, and vegetation by the United States Department of Agriculture Forest Service (USDA, n.d.).

One possible explanation for this could be location. Most of the research showing declines comes from Europe, here, human populations are dense. As a result, much of the natural landscape has been altered. Habitat loss and fragmentation is known to be a major threat to biodiversity and insect populations would be expected to suffer accordingly (Forister et al., 2019; Hanski, 2011). Second, the popular study from Germany used only one capturing method – malaise traps, thereby limiting their samples to flying insects. Several methods should be used to conclude any trends in insect abundance and diversity. Some insect populations may also fluctuate naturally through genetic drift (Davies, 1988). Distinguishing these from recent, human driven declines could be challenging.

#### The future for insect populations

The reassuring results from the North American study show that insect populations are more stable and robust in the US, than previously suggested. It is therefore tempting to assume that insects are resilient enough to withstand any changes made by humans. However, this is a naïve and potentially dangerous way of thinking. While they found no clear indication of widespread insect declines in the US, this does not rule out the possibility of any subtle changes in species composition or local extinctions (Krno et al., 2018). There is evidence that some species are in decline, as previously noted with the monarch butterfly and Bombus occidentalis (Fox et al., 2015; Thogmartin et al., 2017). Changes in species composition, especially when keystone species are involved, could hurt ecosystems, and influence the services they provide (Cardoso et al., 2020; Jordan, 2009). There is also a possibility of more recent declines, but at a subtle rate not yet being detected.

As we have seen, changes in insect populations are a complex issue. The discoveries from these studies underpin the need for further research and monitoring. Almost no quantitative data is available in the tropics where rates of deforestation are high (Wagner, 2019). Many species of insects are yet to be described, with some estimates suggesting that 80-90% of terrestrial arthropods are undescribed (Stork, 2018). As a result, declines and extinctions may be outpacing our effort to collect data (Wagner, 2019). In the future we will need more researchers collecting material at sites all over the world, as well as more awareness of how our actions influence ecosystems. Although some changes in insect abundance and diversity may be occurring, the results from Crossley et al. (2020) suggests a future not as bleak as the media have made it seem. Insects have been around for 479 million years, surviving several mass extinctions, so a complete disappearance any time soon is unlikely (Misof et al., 2014). Still, the disappearance of some species might bring cascading effects, accelerating the extinction of other species (Kehoe et al., 2021). In conclusion, an insect apocalypse is certainly a cause for concern, although the scope and rate of insect declines may be exaggerated by the media.

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Garter snake (*Thamnophis sp.*) Photo: Maren Halvorsen

## Reports



## Spekkhogger Orcinus orca

### Particulate element composition and phytoplankton biomass in Puddefjorden and Store Lungegårdsvannet during spring 2021

Iliana-Vasiliki Ntinou

#### Abstract

Heavy metal pollution poses a severe threat to aquatic environments, especially in urban coastal areas of high value. This study investigated the elemental composition of particulate matter and the possible impact of heavy metals on phytoplankton biomass in Puddefjorden(PUD) and Store Lungegårdsvannet(SLV), Bergen during spring 2021. Concentrations of C, N, P, Ca, Si, Mg, K, S, Fe, Mn, Cu, Hg, Pb, and As were measured using wavelength dispersive X-ray fluorescence spectroscopy combined with documenting chlorophyll-a concentration. The results revealed that a phytoplankton bloom took place on the 18<sup>th</sup> of March, coinciding with Si values marking a 42% (PUD) & 50% (SLV) increase, indicating that diatoms dominated the phytoplankton community. Mn, Cu, Hg, Pb, and As were relatively low and did not fluctuate with changes in the phytoplankton biomass, suggesting that these elements did not affect the phytoplankton biomass during the study period. Overall, this study contributes to further understanding of the complex abiotic (particulate chemical elements) – biotic (phytoplankton) relationships in the coastal ecosystem of Bergen.

#### 1. Introduction

Phytoplankton are a driving force of global biogeochemical cycles and play an essential role in the C, P, N, Si cycles in the food webs of aquatic ecosystems (Munn, 2019). Phytoplankton cells are capable of scavenging metal ions from aqueous solutions because of their characteristic rapid growth rate and high surface-to-volume ratio (González-Dávila, 1995; Mehta & Gaur, 2005). They can influence the residual concentration of metal ions by binding them to the cell surface with strong ligands and transporting them intracellularly (Jardim, 1984). Changes in elemental ratios may create unfavorable conditions for phytoplankton growth which in turn may disrupt the marine ecosystem's natural processes. Some ecological effects of the distribution of heavy metals summarized by Brand et al. (1983) include reduced reproductive rates of some phytoplankton species thereby putting a selective pressure on the phytoplankton species populations.

To understand essential ecosystem dynamics like nutrient cycling and food web interactions, it is crucial to broaden our knowledge on how the relationship between abiotic and biotic factors can impact elemental stoichiometry and vice versa (Erga et al., 2017; Ho et al., 2003; Larsen et al., 2004; Paulino et al., 2013; Price & Skei, 1975). For many decades the Redfield ratio of C:N:P (106:16:1) in phytoplankton biomass and dissolved nutrients has been the holy grail of describing stoichiometric relations of photosynthesis and remineralization (Redfield, 1934). Recent findings, however, show that the ratios of additional chemical elements can reveal information about how phytoplankton biomass is affected by element stoichiometry. The presence of these elements may vary depending on the organisms, the source of detritus or the amount of dissolved organic matter present in the environment, among other things (Arrigo, 2005; Erga et al., 2005; Erga et al., 2017; Erga et al., 2012; Geider & La Roche, 2002; Klausmeier et al., 2004; Larsen et al., 2004; Paulino et al., 2013). For that reason, investigating the elemental composition of suspended particles may unravel a great deal of information about the underlying causes and mechanisms of phytoplankton-elements interactions.

Urban pollution can contribute severely to the input of chemical elements in coastal systems, including essential elements like C, N, and P as well as harmful toxic heavy metals like Hg, Cu, Pb (Andersson & Eggen, 2015; Andersson et al., 2014). More information about the availability of elements is needed to understand the impact of their uptake by phytoplankton. In this study, the particulate elemental concentrations of C, N, P, Ca, Si, Mg, K, S, Fe, Mn, Cu, Hg, Pb, and As were determined through wavelength dispersive X-ray fluorescence spectroscopy in combination with documenting chlorophyll-a concentration in Puddefjorden (PUD) and Store Lungegårdsvannet (SLV), located in Bergen. These areas are excellent study subjects as they are a pollution recipient from human activity (Andersson & Eggen, 2015). The findings presented here contributes to the understanding of the complex abiotic (particulate chemical elements) and biotic (phytoplankton) relationships in the coastal ecosystem of Bergen.

#### 2. Study site

PUD is located in the northern part of the Byfjorden water body, which is 3.5 kilometers long, and stretches from the tip of the Nordnes peninsula to the SLVbay (Figure 1). The north-eastern part of PUD is characterized by intense port activity with large commercial vessels, while the inner part is characterized by smaller boats and marinas. Additionally, metal pipelines extend underwater across the seabed. In 2015 a risk assessment of the Puddefjorden seabed concluded that there are concerning levels of mercury pollution associated with the accumulation of pollutants in marine biota. This can pose a severe threat to human health mainly by uptake through seafood consumption (Bergen\_Kommune, 2020). In 2018, in the inner part of PUD, a new seabed was overlayed with 45 cm of pure tunnel boring machine masses. Covering the contaminated seabed will, in the long run, act as a physical barrier and

will insulate the underlying contaminated sediment. This action is part of the project Cleaner Harbor Bergen which aims to alleviate the urban coastal sites of Bergen Harbor from chronic pollution by organic pollutants and heavy metals so as to limit the content of environmental toxins in fish and seafood from Byfjorden as well as make the area safe for leisure activities (Bergen\_Kommune, 2020).

SLV(Figure 1) is also one of the sub-areas in the Cleaner Harbor Bergen project, where it is planned to implement measures to monitor the seabed heavy metal pollution in 2022. It has a maximum depth of 26 m and is situated at the southeast end of PUD. The site has a long history as an industrial area, including milling in the Middle Ages, rapid urban and industrial development along the shore, increased shipping activity (Andersson et al., 2014), road traffic, old paint, and other façade materials (Bergen\_Kommune, 2020). The water mass of SLV has been dramatically modified over the last 150 years due to continuous land fillings associated with rapid urban development, with the most remarkable one in 1926, when the strait between Lille Lungegårdsvann and SLV was completely filled (Andersson et al., 2014). Today, the connection between SLV and PUD is approximately 3.7 m deep. There is a daily water mass exchange between PUD and SLV during the high and the low tides. As a result of the shallow connection, water exchange is limited, and oxygen in the deepest parts of SLV is depleted (Bergen\_Kommune, 2020). The Møllendal River originating from the Svartediket reservoir is SLV's primary freshwater source. The flow is generally low but can increase significantly after heavy rainfall (Paetzel & Schrader, 2003) which enhances the input of natural minerals in the water.



**Figure 1.** Overview of the study area with the two stations marked on the map, one in front of the Marineholmen Forskningspark in PUD (total depth 5 m) and one in the Møllendal swimming platform in SLV (total depth 11 m).

#### 3. Materials and methods

#### 3.1. Sampling and laboratory analysis

This study was carried out weekly in SLV and PUD from the 26<sup>th</sup> of February to the 28<sup>th</sup> of April 2021. Five liters of water samples were collected from each station using a Niskin water sampler at 5 m depth in SLV and 3 m depth in PUD (Figure 1). The water samples were analyzed to measure the chlorophyll a concentration and the particulate elemental composition.

The salinity was recorded using a Xindacheng ATC handheld refractometer. Phytoplankton was identified using a Zeiss Supra 55VP scanning electron microscope after filtering 250 ml of sample material on 1 µm pore size PC filters (25 mm diameter). The chlorophyll a concentration was determined according to Parsons et al. (1984) and Holm-Hansen and Riemann (1978). For each station, three replicates were obtained by filtering 250 ml of sample water onto 0.2 µm Nucleopore polycarbonate filters (PC) (47 mm diameter) using moderate vacuum pressure (≤200 mmHg). The day before the analysis, 10 ml of 100% methanol were added to the samples for chlorophyll-a extraction (for 15-24 hours at ~4°C). The fluorescence was measured with a Turner Designs Fluorometer 10-AU. The whole process took place away from external light sources as far as it was possible. The concentration of chlorophyll-a was calculated according to Equation 1.

**Equation 1.** Formula for calculation of chlorophyll-a concentration (Holm-Hansen and Riemann 1978; Parsons et al. 1984)

 $Chla = F_d \times \tau \times (R_b - R_a) \times \frac{methanol \, volume}{filtration \, volume}$ Where: Chla: chlorophyll a concentration measured in mg/m<sup>3</sup>  $F_d$ : acid factor equal to 2.27  $\tau: \frac{(R_b - R_a)}{(R_b - R_a) - 1} = 1.62$  $R_b$ : fluorescence value prior to 10% HCl addition  $R_a$ : fluorescence value after 10% HCl addition methanol volume: 10 ml of 100% methanol filtration volume: 250 ml from the sample

Water samples for total particulate element analysis were filtered in triplicate (n=3) on 0.6  $\mu$ m pore size polycarbonate (PC) filters (47 mm diameter), and Whatman glass fiber (GF) filters (57 mm diameter), using  $\leq$ 200 mmHg vacuum pressure. After filtration, the filters were rinsed with 5 ml of distilled water to prevent interference from salt crystals and left to air-dry until analysis. Total particulate element concentration was measured by wavelength dispersive X-Ray fluorescence spectroscopy (WDX-RF) in a Bruker AXE S4 pioneer XRF instrument, based on the method described by Paulino et al. (2013). Data for S, Na, Mg, Cl, K, Ca, Si, Al, Fe, Cu, and Mn were collected from polycarbonate filters, while data on C, N, and P were taken on three dates (10<sup>th</sup> of March, 31<sup>st</sup> of March and 21st of April) from GF filters to avoid interference from the filter's chemical composition. The concentration of all the above-mentioned chemical elements was calculated by Equation 2 and based on the calibration parameters and detection limits explained in Paulino et al. (2013). For Hg, As, and Pb, information about their relative concentrations were recorded manually by looking at the intensity counts (kilo counts per second - kcps) of the K electron shell for Hg and As and the L electron shell for Pb. The criteria chosen for the analysis was the Highest Resolution (HR) which selects the spectral line that favors the best resolution at the expense of intensity. For both methods, at least three blank filters were analyzed for reference. The instrument measures the total amount of an element and does not discern between different chemical forms.

**Equation 2.** Formula and parameters used for the calculation of the particulate element concentration with WDXRF spectroscopy (Paulino et al. 2013).



#### 3.2. Statistical analysis

A two-sample t-test was applied to compare the mean concentrations of elements between the two sites. Additionally, the results were analyzed for significant changes in the measurements within each site and between sampling dates, using a two-way analysis of variance (two-way ANOVA). The response variables were the measured concentrations of elements and chlorophyll a, and the predictors were the date and station. Whenever the interaction term was not significant, a backward elimination technique was used to remove those features that did not significantly affect the dependent variable (p > 0.05). All statistical analyses and plotting of the data were performed in RStudio using R 4.1.0 (R Core Team, 2017).

#### 4. Results

Throughout the study period, salinity levels varied between 25-33‰. From the beginning of March, there was a slight differentiation between the two sites, with SLV having a higher average salinity of 30 in 5 m depth, except for the 24<sup>th</sup> of March, where salinity reached 33‰ in PUD at 3 m depth. During the second half of the study period, salinity in both areas was on average 27‰.

At the beginning of the study, the chlorophyll-a concen-

tration was 1.05 mg/m<sup>3</sup> and 0.51 mg/m<sup>3</sup> in PUD and SLV, respectively. The maximum chlorophyll a concentrations were measured on the 18<sup>th</sup> of March in both sites (PUD: 16.46 mg/m<sup>3</sup>, SLV: 18.83 mg/m<sup>3</sup>), followed by a dramatic drop on the 24<sup>th</sup> of March (Figure 2). Subsequently, the concentration marked an upward trend until the 21<sup>st</sup> of April. A two-way ANOVA revealed that the variations in chlorophyll a were statistically significant within each area between each date (p < 0.05. A two-sample t-test comparing the total average concentration between the two areas showed no statistically significant difference (p > 0.05).



**Figure 2.** Chlorophyll-a concentration from PUD and SLV during 26<sup>th</sup> of February – 28<sup>th</sup> of April 2021.

Particulate C, N, and P average values were 19.76  $\mu$ M, 2.74  $\mu$ M, 0.35  $\mu$ M in PUD and 22.12  $\mu$ M, 3.69  $\mu$ M, 0.43  $\mu$ M in SLV, respectively. The lowest values in PUD were recorded on the 10<sup>th</sup> of March, while for SLV on the 31<sup>st</sup> of March. Generally, C, N, and P concentrations fluctuated in agreement with each other, with N and P remaining relatively stable. On the 31<sup>st</sup> of March, maximum C:N ratios were recorded in both areas (PUD: 8.88 and SLV: 7.62),

which was higher than the Redfield ratio (6.6). The maximum C:P ratios were measured on the 21<sup>st</sup> of April in both sites (PUD: 59.4 and SLV: 68:85). During the study, average values of C:N, C:P, and N:P ratios were 7.34, 7.3, 53.04 in PUD and 6.32, 8.62, 53.15 in SLV, respectively (Figure 3).

Particulate Ca and Si fluctuated between 0.15-0.95 µM and 0.96–4.57  $\mu$ M, noting a maximum on the 28<sup>th</sup> of April and 18th of March, respectively. Particulate Ca varied significantly only between the sampling dates (p < 0.05), while Si was considerably higher in SLV throughout the study (p < 0.05). The maximum particulate Si concentrations observed on the 18th of March coincided with a bloom of the diatom Skeletonema marinoi. S concentration was generally higher in SLV (p = 0.05) but remained low throughout the study. Fe concentration during the first three weeks and the last two weeks of the investigation varied between 0.19-0.74 mM in both locations but marked a maximum of 3.74 on the 31st of March in SLV. Particulate K and Mg remained at low concentrations and peaked during the second half of the study period but differed significantly between the two areas and between each sampling date (p < 0.05). Maximum K and Mg values reached 0.47  $\mu M$  in SLV on the  $28^{th}$  of April and 0.64  $\mu$ M in PUD on the 31<sup>st</sup> of March, respectively (Figure 4).

The particulate concentration of Cu and Mn 0.002-0.004  $\mu$ M and 0.007-0.281  $\mu$ M (Table 1). However, there was no significant fluctuation found or difference between the areas (p > 0.05), suggesting there was little to no variation in the concentration of these metals.

Statistical analysis of the intensity counts for Hg, Pb, and As, revealed no significant fluctuations throughout the study period (p > 0.05), although the



Figure 3. Elemental ratios C:N, N:P and C:P in PUD and SLV during spring 2021.

Bikuben Student Journal • page 24



Figure 4. Elemental composition of seston from 26th of February to 28th of April 2021 in PUD and SLV.

counts of all elements were higher in SLV (p > 0.05).

#### 5. Discussion

### 5.1. Low Redfield ratios explained by natural processes and anthropogenic P input

In spring 2021, in both study sites, a diatom bloom took place on the 18<sup>th</sup> of March, which also occurred during the annual spring diatom bloom in Raunefjorden, which is typically taking place shortly after the 5<sup>th</sup> of March (Howe et al., 2010; Paulino et al., 2013). Phytoplankton play a major role in the carbon cycle as primary producers (Arrigo, 2005). Other essential micronutrients for cell growth are P and N, which are available through biochemical cycling as well (Arrigo, 2005). Thus, seston concentrations of C, N, and P can be particularly informative about the trophic state of an area when inspected in relation to one another. However, the Redfield atomic ratio interpretation can differentiate depending on the location, the season, and the ecological condition (Geider & La Roche, 2002). For example, Norwegian fjords are typically described as P-limited due to the deficit of phosphorus from freshwater sources (Erga et al., 2017; Erga et al., 2012; Paasche & Erga, 1988; Sakshaug et al., 1983; Thingstad et al., 1993). All planktonic organisms contain C, N, and P, and populations of autotrophic and heterotrophic organisms will contribute to these element pools (Erga et al., 2017).

In this study, the C:N:P ratio for PUD was 53:7:1 and 53:9:1 for SLV. C, N, and P peaked around the time of the diatom bloom, suggesting that the contribution by diatoms to the concentrations of these elements was significant. Erga et al. (2017) attained a C:N:P-ratio of 66:11:1 (C:N = 6.2) on a 5 m depth in Raunefjord, but concluded that this evidence did not support any significant nutritional deficiency for N or P. On the contrary, they found that a surplus of P likely was a response to the extreme negative North Atlantic Oscillation (NAO) in 2009-2010. Additionally, they

Date	Cu (Mean ±SD)		Mn (Mean ±SD)	
	PUD	SLV	PUD	SLV
26-Feb	0.0036 ±0.0003	0.0035 ±0	0.0121 ±0.0009	0.0126 ±0.0016
03-Mar	0.003 ±0.001	0.0024 ±0.0004	0.0082 ±0.0012	0.0119 ±0.0015
10-Mar	0.0025 ±0.0001	0.0033 ±0.0002	0.0079 ±0.001	0.0105 ±0.0007
21-Apr	0.003 ±0.0003	0.003 ±0.0007	0.0082 ±0	0.0108 ±0.0003
28-Apr	0.0026 ±0.0006	0.0049 ±0.0007	0.0117 ±0.0008	0.0281 ±0.0014
Average	0.0033 ±0.0005	0.0035 ±0.0004	0.0094 ±0.0008	0.0143 ±0.0011

Table 1. Particulate concentration of Mn and Cu in PUD and SLV during spring 2021.

concluded that great values of C: N and C:P atomic ratios were linked to haline stratification and high chlorophyll-a concentration, whereas high N:P ratios are linked to water column mixing with low phytoplankton biomass. Atomic N:P ratios of seston matter that divert from the Redfield ratio could also be attributed to the different species that may dominate the phytoplankton community (Klausmeier et al., 2004). Therefore, C:N:P ratios depend highly on the biological, physical, and chemical processes in a certain area.

Another explanation of the low C:N:P in PUD and SLV recorded in this study could be due to a P surplus. In their research, Martiny et al. (2013) stated that elemental ratios followed a latitudinal pattern due to variations in the coupling of C, N, and P cycles along the different latitudes and observed a C:N:P ratio of 78:13:1 in cold, nutrient-rich high-latitude areas. According to the hypothesis by Redfield (1934), N:P ratio below 16 assumes N-limitation (Frigstad et al., 2011; Geider & La Roche, 2002). However, as Geider & La Roche (2002) suggested, according to studies comparing marine phytoplankton elemental composition, a ratio of N:P<16 of oceanic particulate matter can imply P-rich waters, since it can range from 5 to 19 under sufficient nutrient conditions. However, more evidence is required to determine whether a low C:N:P ratio could be attributed to P-surplus due to anthropogenic nutrient input or other physical-chemical processes in these areas.

#### **5.2.** Increased silicate as the aftermath of a Skeletonema marinoi bloom

Si and Ca are fundamental components of cellular structures in many plankton taxa like coccolithophorids, foraminifera, and diatoms (Buchan et al., 2014; Howe et al., 2010; Langer, 2008). High concentrations of these elements are linked to blooms of these organisms (Buchan et al., 2014; Howe et al., 2010; Paulino et al., 2013). In April 1973, in Hardangerfjord, particulate Ca and Si values were approximately 0.18  $\mu$ M and 4.5  $\mu$ M (Erga et al., 2017; Price & Skei, 1975). In 2008, Raunefjorden, particulate Si concentrations corresponded well with the two distinct diatom blooms, both dominated by Skeletonema sp. (Erga et al., 2017). In late spring 2010, at Raunefjorden, Ca and Si varied between 0.099-6.28 µM and 0.071-3.63 µM, respectively, coinciding with the high abundance of diatom species like Chaetoceros spp., Skeletonema marinoi, and Pseudonitzschia sp. (Erga et al., 2017). In this study, a strong link between Si concentrations (0.96-4.57 µM) and Skeletonema marinoi dominated bloom was observed. Additionally, the study area is directly influenced by the freshwater input from the Møllendal River on the southeast part of the bay, which is an important source of particulate Si in the fjord (Paetzel & Schrader, 2003). Regarding particulate Ca, biogenic matter is probably the primary source, basically in the form of coccoliths. Emiliania huxleyi is one of the most common calcifying species in Norwegian coastal waters, responsible for blooms following the spring diatom bloom (Fagerbakke et al., 1994; Heimdal et al., 1994). However, it seems unlikely that E. huxleyi would be entirely responsible for the Ca concentrations (0.15-0.95 µM) observed in this study but may be also attributed to the water mixing and/or the riverine input. The results from this study for particulate K and Mg agree with results reported in previous studies at nearby fjords (Erga et al., 2017; Price & Skei, 1975).

#### 5.3. Metal concentrations

Fe is thought to limit primary production in marine oceanic systems (Wells et al., 1995). In this study, Fe concentration ranged between 0.19-3.47  $\mu$ M, which is higher than the values typically observed in Norwegian fjords. Due to a handling error during the measurement process for the samples from 18<sup>th</sup> of March to 14<sup>th</sup> of April, the concentration for elements with high molar weight like Fe, Mn, Cu do not reflect their actual particulate concentration, and are thus is not reliable. That is the primary reason why maximum Fe values documented here greatly exceed the typical particulate Fe concentrations in marine seston. Typical values for fjords on the west coast of Norway range between 0.068–0.23  $\mu$ M (Price & Skei, 1975), however, in areas strongly influenced by terrigenous sources, the concentration of particulate Fe can vary between 0.04–2.8  $\mu$ M (Bazzano et al., 2014). In a study performed on shelf waters of the eastern United States, concentrations of particulate Fe and Al were indirectly linked to salinity since their concentrations in runoff water can be several orders of magnitude higher than in oceanic waters (Atkinson & Stefánsson, 1969). Erga et al. (2017) concluded that the prominent iron sources in the samples from Raunefjorden 2009-2011 were Fe sequestering bacteria (Heldal et al., 1996), Fe bound to phytoplankton cells, as well as Fe fractions associated with certain microalgae blooms. All these factors could explain the Fe values observed in this study, together with some other exogenous sources from contamination in the study area; however, this is subject to further research.

So far, no comprehensive studies have been performed on the particulate concentration of heavy metals in the study area. Most of the studies focus on analyzing the sediment and providing estimates of the pollution state based on these values. Comparison of results from sediment surveys in the entire PUD between 1992 and 2020 (Table 2) shows a remarkable decrease in the sediment concentration of most heavy metals in PUD, especially after covering the seabed in 2018 (Bergen\_Kommune, 2020). In this study, I showed that heavy metal presence (Hg, Pb, As) was significantly different in the two sites (p < 0.05)with PUD having the lowest amount. It is highly possible that the low particulate concentration documented in this study is linked to their concentration in the sediment. A recent follow-up survey in PUD, however, revealed that despite the low level of pollution achieved, the top layer of the seabed is subject to recontamination with environmental toxins from the surrounding urban environment (Bergen\_Kommune, 2020). This highlights that proper control of pollution sources that can lead to recontamination is the prerequisite for a monitoring plan to be successful.

#### 6. Conclusions

This study was an effort to document the particulate concentration of chemical elements and associate these concentrations with the phytoplankton biomass. Chlorophyll-a varied with the concentration of C, N, P, and Si. These elements are important for the development of phytoplankton, especially Si for the development of diatoms that dominated during the spring bloom. Heavy metals were detected in both areas in low levels; however, the concentrations were lower in PUD, suggesting that the municipality's plan to cover the polluted sediment may have resulted in fewer particles in the water column containing those metals. Overall, this study was an example of investigating abiotic and biotic relationships in an urban coastal environment, however, the collection of more observational data is necessary to understand the aftermath of heavy metal pollution and to design effective and sustainable management plans in the long term.

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<b>Chemical element</b>	1992-2003 (mg/kg)	2005-2014 (mg/kg)	2019-2020 (mg/kg)
As	21	18	1.3
Pb	298	242	16
Cu	414	299	13
Hg	6.2	3.3	0.05
Zn	734	479	38

**Table 2.** Sediment data used in the risk assessment for PUD divided into three periods. Data from 1992-2003 and 2005-2014 illustrate the condition in the whole fjord, and it is uncertain on which depth and exact sites they were collected. Data from 2019-2020 were taken from the top 10 cm of the seabed in the most inner part of PUD(Bergen Kommune 2020).

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## Vågehval

Balaenoptera acutorostrata

### Can we use fish scales to identify spring and autumn spawning herring (*Clupea harengus*)? Hannah Røkke

#### Introduction

Norwegian spring-spawning (NSS) herring (Clupea harengus) represents one of the world's most abundant fish stocks and is of great commercial importance for many nations (Bjørndal & Gordon, 2000). The NSS stock size have fluctuated throughout the 20th century. Heavy fishing pressure and environmental influence on recruitment caused declines in the 50s and 60s while recruitment from strong year classes and less commercial exploitation caused a slow increase over the later decades (Nakken et al., 2008; Toresen & Østvedt, 2000). Variation in features among individuals leads to a more resilient population, with a higher chance of survival when exposed to challenges like overfishing or changes in the environment (Markert et al., 2010). To avoid stock collapses in the future, fisheries management aim to maintain diversity of populations, using stock assessments (Smedbol & Stephenson, 2001). Correct population identification and discrimination are essential to perform reliable stock assessments, and therefore it is important to study behavior, life history, genetic differentiation, and morphology (Begg et al., 1999).

Spawning seasonality is an important factor in stock assessment because fish stocks are especially vulnerable to commercial exploitation during their spawning season (Biggs et al., 2021). NSS herring aggregate when spawning, which make them easier to catch in bigger numbers (Erisman et al., 2017; Slotte, 1998). Herring is specifically known for their phenotypic plasticity when it comes to spawning season (Geffen, 2009). Herring usually spawns in autumn (autumn spawning herring) or spring (spring spawning herring), but spawning can be observed at different times throughout the year (Sinclair & Tremblay, 1984). Metamorphosis in Atlantic herring is a process that takes place gradually over weeks, and body size at completion may vary (Sinclair & Tremblay, 1984). Because autumn spawned herring overwinters their first winter and have a longer growth season the following year, they can begin metamorphosis earlier than the spring spawned individuals. There are several methods that are used to identify autumn and spring spawning herring including use of genetic markers, checking maturity development, and analysis of otolith microstructure (Berg et al., 2021). Berg's comparison of the different methods showed that the otolith-typing contradicted the other two methods in 7% of the cases while phenotypical assignment was contradictory 8 % of the time. In the case where the results of the methods contradict each other, it is important to have other methods for differentiating spawning groups. Fish scales are used to identify species and estimate growth and could potentially be used to differentiate between autumn and spring spawning herring since their growth period differs (Bräger et al., 2017; Poulet et al., 2005).



Figure 1. Schematic drawing of a herring scale.

The form of herring scales is almost elliptic, with the size and shape varying between individuals. Herring scales have a median that separate the different sides of the scale. One of the sides is almost transparent, while the other show annual rings describing age and growth patterns of the fish. The side with the annual rings alternates between narrow transparent rings representing physiological winter, and broader less-transparent areas representing physiological summer (Dahl, 1907). A schematic drawing of a herring scale showing the annual growth rings is shown in Fig 1.

Our aim for this report was to investigate if we can use herring scales to distinguish between autumn and spring spawning herring. Our hypothesis was that herring that is genetically fit for autumn spawning (genetic autumn spawners) have a relatively wider first scale increment size than genetic spring spawners, due to longer first growth season. We further hypothesize that this difference could be used to differentiate between the two spawning groups.

#### 2. Method

#### 2.1. Biological data

160 herring were taken from a series of gill net samples at Herdla, Askøy at varying times of the year in the time period from 31.10.2016 to 18.09.2018 (Table 1).

**Table 1.** Overview of dates and locations of trawls where the

 Atlantic herring (*Clupea harengus*) were captured.

Location
Søre Tretthølo, Gandvikje and Købbevågen
Søre Tretthølo and Købbevågen
Søre Tretthølo, Gandvikje and Købbevågen
Gandvikje and Købbevågen
Købbevågen
Søre Tretthølo and Købbevågen
Søre Tretthølo and Købbevågen
Søre Tretthølo, Gandvikje and Købbevågen

The fish were measured (total length (mm), total weight (g)), and otoliths, scales and DNA samples were taken for further analyses. The otoliths were used to determine age and spawning time, while the genetic samples were used to determine genetic spawning season.

The genetic analysis of the DNA identified the genotype at two genetic markers, indicating the spawning season the individuals were fit for. Individuals were identified as Autumn, HeteroAutumn, Spring, Hetero-



**Figure 2.** Picture of a slide with gelatin coating. Each slide had scales from 2 individuals with at least 5 scales from each fish. The fatty skin layer is removed from the scales before they are placed on the slide.

Spring or Heterozygote genetic spawners (Table 2). In this study, the herring were further divided into two genetic groups; Genetic Autumn Spawners and Genetic Spring Spawners. Genetic Autumn Spawners included Autumn and HeteroAutumn individuals, while Genetic Spring Spawners included Spring and HeteroSpring individuals. Heterozygote spawners were excluded from the statistical analysis because we could not divide these individuals into genetic spring and autumn spawners.

Analysis of the otoliths were performed to identify spawning phenotype as well as age. Otolith age was compared to age determined from the scales, while otolith spawning season was used to see whether there were any differences in the scales of autumn and spring spawned herring.

The herring scales were mainly collected from an area dorsally above the lateral line but could also be collected from other areas of the fish body if few remained attached in the original area.

#### 2.2. Scale analysis

The fish scales were placed in NUNC trays covered with water and frozen. When the analyses were performed, the scales were thawed and the fatty surface skin was removed by placing the scale between index finger and thumb, rubbing the layer off. The scales were again dipped in water before being placed on top of a slide covered in a gelatin coating to keep the scales in place. The gelatin coating was made by mixing 5 grams of gelatin (Merck Emprove catalogue number: 1.04078.1000. CAS # 9000-70-8) with 2,5 dl water and heating it up. The gelatin coating was smeared on to the slides and set to dry for 24 hours in room temperature.

3 or more scales from each fish were placed on a slide

Genetic Spawning Seasons Genotypes		
Autumn	Both genetic markers are homozygote autumn	
HeteroAutumn	One genetic marker is homozygote autumn, the other is heterozygote	
Spring	Both genetic markers are homozygote spring	
HeteroSpring	One genetic marker is homozygote spring, the other is heterozygote	
Heterozygote	Both genetic markers are heterozygote	

**Table 2.** Table of genetic spawning season genotypes, using the genotype composition of two genetic markers indicating genetic spawning season. for comparison purposes and to ensure that at least one scale was readable (Figure 2). After the herring scales were mounted, a stereo microscope (Leica M125) in 1x enlargement and a camera (Nikon Digital Sight DS-U3) was used to take pictures of the scales. The scale with the best quality and easiest read was chosen for each individual.



**Figure 3.** Schematic drawing of a herring scale. All fish caught on the northern hemisphere have January 1st registered as their birthday. Because the annual rings of the scale grow throughout the year, a fish caught in August will have a larger outer annulus than a fish caught in April. The outer edge of the scale from a fish caught from January to May will be counted as a year annulus while it will not be counted for individuals caught from June to December. Line used for measurement of growth increments with age corresponding annuli indicated. The different colored lines indicate the measurements of increment 1 (red line), increment 2 (yellow line), radius 1 (purple line) and radius 2 (blue line).

A picture was taken of a calibration scale for use in later measurements of annual ring lengths. The data program ImageJ(1.53a) was used in the length analyses of the scales. The scale was set to 10mm, using the calibration picture that was taken along with the pictures of the herring scales. A reference line going from the middle of the focus and straight up through the scale was drawn on every picture. Every annual ring was then marked by a point and the x- and y-values were noted down using the measure feature in ImageJ. These values were then added to an Excel file where the radius and increments were calculated. All fish caught on the northern hemisphere have January 1st registered as their birthday. Because the annual rings of the scale grow throughout the year, a fish caught in August will have a larger outer annulus than a fish caught in April. The outer edge of the scale from a fish caught from January to May will be counted as a year annulus while it will not be counted for individuals caught from June to December (Figure 3). Radius and increment are indicated in the schematic drawing in Figure 3. Both radius 1 and increment 1 is measured vertical from the horizontal focus line, up to the end of the first annual winter ring. Increment 2 is measured from the end of Increment 1 and up to the end of the second annual winter rind, while radius 2 is measured from the horizontal line at the focus up to the end of the second annual winter ring.

#### 2.3. Statistical analysis

One-way ANOVA tests were performed to compare the different spawning and genetic groups using the R software (Team, 2013). All statistical analysis were performed with a significance level of 0.05.

For statistical analyses, we used scatter plots to get a visual indication on the influence of genetic spawning season on the relationship between otolith age and fish length as well as the relationship between fish length and scale length. We used pivot tables and means plots to look at how genetic spawning season affected radius 1, radius 2, and the relationship between radius 1 and radius 2. The same pivot table and means plot were performed with increment 1, increment 2, and the relationship between increment 1 and increment 2.

#### 3. Results

Fish length in relation to otolith age varied slightly with genetic spawning groups (Figure 4). The two genetic spawning groups start out at the same length of 30 cm, at 2 years of age. Genetic spring spawners grow longer than genetic autumn spawners on average as they get older. The genetic autumn spawners have extreme outliers both on the positive and negative sides of the regression line.

There was no apparent difference between the two genetic spawning groups in the relationship between fish length and scale length (Figure 5). Both genetic spring and genetic autumn spawning herring started out at around 4.5  $\mu$ m at 28 cm, going up to 5.5 – 6  $\mu$ m

	Radius 1 Mean	Radius 2 Mean	Radius 1/2 Mean
Genetic Autumn	3.314	4.026	0.822
Autumn	3.314	4.026	0.822
Genetic Spring	3.025	3.791	0.792
Autumn	2.368	3.432	0.684
Spring	3.078	3.820	0.801
Total	3.087	3.841	0.798

**Table 3.** Table showing mean of first and second radius and mean of radius 1 divided by radius 2 for all genetic spawning groups. The different groups seem to be similar, with no significant differences. The measurements are in  $\mu$ m.



**Figure 4.** Scatter plot of otolith age against fish length (cm) for the two genetic spawning groups. From the plot we can see that there is a difference between the two genetic spawning groups when we look at the length of fish and their age. The linear model equation for genetic spring spawners is y = 28.4329 + 0.8323x and the linear model equation for genetic autumn spawners is y = 29.4508 + 0.3755x. At around age 2 both groups are approximately 30 cm long, and the genetic spring spawners tend to get longer as they grow older that the genetic autumn spawners. The genetic autumn spawners have extreme outliers both on the positive and negative side.

at 36 cm for otolith length and fish length, respectively.

There was a small difference in mean of radius 1 and 2 between the autumn and spring spawned individuals that spawned in their genetic spawning season (Table 3, Figure 6). There was a bigger difference in radius 1 and 2 between the groups where individuals had the same spawning season genotype and phenotype, and in the groups where spawning season phenotype and genotype did not match. The same differences were found when we looked at the relationship between radius 1 and 2 by division.

	Increment 1 Mean	Increment 2 Mean	Increment 1/2 Mean
Autumn	3.218	0.707	5.620
Autumn	3.218	0.707	5.620
Spring	2.966	0.756	4.882
Autumn	2.366	0.915	3.142
Spring	3.024	0.741	5.052
Totalsum	3.020	0.746	5.042

**Table 4.** Table showing mean of first and second increment and mean of increment 1 divided by increment 2 for all genetic spawning groups. The different groups seem to be similar, with no significant differences.



**Figure 5.** Scatter plot of fish length (cm) vs Scale length ( $\mu$ m) for each of the genetic spawning groups. The linear model equation for genetic spring spawners is y = 0.3603 + 0.1466x and the linear model equation for genetic autumn spawners is y = -0.5052 + 0.1805x. There are no significant differences between the two genetic spawning groups when it comes to the relationship between scale length and fish length.

The mean of increment 1 and 2 was between the autumn and spring spawned individuals that spawned in their genetic spawning season (Table 4, Figure 7). There was also a difference in increment 1 and 2 between the groups where individuals had the same spawning season genotype and phenotype, and in the groups where spawning season phenotype and genotype did not match. There also were differences in the relationship between increment 1 and 2 by division.

#### 4. Discussion

There was a difference in radius and increment between genetic spring spawners with autumn phenotype and genetic autumn spawners with autumn phenotype. The mean of the genetic spring spawners was much lower than the mean of the genetic autumn spawners for both increment and radius when their phenotype was autumn spawning. The reason for this could be that individuals with similar genotype and phenotype perform better because they spawn in the season, they are genetically fit for. They could be better fit to take advantage of a longer growth season. We did not have any genetic autumn spawning herring with spring phenotypes, and it would be interesting to see how they fit in with the results of the other groups.

The difference between increment and radius for the two genetic spawning seasons was small and seemed to be unsignificant. Similar studies of herring otoliths show no significant difference in growth pattern between autumn and spring spawning herring (Johannessen et al., 2000). The difference in length of growing season could be too small to give a significant difference between the two groups, or other factor may interrupt the autumn spawn-



Figure 6. Means plot of relationship between the mean of radius 1 and 2 with standard deviation for each otolith spawning group, categorized by genetic spawning groups. There is no apparent difference in the relationship between radius 1 and 2 in genetic spring spawners spawned in spring and genetic autumn spawners spawned in autumn. What we can see, is a difference between individuals that are spawned in the season they are genetically fit for, and the individuals that are spawned in the opposite season of the one they are fit for.

Figure 7. Means plot of relationship between the mean of increment 1 and 2 with standard deviation for each otolith spawning group, categorized by genetic spawning groups. There is no apparent difference in the relationship between increment 1 and 2 in genetic spring spawners spawned in spring and genetic autumn spawners spawned in autumn. What we can see, is a difference between individuals that are spawned in the season they are genetically fit for, and the individuals that are spawned in the opposite season of the one they are fit for.

ing herring from taking advantage of the longer growth season. There is also a chance that there are differences between the two groups, but that these are evened out with other life history factors unknown to science now. We know little about how the herring populations move outside of spawning season, and more research on this topic could give us answers about the questions brought up in this report.

Based on these minor differences, our conclusion is that we cannot use herring scales to identify spring and autumn spawning herring, with the method used in this scientific project.

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# Blåmeis

Cyanistes caeruleus

## The habitat uses and behaviour of Black Grouse (*Lyrurus tetrix*) in western Norwegian heathlands, in winter

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#### Abstract

Human activities keep exploiting lands to the detriment of biodiversity and species that are declining, owing to this habitat loss. Hence, understanding what use a species makes of its habitat contributes to preserving the species by establishing conservation strategies. Black grouse has a huge distribution range, from the Atlantic coast and northern Eurasia to south-eastern Siberia. Therefore, the majority of their distribution area has large climatic variation between summer and winter. Most studies on black grouse winter behaviour describe inland populations, whilst the habitat use of coastal populations is poorly described. We studied the habitat uses and winter behaviour of a coastal population of black grouse (*Lyrurus tetrix*) during winter 2021-2022 in the heathlands of Lygra, a coastal island in western Norway. We quantified vocal territoriality and the use of heathlands as feeding grounds during winter. We find that black grouse are present in the heathlands on the island through winter to mark territory and to feed. We used an acoustic recorder and recorded two types of calls, hisses and coos, proving that this coastal population of black grouse vocally defends their territory in winter, in contrast to inland populations. Black grouse appear to be most active shortly before and up to two hours after sunrise. No vocal activity is found in the afternoon or before sunset. We notice a decrease in activity from December to January, which may be due to increased rainfall and wind. Black grouse keep singing during both positive and negative temperatures but tend to reduce activity at very low temperatures. Fecal analyses confirm that they feed on the heathland shrub *Calluna vulgaris* in winter, and we did not observe any changes in their diet over the winter period.

#### Introduction

According to the 2019 report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, three-quarters of the global land-based environment has been significantly altered by human actions (IPBES, 2019). This land-use change can be the conversion of land cover, changes in the management of the ecosystem or agro-ecosystem, or changes in the spatial configuration of the landscape (IPBES, 2019). Therefore, it damages, divides and wipes out habitats to meet human needs and preferences. As a result, land-use change is the main driver of biodiversity loss worldwide and even species extinction as they lose their habitats (IP-BES, 2019). Hence, a crucial part of restoring, conserving and managing species is to understand the habitat use.

The black grouse (*Lyrurus tetrix*), (Linnaeus, 1758), is a bird whose habitat has been altered for decades now. The

species is widely distributed globally: ranging from Britain and North Eurasia to China (Lawrence, 2004; Zhang et al. 2020). Studies of Wegge & Kastdalen (2008) and Ciach (2015) showed that black grouse avoids dense woodland. The northern population is rather associated with young forest (Swenson & Angelstam, 1993; Gregersen, 2009; Ciach, 2015) or forest edge habitat (Paloc, 2004; Wegge & Kastdalen, 2008; Kurhinen et al., 2009), but coastal populations are also found in open heathlands (Baines, 1994; Starling-Westerberg, 2001). The differences in habitat use and winter behaviour between the inland and coastal populations is poorly described, but there are reasons to expect that there are important differences between these populations, in terms of habitat use and winter behavior. We expect these differences to be especially clear in winter, because of the lower temperatures, more snow cover, and higher predator pressure in the inland compared to the coastal areas.

Black grouse territoriality involves competitive behaviour such as calls and physical confrontations (Brown & Orians 1970). Studies on territorial behaviour is focused on inland populations and are concentrated on activities performed in spring, during the mating season (Rintamäki et al. 1999) when seasonal cackle period doubtless concurs (Kruijt & Hogan, 1967). Females may also show territoriality during the breeding season (Angelstam et al. 1985). Yet, males can have territory defence behaviour in autumn (Rintamäki et al. 1999) and visit leks most of the year (Gregersen, 2014, in Eastern Norway). According to Gregersen (2014), males fight for a position in the flock each day to establish dominance hierarchies. Black grouses also have a largely herbivorous diet (Lawrence, 2004). The diet of inland populations of black grouse consists of leaves and buds of berries, rowans, alders, spruces, and some seeds (Starling-Westerberg, 2001; Darmangeat & Dupérat, 2004; Paloc, 2004) and needles from resinous trees and bilberries (Vaccinium myrtillus) (Wegge & Kastdalen, 2008; Selås, 2019). Specific descriptions of the coastal populations' diets are lacking, but high availability of rather notorious shoots of Calluna vulgaris suggest that this is a relatively more important plant in the coastal populations' winter diet compared to inland populations.

Therefore, the interest of our study was to know more about winter behavior and habitat use (feeding and territoriality) of a coastal population of black grouse in western Norway, focusing on their diet and calls. This would highlight differences with inland populations of black grouse. So, based on frequent observations of black grouse in heathland habitats during winter, we recorded sound at dawn and dusk through winter to explore if the males were expressing territorial calls in a coastal black grouse population. We also studied plant material in feces samples to quantify how important heathlands are for feeding and foraging through winter, and to determine if the diet varies from early to late winter.

#### Material & Methods

#### 1. Black grouse

We studied a coastal population of black grouse (Lyrurus tetrix) in Norway from December 2021 to February 2022. The population size has not been quantified, although observations of 15 males simultaneously (artsobservasjoner.no) suggest a population size of at least 30 individuals (Ellison and Magnani 1985, Marti et al. 2016). Despite the species being ranked as 'Least concern' on both the IUCN and the Norwegian red list for species, the species is nowadays in decline, and has even gone extinct in some distributional border areas (Warren & Baines, 2002). The driving causes for the decline are intensified land-use, climate change, parasite infestation, and predation (Baines et al., 2000 ; Ciach, 2015 ; Jahren et al., 2016 ; Hambálková et al., 2021). Moreover, the reproductive success of the species has also decreased (Ciach, 2015; Jahren et al., 2016). According to Gregersen (2009), a decline in the Norwegian black grouse population has been observed since 1970, especially into the south and in the very north of the country.

Black grouse are most active in the early morning and spend the rest of the day, 94 percent on average, hiding (Marjakangas, 1992 ; Darmangeat & Dupérat, 2004). Black grouses show their territoriality with actual defence (attacks, chases) but also with calls and displays that keep rivals out (Brown & Orians 1970). As Angelstam (1985) described it, "the cackle-call is used as an identifying territorial act and as an aggressive call in actual defence".

#### 2. Study site

The study site is located at Lygra (Fig.1), an 2.5 km<sup>2</sup> large island in Alver municipality in western Norway. Most of the island is covered in agricultural land, ranging from semi-natural heathlands in the northwest to more intensive land-use in the central part, and afforestation in the south-east. Approximately 60 people live in the central part of the island. The heathland is about 0,5 km<sup>2</sup> and reaches up to 20



Figure 1. Location of Lygra, the island with heathlands outlined in red. Source : Google Maps, 2021.

meters above sea-level. Vegetation is dominated by common heather - *Calluna vulgaris*. There are also bushes and shrubs. The heathland is grazed year-round by old norse sheep.

Coastal heathlands are amongst the oldest cultural landscapes in Europe, reaching back 6000 years (Gjedrem & Log, 2020). Today, coastal heathlands have high conservation value throughout their range because of their biological diversity and cultural history. While the heathlands along the European Atlantic coasts are threatened by extinction - as much as 90 percent of European heathlands have been lost in the past 100 years due to cultivation, pollution, and overgrowth (Kaland & Kvamme, 2014) authentic heathland coastal landscape of Lygra are well preserved, as it is a museum and research station used all year round. They are maintained by local farmers through periodical burning and continuous grazing. Once a year, prescribed burning is applied to parts of the heathland, creating a mosaic of fire patches with a fire return interval of approximately 25 years across the landscape (Fig. A1 in the appendix). This mosaic burning maintains fodder value of the vegetation and increases species richness through high microscale vegetation heterogeneity. The periodic burning and year-round grazing are not believed to affect black grouses directly, however this management is necessary to keep heathlands open and provide the habitat where black grouses can find food (heather, bilberry, buds, leaves) and display in open areas (Fig. A2 in the appendix).

#### 3. Audio recordings

We used an acoustic recorder (Song Meter Mini from Wildlife Acoustics) to quantify territorial behaviour of black grouse in the heathland from December 2021 to the beginning of February 2022.

The recorder possesses an omni-directional microphone, has a recording bandwidth from 20Hz to 48kHz and can run 210 scheduled hours. These characteristics make it very suitable for bird recording. We placed it in a suppression in the terrain to shelter it from strong winds (Fig. 2), close to a hill with frequent observations of black grouses, in an early-successional stage of heathlands. The frequent visits to this hill were confirmed by the presence of fresh black grouse feces. The recorder is fixed high up on a wooden post so as not to be disturbed by the sheep. We programmed it to start recording from 7:00 to 11:00 and from 13:30 to 18:00, to capture the time just before and after sunrise and sunset. Every two or three weeks (dates in the appendix), we collected the recordings from the SD Card and changed the battery. Thus, this method is easy to implement, inexpensive and allowed us to study black grouses with little interference in their environment.

Then, to analyse the collected recordings, we used the



**Figure 2.** Location of the recorder in the heathlands. Source : <u>Google Maps</u>, 2021.

version 2.3.3 of Audacity<sup>®</sup> recording and editing software<sup>1</sup>. It gives the spectrogram that enabled us to simply "look" for a sound through visualizing the audio recordings (method in Fig. A3 in the <u>appendix</u>). We knew the shape of a black grouse sound, thanks to literature (spectrograms in the study by Hambálková et al. (2021), Xeno-Canto website (spectrograms and audio recordings) and by recognizing the sound during our analyses. A spectrogram also provides complementary information like frequency, intensity and duration of the sound. Figure 3 illustrates an example of a spectrogram representing the audio recordings from two types of black grouse calls. Figure 3a shows a hissing call. This sound has a wider range of frequencies, often is shorter in duration and it is composed of two notes. Figure 3b illustrates a cooing call (Kruijt & Hogan, 1967). This sound has a smaller intensity, a lower and smaller frequency range, and may last longer because it is composed of several notes as it is a coo, a cackle. One repetition of each sound is visible.

For all of the calls found on the spectrograms, we noted the date, the time of day and the time in the recording in which it occurs and specified the type of call. We

1 Audacity<sup>®</sup> software is copyright © 1999-2021 Audacity Team. The name Audacity<sup>®</sup> is a registered trademark. **Figure 3.** Two sounds produced by a black grouse. A: hissing call. B: cooing call. The horizontal and vertical axes represent time and frequencies, respectively. The horizontal and vertical red arrows represent respectively the duration and the frequency range of the sound.



counted and sorted them. To obtain the length of activity, we defined a period of activity as calls with less than one minute of silence in between. Then we calculated the sum of the periods of activity per day, during recording hours.

We also used weather data from the study period, collected by the bioCEED weather station at Lygra to answer if the activity is affected by temperature, precipitation or wind. First, we summed the amount of precipitation, per day, from 6:00 to 11:00, so it would correspond to the period where black grouse sing, and we looked for the maximal wind speed over the same time slot. Then, we calculated the average temperature, still between 6:00 and 11:00.



Figure 4. A: Collected feces. B: Sorted and dried fragmented feces.



Figure 5. Spectrograms showing the different calls of the black grouse.

#### 4. Faecal collection and analysis

In order to determine the diet of black grouses, to know if it changes during winter and to explore if black grouse also use the heathlands for foraging, we collected their feces. We collected it twice, around ten samples each time, at the end of November and in January to compare feces from the beginning and the end of winter. Each time, samples were collected (Fig. 4a) in two areas where black grouses are frequently observed during winter. Both areas are in the young successional vegetation stage of heathlands, meaning that there is less than 7 years since the last fire. One of the areas is a natural hill, whilst the other area has scientific installations with dimensions 3x3x1 meter (length x width x height) which black grouses have been observed to use for displaying. Then, in the lab, the samples were dried in the oven, weighed with a fine scale balance and placed in petri dishes before being fragmented with forceps and fingers. We looked for recognizable fragments of branches, seeds and leaves. We sorted them in smaller petri dishes (Fig. 4b) and weighted these portions. Thus, we calculated the ratio of their mass to the total mass of the sample and obtained the proportion of branches, seeds and leaves in the black grouse diet. But we also had to consider the part of the samples which remained unsorted, due to the



Figure 6. Number of coos and hisses recorded per day, according to the time slot. A dot represents one day.

tiny size of the fragments, to properly interpret the results. Indeed, the variations in identified sample proportions may have been caused by varying degrees of fragmentation of the plant matter in each sample. For that, we calculated the ratio between the mass of sorted matter and the total mass of the sample. Moreover, the results will be discussed as approximations and not as a direct measurement, because much of the leaves could have been digested already. When the grouse feeds on a plant, it eats a whole section of the shoot, which is a branch covered in leaves.

#### Results

#### Acoustic analysis

#### Spectrogram

Our acoustic analysis reveals the existence of black grouse calls in the heathlands in winter. We heard two types of calls (Fig. 5a): cooing calls within a range of 900-2500 Hz (Fig. 5b) that often overlaps with background noise frequencies, and hissing calls (Fig. 5c), with wider signals in the frequency range of 1000-5000 Hz, sometimes up to even higher frequencies, depending on the intensity of the call. With these frequencies and the fact that they often are louder than coos, they are easier to filter out from background noise. We heard both isolated and grouped calls.



Figure 7. Length of black grouse activity in seconds per day. Some dates are missing due to data gaps in the recordings.

#### Timing of calls

**Calls counting per time slot:** Over the entire recording period, we never heard black grouse calls in the afternoon between 13:00 and 18:00. The results show that black grouses sing during our morning recording session, that is between 7:00 and 11:00. The peak of activity comes between 8:00 and 10:00 (Fig. 6), that is just before and after sunrise that happens between 8:30 and 9:30 from December to February. It also demonstrates that it is the same pattern for the two noises.

Total number of calls and inactive days counting: We recorded 597 hisses and 362 coos, during our study period. We do not have continuous recordings due to the batteries running out that make us miss some days. In December, the maximum number of calls heard was 110 hisses and 79 coos against 12 hisses and 8 coos in January. No calls were heard during 6 days over 21 days of recordings in December and during 16 days over 20 days in January. That is, in December, 28 percent of the number of study days were without audio activity and 80 percent in January. Over the entire period of study, 43,5 percent of the days recorded audio activity. The overview of all the calls recorded is in Fig. A4 in the appendix.

#### Weather data

By comparing the days with black grouse activity with the other days, we detect a trend: the length of activity of black grouse is more important when precipitation is low and the wind quiet (Fig. 8). For example, on December 1, there was apparently no rain, the wind was light and back grouse activity was over 2000 seconds, whereas during the first half of January, when the weather was rainy and windy, there was no black grouse activity. We however point out the absence of some data recordings from the weather station on January 29 between 10:00 and 11:00, on February 2 (all day) and on February 3, from 6:00 to 10:30. Thus, on Fig. 8, the weather values of these days may be higher because they are incomplete.

We find recorded calls during both positive and negative temperatures (Fig. 9). For example, on December 24, the average temperature was -1.8°C and black grouse activity was around 700 seconds. On January 15, the average temperature was 2.5°C and black grouse activity was around 200 seconds.



Figure 8. Length of black grouse activity and sum of precipitation and maximal wind speed per day. Precipitation and wind areas are stacked.

**Length of activity:** By taking all the days of recording into account, the average length of activity is 179 seconds so almost 3 minutes (Fig. 7). By taking all the days where at least one song was heard (the "active days"), the average length of activity is 412 seconds (6 minutes and 52 seconds).

## Diet linked to the surrounding vegetation based on collected feces in the heathlands

At first sight, fecal samples from November 30 and January 25 have a similar texture, color and when sorted, similar fragments. The composition consists of branches and leaves of *Calluna vulgaris*, and some seeds, for these two groups



Figure 9. Length of black grouse activity and average temperature per day.

of collected feces. Branches often represent between 0 and 10% of the sample, except for sample 1 and 7 (Fig. 10a). But these values may be nuanced with Fig. 10b, which shows how much matter was sorted over the total amount of the sample. For example, sample 1 has the biggest amount of branches but because it has probably been more sorted, given that the ratio sorted matter/non-sorted matter is more important. Therefore, there is little difference between November and January and branches were more often found than leaves and seeds. We did not find any seed in the fecal samples from area 2, a hill with scientific installations.

#### Discussion

#### Location of black grouse on Lygra

On Lygra, the coastal population of black grouse lives in the open and this is the first difference in habitat use with inland populations. In winter, the latter can dig tunnels in the snow (Darmangeat & Dupérat, 2004; Paloc, 2004) that allow them to rest by being sheltered from cold (Bocca et al. 2013) and predators such as, red fox, golden eagle, pine marten but mainly the northern goshawk in forest. These predators are common in forest/ inland areas compared to coastal/open areas. However, there are some foxes at Lygra. Then, as black grouse hide in the bushes and shrubs on Lygra when they are not displaying or foraging, it is probably an anti-predator strategy, as it is a common strategy in many other birds. According to Angelstam et al. (1985), cackle-calls could be located from about 600 m, or even from 1 km in open terrain according to Boback & Muller-Schwarze (1968). So, knowing the location of the recorder, we draw circles of 600 and 1000m radius (Fig. 11). They cover the study area and certify that the black grouse heard are found in the heathlands.

#### Winter behaviour

#### Territoriality through calls

The literature of Norwegian black grouses is mainly based on inland populations who live in the forest all year. It is described that males have a strong territoriality in spring and cackle calls start three weeks before the beginning of incubation, late April (Angelstam et al. 1985), or that males and females show fighting behaviour and territory defence in autumn and in spring (Rintamäki et al. 1999). We heard hissing and cooing calls. Then, this study indicates that this coastal population of black grouse show territorial behavior during winter. Indeed, as mentioned before, territoriality is shown through calls that can be described as aggressive calls (Cramp, 1983). Especially hissing calls, that are frequently uttered during threatening, flutter-jumping and fighting (Kruijt & Hogan, 1967).

Our data match with the study of Hambálková et al. (2021), in which they found call frequencies from 352 to 4482 Hz for black grouse populations of Finland and Scotland. Yet, with our analysis, we can not



**Figure 10.** A: Proportion of branches, leaves and seeds in the sorted matter per fecal sample. B: Proportion of sorted matter per fecal sample. Area 1 : Natural hill. Area 2 : Hill with scientific installation.

exactly tell if black grouse calls frequencies fall below 900 Hz as it is mixed with background noises.

#### Winter activity

As for studies before (Angelstam et al. 1985; Kruijt & Hogan, 1967; Marjakangas, 1992), our results show that bird territorial activity is confined in the morning. We did not notice evening activity unlike Hjorth (1970) suggested. The daily total cackle period in winter is around three hours. That concurs with the study of Angelstam et al. (1985) and Marti and Pauli (1985).

Even though black grouse are out and singing in winter, they seem to be less active in January and February. The length of activity is below ten minutes and depends on the weather, especially precipitation and wind. On rainy and windy days, black grouse stay hidden and do not sing. Black grouse also tend to reduce their activity at low temperatures (Keller et al., 1979). According to the study of Marjakangas (1992), length of activity is correlated with ambient temperature but not with photoperiod.

#### *Characteristics of the calls*

The two types of calls that we were able to hear are defensive calls. Black grouse seem to use more often hissing calls over coos. Moreover, there is no hour difference between coos and hisses, both songs follow the same pattern.

#### Foraging and feeding

Black grouse inhabit the heathlands where they can find resources necessary to them. Studies often describe the winter diet of inland populations of black grouse, living in the forest where snow covers the floor (Bocca et al. 2013). Because of snow cover, these birds are forced to feed in trees and taller shrubs, so mountain pine needles or buds of many conifers are found in their diet (Bocca et al. 2013). But Lygra is not often covered by snow and the vegetation in the heathlands differs. The results of this study suggest that the common heather Calluna vulgaris make up most of the diet of the black grouse population at Lygra, as stated in the study of Baines (1994). During winter, their diet does not seem to change. Moreover, as Marjakangas (1992) deduced, if black grouse are only active in the morning, it is probably during this period that they feed. And because of the relatively low nutritive value and digestibility of their diet, they must feed regularly, because they do not accumulate substantial fat reserves (Bocca et al. 2013). This low digestibility explains that we were able to sort the samples and find recognizable fragments of branches, leaves and seeds.

#### Improvement ideas for our recording analysis

**Margin of error:** Even if the recorder is very good, strong wind and rain have been an issue for the acoustic analysis. It creates more background noises and even large signals and makes it more difficult to hear and see calls (Fig. A5 in the <u>appendix</u>). Therefore, one possibility is that we may have missed some calls during bad weather. The second possibility is that during bad weather, black grouse stay sheltered and do not sing anyway so we did not miss any call. Also, some cooing calls may be missing because, due to their low frequency range, they are barely visible and mix with background noises (Fig. A6 in the <u>appendix</u>). We also had to ensure to differentiate black grouse songs from other birds (Fig. A7 in the <u>appendix</u>).

Battery run-time and SD Card capacity: To avoid periods without any recording due to dead batteries or lack of space on the SD Card, we should have checked the recorder more often at the beginning of our study.

**Recording hours:** Some days, calls were recorded on the 10am recording, between 10:30 and 11:00 and even at 10:59. A quarter of the total days of study contain calls between



**Figure 11.** Distance from the recorder, represented by the red circles. Source: Google Maps, 2021. Distance from the recorder. Area 1: Natural hill. Area 2: Hill with scientific installation.

10:30 and 11:00. Therefore, it seems that black grouse can sing late in the morning and it can be interesting to add one or two more hours in the recording session, that is from 7 to 1pm. Moreover, we were not able to know how many black grouse we heard during the audio analyses.

#### Conclusion

On Lygra, the coastal population of black grouse uses the habitat for roosting, marking their territoriality and foraging. The heathlands are then vital for them whereas inland populations inhabit forests and clearings. These coastal black grouse keep their territorial behavior through calls, to a higher degree than inland populations. They show morning activity that decreases during winter and depends on the weather, especially precipitation, wind and temperature. They feed on branches and leaves of *Calluna vulgaris* and seeds, and their diet does not seem to change during the winter. Knowing this information, is important in terms of species management. To go even further in this approach, it would be interesting to correlate the presence of black grouse in Norway with the conservation of Norwegian semi-natural landscapes and ecosystem services.

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Razorbill (*Alca torda*) Photo: Marthe Olsen



# Kvastfinnefisk

Coelacanthiformes

## Naturen i Bergen lagrer mer karbon enn det nasjonale gjennomsnittet

Gina Koulibaly Barry, Vilde Engelsen, Sofie Klem, Johanne Kuhle, Hanna Sannes og Tor Værøy

#### Introduksjon

Karbon er grunnsteinen for alt liv og beveger seg gjennom biosfæren via karbonkretsløpet. Karbonkretsløpet er utvekslingen av karbon, også kjent som karbonfluks, i og mellom tre store karbonlagre: atmosfæren, land og hav (Houghton, 2014). Karbonlagre er deler av økosystem med evne til å lagre og akkumulere karbon. Forstyrrelse av slike karbonlagre vil åpne for utslipp av det lagrede karbonet i økosystemet (Chapin et al., 2011 s.259) - som er et stort problem i den terrestriske biosfæren, det største biologiske karbonlageret vi har (Chapin et al., 2011 s. 407).

Karbonet som er bundet opp i jordsmonnet (Soil organic carbon, SOC) er et viktig terrestrisk karbonlager, og de fleste estimat indikerer at det lagres betydelig mer karbon i SOC enn i biomasse over bakken (Chapin et al., 2011; Houghton, 2014; Scharlemann et al., 2014). Det antas at omtrent 80% av karbonet, som er bundet opp til terrestriske økosystem, finnes i jordsmonnet (Bartlett et al., 2020; Lal, 2008). Resterende karbon lagres i organisk materiale over bakken (Lal, 2008). Mengden karbon i terrestriske økosystemer påvirkes gjennom fotosyntetisk fiksering av CO2, lagring av karbon i biomasse og jordsmonn, og frigjøringer av CO2 gjennom respirasjon og nedbrytning av organisk materiale (Cheng & Gershenson, 2007).

Norge har forpliktet seg gjennom Parisavtalen til å kutte utslippene av CO2 og i stedet øke opptaket av CO2 fra atmosfæren (Bartlett et al., 2020; FN-sambandet, 2020). Menneskelig aktivitet, som bruk av fossilt brensel, forstyrrer den naturlige balansen i karbonkretsløpet og det slippes ut mer klimagasser enn det naturen klarer å fange opp (FN-sambandet, 2021). Økt utslipp av naturgasser til atmosfæren har forårsaket en økning i gjennomsnittstemperaturen med 1°C det siste århundret (Masson-Delmotte et al., 2019). Når SOC i vegetasjonen frigjøres, slippes den ut som klimagassen CO2 (Houghton, 2014). Naturtyper med god evne til karbonlagring bør derfor bevares for å forhindre utslipp av CO2 (Bartlett et al., 2020). En av de større årsakene til utslipp av CO2 er arealendringer, som avskoging og utbygging (Olerud & Lahn, 2020). Slike fysiske forstyrrelser kan føre til at områder slipper ut mer karbon enn de tar opp og kan gi negative effekter på artsmangfold og habitat på globalt nivå (Bartlett et al., 2020). Kartlegging av karbonlagring i SOC og i biomasse over bakken er viktig for å kunne drive bærekraftig arealbruk, og begrense utslipp av CO2 fra viktige karbonlagre i naturen.

Klima påvirker terrestrisk karbonlagring blant annet gjennom temperatur og nedbør. Disse faktorene påvirker karbonfluks, gjennom regulering av netto primærproduksjon, det vil si summen av karbon som systemet tar opp ved brutto primærproduksjon og karbon som går tapt ved planterespirasjon (Chapin et al., 2011; Falloon et al., 2007). Lave temperaturer vil ha en direkte påvirkning på produksjonen, ved å senke hastigheten på kjemiske reaksjoner (Chapin et al., 2011 s. 147-148). I motsatt ende vil for høye temperaturer også redusere primærproduksjon, ettersom planter da må gå over til fotorespirasjon (Chapin et al., 2011 s. 147). Ved økte sommertemperaturer vil det også være en økning i utslipp av næringsstoffer, som kommer av en økning i nedbrytning (Johnson et al., 2011). I en studie gjort på SOC i Alaska, ble det funnet at sommertemperatur kan føre til økt netto primærproduksjon (Johnson et al., 2011). Det er vist at eldre og mer beskyttet jordsmonn er mer følsom for temperatur i forhold til raskt nedbrytbart jordsmonn (Hartley et al., 2021 s. 2). Ved høye temperaturer vil løseligheten til oksygen være lavere, dette fører til en økning i nedbrytning og nedgang i SOC (Chapin et al., 2011 s. 200). Mye nedbør kan føre til at jordsmonnet blir mettet med vann og luftrommene i jordsmonnet fylles (Chapin et al., 2011 s. 147). Dette vil skape et anaerobt

miljø, som reduserer nedbrytningen av SOC (Chapin et al., 2011 s. 147). Vannmetning i jordsmonnet vil også ha negativ effekt på produksjonen hos planter, ettersom det blir lite tilgjengelighet av oksygen. Dette motvirkes dersom jordsmonnet har god drenering (Chapin et al., 2011 s. 77).

Ulike naturtyper har ulik evne til å lagre karbon i jordsmonnet og biomassen. Skog er det største karbonlageret i Norge, og dekker 38% av det totale landarealet og lagrer omtrent 32% av landets karbon (Bartlett et al., 2020 s. 5). Raten for opptak av karbon i skog er proporsjonal med netto produksjon til trærne (Bartlett et al., 2020 s. 19). Produksjonen til trærne vil avta med aldring, og derfor vil eldre trær ta opp mer karbon enn de slipper ut (Bartlett et al., 2020 s. 19). Våtmark dekker kun 10% av landarealet i Norge, men lagrer likevel omtrent 21% av landets karbon (Bartlett et al., 2020 s. 5). Våtmark har høy fuktighet som hindrer jordrespirasjon og fører til redusert nedbrytning av organisk materiale, og har dermed god evne til karbonlagring (Weldon et al., 2016 s. 6). Åpent lavland er klassifisert som arealer under tregrensen og inkluderer naturlige og semi-naturlige vegetasjonshabitater. Denne naturtypen dekker 2,1% av landarealet (Bartlett et al., 2020 s. 28) og lagrer 3% av landets karbon. Tekstur på jordsmonnet vil påvirke naturtypens evne til karbonlagring (Zhang et al., 2022). Reduksjonen i karbonlagring er mer enn tre ganger så stor i jord med grov tekstur og begrenset tilgang til stabiliserende organisk materiale, enn i jord med fin struktur med god stabiliserende kapasitet (Hartley et al., 2021 s. 1).

Det er funnet at Norge lagrer store mengder karbon, men det er gjort lite kartlegging av SOC og karbon i biomasse over bakken på lokalt nivå (Bartlett et al., 2020). Naturtyper og klimatiske faktorer påvirker jordsmonnets evne til å lagre SOC, og ulik vegetasjon har ulik kapasitet til å lagre karbon. Derfor er det viktig med god kunnskap om karbonlagring på lokalt nivå, slik at forstyrrelser av viktige og naturlige karbonlagre kan hindres, og utslipp av CO2 til atmosfæren reduseres. Denne studiens formål er å kartlegge SOC og karbon i biomassen over bakken i naturtyper i Bergen og omegn. Vi tar for oss tre naturtyper i fastlands-Norge. Disse er skog hvor gran, furu, eik og andre løvtrær dominerer, åpent lavland slik som gressmark, jordbruksareal og lynghei, og våtmark som myr og torvmark. Ut fra det vi vet om klima i Bergen og omegn antar vi at det finnes høyere verdier av SOC i disse områdene, sammenlignet med andre områder i Norge med ulikt klima. Vi forventer også at verdiene for karbonlagring over bakken vil være betydelig mindre sammenlignet med verdiene for SOC.

#### Material og Metode

#### Studiested

Bergen ligger vest i Norge og preges av et mildt og fuktig klima. Den normale middeltemperaturen i perioden 19812018 var 8,4°C. Isamme tidsperiode var den normale mengden nedbør 2 511 mm (Bergen kommune, 2021). Prøvene ble tatt fra ulike lokaliteter i Bergen og omegn. Punkter i de ulike lokalitetene ble tilfeldig generert ved bruk av DarkDiv Net sitt interaktive verktøy (DarkDivNet, 2021). Lokalitene omfattet alt fra urbane områder til skog og myr.

Gjennomsnitt	Karbon (kg/kvm)	Standardfeil (kg/kvm)
Over bakken	5,40	1,26
I jorda	23,1	2,47
Totalt	28,5	2,76

Tabell 1. Gjennomsnittlig SOC og karbon i biomasse over bak	ζ-
ken for alle naturtypene.	

#### Feltarbeid

De tilfeldig genererte punktene ble lokalisert ved hjelp av GPS. Punktene var i noen tilfeller lite tilgjengelige, på privat område eller på stein eller asfalt, og de var dermed uegnede til prøvetaking. Der dette var tilfellet ble et nytt punkt markert på et egnet område så nært det originale punktet som mulig, og jordprøver og estimat av biomasse over bakken ble tatt her. Ut fra det markerte punktet merket vi deretter opp et sirkulært område på 100 kvadratmeter, med en radius på 5,64 m. Dersom det markerte området inneholdt mer enn 15 trær som var over 2 m i høyde, ble størrelsen på området redusert til 25 kvadratmeter. Dette ga en radius på 2,82 m. Dersom det markerte området nå inneholdt mer enn 15 trær, ble størrelsen på området redusert til 4 kvadratmeter. Dette ga en radius på 1,13 m. Disse reduksjonene i areal ble gjort av praktiske grunner for den videre estimeringen av biomasse over bakken. Dette fordi det kunne være vanskeligere å få et godt estimat på biomassen i områder hvor det var tett med trær. Når det sirkulære området var ferdig merket, ble det laget en enkel beskrivelse av vegetasjonen i sirkelen som besvarte hvilke og omtrent hvor mye planter og trær som befant seg innenfor det markerte området. Vi målte høyden på alle trærne i sirkelen, samt diameter ved brysthøyde (1,3 m) på alle trær over 2 m innenfor det markerte området. Karbonet lagret i trærne ble estimert for alle disse trærne individuelt basert på målt høyde og diameter i brysthøyde. Estimat av karbon i trærne er basert på funksjoner fra Marklund (1988)

Naturtype	Antall punkt
Furuskog	4
Granskog	6
Gressmark	18
Løvskog	19
Lynghei	4
Myr	2

Tabell 2. Punkter for uttak av jordprøver og estimering av karbon i biomasse fordelt på naturtypene furuskog, granskog, gressmark, løvskog, lynghei, og myr.



**Figur 1.** Spredning og median av mengde karbon lagret i biomasse over bakken i ulike naturtyper. Boksene indikerer intervallet for midtre 50% av datasettet. 25% av datasettet har lavere verdi enn dette intervallet, og resterende 25% av datasettet har høyere verdi enn dette intervallet.

for furu og gran, og Smith (2015) for løvtrær. I områder hvor det ikke var skog gjorde vi et grovt estimat på karbon i vegetasjonen over bakken, basert på en tidligere studie av karboninnhold i vegetasjon (Althuizen I et al., upublisert).

Jordprøvene ble tatt fra et punkt som hadde en representativ jorddybde for hele det markerte området. Vegetasjonen ble ryddet vekk slik at det øverste jordlaget var synlig før jordprøvene ble tatt. Et plastrør med diameter på 45 mm ble ført ned i jordsmonnet så langt ned som mulig, for så å bli trukket opp. For å sikre at jordprøven ikke falt ut av røret ble begge ender forseglet med plast. Hver jordprøve ble merket med nummeret på punktet, koordinatene til punktet og datoen jordprøven ble tatt. Etter innsamling ble jordprøvene plassert på et kjølig rom (4 °C) frem til videre bruk. Kjølelagerets hensikt var å forhindre ulike prosesser i jorda, både biologiske prosesser og fordamping av vann. På denne måten kunne vi sørge for at jordprøven forble uendret.

#### Laboratoriearbeid

Laboratoriearbeidet foregikk over flere dager. Første dag på laboratoriet gjorde vi jordprøven klar til analyse. Vi skar ut 1 cm brede biter av jordprøven, og lot det være 5-10 cm mellomrom mellom hver bit. Om jordprøven var dyp ble intervallene økt slik at det ble tatt 5 jordlag totalt. Mellom hver prøve ble kniven vasket for å unngå kontaminasjon. Bitene av jordprøvene ble plassert i rene aluminiumsbegre og markert med koordinater, prøvenummer og dybde fra jordkjernen. Deretter ble jordprøvene stående i romtemperatur for å tørke før videre analyse. Andre dag på laboratoriet ble alle bitene av jordprøver silt hver for seg (1 mm netting) for å separere røtter og steiner ut fra jordprøven. Røttene og steinene ble sortert hver for seg og deretter veid. De silte jordprøvene ble veid i et

Figur 2. Spredning og median av mengde SOC i ulike naturtyper i Bergen. Boksene indikerer intervallet for midtre 50% av datasett. 25% av datasettet har lavere verdi enn dette intervallet, og resterende 25% av datasettet har høyere verdi enn dette intervallet.



Volume 1 • page 55



Figur 3. Spredning og median av total mengde karbon lagret i ulike naturtyper. Boksene indikerer intervallet for midtre 50% av datasett. 25% av datasettet har lavere verdi enn dette intervallet, og resterende 25% av datasettet har høvere verdi enn dette intervallet.

veieskip. Organisk materiale i den massen som ikke gikk gjennom silen ble veid og antatt å inneholde 50% karbon. Hver prøve ble tørket i romtemperatur, og deretter tørket en natt i ovn ved 105°C. Mengden tørket jord ble målt og deretter brent ved 500°C i seks timer. Vi benyttet metoden "Loss on Ignition." Den brente prøven ble til slutt veid. Forskjellen på massen før og etter brenning representerer mengden karbon. Det frasilte karbonet og det brente karbonet ble lagt sammen. Det totale karbonet under jorden ble omgjort til mengde karbon per kvadratmeter.

#### Resultater

Undersøkelser tatt i Bergen og omegn viste at jordsmonnet i dybdeintervallet 0-100 cm lagrer i gjennomsnitt over fire ganger så mye karbon som biomassen over bakken (Tabell 1).

Vi tok jordprøver og estimerte biomasse over bak-

ken fra totalt 53 punkter. Disse var fordelt på ulike naturtyper (Tabell 2). Vi har valgt å kategorisere punktene der jordprøver ble tatt ut som furuskog, granskog, løvskog, gressmark eller myr, blant annet ut fra vegetasjonen som ble observert innenfor det markerte området.

Furuskog lagrer mest karbon i biomasse over bakken sammenlignet med de andre naturtypene som ble undersøkt (Figur 1). Av naturtypene som ble undersøkt ble det målt minst karbon i biomasse over bakken i gressmark (Figur 1). Under bakken ble det målt mest SOC i myr, og minst i furuskog (Figur 2). Totalt er det myr som lagrer mest karbon, med en spredning fra 40 – 100 kg C/m2 (Figur 3). Gressmark lagrer totalt minst karbon (Figur 3).

Ved sammenligning av gjennomsnittene for total mengde lagret karbon i ulike naturtyper finner en også at myr lagrer mest totalt karbon i gjennomsnitt (Figur 4). Dersom

Figur 4. Oversikt over gjennomsnittlig total karbonlagring i ulike naturtyper. I denne grafen har verdiene for furuskog, granskog og løvskog blitt lagt sammen til en kategori: Skog. Verdiene tilsvarer summen av gjennomsnittlig SOC og gjennomsnittlig karbon i biomasse over bakken for de ulike naturtypene.



en slår sammen kategoriene furuskog, granskog og løvskog ser en at skog lagrer nest mest karbon, mens gressmark og lynghei lagrer lignende mengder karbon (Figur 4). Norsk Institutt for Naturforskning (NINA) utga i 2020 en rapport som ga omtrentlige estimat for karbonlagring i norske økosystemer (Bartlett et al., 2020). Disse estimatene ble gjort på grunnlag av diverse datasett fra tidligere studi-



**Figur 5.** Sammenligning av verdier for gjennomsnittlig total karbonlagring estimert i landsdekkende NINA-rapport vist i oransje (Bartlett et al., 2020 s. 52-54) og våre funn fra Bergen og omegn, i ulike naturtyper vist i blått.

#### Diskusjon

Denne studiens formål var å kartlegge SOC og karbon i biomasse over bakken i naturtyper i Bergen og omegn. Basert på det vi vet om klimaet i Bergen og omegn antok vi at det finnes høyere verdier av SOC i disse områdene, sammenlignet med andre områder i Norge med ulikt klima. Vi antok også at verdiene for karbon i biomasse over bakken er betydelig mindre enn verdiene for SOC. Vi finner at det lagres betydelig mer karbon i jordsmonnet enn i biomasse over bakken (Tabell 1). Etter våre målinger lagrer jordsmonnet i Bergen og omegn i gjennomsnitt 23,1 kg C /m2, noe som utgjør 81% av gjennomsnitt for total karbonlagring som vi finner. Dette vil være viktig å ta i betraktning ved vurdering av eventuelle arealendringer i naturen i Bergen. Våre funn stemmer godt overens med annen forskning vedrørende karbonlagring (Bartlett et al., 2020). Det er funnet at omtrent 80% av terrestrisk karbon er lagret i jordsmonnet (Lal, 2008). Våre undersøkelser viser også at det er store variasjoner i karbonlagring mellom ulike naturtyper. Det er bred enighet om at myr lagrer mye SOC i forhold til andre naturtyper (Roulet et al., 2007; Villa & Bernal, 2018). Vi har gjort korresponderende funn; ifølge våre undersøkelser har myr betydelig mer SOC enn andre naturtyper (Figur 2). Det er også verdt å merke seg at en fant høyere mengder SOC i granskog enn i furuskog og løvskog (Figur 2). En studie i Sverige som sammenlignet SOC i plott med furu (Pinus sylvestris) og plott med gran (Picea abies) fant lignende mønster (Stendahl et al., 2010). De fant høyere SOC-verdier i plott med P. abies, også når data ble gruppert ut fra temperaturregioner (Stendahl et al., 2010).

er av karbonlagring i ulike naturtyper i flere geografiske lokaliteter. De fleste av disse studiene er gjort utenfor Norge (Bartlett et al., 2020). Ved sammenligning av våre funn i Bergen og omegn med nasjonale estimater av gjennomsnittlig karbonlagring per naturtype (Bartlett et al., 2020) finner vi at naturen i Bergen og omegn i snitt lagrer større mengder karbon i alle naturtyper som er undersøkt (Figur 5).

Temperatur og nedbør er viktige klimatiske faktorer som regulerer SOC (Wang et al., 2013). På global skala er det funnet at temperatur har en negativ korrelasjon til SOC og at nedbør er proporsjonal med SOC (Wang et al., 2013). Det er imidlertid funnet regionale unntak som står i kontrast til de globale trendene (Wang et al., 2013). Bergen og omegn preges av et vått og fuktig klima. Lave temperaturer reduserer SOC-omsetningshastigheten som fører til en økning i akkumuleringen av SOC (Liang et al., 2021). Generelt vil nedbrytningen av SOC øke ved et middels fuktighetsnivå og avta ved ekstremt tørre eller våte forhold (Liang et al., 2021). En kombinasjon av lav temperatur og ekstrem fuktighet vil begrense nedbrytning av organisk materiale, og det vil derfor være mer SOC i områder med slike forhold (Liang et al., 2021). Dermed vil mer SOC lagres i naturtypene i Bergen ettersom klima tilrettelegger for det. Våre resultater står altså i samsvar med tidligere funn (Figur 5).

Både våre estimater og de nasjonale estimatene viser at det lagres betydelige mengder karbon i terrestriske økosystem, spesielt i jordsmonnet. Dersom disse økosystemene ikke hadde hatt denne kapasiteten for karbonlagring ville effektene av klimagassutslippene vært mer alvorlige enn de er i dag. Utbygging, forstyrrelser og arealendringer vil føre til utslipp av CO2 til atmosfæren. Derfor er kunnskap om hvordan karbonlagring varierer i ulike regioner viktig. Det kan være store variasjoner i karbonlagring mellom ulike naturtyper og geografiske lokaliteter. I Norge er det begrenset med data på karbonlagring, både over og under bakken (Bartlett et al., 2020). Det kan derfor være hensiktsmessig å bruke data fra andre naturtyper og geografiske lokaliteter utenfor Norge. Det er da viktig å ta høyde for at regionale og klimatiske variasjoner kan gi et upresist estimat for norske økosystemer. Vi finner store forskjeller mellom grove nasjonale estimater som baserer seg på data utenfor Norge, og estimater vi har gjort på lokalt nivå (Figur 5). Dette viser at det er nyttig å få oversikt over karbonlagring i naturen på lokalt nivå i Norge, slik at en kan drive mer bærekraftig arealbruk.

Dersom forsøket skal gjennomføres igjen er det flere ting som kan heve metoden. Jordtyper har vesentlig påvirkning på hvordan jordsmonnet håndterer klimatiske faktorer (Zhang et al., 2022). Eksempelvis har jordtyper med mye leire dårlig drenering, som samsvarer dårlig med mye nedbør. Det hadde derfor vært interessant å ikke bare ta generelle jordprøver, men også undersøke ulike jordtyper. Ved tidligere forskning er det vist at lav pH i jordsmonn kan øke lagring av SOC (Marinos & Bernhardt, 2018; Wu et al., 2016). Det kan dermed være interessant å se på hvordan karbon oppfører seg i jordsmonn med ulik pH og eventuelt hvordan pH varierer i ulike naturtyper.

I denne studien ble det en ujevn fordeling av antall prøver for hver naturtype, for eksempel var det få prøver fra myr i forhold til de andre naturtypene (Tabell 2). I videre undersøkelser kan resultatene styrkes ytterligere dersom en fordeler like mange jordprøver per naturtype. Punktene kan fortsatt være tilfeldig generert, men fordelt jevnere mellom de ulike naturtypene. Våre resultater for karbonlagring i myr korresponderer godt med annen forskningslitteratur. Dermed antar vi at skjevfordeling i resultatene våre ikke har medført betydelig usikkerhet.

Ettersom karbonlagring er en prosess som skjer over lengre perioder hadde det vært interessant å ta jordprøver fra samme sted over lengre tid. Prosjektet kan forslagsvis foregå over flere år. Dette kan gi mer robuste dataserier som kan bidra til bedre kunnskap om karbonlagring i Bergen og omegn. Ved reproduksjon av forsøket over tid vil en også kunne få mer sikre resultater.

#### Konklusjon

Funnene fra denne studien underbygger viktigheten av å ta vare på karbonlagrene, og da særlig i områder med naturtyper med spesielt god evne til lagring. Ved å ta vare på områder som lagrer mye karbon kan vi unngå å forsterke klimaendringene. Studien vitner også om viktigheten av å kartlegge karbonlagringen på et mer lokalt nivå, da vi ser at det er store variasjoner mellom ulike naturtyper og geografiske lokaliteter. Naturtypene i Bergen og omegn har spesielt god evne til karbonlagring, antageligvis grunnet et vått klima som medfører lavere nedbrytningsrate av SOC, og naturen her er derfor spesielt viktig å ivareta.

#### Referanser

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# Opinion piece

Plum flowers (*Prunus domestica*) Photo: Silje Maria Midthjell Høydal

## Decoupling Our Future from Our Past: Reflections on Sustainable Development

Erika Johanna Scheibe

When Sustainable Development is discussed, several issues concerning the term "sustainable growth" come to the fore across scales. First, the term "decoupling" is problematic when used in reference to GDP growth and resource use/ carbon emissions (Parrique et al., 2019). Second, historical and global inequalities remain embedded in Eurocentric epistemologies such as the Modernization Theory, Progressivism and others (Graeber & Wengrow, 2021; Santos, 2012). Finally, diverse, inclusive, and plural worldviews are sometimes excluded in policies for the sustainable transition.

One definition of Sustainable Development is "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). At first, the term was a "compromise between the notions of development and conservation" (Pisani, 2006) whereas they were previously regarded as opposing ideas. Conservation was the protection of resources, and development their exploitation. The compromising term relates to Green Growth Theory, which posits a hypothetical path of economic growth that is environmentally sustainable (Hickel & Kallis, 2020). According to Sustainable Development Goal 8.1, the Sustainable Development Goals assume that Green Growth is possible. Goal 8.1 specifically aims to "Sustain per capita economic growth in accordance with national circumstances and, in particular, at least 7 per cent gross domestic product growth per annum in the least developed countries". The indicator for Goal 8.1 is annual growth rate of real GDP per capita (U.N., 2018).

Striving for Goal 8.1, which assumes decoupling GDP and resource use, carbon emissions, etc., will have many implications for the other 17 goals, Earth, and humanity to meet their needs. These Goals, which are interlinked, assume that both economic growth and environmental sustainability are possible (U.N., 2018). The word "decoupling" refers to two variables where one is driven by the other. When in reference to sustainability, it means that "environmental pressures decline without a corresponding drop in economic activities, or vice versa, economic activities rise without an increase in environmental pressures" (Parrique et al., 2019). However, several recent studies suggest that it is not possible to decouple GDP growth and resource use/ carbon emissions (Hickel & Kallis, 2020; Keyßer & Lenzen, 2021; Menton et al., 2020; Parrique et al., 2019). In a 2020 analysis by Hickel and Kallis, for example, Green Growth Theory was examined as it is modeled in the literature. They focused on feasibility and whether it could be implemented quickly enough to prevent ecological breakdown. In their meta-analysis, they found that according to every study included, under no scenario can growth in GDP be sustained indefinitely while staying within what Rockström et al. (2009) describes as the "safe operating space". Their conclusion: if policymakers continue to operate under Green Growth Theory assumptions, climate crises are unlikely to be avoided. In their discussion, Hickel and Kallis (2020) argue that due to empirical evidence and facts pointing away from the feasibility of green growth, the ecological emergency plans created by the World Bank and OECD are not sufficient. They end their discussion by saying:

"But it might well be the case that, as Wackernagel and Rees (1998) put it, 'the politically acceptable is ecologically disastrous while the ecologically necessary is politically impossible'. As scientists we should not let political expediency shape our view of facts. We should assess the facts and then draw conclusions, rather than start with palatable conclusions and ignore inconvenient facts."

Furthermore, in analyses that analyzed each country's "safe operating space" (Rockström et al., 2009), those that are closest to meeting the SDGs such as Norway and the UK are some of the furthest from environmental sustainability (Hickel & Kallis, 2020; Fanning et al., 2022). These findings suggest the importance of decoupling human well-being and other indicators of standards of living from growth.

How can human well-being, which includes environmental, social, emotional, physical, intellectual, and other aspects, be protected alongside a simultaneous decrease in GDP growth? What is human well-being, and how can it be measured? The article "Providing decent living with minimum energy: A global scenario" by Millward-Hopkins et al. (2020) takes a critical look at how human well-being is currently analyzed:

"Current socio-political organization, economic provisioning systems, and the highly unequal wealth and income distributions that exist, all influence the efficiency with which energy- and resource-use supports human well-being; inefficiencies in the system tend to become embedded within the conclusions of top-down modeling studies. Only rarely do studies look into reducing social inefficiencies that stem from consumption that doesn't satisfy human needs, or even inhibits need satisfaction... Most studies, however, look at topdown approaches... far from cultivating well-being, consumption is often driven by factors such as private profit; intensive and locked-in social practices; employment-related stress and poor mental health; conspicuous- or luxury-consumption; or simply over-consumption in numerous forms."

This quote touches on the complexity of analyzing human well-being within current systems. Later in their discussion, Millward-Hopkins et al. (2020) argue that sufficiency of human well-being (which includes different measures and indicators) and economic equality are incompatible with current economic norms, and current systematic requirements include unemployment and vast inequalities. Waste is considered economically efficient, and permanent economic growth is required for political stability. Furthermore, when sufficiency-levels of consumption do exist, they are overwhelmingly middle-class and white (such as "transition towns" and "minimalism"). While in the Global South, the wealthy have left behind millions in poverty (Millward-Hopkins et al., 2020). Several alternative indicators of human well-being are being debated. These debated indicators may better encompass values, solidarity, and diversity than GDP growth, while acknowledging the historical roots of development and colonialism.

Indigenous delegates at the fifth World Park Congress in Durban, South Africa, 2003, stated: "First we were dispossessed in the name of kings and emperors, later in the name of state development, and now in the name of conservation," (in Dowie 2009). Should the Global South try to emulate the Global North? In an article by Pisani (2006), the historical roots of the term "Sustainable Development" are discussed. Development in the Global North and South is broadly encompassed through the lens of Modernization Theory and Dependency Theory, which have many schools of thought today. Modernization Theory, simplified, is the idea that the Global South should take on Western values like progress and economic growth, and allow the market to automatically spread affluence and solve global inequalities. Dependency Theory argues that the Global South should follow a non-capitalist form of development based on their own values (Pisani, 2006). However, both of these theories were produced in the Global North and may not fully account for the dispossession described by the indigenous delegates at the 2003 World Park Congress.

Along the lines of the Dependency Theory in Pisani (2006), an article by Santos (2012) investigates how to incorporate different worldviews. Outside of Eurocentrism and colonial thought (including Dependency Theory), the Global South might find its own path forward:

"At this point, to account for such diversity involves the recognition that the theories produced in the global North are best equipped to account for the social, political and cultural realities of the global North and that in order adequately to account for the realities of the global South other theories must be developed and anchored in other epistemologies — the epistemologies of the South." (Santos, 2012)

Including diverse views and histories of the world, according to Santos (2012), may facilitate social transformation more in-line with diverse political needs in ending the inequality between the Global North and Global South. The "Global South" can be seen as a metaphor for human suffering (most of the Global South does live in the southern hemisphere, but the Global South also exists in the Global North, as seen in oppressed, silenced peoples such as undocumented immigrants, unemployed, excluded people on the basis of race, sexual orientation, ability, and many others) (Santos, 2012). Including other epistemologies may be more inclusive, because "the diversity of the world is infinite... [it] encompasses very distinct modes of being, thinking, and feeling..." (Santos, 2012), thus minorities' counternarratives could serve as a catalyst for change.

Implementing the Sustainable Development Goals has become the modus operandi for tackling the climate crisis. It is important, therefore, to critically engage with the histories and assumptions surrounding Sustainable Development, and whether proposed actions will be enough to avert climate crises. When the aspirations of minorities are translated, words such as dignity, self-governance, community, respect, care, and the good life, or amor fati, become ubiquitous, as opposed to the language of development and progress (Santos, 2012; Shiva, 1993). Therefore, it is important to co-produce knowledge and social learning that leads to flexible, resilient, and adaptive climate action (Clark et al., 2016).

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# About the authors

Honeybee (Apis mellifera) in plum flower (Prunus domestica) Photo: Silje Maria Midthjell Høydal



### Mads S. Michalsen

Mads finished his bachelor's degree in biology at UIB in 2019. He had already finished his studies in industrial engineering (NTNU) and took up Biology out of curiosity and personal interest for the field. Mads is now living in Oslo, where he works as a management consultant for PwC.

When we asked what motivated him to write about this subject, Mads answered: "The question of why sex (specifically gendered reproduction as a strategy) is prevalent, is a theme I wished to explore because it represents one of my favorite problems: the type where the answer at first seems obvious, but on closer look is a real mystery – in this case an evolutionary riddle. It is also fun to review a field which has a century old history, but where there still remains lively debate and no consensus has been reached. This was also a chance to exercise the practice of robust, evolutionary logic."



### Susanne Zazzera

Susanne is a 23-year-old student from Stavanger who is currently doing her master in "Evolution, ecology and biodiversity" at UiB. She chose this study programme because she finds it interesting and believes that it is of great relevance for many of the problems we humans face today. She says that increased knowledge and awareness in this field is essential to achieve a sustainable development. When she graduates, she hopes to find a job where she can contribute to this development and further communicate the consequences of loss of biodiversity.

When asked about her paper, she answered: "The text is based on an assignment I wrote in BIO233 - Insect diversity and biology. The assignment was to write a comment on a paper by Crossley et al. (2020), "No net insect abundance and diversity declines across US Long Term Ecological Research sites." Writing the paper gave me a deeper understanding of why insects are important to maintain functional ecosystems, and how useful their services are for us humans. Many of their services are often taken for granted, but are key components for our survival and well-being. After reading my essay I hope you too see the fascinating wonders of these strange, but helpful, creatures. The process of rewriting this text as a Bikuben publication has been an educational process for me, something I truly appreciate!"



### Iliana-Vasiliki Ntinou

I am 24 years old and I am finishing my master's degree in Marine Biology at the University of Bergen. My research interests include marine microbial organisms like phytoplankton and their ecological role in the changing marine environment. I am currently working on my thesis about the variation in the elemental composition of particulate matter and the microbial community in the Northern Barents Sea which is part of the *Arven etter Nansen* research program.

The text for Bikuben was originally written for the course BIO299 Research Practice in Biology at UiB during the spring semester of 2021, where we studied the elemental composition of particulate matter and phytoplankton biomass in Store Lungegardsvannet and Puddefjoren together with my supervisors, professor Jorun Egge and researcher Tatiana Tsagaraki. I was very excited and intrigued when we discussed the opportunity to get a better look at the coastal environment of Bergen, not only because it falls within my scientific interests, but also because it is directly linked to our society as most residents are concerned about the water quality of their city. You are very welcome to read my blog "Dark waters in Bergen" to find out more about the background story of the area.



### Hannah Røkke

Hannah is 24 years old and grew up in Nittedal, Viken. She is about to finish her second semester in the master program "Fisheries Biology and Management". The coming semesters she will be writing her thesis on genotype dependent growth and selection in herring larvae at different temperatures and light regimes.

When asked what inspired her to publish, she answered: "This text is based on a report I wrote for my BIO299 – project, which I did during my final semester as a bachelor student. I spoke to my supervisor about how I would like to write about something exciting that I could learn something new from and was then assigned this project. This was a great opportunity for me to work independently at the lab, and to see if this was the kind of work that I could see myself doing in the future."



### Donna Bertrand

I am a 22-years-old student from Brittany in western France. I entered Institut-Agro-Rennes, a French higher education institution of agronomics, to become an engineer in 3 years. After the first year of core curriculum, we have a 6-month internship and for the last year, we choose one of 13 specialization, before getting the engineering diploma. I am currently in the second year which is equivalent to the first year of a master's degree. I was interested in two specializations: ethology and the political field that deals with environmental issues. In order to choose, I took advantage of the 6-months internship to learn more about these two fields. Thus, I went to Malta at the Ministry for Environment and then to Norway at the University of Bergen. Working in Bergen alongside Siri Haugum lived up to my expectations. It enabled me to carry out a real project, to study animal behavior under natural conditions, to work with others and to write a scientific paper. I find studies like this important because they help to preserve the fauna and flora, and to raise awareness of environmental issues. I will always remember this enriching opportunity even though I completely changed my mind and finally chose my specialization: Urban Green Space Engineering.



## Gina Koulibaly Barry, Vilde Engelsen, Sofie Klem, Johanne Kuhle, Hanna Sannes og Tor Værøy

We are Gina (20 years old from Harstad), Vilde (22 years old from Os), Sofie (21 years old from Oslo), Johanne (22 years old from Bærum), Hanna (22 years old from Bryne), and Tor (22 years old from Stord). We are all finishing the second year of a bachelor's in biology at UiB. Within our author group there are varying academic interests, but we are all engaged students that are interested in climate and sustainable solutions for use and management of nature.

Our report is from a project in the introductory course BIO102 during the autumn of 2021. The project was carried out at request from the municipal climate agency in Bergen, which wanted to map out natural carbon storage in Bergen and surrounding areas. While working on this project during the course, we understood how little data there was regarding the subject, and we were therefore thrilled when John-Arvid Grytnes (course leader of BIO102) asked if anyone could write a summary report of all the results from the course.

After reading this report, we hope that you will have a broader understanding of carbon storage, and how disruption of natural habitats can affect the climate. Additionally, we hope to highlight the value in preserving the carbon storages in Bergen. This has been an educational experience, where we have received experience in academic writing and the peer-review process early in our study program.

We want to thank all the students from BIO102 for data collection and lab work, as well as Jon-Arvid Grytnes, Siri Vatsø Haugum and Kristine Birkeli for being great advisors.



### Erika Johanna Scheibe

My name is Erika and I'm originally from Idaho. My academic background includes a B.S. in Biology from The College of Idaho, Master's coursework in Sustainability through the University of Graz with a semester at Hiroshima University in Japan, and now I'm in my first semester for my Master's in Ecology, Evolution, and Biodiversity here at the University of Bergen. I've also completed internships with the National Museum of Natural History, Smithsonian Institution in D.C., where I worked on a project involving DNA barcoding and endangered species, as well as with The University of North Dakota.

I have a broad range of academic interests, supported through my three minors, including Psychology, Technical Skills in the Natural Sciences, and Art, that I completed during my Bachelor's. Recently, I have been reading articles in journals related to the field of Political Ecology. I wrote my piece, "Decoupling our Future from Our Past: Reflections on Sustainable Development" in order to assimilate some of the conflicting visions I've come across related to this important albeit contentious topic. I hope to continue to learn more about Sustainable Development during my time here in Bergen.

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Walrus (*Odobenus rosmarus*) Photo: Johanne Kuhle







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