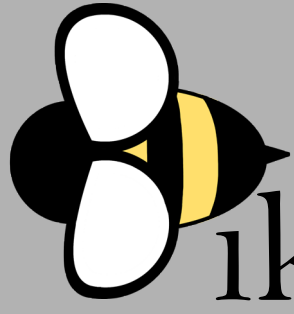


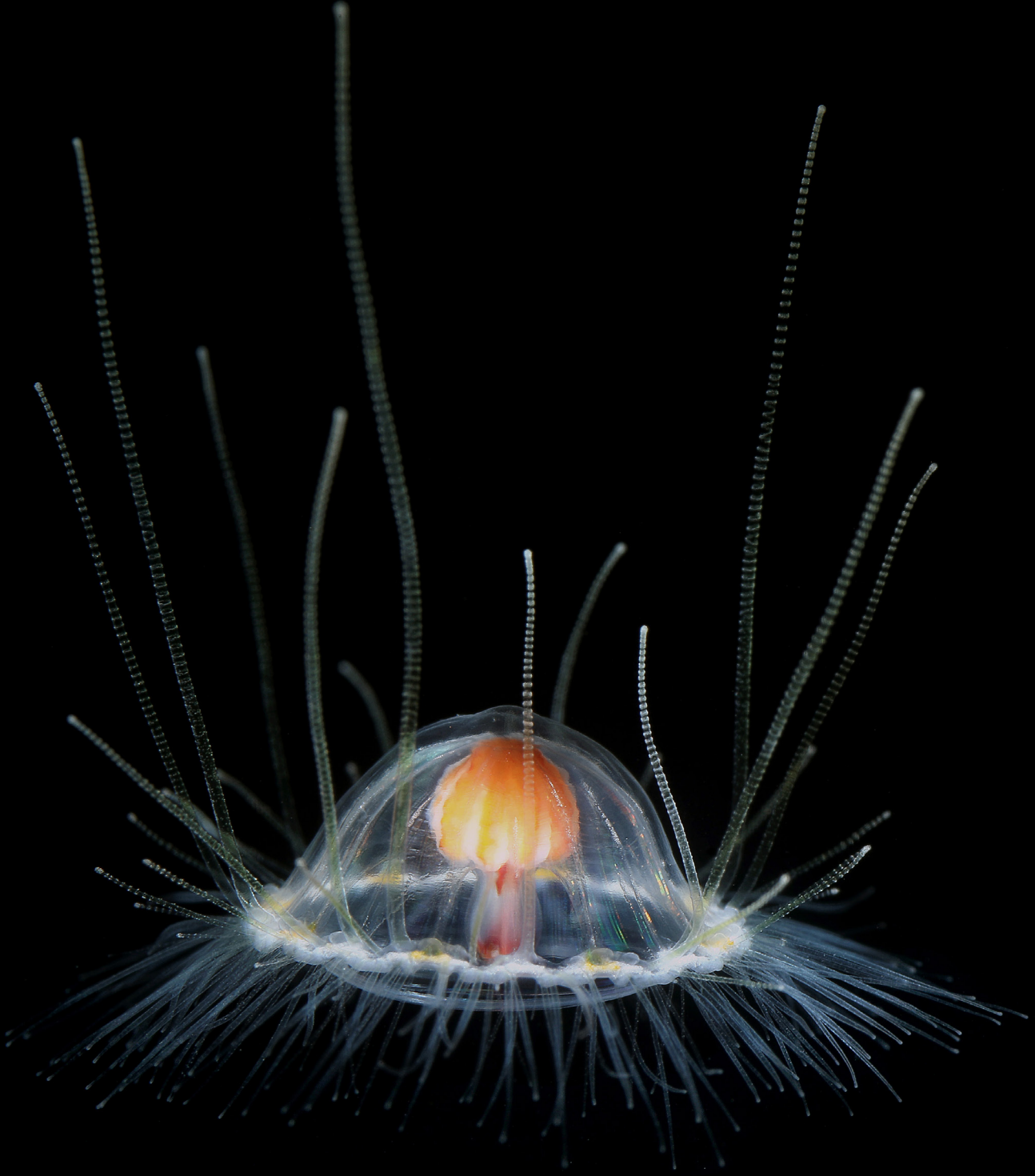
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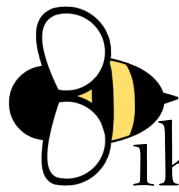
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ikuben Student Journal

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.

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The editors' corner

Dear Bikuben Reader,

While echoing the famous phrase of Neil deGrasse Tyson: “The good thing about science is that it’s true whether or not you believe in it”, we extend a warm welcome into the second volume of Bikuben Student Journal. As you navigate these pages, you are entering a world where discussions, methodologies, and captivating images have been produced with passion and dedication by numerous students here at the Department of Biological Sciences, University of Bergen.

Through these pages, a broad spectrum of scientific exploration awaits you – a testament to the shared enthusiasm that fuels our scientific community. The combined efforts of our dedicated crew and contributors have rendered this second volume of Bikuben not only exceptionally unique but also undeniably significant. As these words are written, the arena of political debate is amplifying, and the culmination of the municipal election is merely a month away. Science and the urgent topic of climate change stand at the front line of this troubled landscape. Regrettably, scientists are increasingly being manipulated to serve the agendas of politicians and vested interests. Following the recent extreme weather event “Hans” in Norway, and the raging fires ravaging Europe and other corners of the world, it has become ever more imperative for us in Bikuben to ensure the proper and honest presentation of scientific endeavors.

As we return to Tyson’s famous wise words, we confront the challenging reality that even the soundest scientific evidence can face skepticism, misunderstanding, and even manipulation by those who prioritize their own agendas. In the face of such challenges, our dedication to fostering honest and reliable scientific communication remains steady. We encourage all our readers to keep celebrating and engaging in the wonders of science; Use the opportunity of Bikuben to share your academic productions with a broader audience. Ask big questions. Seek answers. Convey reliable results.

We, the editors of Bikuben, wish to extend our sincerest gratitude to our authors, reviewers, faculty advisors, designers, and photographers. The outcome of this second volume has left us beyond proud and grateful, and it would not have been possible without any of you. And last but certainly not least: many thanks to the team at bioCEED who have been there with us every step of the way, guiding and helping us whenever we wanted it, but most importantly when we needed it.

Happy reading!

Silje Maria, Hanif & Gina



Review



Lycaena sp.
Photo: Maria Ulvang

Examining Microalgae as a Substitute to Soybean in the Production of Aquafeed

Hannah L.A. Gaustad, Oda Bellika Kjæmpenes and Paulina Pokusa

Introduction

Soybeans are a valuable resource for aquaculture because they can be used in aquafeed. However, some aspects of soybeans are problematic. Due to the issues associated with soybean cultivation and the final soybean-based product, microalgae could be a potential substitute for soybeans which is examined in the following review. Microalgae have several beneficial attributes which make them suitable for aquafeed, for example, balanced nutrition profiles and ease of cultivation. The following review focuses on the attributes of microalgae in aquafeed, microalgae cultivation, and the impact it would have if microalgae were applied in aquafeed.

Soybeans in Aquafeed

Soybeans are a versatile and valuable product in aquaculture, because of their nutritional and protein profiles that make them an ideal component of aquafeed (Park et al., 2017; Shea et al., 2020). Even so, there are two major obstacles that need to be accounted for when applying soybeans in feed – they are methionine deficient and contain trypsin inhibitor proteins. Methionine is an essential amino acid, making it a vital component of feed. If the concentrations of methionine are insufficient, the feed has to be supplemented with synthetic alternatives (Shea et al., 2020). The presence of trypsin inhibitor proteins is also detrimental because they inhibit trypsin, an enzyme that is important for protein metabolism (S.S., 2018). In order to overcome this issue, the product would need to undergo an additional processing step to render these inhibitors harmless (Shea et al., 2020). Other issues associated with soybean production are environmental, societal, ethical, and economic controversies, such as the questions raised about monoculture cultivation, slash-and-burn culture, uncertainty surrounding crop yield, and the industrial structured treatment of producers (Bicudo Da Silva et al., 2020; Edivaldo & Rosell, 2020; Miransari, 2016).

Despite these issues, soybeans remain a highly valuable source of aquafeed. In Norway, 800,000 tons of soybeans were used to produce aquafeed in 2017, which corresponded to 0,2% of the world's total production (NHO, n.d.). This soybean-based feed also requires supplements from marine raw materials such as fish meal, which consists of ground-up fish (Hwang et al., 2014; NHO, n.d.). Whilst discussing this somewhat imperfect product, one might wonder whether there is a substitute where sustainability issues such as monoculture cultivation are avoidable. One possible alternative would be microalgae, which has been established as a strong contender for aquafeed within aquaculture. Not only would it replace finite resources such as fish meal and soybeans, but according to Ingmar Høgøy, the CEO and Chairman of the Board of AlgaePro, it could actually increase the quality of feed (personal communication, 29.10.2021). It has been demonstrated that microalgae-based feed provides several benefits, both animal welfare such as stress resistance and immunity, and consumer quality traits such as pigmentation increase as the fish were fed microalgae-based feed (Fernández et al., 2021).

Microalgae - a sustainable alternative to soybeans

Microalgae are unicellular organisms that can be either auto-, hetero-, or mixotrophic, which means that they obtain nutrition by their own means, through organic compounds, or both, respectively (Pereira et al., 2021). A subtype of autotrophic microalgae, the photoautotrophs, is the general focus of the following examination. Photoautotrophic microalgae convert inorganic materials, such as water, light, and carbon dioxide (CO₂), into oxygen (O₂) and glucose through photosynthesis. Water and CO₂ are easily accessible because they are found in the environment where they are available for absorption. The light source for microalgae growth can be either artificial or solar; the solar light intensity varies depending on the time of day,

weather, and season (Abu-Ghosh et al., 2016). The growth kinetics of photoautotrophic microalgae relies on the availability of carbon, which is mainly derived from CO₂ (Hwang et al., 2014). In addition to inorganic compounds, macro-, and micronutrients, such as sodium, phosphorus, nitrogen, magnesium, calcium, and potassium, are also necessary for optimal microalgal growth (Khan et al., 2018). These might be sourced from fertilisers.

There are many types of microalgae, and several factors determine whether a strain is suitable for aquafeed, e.g., cultivation ease and nutritional content. Protein, vitamin, and unsaturated fatty acid content are especially important to determine the nutritional worth of microalgae (Colombo et al., 2020; Hemaiswarya et al., 2011). To fulfill the nutritional needs of aquafeed, it might be necessary to combine several algae strains because the vitamin profiles of one single algae strain might be insufficient. In addition to covering vitamin needs, the combined strains could balance out other nutritional compositions which would improve animal growth (Roy & Pal, 2015). Optimal fish development and growth rely on a balanced protein intake. The nutritional value of a protein source is considered high if it consists of amino acids that coincide with those that the feeding animal requires (Ammar et al., 2020; Roy & Pal, 2015). Fatty acid composition and concentration also appear to greatly impact the growth of the animal, and therefore, the lipid contents of the aquafeed biomass are vital (Patil et al., 2007). In addition, other factors, such as cultivation systems and growth, processing steps, risks, and potential improvements, should also be evaluated.

Microalgae cultivation and its challenges

Microalgae cultivation has the potential to grow into a powerful industry because it can be used to create a plethora of products. Some microalgae-based products include feed, medicine, pigments, and biogas fuel (Araújo et al., 2021). In order to fully grasp the potential of microalgae, cultivation means and challenges related to biomass production need to be assessed.

Cultivation of microalgae may take place in either open or closed systems. Determining which of these systems to employ is vital when it comes to biomass yield. There are both positive and negative aspects of either system, and the selection largely depends on external factors. The open and closed systems depend on natural light and the availability of the necessary technology. Internal factors, such as which strain of microalgae is grown, also affect this decision (Fernández et al., 2021). Open systems do not require as sophisticated technology as closed ones. However, they are more exposed to external factors such as precipitation, contamination, pH change, CO₂, and O₂ supply, temperature, nutrient availability, and light, which results in low system control (Fernández et al., 2021). Closed systems are more

robust when it comes to these elements because they are not as exposed to their environment, and the parameters mentioned above can be rigorously regulated due to the complexity of these systems (Fernández et al., 2021; Ruiz Gonzalez et al., 2016). This rigorous control is the reason that the closed system is considered to be more efficient, as it is able to overcome challenges such as high temperatures or oversaturation of O₂ (Ruiz Gonzalez et al., 2016). Yet, the yield of closed systems needs to be substantial enough to cover the building and operation costs, which means that it might be less sustainable than open ones (Fernández et al., 2021). Observations made in Spain have shown that the open system has a productivity level of around 27 tons ha⁻¹year⁻¹, while the productivity of the closed system is between 34-60 tons ha⁻¹year⁻¹, indicating that the closed systems result in a higher biomass yield (Ruiz Gonzalez et al., 2016). Closed systems are also beneficial when it comes to risk mitigation, as they have a lower risk of spillage. The consequences of spillages from large-scale facilities are elusive, and both the short- and long-term impacts of spillages need to be assessed. Some of the risks that need to be considered are whether the spillage will introduce invasive species, and how this will affect the surrounding ecosystem (Gressel et al., 2013). There are several means of risk mitigation that reduce the effects of a spillage, for example, a so-called safety switch in which the species that is being cultivated cannot survive in the surrounding environment (Asveld et al., 2019).

Another important fact to consider when selecting a system is which microalgae to grow, because different strains might require different growth conditions (Barkia et al., 2019). There are also major constraints that regulate the cultivation of certain microalgae. Administrative issues are one such constraint, and an example of this is the process required to cultivate species that have not been grown commercially yet. To get permission to grow these strains, there is a consulting process, in agreement with Novel Food regulation (EU), which is an expensive and long process. Another issue is the fact that microalgae vary greatly in their composition. This leads to a divergent potential for deriving functional compounds such as pigment, long fatty acids, antioxidants, and polysaccharides, and as a result, one microalga might not satisfy the end goal of the final product (Araújo et al., 2021). This issue could be overcome by mixing strains or genetically modifying a strain. However, both approaches can be problematic, and especially gene modification is highly regulated by international laws, such as "Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed" (European Union, 2003). Cultivation of genetically modified microalgae would also increase the risk that is associated with large-scale spillage, because the modified algae might spread and cause irreversible effects on local ecosystems, for example by out-com-

peting native species (Beacham et al., 2017). To mitigate the effects that could occur due to spillage, genes that make the organism unfit for the surrounding habitat can be introduced alongside the desirable genes (Gressel et al., 2013).

A final issue that needs to be assessed is the harvesting step, which is considered to be one of the more costly steps (Pereira et al., 2021). Microalgal growth can be described as a cycle in which the growth has a lag, log, deceleration, stationary, and death phase. When introducing nutrition into the cultivation culture, the time duration of the lagging phase decreases. Hence, microalgae grow faster and obtain an optimum concentration, where it is at its most efficient point in the cycle. Once the optimal concentration is oversaturated, the growth rate decreases (Vasumathi et al., 2012). The goal of the harvesting step is to extract biomass from the culture medium to obtain as much high-value microalgal biomass as possible. There are several different techniques that allow the high-quality yield of biomass, such as electrical, chemical, biological, or physical. Still, there are disputes about which harvesting method is the most effective one (Mathimani & Mallick, 2018).

Applying food waste as a feedstock for microalgae is one addition that could make the industry more sustain-

able both economically and environmentally. Food waste has been shown to be a valuable option as a feedstock in microalgae cultivation, because of its rich and favourable nutrients, such as nitrogen, glucose, and phosphate (Pleissner et al., 2013). It is also a large and underused resource as there are about 88 million tons of food waste in the EU per year (approximately 173 kg per person), which represents about 20% of the food produced. Almost 70% of the food waste comes from households, food services, and retail stores, and the remaining percentage comes from the production and processing sectors (European Science Foundation, 2020; Sanches Lopez et al., 2020). If food waste was to be used as a feedstock, it could replace fertilisers, which are more expensive and less sustainable to use in large-scale microalgae cultivation (Usher et al., 2014). Cultivation on wastewater is also beneficial because organic wastewater from industries such as dairy and meat production cannot be added directly into freshwater sources due to eutrophication. However, this could be mitigated by using the wastewater in cultivation prior to releasing it (Ummalyma et al., 2022). This would promote a circular economy in which food waste collection and processing is connected to aquaculture, promoting industrial symbiosis (Figure 1). Yet, the application of food waste in microalgae

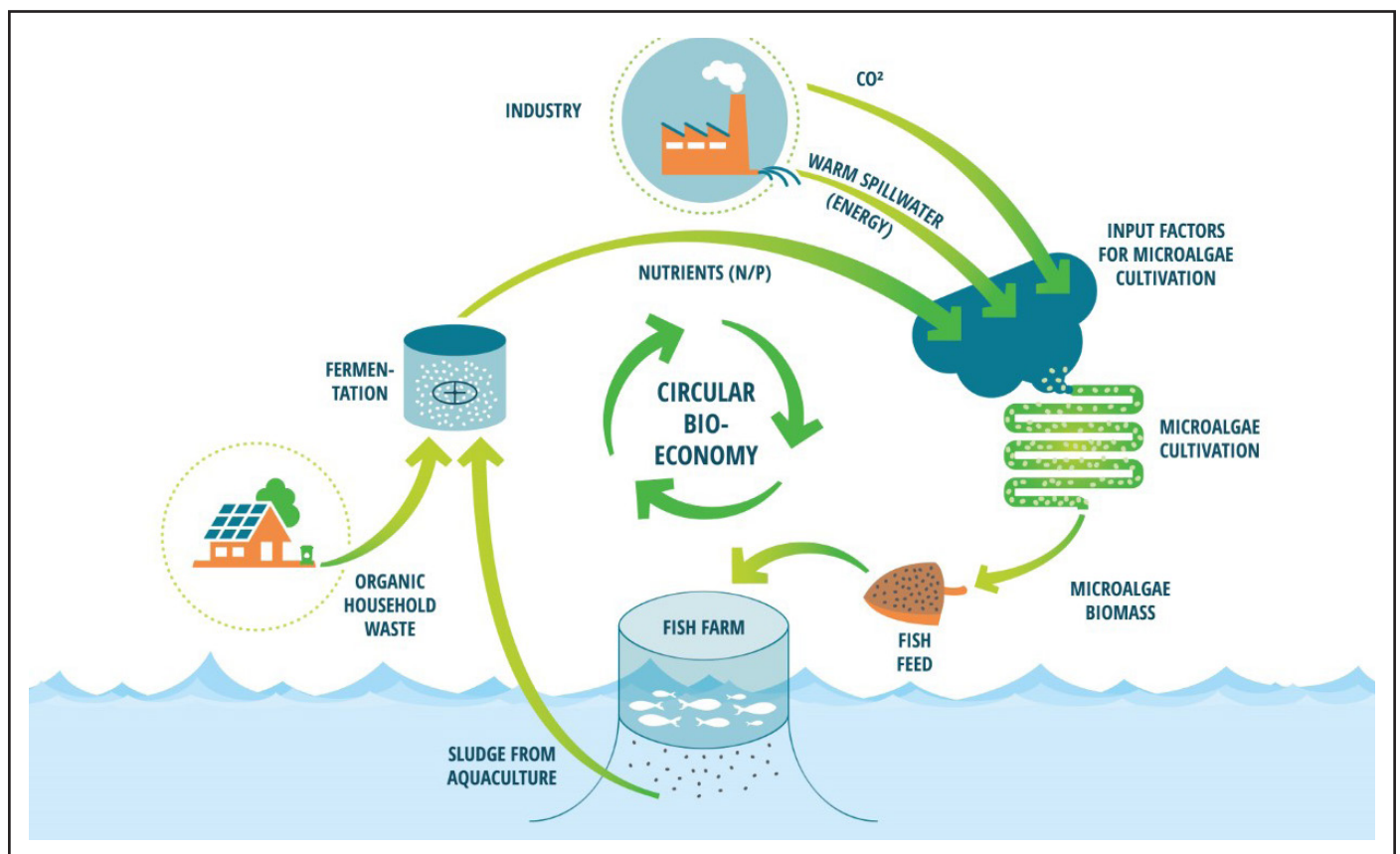


Figure 1. Circular bioeconomy. The figure was provided and given permission to use from AlgaePro, 2022. Household waste and other sources of waste, as well as bi-products from other industries, can be funnelled into aquaculture with microalgae-based aquafeed as a steppingstone. This would be more sustainable and could also cut down costs.

cultivation can be challenging due to its collection, contamination, and processing. These can be overcome by developing a system in which food waste is carefully sourced from households and industries, to then be processed by fermentation or other processes that allows the waste to be circulated back into production processes. According to Toralf Igesund, who is the FoU leader from BIR, this would be more sustainable than disposal by combustion or deposition, because reusing food waste results in reduced emission and yields applicable products (personal communication, 05.11.2021).

In addition to funnelling food waste into cultivation, the cultivation of microalgae in general requires development in both economic and technical departments. Producing biomass is very expensive, due to both the resources and technology it requires, making it less economically sustainable (Pereira et al., 2021). The cultivation means are based on laboratory-scale studies, and up-scaling might be complicated, time-consuming, and could require government funding. Up-scaling will also necessitate stricter risk assessment because safety and environmental effects on a smaller scale might not coincide with the risks of a larger facility. The increase in risk might result in more stringent legislation that restricts cultivation facilities and the introduction of microalgae strains. In addition, the market for algae-based products needs to grow to make room for large-scale cultivation. Therefore, the future of cultivating microalgae depends on the government, researchers, and industries (Agency, 2020).

Environmental, societal, and economic impact

The impact of the research in algae cultivation affects not only the environment, but also society, economy, and science both in the long- and short-term, regionally as well as internationally. The growth of the algae industry can be seen in the context of the UNs Sustainable Development Goals (SDGs), and it will especially have an impact on the following goals: SDG number 9 “Industry, innovation, and infrastructure”, SDG number 12 “Responsible consumption and production” and SDG number 15 “Life on land” (FN Sambandet, 2021).

Environmental impact

Both positive and negative outcomes need to be examined when discussing the environmental effect of microalgae cultivation. One of the advantages of using microalgae is that they are capable of CO₂ fixation by photosynthesis (Pereira et al., 2021). Other benefits include the fact that algae do not require freshwater to grow, as they can be grown in brackish waters, and water that is left over from industrial processes in both food cultivation and dairy production (Ummalyma et al., 2022). Using bi-products from industries as a resource promotes a circular economic stance,

which can be more ecological because these bi-products are being reused to yield another product - this is a step toward reaching the UNs SDG number 12: “Responsible consumption and production” (Compass, n.d.). There are also negative aspects related to the use of CO₂ in algae cultivation, which could potentially harm the environment. CO₂ demand and the need for fertilisation drive up the ecological footprint of algae cultivation; this burden can be lightened by using wastewater or food waste as a fertiliser (Usher et al., 2014). However, there are several aspects that need to be considered when food waste or wastewater is applied for cultivation. One example is that the characteristics of the wastewater affect the choice of cultivation system, microalgae, and final appliances of biomass. It is also important to evaluate the source of the wastewater, where water from food industries is considered less hazardous than water from other industries as it does not contain toxic metals or other toxins (Ummalyma et al., 2022). An additional problem associated with CO₂ demand is that it is not readily available as a substrate due to challenges related to dilution in water, as well as the uncertainty regarding how much CO₂ microalgae actually fixate. Some articles state that algae’s fixation of CO₂ decreases the atmospheric concentration, whilst others argue that algae might release more CO₂ than they absorb (Clarens et al., 2010; Pereira et al., 2021). These problems associated with large-scale cultivation suggest that cultivation might contribute to emissions at an unknown scale. However, some of these issues may be reduced by alterations made in production protocols and by applying regulations that mitigate the chance of these mishaps.

One subgoal of SDG number 12 is to reduce food waste per capita by half and to reduce food loss during production by 2030. By funnelling food waste into microalgae production, the amount of unprocessed waste can be sustainably lowered (Compass, n.d.). According to Toralf Igesund, the application of food waste in industries would decrease pollution related to waste disposal, such as methane gas from deposition or gases released during combustion (personal communication, 05.11.2021). In addition, it could also decrease the net amount of food waste that winds up in landfills (Pereira et al., 2021). Another advantage is that microalgae do not require fields to grow, meaning that they do not compete with food crops, terrestrial plants, or other native species. In contrast, other aquafeed sources such as soybeans require large fields of land which negatively affects biodiversity because native plants and insects might not thrive in these monocultures that are likely treated with pesticides (Wright et al., 2021). Since the microalgae industry competes and provides higher yields than terrestrial feed industries, it will likely impact “Life on land” (SDG number 15) because it might free up terrestrial areas.

Societal impact

Examining the societal effects of the microalgae industry can be better understood within the framework of how the aquaculture industry affects the labour market and smallholders. Development within the aquaculture industry is largely driven by commercial objectives, and when clusters of production and processing become prosperous, job opportunities may increase. This industrial growth also increases competition, which in turn may lead to higher product quality. However, it can also negatively affect smallholders because they cannot compete with large-scale producers. The fact that large corporate entities control the seafood industry negatively impacts small manufacturers which generate the best opportunities for rural communities (Belton et al., 2015; Little et al., 2016). Algae-based aquafeed would compete against other aquafeed sources, which could generate job opportunities within the algae industry but could decrease possibilities within other aquafeed industries such as soybean cultivation.

Since algae depend to some degree on the environment, the growth of industries will likely be local where enough water and sunlight are easily available. According to the EU Blue Bioeconomy report from 2019, this industry has great potential for the development of employment in coastal and remote areas (JRC, 2020). The decline of monocultures in this industry may also have a positive impact from a socioeconomic viewpoint. Since aquafeed production will not rely solely on soybeans, it might enhance the availability of products and raw materials.

The rise of microalgae cultivation would likely affect other niches than aquafeed, and products such as bio-oil and other algae-based products would likely be integrated into the current market. Applying food waste would also affect society by changing how waste is handled, both on an industrial and personal basis because traceability of waste funnelled into production is vital. According to Toralf Igesund, traceability from households can be challenging, but tracing waste delivered from larger food facilities is fully possible and can be done in accordance with the regulations of the Norwegian Food Safety Authority (personal communication, 05.11.2021).

Economic impact

Modernization and building new infrastructure for the future of microalgae cultivation also impacts the economy and is a step toward SDG number 9: "Industry, innovation and infrastructure". The EU algae sector has an annual turnover of €1.5 billion as stated in the EU Blue Economy report of 2019 (JRC, 2020). According to Ingmar Høgøy, applying food waste in aquafeed production has an estimated turnover of 1.3 billion tons yearly, which highlights the potential for economic growth within this industry

(personal communication, 29.10.2021). Another economic improvement is the fact that this industry, through its development within technology and competence, will stimulate other industries. If industrial symbiosis and regional clustering are achieved, economic strength would increase because it would allow several industries, such as food waste management, aquafeed, and aquaculture to grow together, and costs included in storage and transportation could be reduced. According to Toralf Igesund, establishing facilities close to each other could stimulate a circular bioeconomy, which again would strengthen the region where these industries are being settled (personal communication, 05.11.2021). This might make production more efficient and sustainable, as well as open the labour market regionally.

Summary

All things considered, microalgae show potential as a source of aquafeed and are likely to gain a stronger foothold in the aquaculture industry as it develops. However, it is important to keep the risks and regulations of microalgae cultivation in mind since it will likely impact how this field develops. The evolving industry of microalgae production might cause challenges for industries that normally filled niches such as soybean-based aquafeed production. Nevertheless, the foothold of these enterprises may be strong enough to withstand the competition which might cause stimulation of the industries to provide better products.

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Syrphidae
Photo: Thomas Alexander Bondevik

The Hidden Pollinators of Inner Hardanger: a Study in Hoverfly (Syrphidae) Diversity, Abundance, and Phenology

Sara Rodrigues de Miranda

Abstract

Hoverflies (Syrphidae) are a family of flies of the Diptera order that provide a range of ecological services to agriculture, including pollination services. Most crops are reliant on animal-plant interactions to aid their pollination, including those intended for human consumption such as apple trees. While Hymenopteran species are the most well-known insects that provide pollinating services, hoverflies are the second most important pollinators. However, hoverflies are often partly overlooked in scientific research, commonly only identified to family. Little is therefore known about the diversity, abundance, and phenology of this family of insects. This report will give an overview of what hoverflies are, their importance to agriculture, and subsequently what services they provide to aid pollination and ecosystems. A description of species diversity, abundance, and phenology in inner Hardanger will be provided. Additionally, this text will discuss i) the differences in hoverfly diversity of different sites in inner Hardanger, ii) what hoverfly diversity tells us about the ecological integrity of these sites, and iii) the phenology of hoverflies in Hardanger and what the differences in species phenology can tell us about their species-specific importance as apple flower pollinators in apple orchards in Hardanger. The data from this report has been collected in connection with the AP-LECore project, specifically for Silje Maria M. Høydal's master thesis on bee pollinators in apple orchards in Hardanger, Norway. Hoverflies were not the intended insects for capture in this project, and it is therefore noted that the methodology is not streamlined for the capture of hoverflies.

Introduction

Pollination is an ecological service that is essential to the success of agricultural practices and is provided by a range of different animals. Amongst animals that provide polli-

nating services, insects are the largest and most important group (Doyle et al., 2020). Pollinating insects are insects that aid in the fertilization of plants (Berner & Sunding, 2021). This can occur in differing ways, the most common being a visit to a flower by an insect foraging for nectar or pollen (Berner & Sunding, 2021). The insects most famous for pollinating services for fruit-bearing plants are insects of the Hymenoptera order and the flies of the Syrphidae family. Although many plants do not require insect-specific pollinating services, up to 76% of crops intended for human consumption are reliant on animal-crop interactions to aid the pollination process (Bates et al., 2011; Doyle et al., 2020). Agriculture is a fundamental part of food production and has been a part of the foundation of modern societies. For the region of Hardanger, the production of apples has been a cornerstone of their economy and history for hundreds of years (Thorsnæs, 2021).

There are several factors that contribute to effective fruit yield, an essential one being the success of pollination during the spring (Ramírez & Davenport, 2013). Fruit trees, including apple trees, are reliant on animal-plant interactions, in particular visitations of insects on their flowers to aid their pollination (Berner & Sunding, 2021; Ramírez & Davenport, 2013). One of the reasons why apple trees specifically need insect visitations is due to insects being effective cross-pollinators, on which apple trees are reliant (Berner & Sunding, 2021; Ramírez & Davenport, 2013). Cross-pollination is a process in which pollen is transferred between the main cultivar and a polliniser cultivar (Berner & Sunding, 2021; Ramírez & Davenport, 2013). Farmers have long practiced keeping colonies of pollinating insects in the vicinity of their crops to aid pollination (Delaney & Tapy, 2008). Honeybees (*Apis mellifera*, Linnaeus, 1758) is a species of social bees which have been domesticated and is amongst other things used for pollination of com-

mercial fruit trees (Delaney & Tarpy, 2008). However, recent research shows that the pollination services provided by wild pollinators is more effective than those provided by domesticated bees (Bates et al., 2011; Berner & Sunding, 2021; Doyle et al., 2020). Wild pollinator diversity and abundance is therefore important for the increase in crop yield in apple orchards (Bates et al., 2011; Delaney & Tarpy, 2008; Ramírez & Davenport, 2013).

The Diptera order is amongst the most important wild pollinators. In fact, by regularly visiting 72% of crops, it is the second most important pollinating wild insect (Boyle & Philogène, 1983; Doyle et al., 2020). Hoverflies (Syrphidae) are a family of the Diptera order, which represent 52% of crop visitations attributed to all flies, displaying the importance of this family for the pollination of apple trees (Doyle et al., 2020; Ottesen, 2021; Ramírez & Davenport, 2013). They are popularly known for their Batesian mimicry of the insect order Hymenoptera which includes bees, bumblebees, and wasps (McLean et al., 2019; Nottingham, 2000; Penney et al., 2014; Wilson et al., 2013). Hoverflies are abundant and diverse in Norway and are known to visit many of the same fruit plants as bees (including bumblebees) (Ball & Morris, 2015; Bengtson et al., 2022; Djellab et al., 2019). Furthermore, the larvae of the subfamilies Syrphinae and Eristalinae have other ways to contribute services to their ecosystems; Syrphinae larvae are a natural enemy of the crop pest's aphids (Aphidoidea) and have showed to lower pest populations by 70%, whereas the saprophagous Eristalinae larvae are known to decompose dead plant matter (Ball & Morris, 2015; Djellab et al., 2019; Doyle et al., 2020). Because of the aforementioned, they are an asset to their surrounding environments throughout their entire life cycle (Ball & Morris, 2015; Djellab et al., 2019; Doyle et al., 2020).

As a highly migratory species, hoverflies will travel great lengths throughout the year, providing a large geographical spread of pollinating services that widen ecological gene pools significantly (Doyle et al., 2020; Wotton et al., 2019). Throughout their life cycle, hoverflies make use of a wide range of habitats, and it has been shown that they thrive in specific microhabitats (Ball & Morris, 2015; Bengtson et al., 2022; Gittings et al., 2006; Lucas, 2017). They often make use of various forms of faeces, composts heaps, tree bark and herbaceous plants for laying eggs (Ball & Morris, 2015; Bengtson et al., 2022). Adult hoverflies that will specialize in a single plant type are typically early season fliers (Lucas et al., 2018). Of species that fly during late summer months, only a small portion are generalists that will inhabit a range of different types of habitats (Lucas et al., 2018). As such, these insects are often uniquely suited to correlate species diversity with ecological diversity (Ball & Morris, 2015; Bengtson et al., 2022; Gittings et al., 2006). Because of the close connection between hoverflies

and specific plant types, hoverfly diversity can thus directly tell us vital information about the ecological integrity of an ecosystem (Djellab et al., 2019). Hoverflies undergo full metamorphosis, and have four main life stages of egg, larvae, pupae, and adult (Ball & Morris, 2015; Bengtson et al., 2022). The lifespan of most adult hoverflies is about 35 days, and they seldom become active at temperatures below 15°C (Ball & Morris, 2015; Bengtson et al., 2022). As conservation strategy, they will therefore spend up to nine weeks during winter months and three weeks in summer months in their pupal stage (Ball & Morris, 2015; Bengtson et al., 2022; Weems, 2000). Hoverflies visit flowers to feed on nectar and pollen which aids them in ovulatory production (Doyle et al., 2020). Largely due to this, phenological activity for species have been found to peak during flowering seasons of plants (early April-late August), which usually coincides with the same seasons and locations as bees, bumblebees, and wasps (Ball & Morris, 2015; Bengtson et al., 2022; Djellab et al., 2019; Howarth et al., 2004). Therefore, it is generally possible to observe an approximate positive correlation between the appearance of hymenopteran species and their syrphid mimics (Howarth et al., 2004; Penney et al., 2014).

Pollinator diversity and species richness have been shown to follow an urbanisation gradient, where bee and hoverfly diversity was found to be most diverse and abundant in rural areas, as opposed to being the least diverse urban areas (Bates et al., 2011; Luder et al., 2018). As such, conserving rural areas with highly diverse insect populations is expected to also benefit and support their surrounding habitats (Bates et al., 2011). Applying knowledge about the intrinsic connection between insect diversity and plant diversity will allow us to help increase insect populations in urban areas (Bates et al., 2011). One could therefore pose the question as to why a family of insects that provide such a wide range of agricultural and ecological services are relatively unprioritized both in academia and the media. The pollinator crisis has been central in media during the last 20 years of discussions surrounding environmental conservation (Balfour et al., 2018). Due to the decline in pollinator populations, research pertaining to the study of the Hymenoptera order has increased dramatically. However, there is little research focused on hoverflies on the west coast of Norway, or indeed worldwide. Due to this, there is a gap in knowledge related to the diversity, abundance, and phenology of hoverflies in Norway, specifically to the hoverflies in inner Hardanger.

Purpose

The purpose of this study is to provide an overview and analysis of diversity, abundance, and phenology of the hoverflies (Syrphidae) in a selection of apple orchards located in inner Hardanger from data collected in connection with the APPLECore project. The APPLECore project is led by

the Norwegian Institute for Nature Research (NINA) in collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO), where they are investigating a series of questions surrounding pollination and ecology (APPLE-Core, 2021). The effect of pollinator diversity in apple orchards on autumnal fruit yield is among the research questions of the APPLECore project (APPLECore, 2021).

Material & methods

Choice of data analysis

The data analyses for this study included: i) Shannon-Weaver diversity index and ii) species accumulation plots.

The Shannon-Weaver diversity index was used as Alpha Diversity estimate used for the evaluation of species diversity for the three sites. Alpha diversity is an evaluation of how diverse a sample is (Willis, 2019). The Shannon-Weaver diversity index gives a numeric estimate of species diversity and richness for a location (Bobbitt, 2021; Ortiz-Burgos, 2016). The formula for calculating Shannon-Weaver diversity index is:

$$(H'): H' = -\sum(p_i * \log_2 p_i)$$

where:

p_i = proportion of each species in sample

$\log_2 p_i$ = natural logarithm of p_i proportion

Shannon-Weaver diversity index gives a value between 0-4.5, where the usual values range between 1.5-3.5 (Bobbitt, 2021; Ortiz-Burgos, 2016).

Species accumulation curves were used to estimate species diversity and richness and is a representation of sampling effectiveness for a site (Deng et al., 2015). If the curve of a species accumulation plot is flattened, the samples analysed are representative of the whole species diversity for a site (Deng et al., 2015). If a species accumulation plot is still rising, the data analysed is an underrepresentation of site-specific species diversity, and further data is required to accurately represent all species that are discoverable (Deng et al., 2015). The shaded area of a species accumulation curve shows a 95% confidence interval (Deng et al., 2015).

Sampling design

Triplets of pan traps (yellow, blue, and white) and blue vane traps were placed in the locations Djønno, Urheim and Opedal in Hardanger, Western Norway between late April – mid-June 2022. Djønno and Urheim are located in relatively rural areas, whereas Opedal is located in a more urbanized area. Six triplets of pan traps and three blue vane traps were placed in each of the three locations (Høydal, 2022). Both types of traps contained a soap water mixture to break the surface tension of the water to ensure

insects drowned when caught (Høydal, 2022). The insects were sampled through four sampling periods separated by 14 days to prevent over trapping too early in the season (Høydal, 2022). In every sampling period the traps were placed in the field for four days and only emptied every two to three days (due to limitations in available workforce) (Høydal, 2022). Trap contents were bagged and labelled and stored in a freezer prior to identification.

In the laboratory, insects were defrosted and all insects with a false wing vein Vena spuria were selected. After hoverflies were identified to species, they were pinned through thorax with a size 000 pin needle. Individuals identified were given a species_ID. Every individuals' identification was double checked using materials provided by The University Museum of Bergen, of which included Swedish identification literature and pinned museum examples of the most abundant species identified (Bartsch, 2009b; Bartsch, 2009a; Gammelmo, 2017).

Data Analysis

Data summarised in the excel sheet was exported and loaded into an R Studio workspace where the software R version 4.0.3 was used to process the data (The R Foundation, 2020). To visualise species diversity and abundance, phenological activity and trap colour and trap preference we used the `plot()` and `barplot()` functions. The values of Shannon-Weaver diversity index for each of the three sites were calculated and were used as a numerical estimation of diversity. Shannon-Weaver diversity index was calculated using the `diversity()` function from the `vegan` package version 2.5-7 (Oksanen et al., 2022). To check if the sampling portion was an adequate representation of species in the areas, we produced a species accumulation curve. The species accumulation curve was produced by firstly collecting data for species and all traps as a value using the `table()` function. Secondly, using the default extract method for the `specaccum()` function on the values sorted into a table, a polygon plot was made by using the `plot()` function with the specification of `ci.type` being "polygon".

Results

Phenological activity

Hoverflies were found to be mostly active during the summer months of June (Figure 1). The highest abundance of hoverflies was found to be mid-June in post-flowering period of apple trees (Figure 1).

Shannon-Weaver diversity index

The Shannon-Weaver diversity index for Urheim was approximately 2.66 and was the most diverse site. Close to Urheim on the diversity index was Djønno with an approximate value of 2.52. The least diverse site with an approximate value of 1.71 was Opedal.

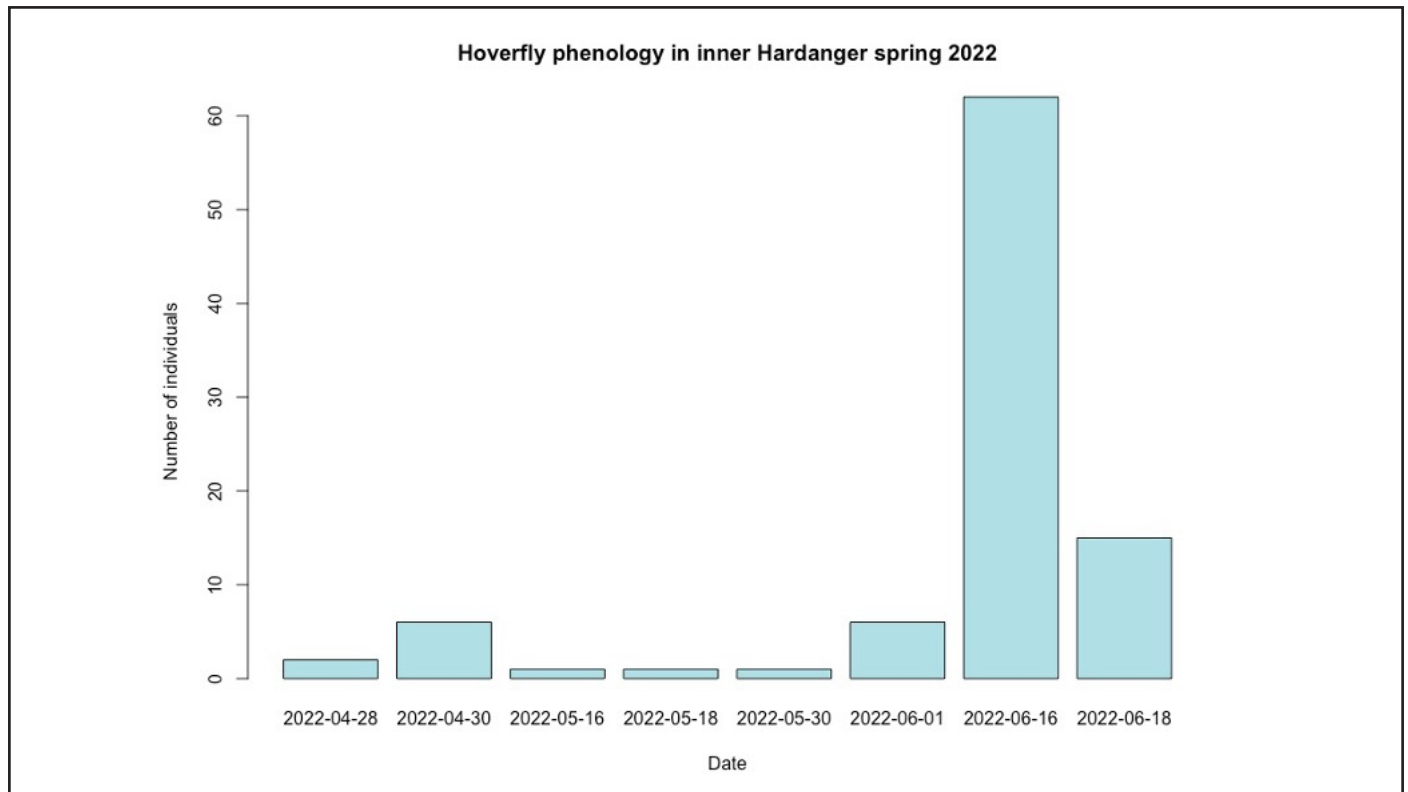


Figure 1. Bar graph showing hoverflies caught on dates throughout the field season in total for all locations.

Species accumulation curve

The species accumulation curve did not flatten (Figure 2). Due to the rising graph, individuals of the hoverfly family Syrphidae collected was likely an underrepresentation

of the available hoverflies in Djönno, Urheim and Opedal (Figure 2). The species accumulation curve did not represent the total species richness for inner Hardanger (Figure 2).

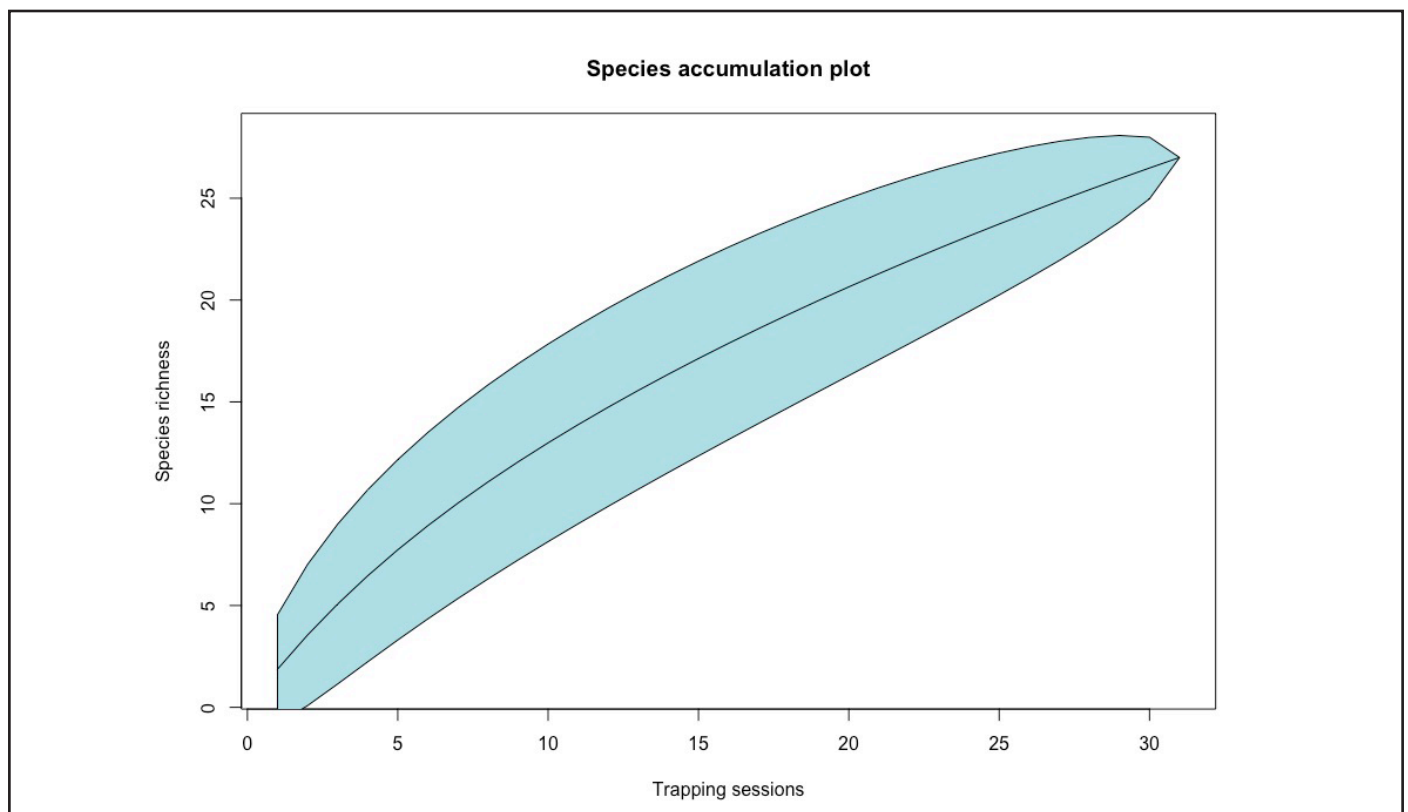


Figure 2. Species accumulation curve for hoverflies caught in all trap types throughout the entire field season.

Species abundance

The most abundant subfamily was found to be Eristalinae, representing 65.96% of all the individuals caught (Figure 3). Syrphinae represents the remaining 34.04% of the individuals caught (Figure 3). There were no individuals caught in the Microdontinae subfamily, and it is therefore not represented in this sampling.

Cheilosia spp. were found to be the most abundant hoverflies, representing 26.6% of all individuals collected. Collectively, *Xylota* spp. was nearly as abundant as *Cheilosia* spp., with a representation of 22.34% of individuals.

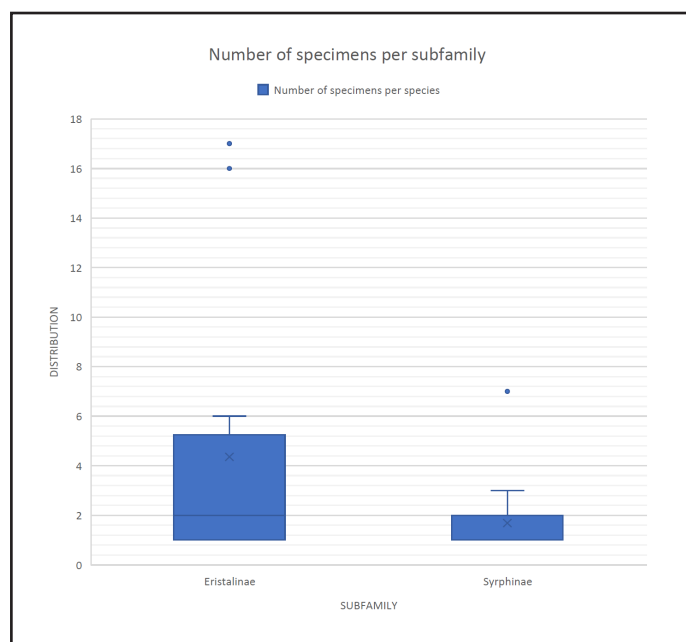


Figure 3. Boxplot displaying the distribution of specimen found for subfamilies Eristalinae and Syrphinae. No data for subfamily Microdontinae.

Site-specific Species abundance

The highest abundances were found in Djonno, with 63 individuals (25 females, 38 males) (Figure 4). Urheim and Opedal were both similar in terms of hoverfly abundance (Figure 4). Identified from Urheim were 15 individuals (6 females, 9 males) (Figure 4). From Opedal, 16 individuals (11 females and 5 males) were identified (Figure 4). A disclaimer is placed here to inform that there were dates with missing trap materials for both Djonno and Opedal.

Methodological effectiveness

Insects collected using pan traps and vane traps under-sampled the available species for all sites (Figure 5). Pan traps were more effective than vane traps (Figure 5). Yellow pan traps were more effective than white and blue pan traps (Figure 6). 94 individuals of the hoverfly family were identified from this field season.

Discussion

Phenological activity

The results show that hoverflies in Hardanger become active at the same time as the beginning of apple tree flowering season, where they peak in activity in summer during mid-June (Figure 1)(Djellab et al., 2019). A peak around June is consistent with both optimal temperature levels for activity and hoverflies need for pollen during ovulatory production (Figure 1)(Djellab et al., 2019). As their life span lasts about 35 days, the peak in activity in June would be expected and is consistent with the results (Figure 1). As the availability of pollen rises over time, a correlated abundance of hoverflies can be observed (Figure 1).

To adequately provide a true representation of the phenological activity, more consistent sampling would be needed. The 14-day period between trapping sessions potentially allowed for hoverfly species that are active between these periods to go undetected. In addition, one must also consider a different methodology. Sampling with an entomological net every day would be an alternative method that would allow for a line-graph to be produced and would likely yield a higher degree of certainty about the activity of hoverfly species (Bates et al., 2011; Gittings et al., 2006; van Steenis, 2016).

Shannon-Weaver diversity index

The hoverflies were found to be most diverse in Urheim and the most abundant in Djonno, the two most rural of the three sites. This is consistent with findings of earlier studies of pollinator diversity under urbanisation gradients (Bates et al., 2011). All Shannon-Weaver diversity index values are consistent with the estimated Hymenoptera order diversity for the sites as well. Additionally, diversity metrics for all three sites also corroborate with their urbanisation gradients (Bates et al., 2011). Opedal being a relatively urban site, roads and the cropland nearby are cultivated and relatively monocultured both in diversity of insects and plants. The diversity of Djonno was equally consistent with urbanisation gradients, as the site was far away from heavily trafficked roads, and the surrounding ecology was not as heavily modified from natural habitats. Urheim has median value with an index of 2.66, which corresponds with the site being in a relatively rural area. While the Shannon-Weaver diversity value found for Djonno was not as high as Urheim, the difference between the two sites is only a numerical value of 0.14, and as such, they are approximately as diverse. Nevertheless, there is a clear numerical division in the diversity index between the most urbanised site (Opedal) and the most rural sites (Urheim and Djonno).

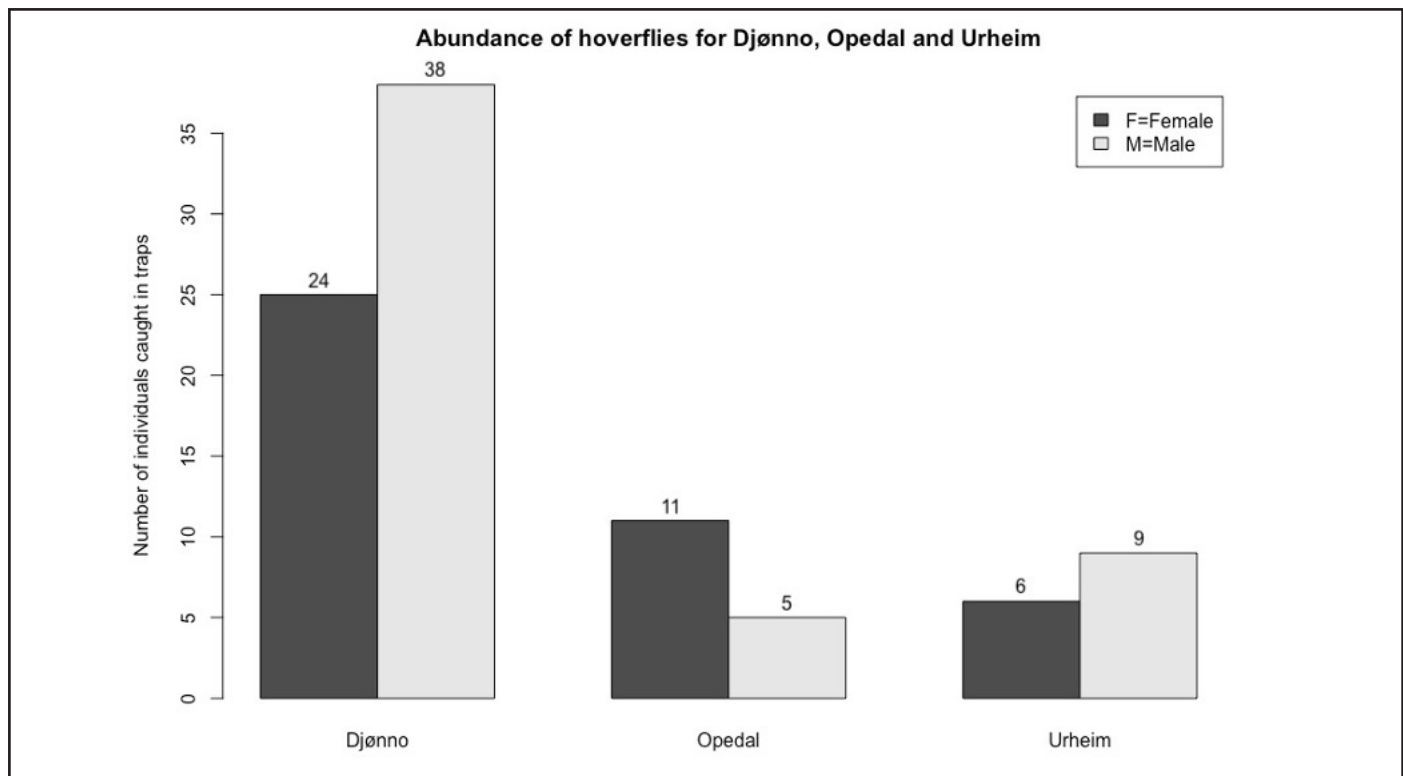


Figure 4. Species abundance for Djønno, Opedal, and Urheim with gender distribution.

Species accumulation curve

The species accumulation curve is still rising and shows no flattening (Figure 4). We can therefore ascertain that the data was an underrepresentation of the available hoverfly species diversity and richness for Djønno, Urheim and

Opedal. Methods used for capture of insects are in a higher degree streamlined for the capture of Hymenoptera. To get a sample size that is properly representative for the area, one would need to be consider alternative methods for hoverfly capture, such as entomological netting.

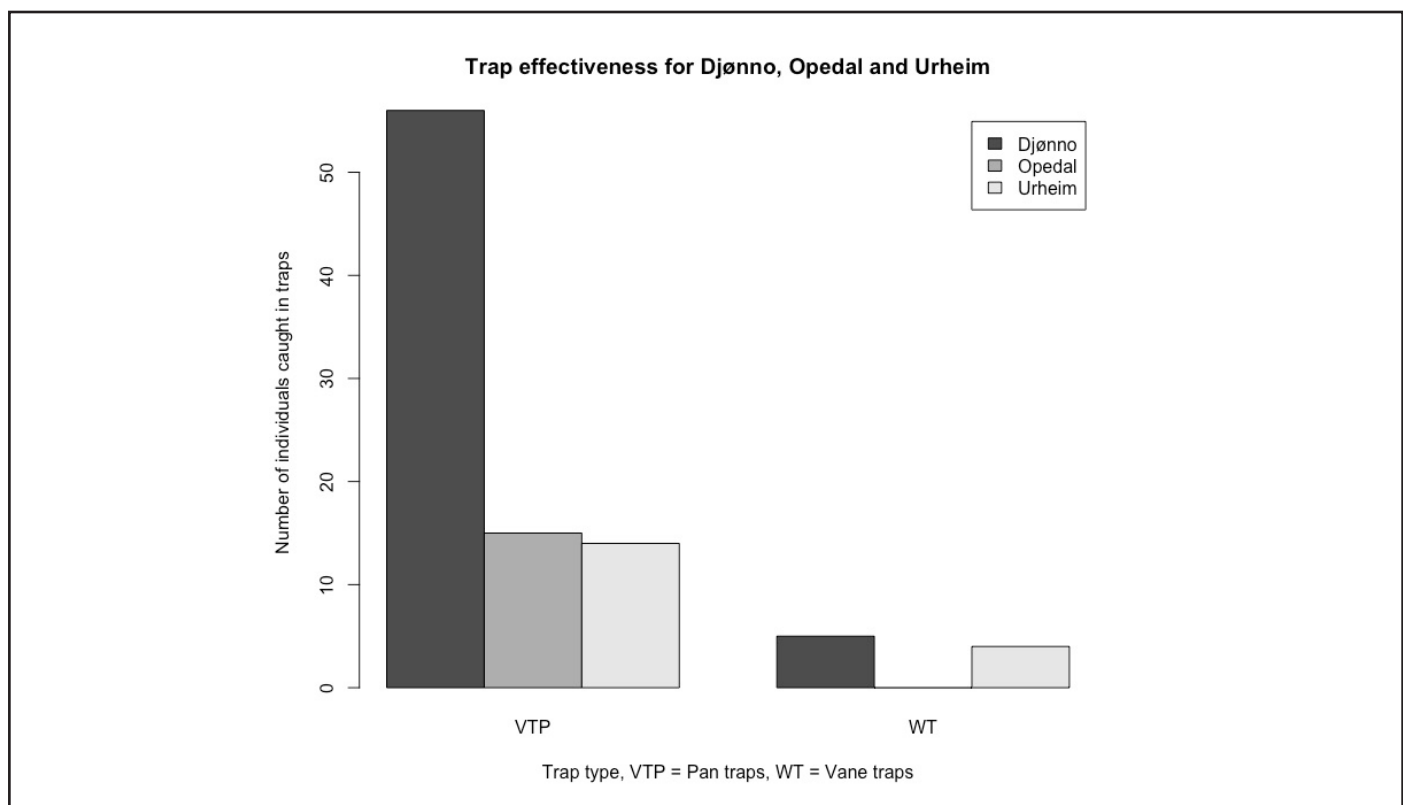


Figure 5. Distribution of individuals captures in 1) pan traps (VTP) and 2) vane traps (WT).

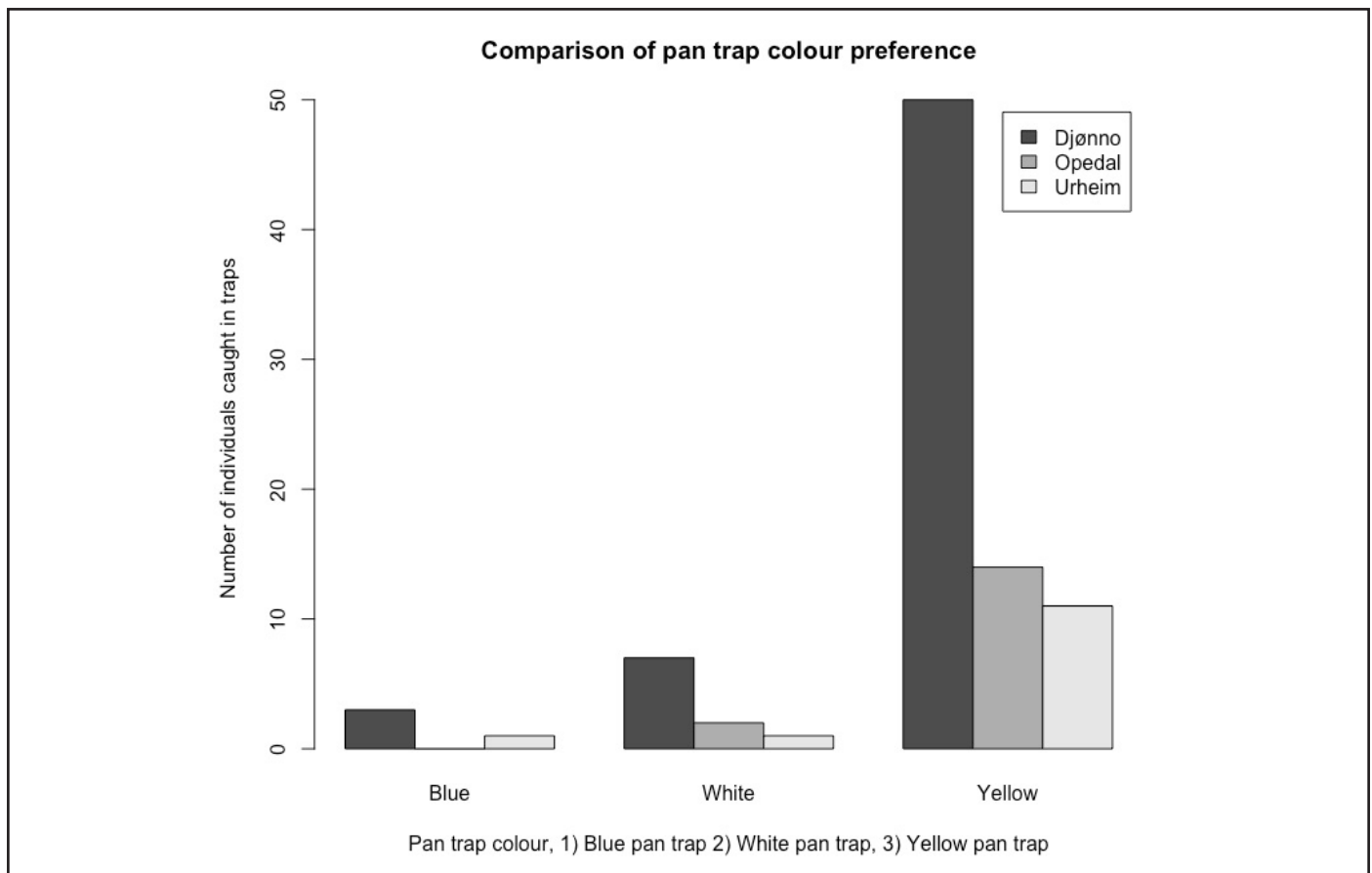


Figure 6. Distribution of individuals per trap colour for all three locations.

The period where the traps were placed was broken into four periods. To obtain a greater representation of what the phenological activity of hoverflies looks like, one would need to have a consistent and continued sampling of all three areas. A greater sample size over a longer time period would therefore likely show a better representation of the diversity, abundance, and phenology of hoverflies in the area. Common methods for hoverfly capture used in studies of hoverflies are entomological nets or Malaise traps. Capture is usually done consistently at the same times and locations every day over a time period of between 5-12 months, with variations depending on the study (Bates et al., 2011; Gittings et al., 2006; van Steenis, 2016). Recognized here is that the main purpose of the trapping of insects was not to trap hoverflies, but rather to sample bees and bumblebees in the area.

Species diversity and abundance

All species identified were registered as having strong populations (abundant) in Norway (Artskart, 2022). Generally, hoverflies become active at around the same temperatures as the start of the flowering season of apple trees (~15°C) (Bengtson et al., 2022; Ramírez and Davenport, 2013). In regard to the Hardanger region, a wide range of syrphids can be observed. The different species become active at different times during the year. Finding the species *Melangyna barbifrons* (Fallén, 1817), was particularly interesting,

as this is a species of hoverfly that becomes active early in the year. The timing of *Melangyna barbifrons* activity coincides with the beginning of the flowering season of apple trees, and this species is therefore likely especially important for the pollination of apple trees (Ramírez and Davenport, 2013). There are no earlier registrations of this species in this area, and this species is therefore a particularly important find for Djønno (Artskart, 2022).

Other species found to be important for pollination are species of *Eristalis* spp. which have shown to carry similar pollen loads to *Apis mellifera* (Ramírez and Davenport, 2013). It is positive that an individual of *Eristalis pertinax* (Scopoli, 1763) was identified, where the region of Hardanger already have seven sightings recorded (Artskart, 2022). Uncommon species that were observed and not previously registered in Hardanger but are represented in our dataset include species such as *Cheilosia proxima* (Zetterstedt, 1843), *Cheilosia pegana* (Meigen, 1822), *Chrysotoxum fasciolatum* (De Geer, 1776), *Orthonevna geniculata* (Meigen, 1830) and *Parasyrphus nigrifrons* (Zetterstedt, 1843). Of hoverflies identified through this study, the species that have previously been found to be the most abundant in inner Hardanger area are the species *Cheilosia sahlbergi* (Becker, 1894), *Platycheirus albimanus* (Fabricius, 1781), *Syrphid pipiens* (Linnaeus, 1758), *Syrphus ribesii* (Linnaeus, 1758), *Syrphus vitripennis* (Meigen, 1822) and *Syrphus*

torvus (Osten-Sacken, 1875). These species are therefore expected to see in our dataset (Artskart, 2022). Notable mentions are species such as *Ferdinandea cuprea* (Scopoli, 1763), *Cheilosia albitarsis* (Meigen, 1822), and *Xylota jakutorum* (Bagathanova, 1980), which are species of which only a few sightings are recorded. They additionally represented some of the highest abundancies of specimens per species in our results (Artskart, 2022).

The results show a large variation of species that emerge and are active as adults at different parts of the year with a mix of species that are both early season fliers such as *Melangyna barbifrons* and late season fliers such as *Melanostoma mellinum* (Linnaeus, 1758), *Syrirta pipiens* and *Xylota segnis* (Linnaeus, 1758) all of which are saprophagous late spring-early autumn fliers. There are species of hoverflies identified in this study that one would expect to see, such as *Syrirta pipiens* which is a species known to inhabit compost heaps and a species that we could expect to find in areas that require farmers to use fertilizers on their crops (Bengtson et al., 2022). *Melanostoma mellinum* are species known to inhabit areas that include grasslands as they are a species that feed on grass pollen (Artsdatabanken, n.d.).

Species activity and abundance are both variables that can differ between years, and seasonal activity is a variable that also depends not only on flowering plants, but also on temperature and weather conditions (van Steenis, 2016). As such, it is recognized that there is a limitation to the dataset that is used in this study, and to be able to ascertain to a greater degree what species are expected in inner Hardanger, we would need to study these insects over a multitude of years. Hoverflies are likely a species that are underrepresented in The Norwegian database for species *Artsdatabanken*. Attention is drawn to this due to some species were found to be highly abundant in this study. Examples of such species are *Cheilosia albitarsis* and *Xylota jakutorum* which are rarely reported for the Hardanger area in the species register (Artskart, 2022).

Conclusion

Given the materials sampled, the peak phenological activity of hoverflies in inner Hardanger region was mid-June. Pan traps sampled hoverflies in greater degree than vane traps. Shannon-Weaver diversity metric showed that the rural areas of Djønno and Urheim were the most diverse, while Opedal, the most urbanised area, was the least diverse. Due to the missing dates and trap materials in data, there is uncertainty in the Shannon-Weaver diversity index for both Opedal and Djønno as opposed to Urheim. Species accumulation curve showed that traps underrepresented the available species, and the data collected is therefore unlikely a true representation of what the hoverfly community looks like. To obtain a better representation of diversity, abundance, and phenology of hoverflies in Har-

danger, we would need to reassess the methodology used for capture and sample insects over a greater and more consistent time period.

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Bee beetle (*Trichius fasciatus*) & bumblebee (*Bombus* sp.)
Photo: Maria Ulvang

The Beetles and the Beasts

Ingrid Vaksvik

Abstract

This study explores the interactions between domestic herbivores and beetles in the fjord system of the Nordhordland Biosphere Reserve. Fields grazed by sheep had overall higher beetle abundance, even if the number of families did not vary. I also found that beetle diversity and abundance were affected by the ecological zone: coastal sites showed higher beetle abundance, particularly including hygrophilous families, compared to fjord sites. The type of livestock seems to have an influence on the composition of beetle assemblages, but it may be covered by stronger environmental effects.

Introduction

Small-scale farming and versatile land use have been practiced in Nordhordland Biosphere Reserve (NBR) for more than 6000 years (Hjelle et al., 2018). This has resulted in an assemblage of diverse landscapes, including “semi-natural” grasslands which are defined by the modification of vegetation (e.g., using domestic livestock for labour, grazing, and food) and the need for cultivation (e.g., creating artificial plant communities) (Kaland et al., 2018). Fodder production (plant biomass) for animals supports the livelihood of humans and can provide a rich habitat for insects that may serve as pollinators and/or natural pest control (Bengtsson et al., 2019). However, agricultural intensification has contributed to species extinction and lower diversity in both pollinators and European carabids, such as ground beetles. This is eligibly caused by the use of artificial pesticides, along with the removal of hedgerows and other natural habitat features (Sánchez-Bayo & Wyckhuys, 2019). In coastal Norway, changes in agricultural practices can be illustrated by the diversification of livestock and the grazing intensification at a local scale (Austrheim et al., 2011). For instance, the main sheep breed used in Norway in the

last decades is heavier than other breeds and forages more on graminoids than woody species. The number of grazing sheep was in decline between 2004 and 2014, a change that may also have an indirect effect on invertebrates and grassland structure (Ross et al., 2016).

Different types of livestock have different specificities regarding fodder preference, foraging pattern, trampling, and dung dropping. It is possible that this affects the local environment differently, including soil, litter, plant, and arthropod communities (Kotze et al., 2011; Liu et al., 2015; McFerran et al., 1994; Zhu et al., 2015; Garrido et al., 2019). The assemblage of insects associated with dung, such as the paraphyletic group of “dung beetles” (Scarabaeidae), will be particularly influenced by the presence of cows (van Klink et al., 2015; Zhu et al., 2015). Species in the families of rove beetles (Staphylinidae), ground beetles (Carabidae), and scarabs (Scarabaeidae) are attracted to fresh cattle dung in both open and wooded pastures (Galante et al., 1995). Carabidae are particularly sensitive to changes by both natural and human-induced disturbance and thus were shown to be a good indicator of the health of an ecosystem (Koivula, 2011). The grazer species will however not be the only factor affecting dung beetle assemblages. Requirements for moisture, pH, light exposure, and soil type e.g., differ between families, implying that the local environment plays an important role in shaping beetle assemblage. The fjord system of the NBR offers very complex and intertwined climatic, geomorphological, and ecological conditions. The coastal areas are flat and moist, exposed to strong winds, salts from sea spray, and high precipitation. The coast also has milder winters and colder summers than the inner fjords (Hjelle et al., 2018).

A healthy ecosystem should contain species communities that are diverse and abundant. Certain beetle families (such as carabids) function as indicators of overall

biodiversity and ecosystem health. They show predictable responses to both small- and large-scale variations of management and disturbance. These variations can be indicated by the presence or absence of carabids – which may also reveal the condition of certain beetle species and/or other animal taxa (Kotze et al., 2011). Ground beetles are affected by pollution such as heavy metals in soil or by pesticides, and their mean individual body mass can be linked to succession in numerous habitats (carabids being habitat-generalists) (Kotze et al., 2011). Furthermore, carabids include keystone species whose abundance may impose dramatic effects on pests and crop production - as they are responsive to both agriculture and forestry disturbance (Kotze et al., 2011). Some carabid species may even signal environmental change by e.g., shifting the altitude of habitat residence (Kotze et al., 2011). However, these responses should be addressed individually unless otherwise proven for that taxon and/or for conditions that are hard to observe. By using these indicators, we can potentially enhance conditions for carabid families and their living habitats (Kotze et al., 2011; Sánchez-Bayo & Wyckhuys, 2019).

In this study, the overall impacts of different grazers (cows, sheep, goats) are compared using the total abundance of beetles, abundance in main families of beetles, and family diversity of beetles.

By comparing different grazers across different sites of the Nordhordland Biosphere Reserve, I hope to find the effect of grazers and geographical location on beetle diversity and abundance. For each study site, I will explore the total abundance of beetle, abundance in main families of beetles, and family diversity of beetles. I seek to answer two hypotheses:

H1: More dung in cow sites will result in a generally higher abundance of dung beetles than in sites grazed by sheep and goats.

H2: Geographical location will have an impact on beetle diversity and abundance. For instance, coastal areas may get more precipitation and higher levels of salinity in the soil – which can have indirect effects on the beetles.

Material & methods

Study Area

The study area is the Nordhordland Biosphere Reserve (NBR), including nine grassland study sites (Figure 1). Three of the sites were sampled closer to the coast (O' coding/outer fjord, Table 1), while the other six sites were in the fjords (I' coding/coast, Table 1). All sites were grazed by either cows, sheep, or goats. Additional details

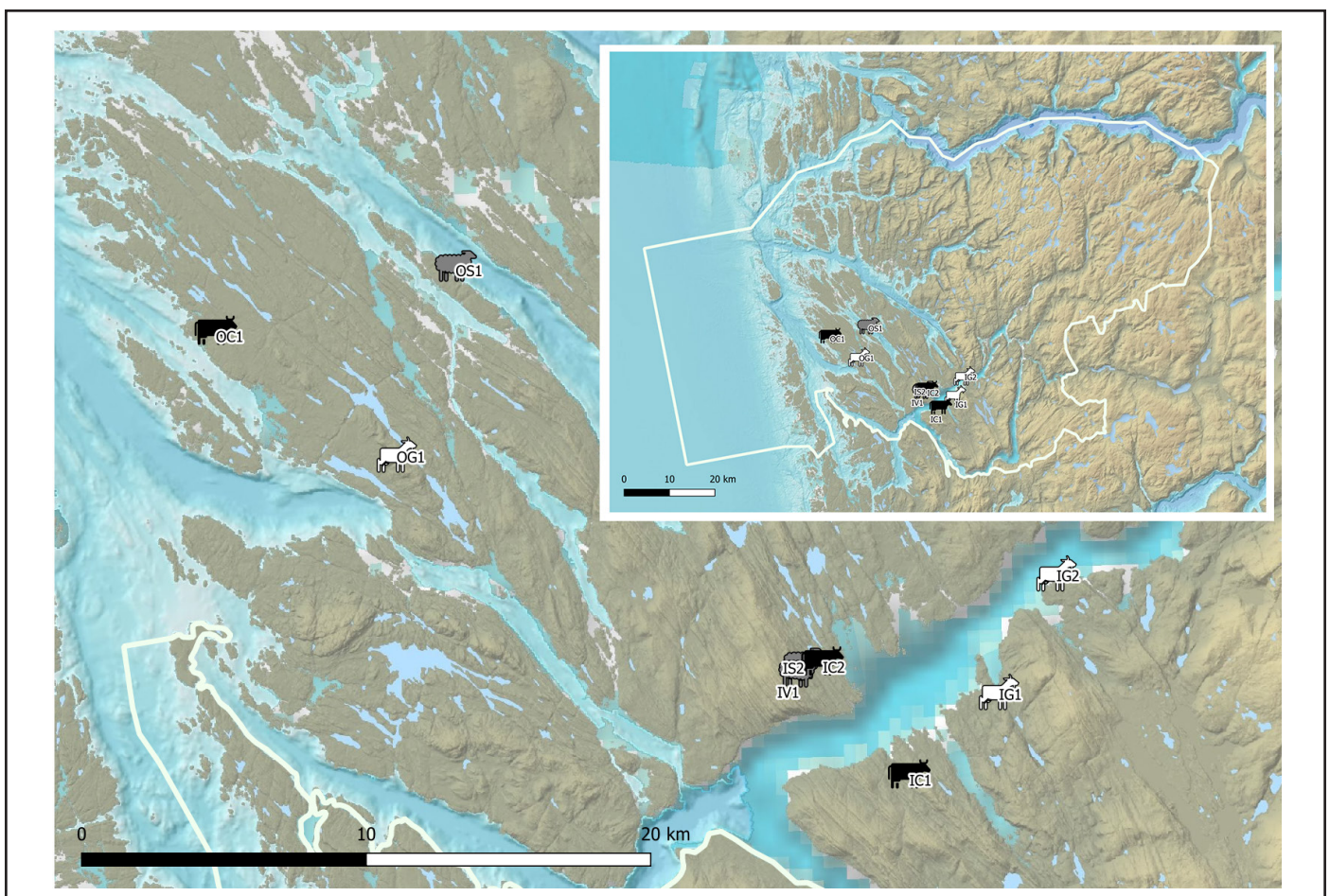


Figure 1. Location of the nine sites grazed either by sheep (grey), goat (white) or cows (black). Credits: Morgane Kerdoncuff.

Table 1. Description of the nine study sites in Nordhordland Biosphere Reserve.

Site	Sampling period	Grazer	Location	Municipality	Area	Aspect
IC1	02.08.19-09.08.19	cows	Raknesveien	Osterøy	Fjord	NE
IC2	02.08.19-09.08.19	cows	Hodnesdalvegen	Hjelmås	Fjord	NE
OC1	20.06.19-27.06.19	cows	Nedre Vågenes	Manger	Coast	NW
OS1	26.06.19-07.07.19	sheep	Lygra	Seim	Coast	NW
IV1	02.08.19-09.08.19	sheep	Hopdalsvegen	Hjelmås	Fjord	E
IS2	02.08.19-09.08.19	sheep	Hopdalsvegen	Hjelmås	Fjord	SW
IG1	02.08.19-09.08.19	goats	Hole	Lonevåg	Fjord	NE
IG2	02.08.19-09.08.19	goats	Bernes	Osterøy	Fjord	SW
OG1	12.06.19-19.06.19	goats	Nottveit	Manger	Coast	S

are listed in Table 1. We collected the samples in systems with low-intensity grazing, and low to no pesticide usage.

Data Collection

Sites were defined as sampling areas of 20 x 20 m², with homogeneous grassland vegetation cover representative of the surrounding grazing field area. Wet zones (e.g., with peat moss or cotton grass) or bare rock were avoided. The aspect and elevation of the sites were recorded. In each site, we installed three sets of four dung-baited pitfall traps. One set was made of a 1 m² square, with one trap on each corner. Sets were separated from each other by a minimum distance of 6 m². There was a total of 12 traps per site, which resulted in 108 samples for the overall survey. Each trap had a diameter of 11.5 cm and was covered by a wire mesh and baited with cow dung to attract dung beetles.

The samples in this project are from different periods during summer 2019, with traps being active for one week each (Table 1). All beetles were then sorted by family in the lab and stored in 70 % ethanol. For family identification I used Duff & Smith (2012) and Unwin (1985).

Data Handling

The survey will address the following response variables: total abundance of beetles, abundance per family and family diversity. The variables were visualized as bar plots using Microsoft Excel.

Results

My results showed that different grazers had different impacts on beetle communities. It seems that beetle abundance may not be related to beetle diversity.

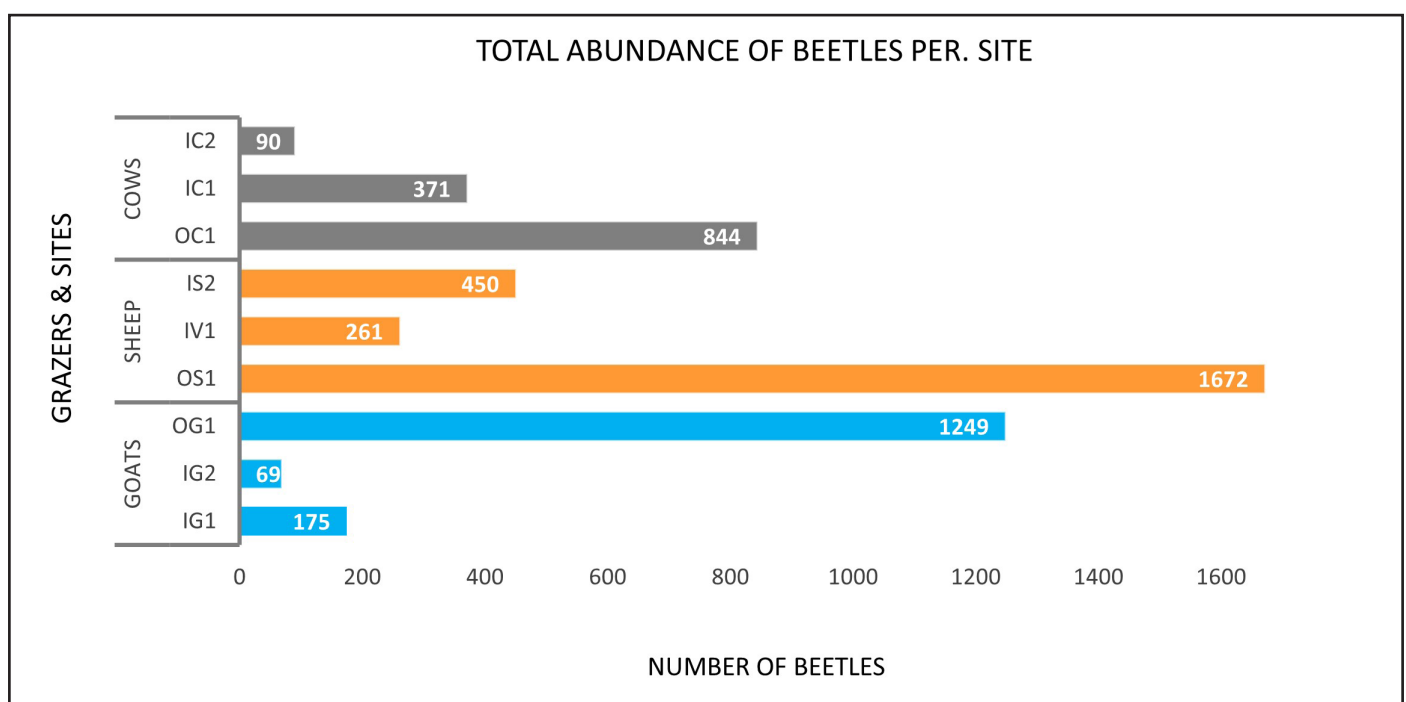


Figure 2. Total abundance of beetles for each site, classified according to livestock type (cows, sheep, goats).

The abundance of beetles per. site ranged from 69 (IG2) to 1672 (OS1) individuals (Figure 2). The sites grazed by sheep had an overall greater abundance of beetles but also showed the highest variability across sites. The lowest abundance of beetles is found in a goat site (IG2). On average I found a total abundance of beetles of 157 for sheep, 97 for goats, and 77 for cows. There is a clear difference between fjord and coastal sites, especially for sheep and goats – reflected in e.g., four times as many beetles in the OS1 site than in the IS2 site.

The coastal sites (OC1, OS1, OG1) had an overall higher abundance of beetles, particularly for Staphylinidae (rove beetles) but also for Hydrophilidae (water scavenger beetles) (Figure 3). Staphylinids are most abundant for all sites, except the IC2 site grazed by cows. Carabidae (ground beetles) and Scarabidae (dung beetles) are the least abundant families throughout nearly all sites. The sheep sites IS2 and OS1 stood out by having the highest abundance of dung beetles. The goat sites in fjords had the lowest abundance of beetles per. family of all the fjord sites in the survey.

The total abundance of beetles is highest in sheep sites (Figure 2). These sites also have the highest abundance of beetles in different families (Figure 3). This is true for three out of the five families analysed, respectively the families of Scarabidae, Staphylinidae, and Hydrophilidae. Ptiliidae (featherwing beetles) were most abundant in OC1 (Figure 3), however, they seemed to thrive in any habitat.

The diversity ranges from 5.0 to 9.0 families per site. Cow and goat sites have 9.0 families in two out of three

sites. The average number of families is highest in cow sites by 8.0, closely followed by goats with 7.7 and sheep sites with an average of 7.0 families per site (Figure 4).

From figure 2, 3 and 4 we can observe that beetle abundance and diversity do not always follow the same trends. For instance, beetle abundance is substantially lower on IG2 and IC1, yet the number of families identified is quite similar to other sites.

Regarding geographical location, the abundance in main families is highest in coastal sites – as with the overall abundance.

Discussion

The negative impact of intensified agriculture involves the abandonment of small-scale farming and semi-natural grassland, which has disadvantages for biodiversity. Yet, grazing in semi-natural grassland affect keystone species such as beetles of the carabid family. They are immensely affected by their environment and may respond to grazing regimes by grazer species (Sánchez-Bayo & Wyckhuys, 2019; Zhu et al., 2015; Pozsgai et al., 2022). We collected beetles from nine locations with semi-natural grassland in Nordhordland Biosphere Reserve. The abundance and diversity of beetles were then compared to the type of grazer in their habitat and habitat location. From my analysis, I could observe the following trends: the total beetle abundance in the fjords was lower than in the coastal areas, and it was higher in sites grazed by sheep. Beetle abundance and diversity were not consistently following similar trends:

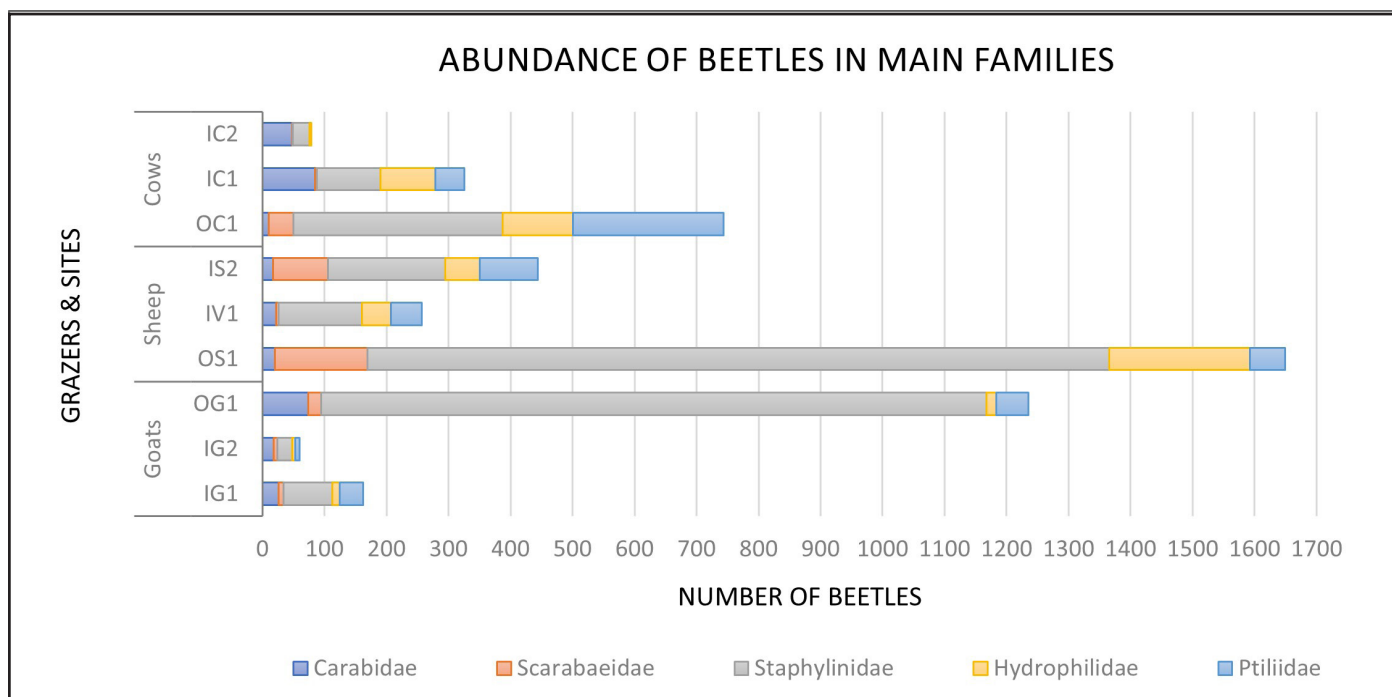


Figure 3. Total abundance of beetles for the main families (Carabidae, Scarabaeidae, Staphylinidae, Hydrophilidae and Ptiliidae) in each site, classified by livestock type.

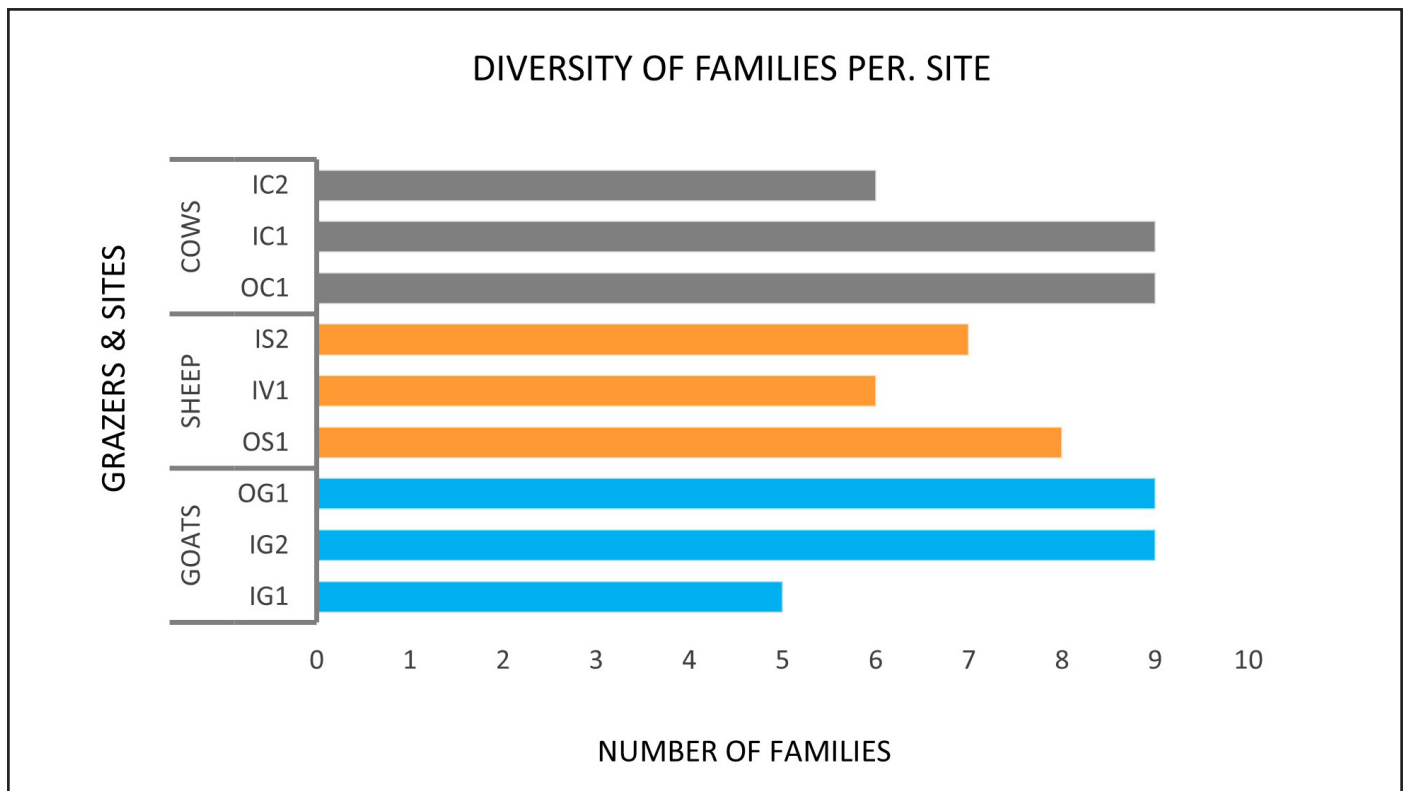


Figure 4. Total number of beetle families per site, classified according to livestock type.

very low abundance was not followed by lower diversity. The first hypothesis of this study was not supported by my results, as sites grazed by cows do not obtain a higher abundance of carabids (Figure 2-3). My second hypothesis is verified, as there are some clear patterns when it comes to carabid abundance in different sites (Figure 2-3). Diversity is slightly higher in coastal sites (Figure 4), even though it doesn't appear to be related to any particular factors.

Diversity and Abundance are not always Mutually Consistent

It is important to check both diversity and abundance because more abundance does not necessarily mean that a beetle community is thriving under the specific circumstances of that area (Koivula, 2011). Although grazing has an impact on the diversity of plants, plant diversity is not shown to have a direct impact on arthropod diversity (Borer et al., 2012). On the other hand, there is support for a link between the biomass of plants and arthropod diversity. That said, arthropod abundance does not have to be affected by the increase in plant production (Borer et al., 2012). High species abundance can serve as a reflection of trees and shrubs in a heterogeneous landscape. Small-scale grazing is one such system, with a mosaic of open habitats and woodland. This vegetation may function as a foraging resource and microclimate refuge in times of environmental stress (Söderström et al., 2001). Vegetation is in turn affected by the type of grazer and grazing patterns. Sheep mainly graze on grass and occasionally woody species and forbs,

goats graze on woody species and grasses while cows prefer grass but may eat forbs. These preferences lead to grassland with diversity in structure, reflecting what type of plants that are not grazed on (Mphinyane et al., 2015; Scohier & Dumont, 2012). To reflect the grazing intensity, we collected dung and recorded the amount thereof in all sites. OS1 contained the absolute highest proportion of dung, which may explain the high abundance of beetles in OS1. However, it does not explain the high diversity as this is expected to be lower in systems with high-intensity grazing. Neither does it explain the relatively high abundance in the OG1 site as it has low amounts of dung, but it does explain its high diversity as the site has low grazing intensity.

To understand why the abundance of beetles and families of beetles are higher in sheep sites, while the diversity of families is lower – we first need to look at the grazer. In small-scale semi-natural grassland, the populations of arthropods are highly affected by local management as well as the grazing herbivores. Light grazing leaves the “plant-herbivorous insect” interactions alone, which increases insect diversity. The same applies to short grazing periods (Scohier & Dumont, 2012). A study conducted by Borer et al. (2012) states that plant production changes the relationship between the diversity of arthropods and plant diversity, and thereby that plant diversity does not control arthropod diversity directly. On the other hand, plant abundance influences arthropod abundance. The low beetle diversity in the sheep sites could result from intense grazing, reducing plant biomass and diversity. Sheep are

selective grazers and create short, homogenous grassland (Schoier & Dumont, 2012) that may only be attractive to certain families of beetles. These families could be high in numbers simply because they live on unspoiled vegetation, and that they are amongst few families that thrive with that certain type of land. High abundance can also be the result of size, such as the Staphylinids I collected which were dominantly small in size. This makes them less affected by changes than bigger specimens like Carabidae and Scarabaeidae (van Klink et al., 2015).

The relation between grazing, beetle abundance, and diversity are investigated by Kruess & Tscharntke (2002) supports the results of this survey: species diversity is lower on intensively grazed pastures than on extensively grazed or non-grazed grassland, while abundance is higher on the latter. Abundance on intensively vs. extensively grassland did not differ significantly.

Effect of Climate and Ecological Conditions

Beetle abundances in OG1, OS1 and OG1 were substantially higher than in other sites, while the same sites also had higher diversity. Both fields are closer to the coast, which implies that geography may affect beetle communities. The high abundance of staphylinids and hydrophilidae in coastal sites, indicates that hypothesis number two may be supported, implying a connection between abundance and location. The difference in the total abundance of beetles in these sites is explained by the high numbers of Staphylinidae. Fjord sites are lower in beetle abundance, and most of them are lower in diversity than sites closer to the coast. The latter sites are more exposed to conditions such as high winds and waves - but may also get better conditions in terms of higher winter temperatures, soil, nutrients from saltier water, less precipitation, and more (sun)light. The sites with proximity to the coast were also sampled earlier than other sites, which may have affected the results. Differences in abundance and diversity of beetles between sites could also be the result of pesticides or the type and sequence of crops (Goulet, 2003), but our samples were collected in low-intensity systems with low to no pesticide usage. One specific family of beetles makes up most of the abundance in the OS1 site. This is the world's largest family of beetles, Staphylinidae. The family covers a wide range of habitat types and functional groups. Rove beetles may be more abundant because of their versatile feeding habits, and by being the most successful group of Coleoptera - considering their ability to live in almost any habitat (Betz et al., 2018). Overall, there is slightly higher diversity in coastal sites but no clear patterns. Hypothesis number two can be confirmed based on both generally higher abundance and diversity in coastal sites.

One of the questions yet to be answered is why the results differ between goat and sheep sites. Is the difference

created by the animal itself, or perhaps by the management strategy of the farmer who moves the goats closer to shrub-encroached habitats? Goats graze on shrubs and are used for this purpose, while sheep prefer grass and herbs. Crawley (1983) made an interesting observation in that regard: "Larger herbivores were predicted to increase plant diversity through grazing on dominant plant species, whereas smaller herbivores were predicted to reduce plant diversity by grazing on rare plant species". Beetles in goat sites are less abundant but more diverse than in sheep sites, which can be the result of less intense grazing. Lower disturbance by sheep may explain higher abundance in those sites, but higher site heterogeneity created by goats and cows may explain the higher diversity there. However, the overall trend shows only small differences. And why do cow sites have more diversity and less abundance of beetles than other sites? The heavy trampling of cows on grassland may create suitable microclimates of sheltering tufts, which could increase beetle diversity. Contrary to my first hypothesis, the cow sites may have a lower overall abundance due to less intense grazing than sheep and goat sites, or by heavy trampling and solidification of the soil (van Klink et al., 2015; Zhu et al., 2015).

Limitations of the Study and Research Perspectives

Parameters that were not thoroughly examined are the timing of sampling and the site aspect. There seems to be some patterns connected to the period of sampling (Table 1). The sites showing most abundance was sampled in June and July (OC1, OS1, OG1), while the diversity across sites fluctuated throughout the summer. Although the four least diverse sites were found in August (IG1, IV1, IS2, IC2), two of the sites (IC1, IG2) with highest diversity were also found in August. The general pattern is that both abundance and diversity declined from about mid-July. Another parameter of interest is the positioning of sites (Table 1). South faced sites are exposed to more sun and less humidity than north faced sites, which may provide better living conditions. The most abundant sites faced northwest (OC1 and OS1) and south (OG1), and the least abundant faced southwest and northeast/ east (Table 1). There is no pattern at all for high diversity, while low diversity is prevalent in northeast/ east facing sites (IG1, IC2, IV1). The most interesting pattern is the positive trend between early sampling date and the northwest and south facing sites. They have the highest overall abundance of beetles per. site and per. family, and this also happen to be the coastal sites. This may also be a result of factors such as the coastal habitat, the sampling period etc. As for low diversity and abundance, the northeast/ east sites stood out. The northeast/ eastern sites may provide a poor habitat for beetles, with e.g., little sun exposure and cool temperatures. This may be the reason why there is low abundance and diversity in these sites. For future studies it would be interesting to investigate the

importance of site position to beetle communities, in comparison to time of sampling and the aspect of sites.

I would also like to explore the specific location of OS1 and OG1. These sites have a unique history in terms of management and geography, because of the proximity to heathland and being part of the two traditional cultural landscape areas in the NBR (Kaland et al., 2018). I would also like to dig deeper into the effects of trampling – especially in cow sites. As for practical adjustments I would use high-quality plastic boxes for traps because the ones used in this study were fragile and damaged the samples during preservation. Also, the data would have been more precise with additional sites - leaving the traps out for longer and having the same number of sites in fjord and outer fjord areas. More parameters need to be addressed to make better estimates, such as soil composition, inclination, temperature, precipitation, plant height, nutrients, and latitude.

Since this survey does not have the same number of sites from the fjord (six sites) as sites closer to the coast (three sites), no explicit conclusions can be drawn. As mentioned earlier, it is known that grazing plays both a direct and an indirect role in the amount of diversity and abundance of beetles. Although, the interpretation is different between the two. Some assumptions can be made from comparing outer fjord sites to coastal sites, concerning the influence of geography, plants, and dung. In general, beetle abundance and diversity are connected to the type of grazer - and the indirect effects concerning dung, plant bloom, diversity, and abundance. The main result from this study is the clear connection between the grazer and the beetle community. Other important results are the high numbers of rove beetles and the difference in abundance between fjord sites and coastal sites.

In summary, this study analyses some important processes connected to semi-natural grassland. The main process examined was the impact of grazers on the total abundance of beetles, abundance in main families of beetles and family diversity of beetles. Compared to fjord sites, the sites closer to the coast had higher diversity of beetles, higher abundance of beetles in general and especially higher abundance in the Staphylinidae family.

The amount of grassland is declining worldwide, while the demand for food production is increasing (Bengtsson et al., 2019). Since the agricultural revolution, the demand for high productivity has left only the most productive areas for grazing animals. This has led to an overgrowth and expansion of woods in leftover, abandoned land and a decrease in summer dairy farms (Kaland et al., 2018). These changes have vast implications for local biodiversity. Different grazing mechanisms made by cows, sheep and goats has the capacity to benefit these complex systems (Garrido

et al., 2019; Zhu et al., 2015). We need to keep making better estimates of biological interactions and provide optimal management of vulnerable ecosystems and biodiversity.

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Gowidon sp. - Alice Springs, Australia.
Photo: Brage Seime Øygard



Rock ptarmigan (*Lagopus muta*)
Photo: Eirik Laksemo

Characterizing Expression Patterns of LexA and LexAop Lines for the Development of a *Drosophila* Cancer Model

Nora Solheim

Abstract

Drosophila melanogaster (the fruit fly) is a very well-suited model organism within the genetic fields of biology. It has a short generation time and a simpler genome than that of humans, providing a good opportunity to control gene expression within the organism. The genome of *Drosophila* is closely related to that of humans where 75% of disease-related genes overlap. Cancer is a well-known disease in which some of the cells in the body grow uncontrollably, and per today there is no cure. Malignant tumour growth and spread are the cause of mortality in more than 90% of all cancer patients, and the mechanisms of what causes malignancy are poorly understood. This experiment is one of many steps necessary to obtain a greater understanding of the communication between cancer cells and healthy surrounding tissue cells. *Drosophila* offers a system where a tumour can be induced in a restricted region using RET/Stit combined with LexA for controlling expression patterns. The surrounding neighboring cells can also be controlled by using a second binary system which *Drosophila* provides. We were provided with a selection of different LexA driver lines and have through this experiment identified their expression sites, finding suitable LexA drivers which can be used to compare RET/Stit expression to tumour development. Two of the lines were exposed to Stit to see which effect this might have. The results indicate that Stit promotes the spreading of cells as the expression sites altered from the control samples.

Introduction

The body size of an organism is an important trait developed over time to adapt to a specific environment (Layalle et al., 2008). Cellular and organismal growth in animals depends on two factors: the rate of growth, and the duration of the growth period. Both these factors are regulated

and modulated by environmental factors such as nutritional and hormonal cues (Layalle et al., 2008; O'Farrell et al., 2013). Within many species, the insulin/IGF family plays an important role in setting the growth rate as it is one of the factors connecting nutrition intake and growth (Layalle et al., 2008). In all eukaryotes studied, the conserved protein complex target of rapamycin complex 1 (TORC1), is the link between the insulin/IGF family obtained via nutritional cues and the regulation of cellular growth and proliferation (O'Farrell et al., 2013).

The TORC1 system is initiated by the binding of insulin to the insulin receptor (InR) activating the insulin substrate (IRS), which in turn activates a downstream signalling process. This signalling process includes phosphatidylinositol-kinase class 1 (PI3K-1), which in turn phosphorylates and activates the protein kinase Akt, promoting TORC1 activation (O'Farrell et al., 2013; Schmelzle & Hall, 2000). Recent research has found that the proto-oncogene RET (Rearranged During Transformation) has an impact on the TORC1 system, resulting in cell and tissue overgrowth (O'Farrell et al., 2013). The RET proto-oncogene encodes a receptor tyrosine kinase which is expressed in tumours and tissues originating from the neural crest (Eng, 1999). Different rearrangements of RET have been detected in several different varieties of human cancers such as lung and thyroid cancer (Takahashi et al., 2020).

Malignant tumour growth and spread are the cause of mortality in more than 90% of all cancer patients. It is a disease in which the abnormal cells divide uncontrollably and can spread to nearby tissues and sometimes other regions and organs of the body through the blood and lymph systems (National Cancer Institute, 2022). A homolog of RET has been identified in *Drosophila melanogaster* (the fruit fly), first used as a model organism by Thomas Hunt Morgan (Markow, 2015), called Stitcher (Stit). This togeth-

er with the fact that downstream signalling processes are widely conserved makes *Drosophila* a good model organism for cancer research. Stit encodes a RET-family receptor tyrosine kinase which is required and activated during epidermal wound healing in *Drosophila* embryos (O'Farrell et al., 2013; Wang et al., 2009). As a consequence of wound healing, it is also found to promote growth in the *Drosophila* epithelial imaginal wing discs, where it controls the balanced growth of the dorsal and ventral wing disc compartments. Stitcher is therefore required for optimal growth and activates the TORC1 downstream signalling pathway (O'Farrell et al., 2013; Wang et al., 2009). Both oncogenes RET and Stit show stimulatory effects on cell migration, a process allowing the movement of individual cells or a group of cells from one location to another (Boekhorst & Friedl, 2016).

Understanding the relationship between the microenvironment and the tumour itself requires a complex that enables the induction in one cell while still allowing the surrounding neighbouring cell's gene expression to be regulated, in other words, two independent binary systems (Lai & Lee, 2006). The combination of the binary systems can therefore be utilised to find out what causes the transition to malignancy, and to what extent the surrounding tissue of a tumour prevents or promotes the migration of aberrant cells. The *Drosophila* model organism provides such a system, using RET/Stit which promotes tumour combined with LexA for controlling the expression patterns of these cells (Lai & Lee, 2006; Boekhorst & Friedl, 2016).

Drosophila is a great genetic model organism due to a variety of benefits. *Drosophila* generates a large number of externally laid embryos that are transparent throughout the larval stages of development, have a quick generation time of only 10 days, and is simple and inexpensive to maintain in the lab (Jennings, 2011). Humans and *Drosophila* share a strong genetic relationship; between the two species, 60% of the genes in general and 75% of disease-associated genes are shared (O'Farrell et al., 2013). *Drosophila* has a simpler genome, providing a better opportunity to control gene expression. The imaginal wing disc, which becomes the wing of the organism, is easily accessible within the larval stage of development and the genetic expression within this area is easily controllable, making it a suitable organ for characterizing genetic expression (O'Farrell et al., 2013). *Drosophila* has four chromosomes which make site-specific insertions easier. To ensure that the whole gene (and chromosome) of interest is passed on to the following generation, balancers are used on entire chromosomes to inhibit recombination (Miller et al., 2019).

Drosophila genetics provides the binary expression system GAL4/UAS (Upstream Activation Sequence), con-

sisting of two components GAL4 transcriptional activator which is inserted in a combination of an enhancer. When activated, it expresses GAL4. The UAS promoter is activated in the presence of GAL4, promoting transcription of a gene of choice downstream, in this case being Green Fluorescent Protein (GFP) (Rodriguez et al., 2011). All RNAi (interfering RNA capable of reducing gene expression) lines, as well as the oncogenes RET/Stit, rely on the binary system GAL4/UAS. To knock down the genes within the surrounding cells of the oncogene independent of RET/Stit expression, a separate system is required; one system for controlling the expression of RET/Stit, and one system controlling the expression of the surrounding tissue (Rodriguez et al., 2011). *Drosophila* provides a second binary system, LexA/Aop. This system has the same mechanism as GAL4/UAS, where LexA binds to and activates the Lex-Aop, the LexA operator (Rodriguez et al., 2011). Further, the two systems are not affected by one another and can work simultaneously within the same tissue, allowing researchers to perform two manipulations of gene expression in vivo (Rodriguez et al., 2011).

The aim of this study is to find suitable LexA driver lines and compare RET/Stit expression to tumour development within the imaginal wing disk of the *Drosophila*. To achieve this, different genetic lines had to be created by performing numerous crosses of *Drosophila* flies tagged with different phenotypes to ensure the presence of the desired gene. Our hypothesis is that some of the LexA driver lines will provide suitable and restricted expression patterns, which can later be used to understand the mechanisms of tumour growth and communication within the microenvironment of a tumour. We further hypothesise that the strength of expression will correlate with the penetrance of the tumour phenotype.

Material & methods

At the University of Bergen's fly lab facility, flies were cultivated in an incubator at 25 °C and with a light regulation that mimicked the daily rhythm of the sun in the flies' natural environment. The light turns on at 9.00 in the morning and off at 9.00 in the evening. Flies were stored in tubes containing a food medium and were flipped¹ once a week to a fresh tube of food. All different genetic fly lines were provided by Fergal O'Farrell, associate professor at the University of Bergen (Table 1). Table 1 abbreviations will be used to refer to the genetic lines throughout this paper.

¹ Flipping is the act of transferring flies between two tubes. Removing the lids from both tubes - one containing fresh food and the other one with flies - and fast stacking them on top of one another and rotating them. Next, pounding the stacked tubes on the table, forcing the flies down into the new tube. When completed, replace the old lid and discard the empty bottle with its lid still on.

Table 1. An overview of the *Drosophila* genotype of original genetic lines provided at the beginning of the experiment. The genotype and the corresponding stock number are aligned. The table also provides an abbreviation for each genotype and which chromosome the gene of interest is inserted/present. N.A. indicates information Not Available.

Abbreviation	Stock Number	Complete genotype	Location (Chromosome)	Homozygous
LexAop lines (promoter)				
AopmyrGFP, 3 rd	32209	w[*]; P{y[+7.7] w[+mC]=13XLexAop2-IVS-myr::GFP}attP2	3 rd	Yes
AopmyrGFP, 2 nd	32210	w[*]; P{y[+7.7] w[+mC]=13XLexAop2-IVS-myr::GFP}attP40	2 nd	Yes
LexA enhancer lines (transactivator)				
bx-lexA	54414	w[1118]; P{y[+7.7] w[+mC]=GMR34G06-lexA}attP40	2 nd	Yes
wi ap-lexA	54268	w[1118]; P{y[+7.7] w[+mC]=GMR42A06-lexA}attP40	2 nd	Yes
wi ap-lexA	53641	w[1118]; P{y[+7.7] w[+mC]=GMR42D11-lexA}attP40	2 nd	Yes
nub-lexA/CyO	54415	w[1118]; P{y[+7.7] w[+mC]=GMR78A01-lexA}attP40/CyO	2 nd	No
ptc-lexA	54926	w[1118]; P{y[+7.7] w[+mC]=GMR69F10-lexA}attP40	2 nd	Yes
ptc-lexA	N.A.		2 nd	Yes
kn-lexA	48576	w[1118]; P{y[+7.7] w[+mC]=GMR13F08-GAL4}attP2	3 rd	Yes
trx-lexA	61615	w[1118]; P{y[+7.7] w[+mC]=GMR83F09-lexA}attP40	2 nd	Yes
salm-lexA	61619	w[1118]; P{y[+7.7] w[+mC]=GMR85E08-lexA}attP40	2 nd	Yes
LexAopStit lines (promoter)				
F1-M	N.A.	LexAop-Stit	X	Yes
F2-M	N.A.	LexAop-Stit	X	Yes
Control (wildtype)				
WT	N.A.	W ¹¹¹⁸		Yes
Double balancer				
DB	N.A.	$\frac{Sp}{CyO} ; \frac{Dr}{Tm6C}$		No

Selection of Flies - Fly Pushing

The balancers had different phenotypic expressions, enabling us to sort out the flies containing the genetic lines of interest with the use of a microscope (Figure 1). The technique used is called fly pushing and refers to the daily sorting of flies. Flies are emptied from their tube onto a CO₂-perfused pad, knocking out the flies instantly. A small paintbrush is then used to 'push' the flies around to select for or against anatomical characteristics and phenotypic expressions. A Leica light microscope with additional spotlights was used for fly pushing.

Setting Crosses

Crosses were set on a Friday, ensuring that the F1 generation started to emerge on a Monday (10 days after fertilization). In order to make a cross, 4-8 virgin females and 2-4 males were required. Virgin females have specific characterisations visible with the use of a microscope. They

are more transparent, paler in colour, and possess bloated abdomens. The crosses were flipped to a new tube 4-5 days after the cross had been made, preventing the parent generation and F1 generation to be mixed.

Generation of Double-Balanced Stocks

The goal was to create double-balanced stocks containing a LexA enhancer line on the 2nd chromosome, and LexAopmyr::GFP 3rd chromosome (Figure 2). There was one exception, that of the KnLexA, where the aim was to have both the LexA and LexAopmyr::GFP on the 3rd chromosome. The differentiation was caused due to the position of the KnLexA enhancer on the 3rd chromosome (Figure 2). The goals required a multistep crossing scheme. A short summary of the original genetic lines needed to create each double-balanced stock is included (Figure 2). A slash (/) indicates that the genes are on the same chromosome whereas a semicolon (;) indicates that the genes are on separate chromosomes of *Drosophila*.

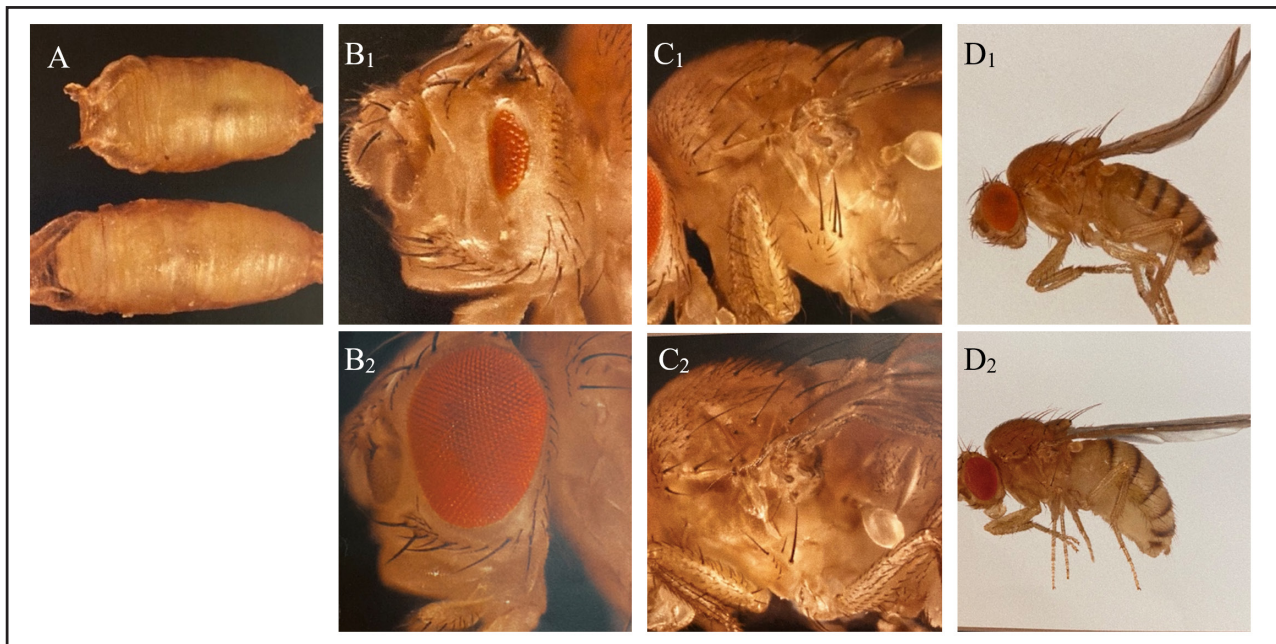


Figure 1. Phenotypic expressions of balancers and corresponding wild type of *Drosophila* used in the experiment. A: C: Tb (Tm6C), reduction of body size (above), and wildtype (below). B: Dr, reduction of eye (B1) and wildtype (B2). C: Sp, additional hairs behind the first anterior leg of the fly (C1) and wildtype (C2). D: CyO, curly wings (D1) and wildtype (D2).

Simultaneously as the multistep process of creating double-balanced stocks started, direct crosses of each LexA promoter line and Aopmyr::GFP were also made (before the enhancers had been balanced). This was done to check if there was a GAL4 expression pattern, shown with GFP-positive cells if present. If the results were negative, meaning no expression pattern, the given LexA enhancer line would not be used further in the experiment.

Microscopy and Dissection

The imaginal wing disk of wandering third-instar larvae was removed by dissection and fixed in 500 µl 4% formaldehyde/PBS (Phosphate-Buffered Saline) for 20 minutes in a well plate. Then the disks were rinsed with PBS for 3x1 minute before being fixed in 500µl PBS containing Hoechst 10 µg/ml with a 1:10 000 ratio for 20 minutes. Lastly, the disks were rinsed with PBS.

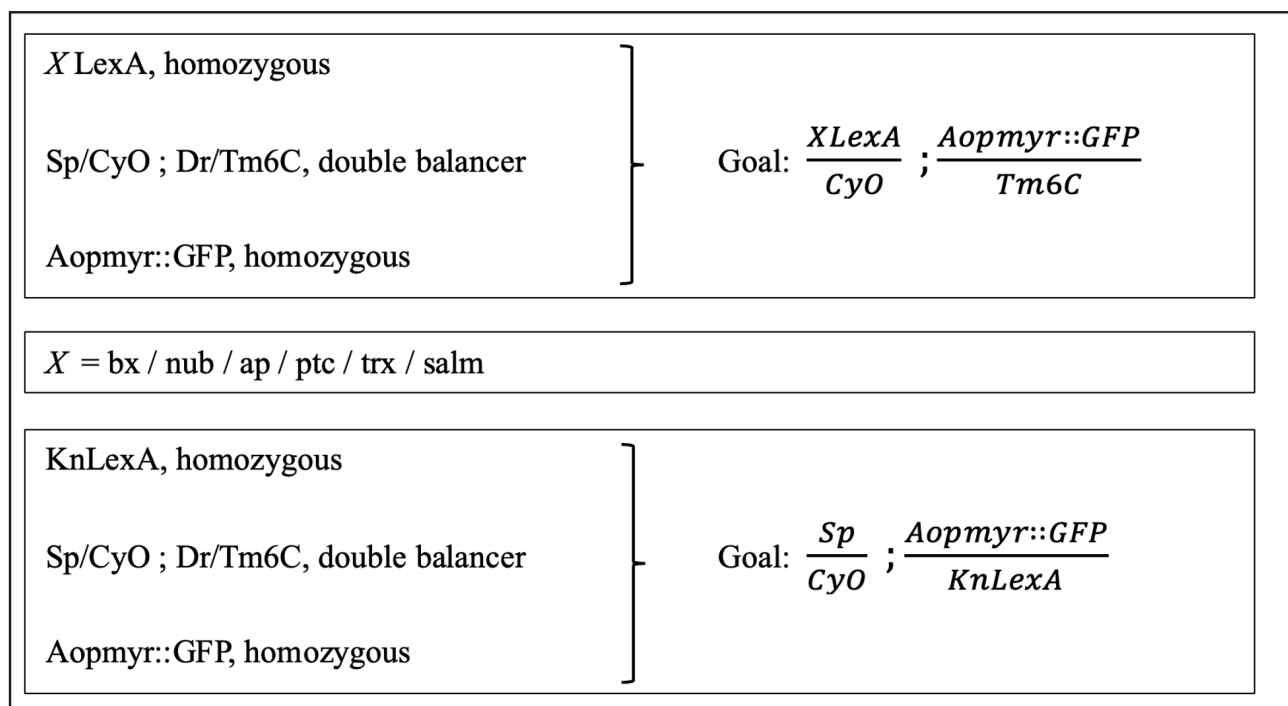


Figure 2. Presentation of the crossing scheme aim for obtaining double balanced stocks. The original lines (left) that were used to create the goal (right). The procedure was consistent for all different LexA enhancer lines; therefore, the X is used in order to indicate each LexA enhancer line. The goal and original lines needed for KnLexA are also shown.

A drop of glycerol mountant was added to an objective slide by using a pipette. The imaginal wing disks were then transferred to the mountant, and an objective glass was placed on top. The objective slides were labelled immediately afterward with the genotype of the imaginal disks. All objectives were stored in a fridge until microscopy of the disks. The dissection was performed using a surgical needle and forceps. A minimum of three discs showing the same result for each genotypic expression was required before conclusions could be drawn.

Illumination microscopy imaging was performed using a Leica inverted microscope and the LasX Leica software. The disks were illuminated with 420nm and 488nm wavelength light produced by an LED laser light source for the detection of Hoechst and GFP-positive cells/expression, respectively. Following image acquisition samples were compared using ImageJ Fiji.

Double-Balanced Enhancer LexA lines exposed to Stit

The completed double-balanced LexA;AopmyrGFP stocks were crossed with LexAopStit (Stit). The goal was to detect any changes that might occur within the GAL4 expression pattern when being exposed to Stit. At least two samples of each Stit;LexA;AopmyrGFP as well as a control, not crossed to Stit, were made for each completed line following the same procedure as the microscopy and dissection before (section “Microscopy and Dissection”). Due to limited time, only the LexA enhancer lines PtcLexA and ApLexA was exposed to Stit.

Results

Direct Crosses of LexA Promoter lines for GAL4 Expression

The expression patterns of all results are shown with GFP-positive cells, if present, within the imaginal wing disc of *Drosophila*. The direct cross of different LexA pro-

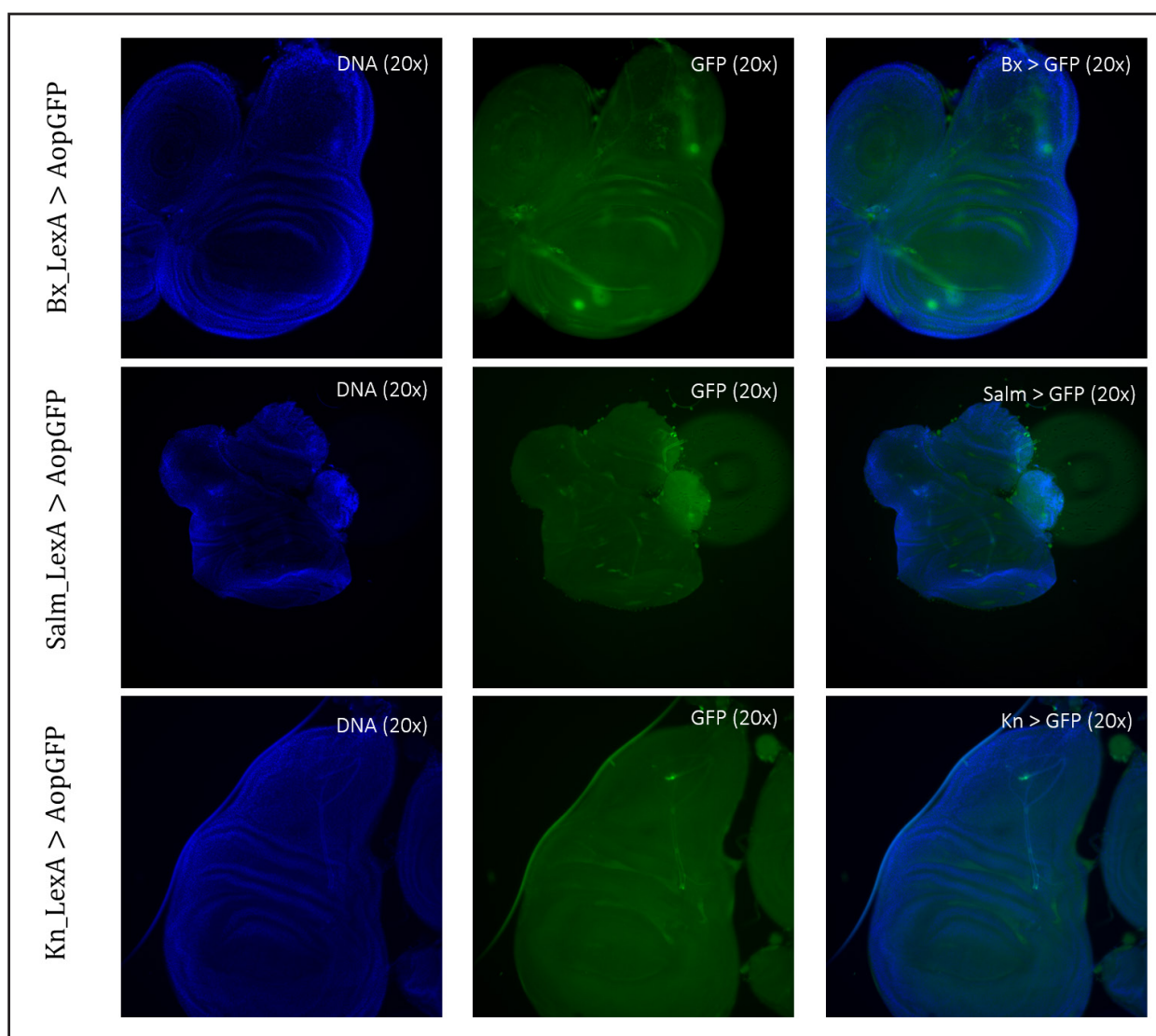


Figure 3. Negative GFP-cells in tissue samples from LexA promoter for GAL4 expression in *Drosophila*. BxLexA, SalmLexA, KnLexA, showed no expression sites of GFP-positive cells within the imaginal wing disk of *Drosophila*. The GFP scan shows that there is only background colouring for these driver lines.

motor lines for GAL4 expression is presented in Figures 3 – 5. GFP-positive cells were observable in the LexA promoter fragmented genetic lines of Trx, Ap, Ptc, and Nub (Figure 4 – 5), whereas the LexA promoter fragmented genetic lines Bx, Kn, and Salm were GFP-negative (Figure 3). The GFP-positive expression sites varied within the imaginal wing disc among the different genetic lines, however,

all lines had some expression within the wing pouch area. Further NubLexA only showed expression in 50% of the imaginal discs dissected due to this line not being homozygous. To confirm GFP-positive cells a tube with larvae was held under a LED light where GFP was visible as a brighter region in the anterior region of the larvae (Figure 6).

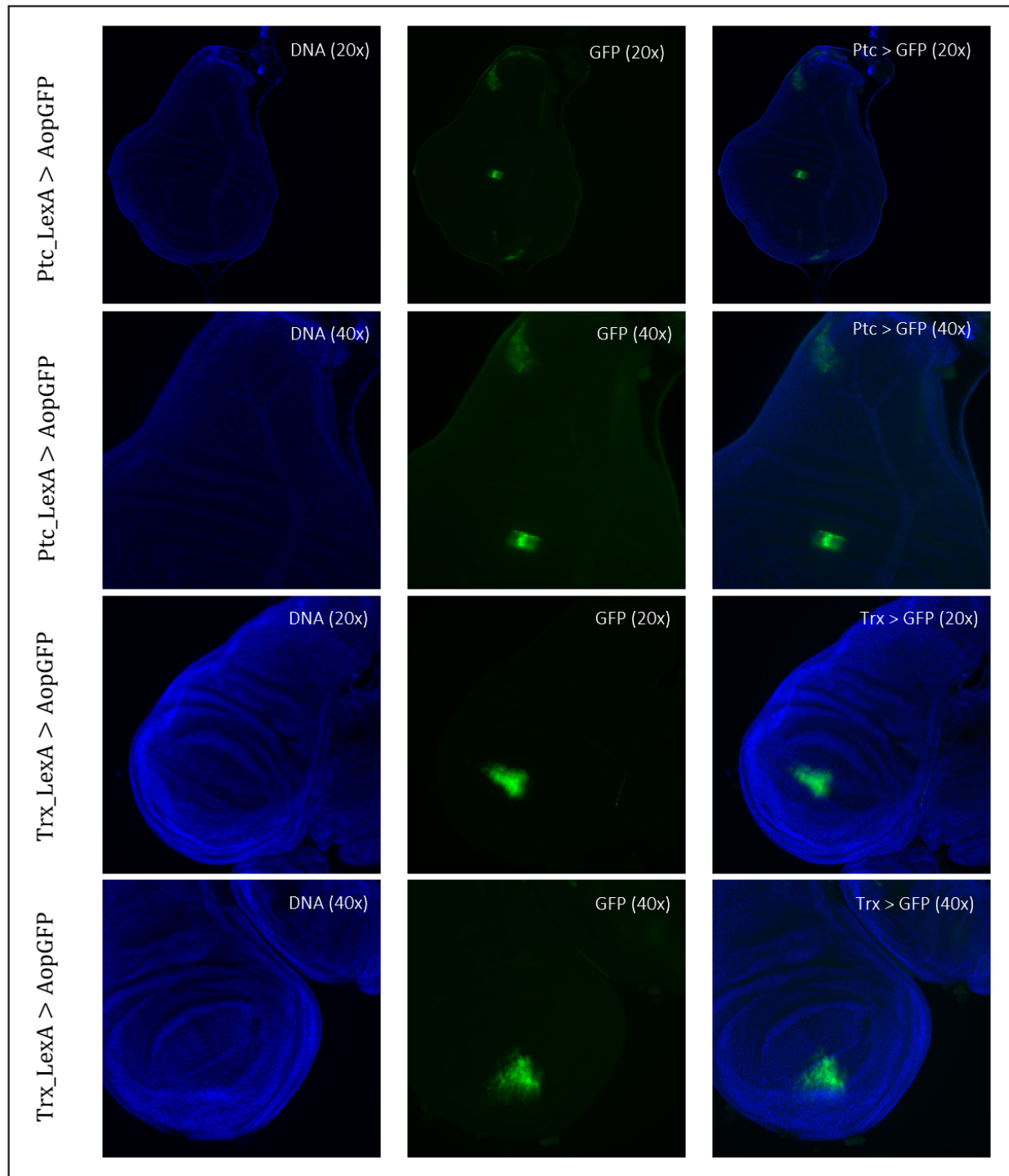


Figure 4. Tissue samples from LexA promoter for GAL4 expression in *Drosophila*. GFP-positive cells were observable in different regions of the imaginal wing disk of *Drosophila* for driver lines Ptc(54926)LexA and TrxLexA. PtcLexA showed fragmented expression within the wing disk, including the wing pouch and notch. TrxLexA showed a restricted region of positive GFP cells in the pouch area of the imaginal wing disk.

Crosses of Double-Balanced LexA promoter lines for GAL4 Expression when exposed to Stit

Crosses of double-balanced lines Ptc>GFP and Ap>GFP with Stit-transformation of cells showed abnormal expression patterns. Two different positioned Stit-transformations were used, Stit FM1 for Ptc expression (Figure 7) and Stit FM2 for Ap expression (Figure 8). Stit>Ptc>GFP,

Stit-transformed cells, showed an elongated expression fragment within the wing pouch of the first sample, less centered than that of the control (Figure 7). The second sample showed more centered fragments similar to that of the control. The region of expressions for both samples were overlapping with that of the control.

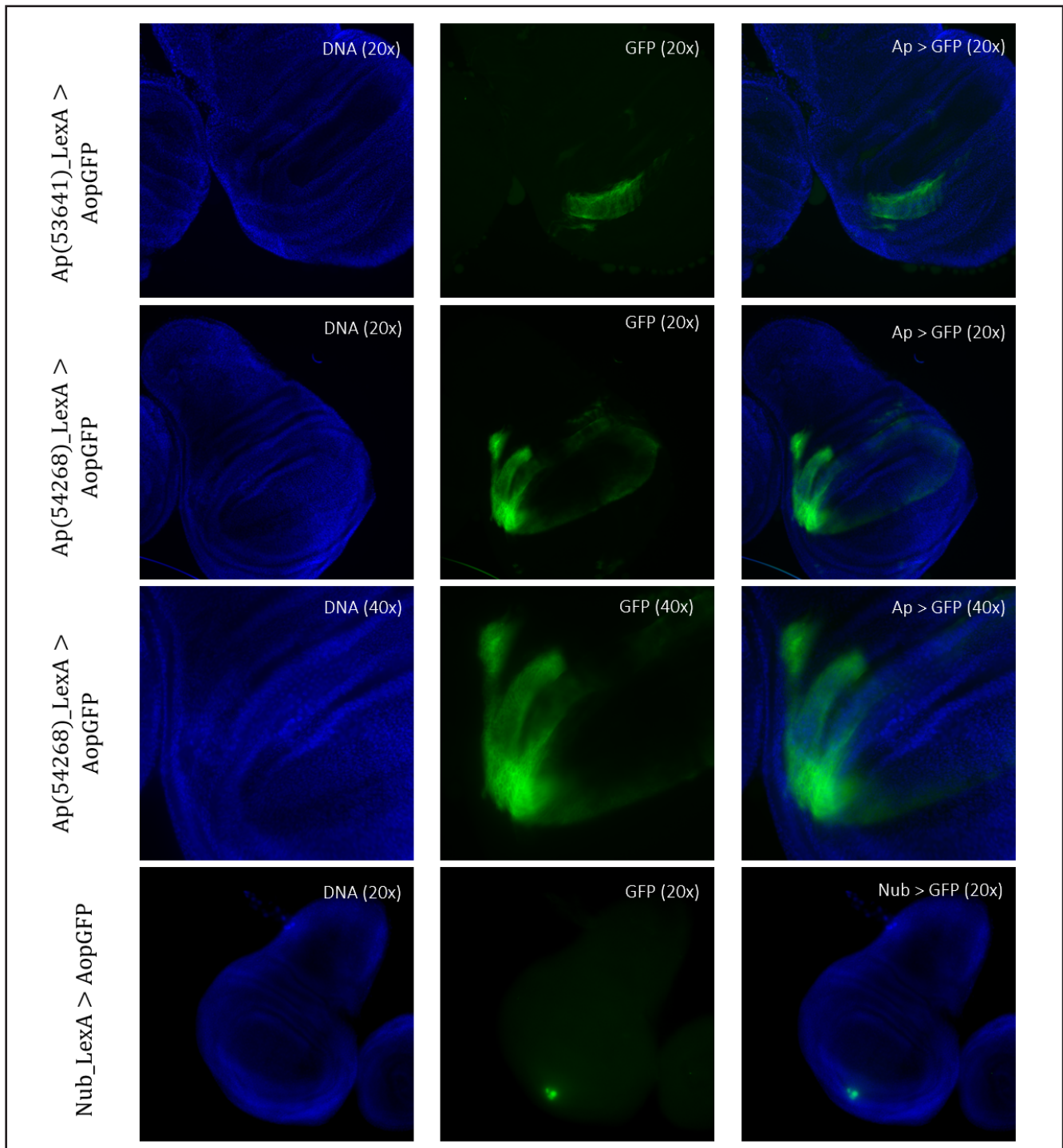


Figure 5. Tissue samples from LexA promoter for GAL4 expression in *Drosophila*. GFP-positive cells were observable in different regions of the imaginal wing disk of *Drosophila* for driver lines Ap(53641)LexA, Ap(54268)LexA, and NubLexA. Both ApLexA lines had GFP-positive cells in the dorsal region of the wing pouch, where the expression pattern of Ap(54268) was also present in the wing thorax. NubLexA showed three small fragments of GFP-positive cells within the ventral region of the wing pouch.

Figure 6. 3rd instar larvae with NubLexA promoter for GAL4 expression in the imaginal wing disc. The GFP-positive region was identified in the anterior region of the larvae as a small circle with a brighter colour on both the left and the right side of the organism. The figure shows one larva to the left (A) and the same larva but at a higher magnification to the right (B). The areas with expressions are highlighted with arrows and circles.

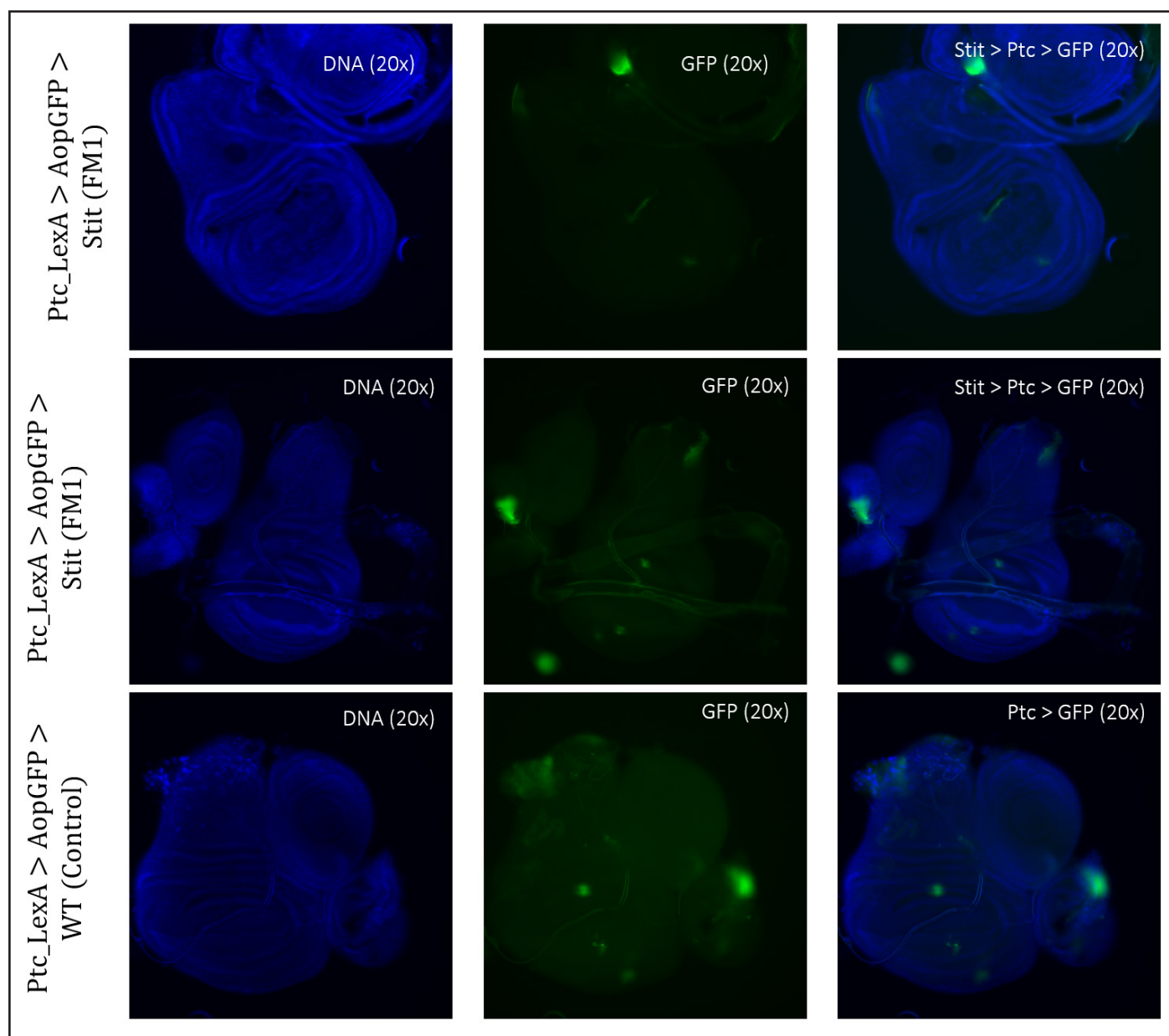
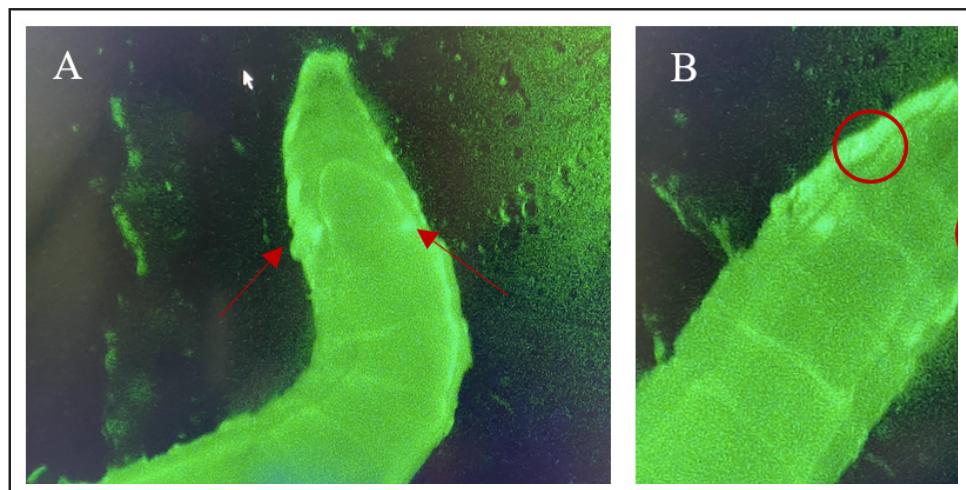


Figure 7. Tissue samples from PtcLexA promoter for GAL4 expression when exposed to Stit in *Drosophila*. Wing disk where PtcLexA>GFP is shown as fragments within the pouch and notch. Stit-transformation of cells (Stit>Ptc>GFP) leads to more diluted fragments of GFP-positive cells. The areas of GFP expression are consistent with that of the control.

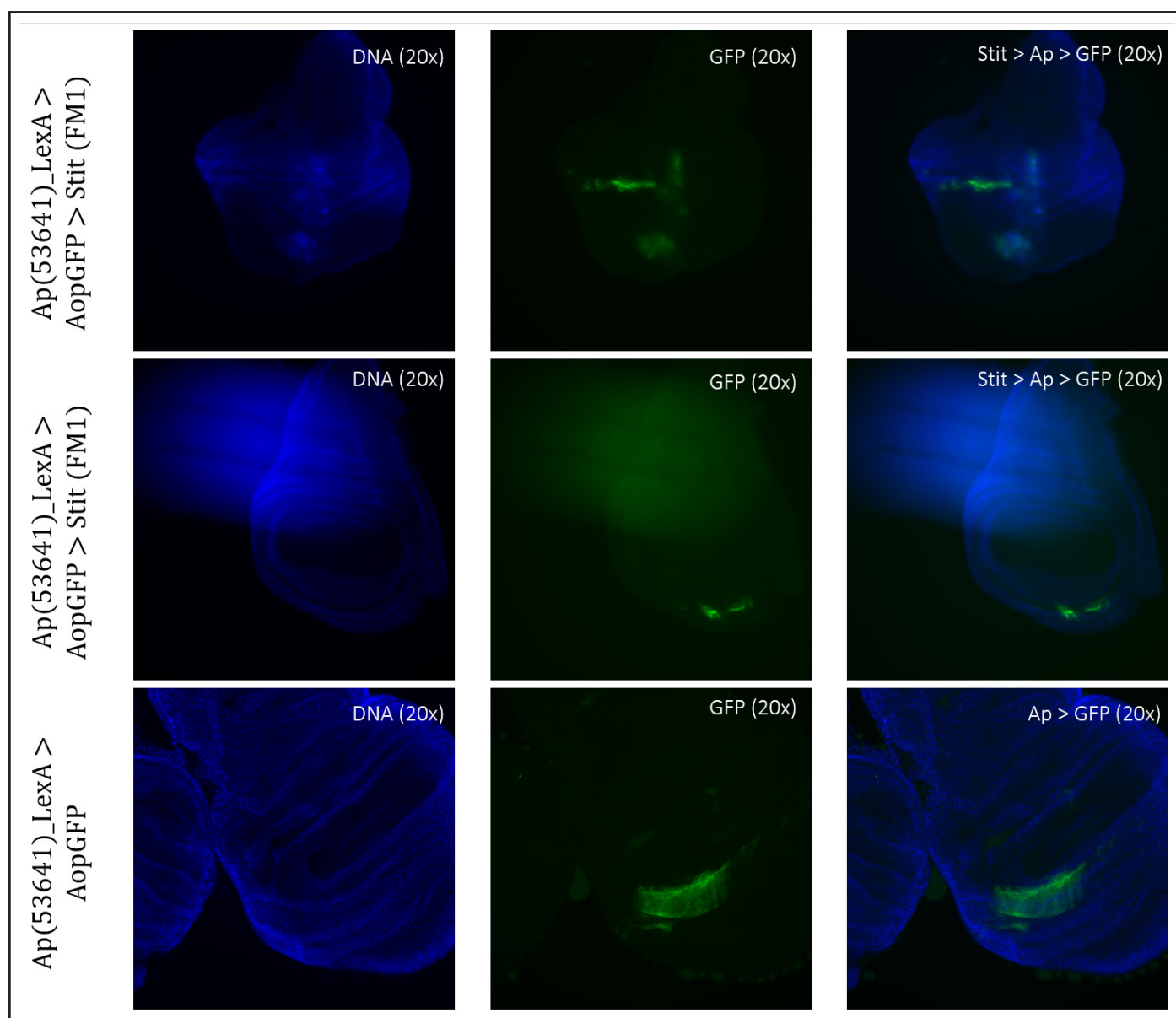


Figure 8. Tissue samples from ApLexA promoter for GAL4 expression when exposed to Stit in *Drosophila*. Wing disk where ApLexA>GFP is shown as fragments within the pouch. Stit-transformation of cells (Stit>Ap>GFP) leads to more diluted fragments of GFP-positive cells within the pouch of the imaginal wing disk. The areas of GFP expression are somewhat consistent with that of the control.

Stit>Ap>GFP, Stit-transformed cells, showed a smaller area of GFP-positive cells than that of the control. This result was consistent for both samples of Stit>Ap>GFP (Figure 8). The first sample was disturbed due to the positioning on the objective glass, however, there were still GFP-positive cells present within the wing pouch, which were more elongated than the control. The second sample showed a strongly reduced expression region in the pouch, with two separate fragments instead of one larger fragment.

Discussion

Cancer, characterized by uncontrolled growth and spread of abnormal cells, is a major public health concern globally and is one of the leading causes of death (National Cancer Institute, 2023; World Health Organization, 2022). The rise of cancer incidence is attributed to various factors,

including changes in lifestyle, exposure to environmental variables, and the aging of the population (National Cancer Institute, 2023). With numerous types of cancer, each having distinct risk factors, symptoms, and therapies. Understanding its causes, preventing its incidence, and finding effective therapies require continuous research and attention.

To investigate the effects of RET/Stit expression on tumour development, we aimed to identify suitable LexA driver lines with restricted expression patterns within the imaginal wing disc of *Drosophila*. Out of eight LexA drivers tested, five were identified as suitable, and two of them, PtcLexA and ApLexA, were further exposed to Stit for an insight into the effects this might have on the cells. Using the binary system LexA/Aop transcribing GAL4, we identified the expression sites within each genetic line, leading

to GFP-positive cells if the gene was present. This way of experimenting along with the use of the two different binary lines is considered to be a decent method for identifying the expression sites based on our results and earlier research within the same scientific field (Rodriguez et al., 2011). Further, the use of balancers has been advantageous as it ensures the whole gene of interest is passed on to the next generation. This mechanism played a crucial role in the project and is a common technique when working with *Drosophila* gene manipulation (Miller et al., 2019).

Expression of LexA driver lines within the imaginal wing disc of *Drosophila*

For detection and identification of the expression within the imaginal wing disc of *Drosophila*, direct crosses of the driver LexA lines to AopGFP were made. The results showed that the lines ApLexA, TrxLexA, PtcLexA, and NubLexA (Figures 4 – 5) had GFP-positive cells within the wing disc of *Drosophila*, whereas the lines BxLexA, KnLexA, and SalmLexA (Figure 3) were GFP-negative. The GFP-positive cells of Ap-, Trx-, Ptc-, and NubLexA were expressed differently in correlation to the region of expression and total area of expression. NubLexA and PtcLexA had the most fragmented and restricted expressions of the lines (Figure 4 – 5). PtcLexA had a clear fragment in the pouch area and another more elongated expression in the thorax of the wing disc (Figure 4). The NubLexA was highly restricted and showed a lower intensity expression compared to that of the others present in the lower region of the wing pouch (Figure 5). As the NubLexA/CyO was not homozygous (Table 1), only 50% of the larvae had the correct genotype, NubLexA;AopGFP, and the other 50% would be CyO;AopGFP. A higher number of samples to confirm expression was therefore required to confirm GFP-positive cells. An additional control by holding the tube with larvae under a GFP light was also performed (Figure 6). This check confirmed our microscopy dissection results that there were GFP-positive cells in the imaginal wing disc.

TrxLexA had a relatively large expression site compared to that of Nub and Ptc. The GFP-positive cells were restricted to the wing pouch of the imaginal wing disc (Figure 4). Both of the ApLexA lines had high-intensity expression sites. Since these lines were different fragments of the same gene, the expression differed between them. The fragment of Ap(54268)LexA had a larger area of GFP-positive cells than that of Ap(53641) present in both the pouch and the thorax of the imaginal wing disc (Figure 5). Ap(53641) was only present in the pouch of the imaginal wing disc. It is difficult to say whether these fragments have some areas where their expression might overlap as the samples of Ap(53641) were of a later larvae stage. This can be concluded as the pouch has started to fold in this sample (Figure 5).

Exposing selected LexA Driver Lines, PtcLexA and ApLexA, to Oncogene Stit

Exposing PtcLexA and ApLexA to Stit resulted in changed expression sites of GFP-positive cells within the discs when compared to that of the control (Figure 7 – 8). It has been shown through previous studies that Stit is a promoter for migratory of cells (Boekhorst & Friedl, 2016). There are indications of migratory cells of both GFP-positive regions of the PtcLexA and ApLexA when exposed to Stit (Figure 7 – 8). Two different samples of each LexA line exposed to Stit were presented in this study, where the two samples in both cases differed slightly from each other. Stit>Ptc>GFP resulted in more diluted fragments of GFP-positive cells. The GFP-positive cells of the wing pouch had a more elongated pattern reminding of a line rather than a centered fragment like it was in the control (Figure 7). This was only the case for the first sample (Figure 7, column 1); the second sample's GFP-positive cells in the pouch region were more similar to that of the control, whereas Ptc>GFP. Ptc>GFP also showed a region of GFP-positive cells within the dorsal region of the thorax. This area was also altered when exposed to Stit in the second sample. Here, it looked like the expression was covering a larger area than that of the control (Figure 7, column 2).

Stit>Ap>GFP resulted in more elongated regions of expression, especially within the first sample (Figure 8, column 1). Since this sample was disrupted when mounted, it is not possible to draw a conclusion. However, there are clear indications that the expression changed within the region, resulting in a more long-stacked GFP-positive region. The second sample (Figure 8, column 2) shows two separate fragments of expression in the pouch instead of one completely larger region like in the control, Ap>GFP. Given that the Stit>Ap>GFP wing disc is at an earlier developmental stage than the Ap>GFP wing disc, it is plausible to conclude that the areas of expression are shared, allowing for direct comparisons. (Figure 8). The second sample (Figure 8, column 2) shows signs of movement because there are several small GFP-positive 'stripes' elongating from the higher intensity fragment of GFP-positive cells (Figure 8).

Summary of Observations when Exposing LexA Driver Lines to Stit

The images of the samples from both the Stit>Ptc>GFP and Stit>Ap>GFP indicate that there is a movement of cells when exposed to Stit as the expression of GFP-positive cells differs from the control sample results. These findings support the fact that Stit is a promoter for the migration of cells and is further supported in earlier research on Stit (Boekhorst & Friedl, 2016; O'Farrell et al., 2013; Wang et al., 2009). During this experiment, two different Stit lines were used, StitFM1 and Stit FM2. It is not possible to draw

comparisons between the two in this case due to the expression with different LexA lines.

Conclusion

In this experiment, we aimed to identify suitable LexA driver lines with restricted expression patterns within the imaginal wing disc of *Drosophila*. We successfully identified five out of eight LexA drivers to be suitable for comparing the effects of RET/Stit expression in tumour development. Two of the appropriate driver lines, PtcLexA and ApLexA, were further exposed to Stit to gain insight into the effects on the cells. The expression sites within each genetic line were identified using the binary system LexA/Aop transcribing GAL4, which ultimately resulted in GFP-positive cells when the gene is present. Based on our findings and previous research in the same scientific field, this method of experimenting, along with the use of two different binary lines, is regarded as a reasonable method for identifying expression sites (Rodríguez et al., 2011). Our findings suggest that the strength of expression correlates with the penetrance of the tumour phenotype, but additional research is needed due to inconsistencies in expression and a lack of repeated experiments.

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Odonata
Photo: Noemí Segura

Investigating the Number and Colour of Photophores of *Maurolicus muelleri* in Two Norwegian Fjords

Anette Aune, Kristian R. Fjeld, Joseph C. Lawrence
and Adam Mortensen

Abstract

Maurolicus muelleri is a dominating mesopelagic fish in Norwegian fjords, yet little is known about its photophores in relation to life history and environment. *M. muelleri* from Masfjorden and Fensfjorden were investigated to see whether body condition, age, environment, or depth have any effect on photophore number or colour. We found no relationship with body condition or age. However, our findings suggest *M. muelleri* higher in the water column (0–100m) have more photophores, and a higher proportion of them had green photophores than deeper down (200–300m). The observed proportion between pink and green photophores in total was 1:4. The total number of ventral photophores among individuals varied, with a mean of 87.9 ± 2.62 , which is inconsistent from a constant amount of photophores previously reported in the literature. A higher proportion had pink photophores in Masfjorden than in Fensfjorden. Further research concerning the topic is necessary for a better understanding of this fascinating trait, and the next step seems to be investigating the relationship between photophores and light attenuation.

Introduction

The vast mesopelagic zone, often known as the twilight zone, is located underneath the sunlit euphotic zone and receives just enough light for vision (Christiansen et al., 2021). Irigoien et al. (2014) estimated the global biomass of mesopelagic fish to be between 6 000 – 200 000 million tons. This estimation is one order of magnitude larger than the earlier estimations, raising questions and interest about their global ecological importance in the world's oceans, and the possibilities of potentially exploiting them as a novel marine resource. The exploitation of this resource would require more knowledge and understanding of both the ecology of these communities and the biology of the species involved.

The distribution of mesopelagic organisms in the water column depends on several abiotic factors (Aksnes et al., 2009). Many species, therefore, perform diel vertical migration, ascending to the surface at dusk and descending back into the depth at dawn (Staby et al., 2013). This allows mesopelagic organisms to stay undetected, whilst still staying at light levels which allows foraging, usually referred to as the antipredation window (Christiansen et al., 2021).

Life history is a term used for traits and strategies describing aspects of an organism's life (Ratikainen, 2018). Examples of this are a species' length, weight, sex, age, maturity stage, body condition, and eye size. It is pertinent to investigate the influence certain life history traits have over others, for example, how weight limits size, how sex can influence total length, and so on. These life history traits and strategies may even influence the development and function of specific organs (Stearns, 1992).

Maurolicus muelleri (Gmelin, 1789)

In the oceanic environment, the only light source apart from downwelling irradiance is bioluminescence, produced by marine organisms themselves (de Busserolles & Marshall, 2017). In fishes, bioluminescence is a common feature, with around 90% of fish exhibiting this trait (Herring, 1978). For many mesopelagic fishes it is used as a mechanism for communication, mimicry, attracting prey, and predator avoidance (Cavallaro et al., 2004).

Maurolicus muelleri is one species that use bioluminescence for counter illumination, hiding its silhouette from predators below. What defines this species are the distinct rows of photophores (bioluminescent organs) that run along the ventral side of the fish. Little is known concerning how life history strategies may control these organs' development and function.

M. muelleri (Stomiiformes: Sternoptychidae), commonly known as Mueller's pearlside, or simply pearlside, is a cosmopolitan fish species, and is one of the most abundant mesopelagic fishes in Norwegian fjords and the Northeast Atlantic Ocean (Grimaldo et al., 2020; Giske et al., 1990; Gjørseter & Kawaguchi, 1980). They tend to be short lived, living no longer than 5 years, and typically grow to a size of 4-5 cm with a mean weight of 0.9g. (Giske et al., 1990; Folkvord et al., 2016). *M. muelleri* plays a key role as both a planktivore and as prey for large fish, like saithe (*Pol-lacius virens*) and blue whiting (*Micromesistius poutassou*) (Giske et al., 1990).

Individuals of *M. muelleri* distribute according to size in the water column (Staby & Aksnes, 2011) and this has been attributed to the conspicuousness of individuals in different life stages. Fry and juveniles are often small, transparent, and occupy shallower depths (from surface to ~100m) of the water column, where they can avoid predators. It has been described that *M. muelleri* performs diel vertical migration (Staby et al., 2013) and has specially evolved eyes. In the place of cone-cells, or rod-cells, it has rod-like cone cells, a combination of both, which allow individuals to see extremely well in low light, used as a mechanism to migrate alongside larger fish, whilst still avoiding predators (de Busserolles et al., 2017).

Photophores & Bioluminescence

Many marine species produce light (bioluminescence) from specialised light organs called photophores. Photophores are complex organs, consisting of light cells (photocytes) that produce the light through a protein-enzyme reaction of luciferin and luciferase, in addition to a reflector, and a lens (Cavallaro et al., 2004). The reflector covers the inner layer of the photophore, reflecting light produced from the photocytes towards the lens. The lenses functions to concentrate the light emitted towards the opening of the photophore (Cavallaro et al., 2004). In addition, some mesopelagic fish can utilize a masking pigment to cover the lenses of their photophores, thus regulating colour and intensity of light emitted (Clarke, 1963).

The photophores of *M. muelleri* can make up 10% of an individual's body mass (Cavallaro et al., 2004). This heavy investment of resources may indicate that the trait is an integral part of their development. However, the metabolic cost of photophore production is unknown (Folkvord et al., 2016), and if this is a heavy cost, the fish must optimise the amounts of photophores regarding other life history traits, such as reproductive effort and body growth.

Individuals of *Maurolicus* species have usually developed most of their photophores when between 5-20mm (Rodrigues-Ribeiro et al., 2022). Adult *M. muelleri* have consistently been observed to have a maximum of 138

photophores (Cavallaro et al., 2004). This consistent maximum may be genetically determined, but the development of photophores from larvae to adult is yet to be documented. Folkvord et al. (2016) noted a relation between the development of photophores and the nutritional state of larval *M. muelleri* as their slowest-growing cohort tested seemed to have the lowest amount of photophores. Similarly, photophore number and life history traits have not been explicitly compared in the Norwegian fjords, an area in which *M. muelleri* is very abundant. The species is isolated from other *Maurolicus* species in this area, and the effects of interspecies isolation has yet to be described, whether there is any.

Aims & Objectives

M. muelleri is currently targeted by Norwegian trial fisheries for fish meal and oil, to be used in the fish feed industry (Fjeld et al., 2023). As there is a lack of knowledge regarding the biology and ecology of the zone, synthesizing more information on the species living there is vital before further exploitation occurs, to ensure sustainability and precaution regarding this understudied environment (Hidalgo & Browman, 2019; Fjeld et al., 2023). Despite their global abundance and importance in oceanic food webs, *M. muelleri* is an under-studied species, and especially regarding their light organs. Therefore, it is important to cover these gaps in the knowledge, as developing knowledge of a species allows the establishment of effective fisheries.

In this study, *M. muelleri* are sampled from two well-studied western Norwegian fjords, Masfjorden and Fensfjorden. From previous research cruises to Masfjorden and Fensfjorden, there was an observed photophore colour difference between individuals, whereas some individuals had pink coloured photophores, whilst others were green. As of now, there is no clear explanation for why this occurs, or how frequent it is.

There are many aspects of the *M. muelleri* life history that should be studied, especially in relation to photophores. In this study we focus on how photophore number and colour change in relation to depth and the life history traits, namely body condition and age. In addition, we compare *M. muelleri* from both fjords to see whether there are any differences between them.

Our main hypothesis of this study is that the number of photophores will increase with age, until maturity, where a maximum number of photophores will be reached (138). In addition, individuals with good body conditions will afford to maximise their number of photophores. Conversely, we expected those with a worse body condition to have less, as a trade-off between photophore development and survival. Moreover, we expect deeper residing *M. muelleri* possess more photophores both due to their larger sizes

and to better perform counter-illumination.

Material & methods

Research Area

The study was carried out between 24–30 September 2022 in two fjords on the west coast of Norway: Masfjorden and Fensfjorden. Masfjorden is a 494m deep, 24km long arm of Fensfjorden, which at its longest point is 50km and 583m deep. These fjords are separated from each other by a 75m deep sill. Data was collected from both fjords to provide the possibility to compare the influence of environmental conditions on the photophores and *M. muelleri* itself. The vessel used for transportation and data collecting was G. O. Sars, a research vessel shared by the Institute of Marine Research and the University of Bergen.

Field Methods

Individuals of *M. muelleri* were collected using a pelagic trawl fitted with a multisampler, with three codends at fixed depths (sub-layers) of 300m–200m, 200m–100m, and 100m–0m with an oblique haul through each layer. We trawled for approximately 10 minutes per codend, with an average speed of 2.5–3 knots. The multisampler had a mesh opening of stretch 22mm. Two trawl hauls were done in each fjord and each trawl was done between 8pm and 4am. We took a semi-random subsample of maximum 34 individuals of *M. muelleri* from each codend. If individuals were damaged to the point where it would have been impossible to count the photophores, they were discarded, and new individuals were selected, based on whether counting the number of photophores appeared possible or not. When the total number of individuals was 34 or below, all individuals were taken. In addition, we collected a random sample of *M. muelleri* from each codend, to compare with our handpicked individuals showing whether they are representative of the total catch. The subsampled individuals were put on a laminated sheet and scanned with Canon CanoScan LiDE 400. The scans were measured in ImageJ, using standard length. We registered whether the fish had green or pink photophores, and individuals with at least 40% pink photophores were assigned 'pink'. As individuals had several shades of pink, from pale pink to purple, we simplified their colour to only pink, reducing errors due to human subjectivity.

Lab Methods

In the lab, we defrosted and weighed each fish individually (wet weight) in its plastic bag and subtracted the average weight of the bags. We then registered the number of photophores for each section of the fish, following the protocol described by Sutton et al. 2020 (p. 68) for the first 25 individuals of each codend. In the case of individuals being very damaged, we discarded that fish and used one of the

remaining 9 individuals. After counting photophores, we extracted sagittal otoliths to determine age. We determined the age by counting increments on the otoliths, starting at age 0, with no, or a very small increment and assuming formation of the rings begins at hatching and continues until the adult stage. Some fish over 1 year were described as 1+ (written Age 1.5), due to the outer winter ring being the same size as the outer summer ring. Age 2 had two clear winter rings. The data was processed in RStudio, R version 4.2.1 (2022-06-23). We used data collected from the same field course, conducted in previous years and in addition to the data collected this year on size distribution for *M. muelleri*, we checked whether our semi-random sample was a good representation of the population.

We made two models in R, one linear model describing the total number of photophores, with total number of ventral photophores as the response variable, and the fjord, depth, body condition and age as the predictor variables. The second model was a binomial model which used photophore colour as the response variable with the same predictor variables as the first model. Both models were made by finding the best fit through removing parameters in order of least significance.

An ANOVA was performed to check for significant relationships between the different parameters. This was repeated for other comparative analyses. Post hoc pairwise comparisons were also made to further investigate significance found in the ANOVA tests performed.

Body condition was calculated using Fulton's body condition factor K (Fulton, 1904),

$$K = 1000 * W/L^3$$

where W is weight (g), and L is length (mm). 1000 is a scaling factor. This condition factor was used to compare against other measurable parameters, rather than estimating the body condition itself. In total, we weighed, registered colour, and counted photophores of 258 individuals, 138 from Fensfjorden and 120 from Masfjorden. 75 fish were not counted, only weighed and colour registered. During our analysis, we found that the number of photophores on the head was constant on all individuals. The only notable variation in photophore number came from the ventral section on the fish (PV, VAV, AC, as given by Sutton et al. 2020, p. 68). By only analysing these photophores, we can include a larger number of fish due to ignoring the instances where it was not possible to record the photophore number. In total, we have 192 individuals with counted ventral photophores, 120 from Fensfjorden and 72 from Masfjorden.

Depth (m)	Masfjorden (individuals)	Fensfjorden (individuals)	Total (individuals)
0 – 100	43	49	92
100 – 200	48	45	93
200 – 300	14	26	40

Table 1. Depth distribution of individuals of *Maurolicus muelleri* used in the analysis of numbers of photophores. Numbers from both fjords and total count are included.

Results

The comparison between the length distribution of our data and the total length distribution of *M. muelleri* caught during night-time on the cruise showed our samples represented the total range of lengths.

Photophore Number

Only 124 of the counted individuals were in a condition where we could count all photophores. The mean number of total photophores for these individuals were 138 ± 2.61 . Of all counted individuals, we were able to fully count the ventral photophores of 225 (Table 1). Of these, 92 where from 0-100m, 93 from 100-200m, and 40 from 200-300m depths. The mean number of ventral photophores for these fish were 87.9 ± 2.62 .

Most individuals sampled in the study were of age class 1 ($n = 202$), with much fewer samples from age class 0 ($n = 2$), age class 1.5 ($n = 15$), and age class 2 ($n = 6$).

There was no significant variation in photophore number related to body condition (ANOVA; $F(1, 221) = [0.83]$, $p = 0.36$), between the two fjords (ANOVA; $F(1, 220) = [0.39]$, $p = 0.53$), or with age (ANOVA; $F(2, 217) = [0.35]$, $p = 0.412$). During model selection, we found the only explanatory variable to be depth ($p = 0.02$), so further downstream analyses were performed using this best fitted model. We found a significant difference in number of photophores in relation to depth (ANOVA; $F(2, 222) = [3.78]$, $p = 0.02$), with the mean number of ventral photophores of individuals at each depth being: 0-100m = 88.4 ± 2.67 , 100-200m = 87.7 ± 2.50 , 200-300m = 87.1 ± 2.59 .

A post hoc pairwise test between total number of ventral photophores and individual codends showed no significant results between the depth layers 0-100m and 100-200m (Post hoc; t ratio = -1.85, estimate = -0.70, $df = 222$, $p = 0.16$), or 100-200m and 200-300m (Post hoc; t ratio = -1.16, estimate = -0.57, $df = 222$, $p = 0.48$). There was, however, a statistically significant result between the shallowest

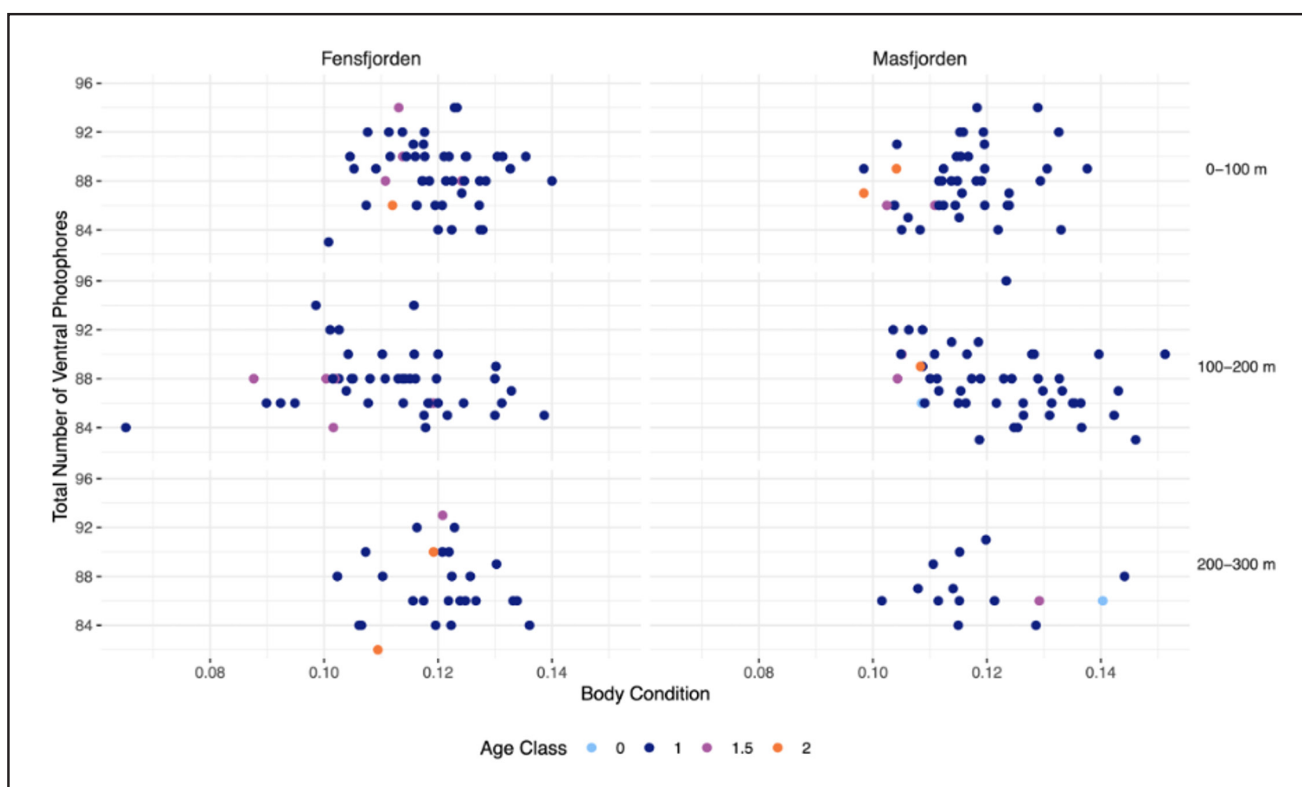


Figure 1. Number of ventral photophores in relation to body condition, depth, and fjord. The relationship between body condition and number of ventral photophores of *Maurolicus muelleri* in the two fjords Fensfjorden and Masfjorden. The depth at which the *M. muelleri* were caught are also included. The age of individuals is shown by the distinct colours of the points. Age was decided by counting otolith increments.

Depth (m)	Masfjorden (individuals)	Fensfjorden (individuals)	Total (individuals)
0 – 100	68	67	135
100 – 200	34	67	101
200 – 300	5	47	52

Table 2. Depth distribution of individuals of *Maurolicus muelleri* used in the colour analyses. Numbers from both fjords and total count are included.

0-100m and deepest 200-300m layer (Post hoc; t ratio = -2.59, estimate = -1.27, df = 222, p = 0.03).

In total, there was a trend of higher number of total ventral photophores in the shallowest layer, but no trends or relationships between age, body condition or fjord (Figure 1).

Photophore Colour

In total, we registered the colour of 289 individuals from all depth layers (Table 2). 217 of the individuals were green (75%) and 72 were pink (25%).

182 of the counted individuals came from Fensfjorden; 143 were green (78%), and 39 were pink (22%). The remaining 107 were from Masfjorden, of which 74 were green (69%) and 33 (31%) pink.

Green coloured photophores occurred in higher numbers in both fjords and depths, except for the deepest depth where the proportion of pink is greater (Figure 2).

Using the best fitted model including only depth and fjord, a chi-squared test showed significant relationships between the colour distribution and fjord (χ^2 (1, N = 289)

= 16.67, p = <0.001), depth layers (χ^2 (2, N = 289) = 44.50, p = <0.001). Age (χ^2 (1, N = 289) = 2.11, p = <0.15) and body condition (χ^2 (1, N = 289) = 1.91, p = <0.17) showed no significant effect on the photophore colour.

A post hoc pairwise test between the observed colour of ventral photophores, and the depths sampled showed a significant difference in colour between the deepest (200m-300m) and middle layer (100m-200m) (Post hoc; p = <0.001 z ratio = 4.71, estimate = 1.92), and between the shallowest (0m-100m) and the deepest (Post hoc; p = <0.001, z ratio = 6.11, estimate = 2.73). There was, however, no significant difference between the shallowest and the middle layers (Post hoc; p = 0.06, z ratio = 2.24, estimate = 0.81).

Discussion

This study is among the first on photophore colour and number, and how these varies in relation to aspect of the species' life history. We found that *M. muelleri* at 0-100m depth have more photophores than those at 200-300m, yet there was much variation in the number at all depths. In total, green photophores was more common than pink,

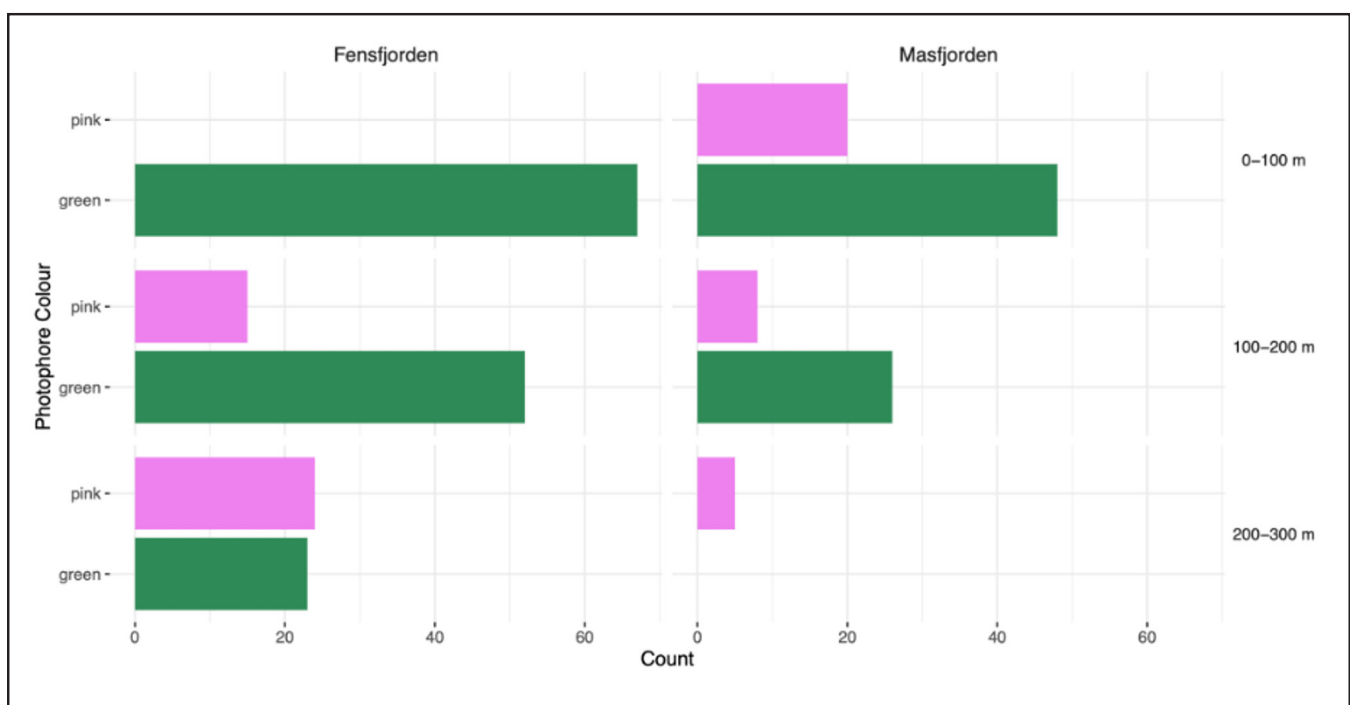


Figure 2. Count of photophore colour in relation to depth and fjord. The number of *Maurolicus muelleri* with green and pink photophores in the two fjords Fensfjorden and Masfjorden. The depth at which *M. muelleri* were caught is also included.

but the relationship between the colours changed between the depths. A higher proportion of pink photophores were found in the deepest layer compared to both shallower. In addition, Masfjorden contained a higher proportion pink photophores than Fensfjorden. In general, it looks like depth is the most crucial factor affecting both aspects of the photophores.

Photophore Number

It was interesting finding a higher number of photophores in the shallowest depth compared to the deepest. This was opposite from what we hypothesised, yet our hypothesis was based on knowledge of larger individuals preferring greater depths (Staby & Aksnes, 2011), which we did not find either. It could be explained by differences in light levels between these depths. The shallowest layer receives more light, and therefore *M. muelleri* here might need more photophores to produce sufficient light to hide its silhouette. However, no literature mention anything about such being found in any species. It might be a result of small sample size, especially for the deepest layer, as mean number of photophores per individual for each layer with standard deviations are quite similar and overlapping. This should be further tested to see if our pattern is indeed there or not.

The literature has consistently reported 138 photophores (Cavallaro et al., 2004), this is including a set of two photophores we did not count (ORB1 in Sutton et al. 2020, p. 68), meaning our mean is higher than the literatures' in addition to showing more variation. Many species may use distinct photophore patterns in species recognition, and the number should therefore stay constant (Clarke, 1963). Davis et al. (2014) found that variation in photophore number within genera of Lanternfishes (Myctophidae) is not sufficient to be used in species recognition. Both findings suggest *M. muelleri* do not use number of photophore in species recognition.

The total number of ventral photophores of *M. muelleri* was not constant, having large variations (82 – 94). These variations are similar, although a bit larger than results of ventral photophore counts from the Red Sea (80 – 90) (Dalpadado & Gjøsæter 1987). Moreover, we observed that some individuals had ventral photophores arranged asymmetrically, with one less photophore on one side. Further analysis to explain this asymmetry was not performed, as it is outside the scope of our project.

It has been postulated that the strict number of photophores is a mechanism of conspecific recognition. *M. muelleri* is the dominating species in these fjords, but also is the only species of the genus in these habitats (Giske et al., 1990; Rasmussen & Giske, 1994). It is very unlikely, if not impossible, they would encounter a species other than *M. muelleri*. This suggests that there would be no selection

pressure to uphold a distinct amount of photophores, as in, if a neutral mutation entered these populations (if they are distinct populations), there would be no positive, and no negative effect if there is no need to discern between species.

However, since our counts from Norwegian fjords have comparable variation to counts from the Red Sea, it could indicate that variation in number of photophores of *M. muelleri* is a universal trait of the species, that can be observed in all the world's ocean. For instance, if a constant number of photophores is inconsequential for counter-illumination functioning, variation in photophore number could arise from neutral mutations.

We observed significant difference in number of photophores between the shallowest and deepest depth layers. The hypothesis here was that individuals in the deeper layers would have more photophores, however, the opposite was observed, and that those in the shallower layers had more, and deeper had less. Following the idea that those deeper in the water column would need more photophores to visualize prey or communicate between conspecifics would not fit this as an explanation. A plausible reason for this observed trend could be that individuals living in shallower depths periodically encounter more predators. It has been described that the eye of this species allows it to exist amongst predators when performing diel vertical migration, having more photophores would perhaps allow them to better perform counter-illumination as a predator-avoidance technique, as such, those with more photophores are more likely to survive (Paitio et al., 2016; de Busserolles et al., 2017).

This could be another example of trait selection, although this case could be evidence for positive selection. Nothing more can be known about this unless genomic investigations are carried out. Similarly, following the trend identified in this study, these concepts do not fit with the established idea that the species distributes due to size (Rasmussen & Giske, 1994).

Photophore Colour

We have not found any literature concerning the colour of photophores of *M. muelleri*, meaning little is known about why we see different colours and what role it plays. In fact, there are pockets of research believing there to be only one colour, green. While it was impossible to comment on the cause of the colour difference, this study can state there were at least two colours in these specimens of *M. muelleri*, green and pink. An explanation for the pink colour could have been damage to the photophore structures, causing blood to stain the films. Another explanation is that *M. muelleri* may use masking pigments to regulate their emitted colour (Clarke, 1963).

On average, the ratio of pink individuals to green was 1:4. This is the first estimation of the occurrence of colour variation in photophores of *M. muelleri*. We do not know whether the colour is a static or dynamic trait. If *M. muelleri* can regulate photophore colour continuously, this estimation only provides a snapshot into how their colour varies during night-time in September, in these fjords. Potentially, pink and green could appear with different proportions throughout the time of day and could also vary with different seasons. In addition, we cannot know how photophore colour is affected by trawling, thus causing bias in our observations. A study conducted on live *M. muelleri* might be necessary to establish this.

Moreover, we observed that when photographing the photophores with flash on, the reflected light from pink photophores came out as blue, meanwhile reflected light from green photophores came out as green. This suggests that in nature, when light is produced from photocytes within a pink photophore, the visible colour emitted is blue, not pink. As blue light penetrates deeper into the depths than green light (Johnsen & Sosik, 2004), this finding makes us hypothesize that pink photophores are an adaptation to greater depths, making the *M. muelleri* better at hiding their silhouette as they better match the light conditions of their surroundings. Higher in the water column, more green light is present and green photophores would be preferable. The difference in photophore colour between the fjords could be related to this as well. Light attenuation of fjords increases when oxygen and salinity decline (Aksnes et al., 2009). If Masfjorden has less oxygen and salinity, we could assume it is darker at specific depths than Fensfjorden. If we had time and resources to investigate this as well, our colour hypothesis might have gotten a stronger validation.

Limitations

All trawling was done during night when *M. muelleri* from different layers are mixed. This could have affected the trends we found as it might not have been completely random which individuals migrated furthest and how they mixed. Repeating the study during daytime would test for this potential bias. Trawling in observed echo layers rather than fixed depths should provide data more representative for the layered vertical distribution of the species. In addition, our age data did not contain sufficient variation to investigate age properly and a relationship might still be found if this study was repeated with a larger sample of each age.

Sub-random sampling could bias our result, however our comparisons with total amount of *M. muelleri* caught during the field course showed our data was similar in distribution of sizes and covered the total length spectre caught. When counting photophores however, we should

have been more critical, and discard all individuals with uncountable photophores to get a fuller dataset and to use total number of photophores instead of just ventral. The size of our sample should be larger, especially for colour from the deepest layer of Masfjorden.

We used Fulton's factor as a measure of body condition even though *M. muelleri* seem to have a weight-length relationship parameter, $b < 3$, while Fulton's factor assumes isometric growth ($b = 3$, Gubiani et al., 2020). This could result in over/underestimates of the condition of the smallest/largest individuals. Even though we found the log-relationship between weight and length to be ~ 3 it was lower, and another measure of body condition could be used to ensure our findings are valid and not a result of biased estimates.

Future Projects

To further investigate photophores and life history of *M. muelleri* this study should be repeated with larger samples and on a larger scale, allowing to compare populations in different ends of the species' distribution. Measurements of light attenuation at different depths should be included to look further into the possibility that both number and colour are affected by the light level at the depth where the fish reside.

Sex determination of *M. muelleri* in our samples was not performed, so it is possible that number/colour of photophores have undiscovered correlations with sex. Species like ponyfishes (Leiognathidae) have been found to have differences between sexes in their bioluminescence organs (Sparks et al., 2005). The biochemistry of the photophores was also not covered in this study. Dissection and chemical analysis of differently coloured photophores could have helped uncover the mechanism and production behind the pink and green colours.

We noted during our study that the photophore colours were of varying intensities. Some had a strong pink/purple colour, while others had a lighter hue of pink. Similarly, some green photophores were strong and vibrant, others were dimmer. In our study, we assigned them as the categorical variables 'green' or 'pink'. However, the colours could be explained better as a continuous gradient going from strong pink to bright green. We did not notice any pattern of different colour intensities with different depths, but this could be studied further. For example, through assigning colours as continuous variables through a digital program capable of registering colour and light emission from photophore pictures, rather than colour determination based on human vision.

Photophore number varied independently of body condition and environment (fjord), which is not expected for a

costly trait. If the number are genetically determined, this could explain why. Based on findings by Davis et al. (2014), photophore number and patterns seem to be closely related to genetics in several species. At least from a genetic viewpoint and the data presented in this research, it is logical to assume many of these observations could be explained by neutral mutations present in these fjord populations of *M. muelleri*. Firstly, by defining populations between the open ocean, and even within the fjords, could be an effective way to investigate whether there is a distinct change in photophore number between different community assemblages (mixed with other *Maurolicus* species, or prolonged isolation as in the fjords).

Biogeography investigations could be used to identify for how long these individuals have been isolated within the fjords, or even if they have ever exchanged with open ocean populations. These isolated populations, and the differences observed from the standard, could even suggest a drift towards subspeciation, but for now at least it seems their isolation from other *Maurolicus* species has allowed an accrument of neutral mutations, that can be afforded. Using a retrospective model such as coalescent theory could identify when these mutations arose and give some context to the ecological influence and presence of these traits. Especially concerning photophore colour, investigating whether differences are observed in the individuals living with other *Maurolicus* species in the open ocean.

Authorship Contribution Statement

All the authors listed confirm that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

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Parrot crossbill (*Loxia pytyopsittacus*)
Photo: Eirik Laksemo

The Proximate Mechanisms behind Fear: an IBM Approach to Understanding Behavioral Changes in Songbirds and the Subsequent Evolutionary Trajectory

Hanif Kawousi, Sergey Budaev and Jarl Giske

Abstract

In this article, we present an individual-based model (IBM) investigating the proximate mechanisms behind phenotypes “boldness” and “fearfulness” in songbirds. Two selection pressures, “hunger” and “predator-induced risk”, are reflected in the genotype of the model’s digital songbirds. In our model, these two emotions work as opposites: when the songbirds are hungry passed a certain point, they are not afraid of predation and vice versa. Ergo, the genes dictate the hard limits for the phenotypic variations possible. Other limitations, such as bird mass and environmental factors, affect them as well. By running the model code through many generations of a population of songbirds, we investigated how these phenotypes evolved despite limitations, and which one(s) would become predominant in our population. Our results show rapid evolution, despite the limitations of bird mass set by the algorithm. The phenotype expressed as “moderate amount of fear” became predominant before ten generations had passed. For the future, we propose further development of the model, as it is quite simplistic. Still, we believe this can add to the explanation of proximate mechanisms behind phenotypes, as well as satisfy the demands of ethical practice regarding animal welfare in science.

Introduction

Since the publication of *The Origin of Species* (Darwin, 1859), the study of animal behavior has revolved around the idea that behavior has evolved through adaptation. For nearly a century, quantitative evolutionary ecologists have simplified the inquiry into adaptive behavior by focusing on the concept of optimal behavior (Fawcett et al., 2013; Lotka, 1925). Darwin’s theories were presented in the form of the English language and not numerically through mathematics. Therefore, in the past century, mathematical

models have been developed in an effort to describe these theories. In the pursuit of modeling behavioral ecology, inspiration was drawn from other disciplines such as physics, and its presumed simplifications of natural phenomena, and economics, with its concepts of costs and benefits. Both, as described below, valuable concepts regarding optimization methods in behavioral ecology.

The primary issue with a mathematical approach was the simplification needed to explain evolution. This need for simplification was addressed by Alfred Lotka in 1925. Lotka was the first to model evolutionary adaptation and behavior, by turning Euler’s population growth equation (1767) into an equation for fitness, now known as the Euler-Lotka equation (Lotka, 1925). Here, Lotka willingly simplified nature by combining the anatomy, behavior, and life history of an organism into a common currency, describing all that either adds to or diminishes an individual’s reproductive rate (r) during a lifetime. Due to the lack of computational technology at the time, these calculations were intentionally made to reach a point of optimal behavior in animals, stripped of realities’ imperfect nature and complexity. In this way, numbers could be easily produced and used in the early models.

The implementation of optimization methods, to forecast how organisms would optimally respond to environmental factors (Fisher, 1930; Lotka, 1925), was therefore both willingly and knowingly done without accounting for the constraints usually present in nature. Still, as those who model nature must keep in mind, these constraints are highly present in the lives of real-life organisms: Imperfect information, lack of analytical skills, limited foresight, physiological and cognitive limitations, etc. Consequently, while modeled organisms may use all relevant environmental and physiological information to assess and determine the behavior that would contribute the most to

fitness, real organisms must contend with many inherent limitations, the above-mentioned included.

More than half a decade later, Allen Grafen built upon William D. Hamilton's influential work (Hamilton, 1964a, 1964b, 1970) and proposed the concept of the "phenotypic gambit" (Grafen, 1984). A "Gambit" is a term originating from the game of chess, representing the sacrifice of a smaller component for an advantageous, larger gain. The phenotypic gambit is in this way designed to "sacrifice" the proximate mechanisms (i.e., genes) for the advantage of not having to do genetics when exploring phenotypes. Despite the continuing increase in computational power available to behavioral ecologists, the simplification does little, if anything, to reveal the mechanisms behind the phenotypes examined.

This presents us with a paradox within evolutionary ecology. Behavioral ecologists have dedicated substantial efforts to devising complex theories and models that identify the behavioral strategies anticipated to optimize lifetime fitness under certain constraints. Contrary, the field of evolutionary ecology remains largely unexplored in terms of shedding light on the proximate mechanisms behind decision-making processes or the specific implementation of fitness maximization within the nervous system (Budaev et al., 2019; Fawcett et al., 2013). The paradox is enforced by the fact that, unlike the assumptions of early models of animal behavior, all nature's solutions have been restricted by proximate mechanisms, i.e., genetic limitations to phenotypic expressions (Andersen, 2014).

The model presented in this article also provides additional push-back against the phenotypic gambit by showing that a single gene can be expressed through a range of phenotypic expressions, meaning plasticity. Phenotypic plasticity is how a gene "comes to show": a manifestation formed not only by its genetic architecture but also by the environment surrounding it (Scheiner, 1993; Via & Lande, 1985). In our model, the songbird is given a single gene. However as shown in the Results section, this single gene can provide a range of phenotypes. Even if they are genetically limited, these results help to solve the above-mentioned paradox. Where the phenotypic gambit intends for the proximate mechanisms to be sacrificed in pursuit of showing fitness by phenotypes, we provide results indicating otherwise.

Modeling Songbirds

In nature, it is shown that the sound of predators alone is sufficient to make songbirds produce fewer offspring (Allen et al., 2022). In their paper, Allen and co-authors showed that through intermittent broadcasting of predator sounds, the fear produced in songbirds were sufficient to cut the population in half after five generations. While

the ultimate explanation based on phenotype in their paper is clear, it is not clear how the genetic constraints nor the phenotypic plasticity affected survival. Why did some songbirds make better choices than others? What was the genetic basis of this advantageous phenotype? Why aren't all the songbirds learning through plasticity and/or heuristics to respond differently to the sounds, when the predators themselves are not present?

To answer this, we have developed a model that aims to investigate the genetic and plastic mechanisms that underlie these fearful expressions in the songbirds' behavior. In our model, we assume *fear* to be an emotion and that the emotion is coded for by a *single gene*. Thereby making a large number of genes into something calculable. We can therefore by this simplification investigate how the gene responds to selective pressures given by the environment in our simulations. Although this might be considered a gambit as well, by doing so we may investigate the components between the gene and fitness such as emotions, plasticity, and norm of reactions.

By using an individual-based model (IBM) with the incorporation of a genetic algorithm (Grimm, 1999; Grimm & Railsback, 2013; Holland, 1992), we suggest a bottom-up approach to investigating the proximate mechanisms behind the emotion *fear*. By assuming *fear* to be an emotion and that the emotion is coded for by a gene, we investigate how the gene responds to selective pressures given by the environment in our simulations. IBMs cannot produce theories on a systemic level (Grimm, 1999). Still, a paradigmatic model that refers to theoretical ecology is ideal for modeling. By using objects (individuals) and studying their interactions as a population, one could study how properties such as specific phenotypes emerge.

Through the use of heuristics (Hutchinson & Gigerenzer, 2005) our digital birds make decisions based on the choices made by their parents. *Heuristics* can be explained as certain rules of thumb, "where the proximate mechanism (the decision-making process) has an architecture that allows efficient information use and decision-making." (Eliassen et al., 2016, p. 90). Following certain rules of thumb, the songbirds change their behavior by perceiving their environment through sensory mechanisms. In our case, the songbirds are presented with a trade-off: If they do not feed, they will reduce in mass and die of starvation, but being too bold in foraging means that they are less safe from predation. The songbirds are therefore subjected to "choosing" the ideal path, in which a simple form of heuristics is needed. These heuristics may therefore consist of building blocks (Hutchinson & Gigerenzer, 2005) that exploit learned behavior through the inheritance of genes and phenotypes. Ultimately, leading to better and quicker decision-making.

The ethics of biological computer science

The ethical reasons for using modeling and computer science in biology should also be mentioned. There are costs and benefits when invasive interventions are done to wildlife in the name of science. As great costs can be disregarded by achieving high-quality answers to important questions, one must still acknowledge the disturbance that is inflicted upon free-living wildlife. According to “The 3Rs principles within experimental animal biology” (ASAB Ethical Committee/ABS Animal Care Committee, 2023; Sneddon et al., 2017), the modeling of such events given by Allen and co-authors is suggested to be the more ethically sustainable choice of research. In the inquiry of how fear affects animals in the wild, interventions, such as Allen and co-authors (2022) presented as their research method caused the death of numerous songbirds. Even though the population regained its numbers after the intervention was removed, there are still reasons to question the ethics of this study’s interventions. Although the subject of animal welfare is sometimes criticized as not being scientifically objective, remaining agnostic of the subject would be a utilitarian approach to animals: the main reasons animals are needed are for companionship and farming (Budaev et al., 2020). Therefore, we present this model as a contribution to act against this utilitarian view of animals. By connecting animal welfare and computational biology, we hope to reduce the cost on nature in the name of science.

Material & methods

The architecture of our model is depicted through Figure 1. For each iteration, aka generation, the digital birds are sent into an array consisting of 100 cells along the x-axis. Each cell represents a habitat consisting of two values: food availability and risk of predation. These values are randomly generated so that each cell/habitat is unique. The birds “fly” at random to a cell. The actions of the bird upon landing at a specific cell are dictated by both their gene, and how their emotional state is at that moment. If they are sufficiently afraid, they will not eat. If they are sufficiently hungry, they will not be afraid. This can either aid them in gaining mass or harm them upon meeting a predator. If they are too hungry to mind the risk of themselves being killed by the predator, they are at risk of being killed. The phenotypic range of their actions is constricted by their genetic value, which is randomly sorted throughout the population at the beginning of each simulation. Therefore, there will be a genetic diversity in place and selection of an advantageous gene will be possible.

In our model, we used elements from the theory of the global organismic state (GOS) (LeDoux, 2012) in the following ways: If our bird is beyond an emotional threshold where it becomes fully fearful, the bird will stay put and not fly from cell to cell in search for food. After a while, it

will grow hungrier, and fear will reduce to a point below the threshold. Mathematically, this can be expressed in the following way:

$$k = \frac{H_{max} - H_{min}}{T_o - W_m}$$

$$b = \frac{H_{min} * T_o - H_{max} * W_m}{T_o - W_m}$$

Here, k represents the slope defining the temporal aspect of the transition between fear and hunger, and b is the intersection point of which the birds’ emotional state is at a certain point in time. H_{max} and H_{min} refers to “maximum hunger” and “minimum hunger” that the bird experiences. T_o is the weight-threshold of the birds and is set to -25% of the initial weight of the digital songbird. If a bird’s mass drops below this threshold, it is considered dead from starvation. W_m on the other hand, gives the value for the birds’ maximum mass, which in our model is 25% larger than its initial starting weight. We have not considered the weight of chicks since we, in our model, assume all birds to be adults.

Object-oriented modeling

The model consists of “objects” (figure 1). Each of the objects are representations of agents in our model, or the environment the agents act within. The agents are the songbirds (BIRD) and the predators (PREDATOR). Each of the objects have their own characteristics that define what qualities the objects possess; *where* the objects are (i.e., which habitat is the bird in), and *how* the objects’ interactions affect their state. The qualities mentioned are assigned values set by parameters in the code. An example is the object “BIRD”, which has the real value of “weight”. In the code, we set the initial weight to “20.0” (grams), which will be the initial value of the birds every time we initialize the code. By using an object-oriented model, we can better mimic the encounters a bird might have with food and risk in nature.

The Genetic Algorithm

In our model, we also created a genetic algorithm (GA) as stated by Holland (1992). When each of our objects: “Environment”, “Predator” and “Parent population” (Figure 2), are initialized and iterations for each generation are done, the GA sorts the songbirds and calculates their fitness”. It does so in the way illustrated by the smaller circle in Figure 2: each parent population undergoes a series of *timesteps*, which gives the order of their actions. After the songbirds’ actions are completed, the fitness of the remaining songbirds is calculated based on their gained mass. Of all the

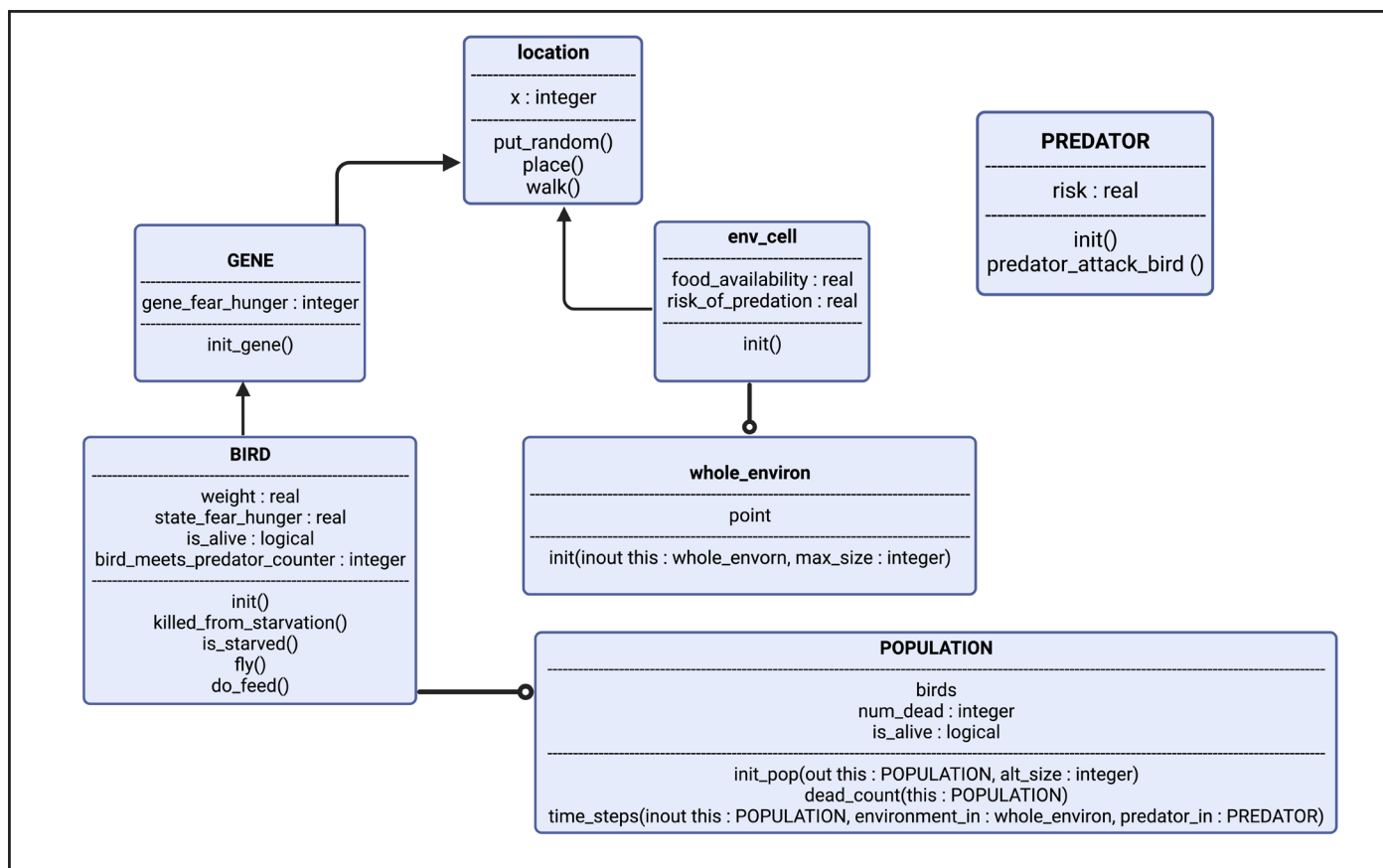


Figure 1. Visualizing the model: the object “BIRD” has attributes `weight`, `state_fear_hunger`, `is_alive` and a counter for when it encounters a predator. It has certain actions as well, such as: `is_starved` and `fly`. “BIRD” is controlled by “GENE”, which gives it the basis of its phenotype. “BIRD” has now a genetically defined personality, and expresses this in “location”, which is the exact point in the “env_cell” (short for environment of the cell), which is a part of “whole_envirn” (the entire environment consisting of 100 cells, i.e., habitats.). In each cell there are also a few instances of “PREDATOR”, which can spawn randomly based on parameter-set probability. Each simulation spawns several thousand “BIRD”, which gives us the “POPULATION”.

songbirds, 25% of the fittest are automatically chosen for reproduction by the code. To further genetic variation in the offspring generation, we have also included an additional 25% chosen at random from the remaining population. Therefore, the next generation in our model will have genes from these 50%. This process is shown in Figure 2 as “select_reproducing”. After this process of selection based on fitness, we submit our new genes to mutation. According to the literature, we chose the parameter for the mutation probability rate to be 4.6×10^{-9} (Smeds et al., 2016). The selection with added mutation results in the parent populations’ offspring (“offspring_population” in Figure 2). The offspring will become the main population for the next generation, completing our single iteration of the GA.

In our model, we chose 100 iterations of the GA, meaning we ran the simulation for 100 generations of songbirds. Since the evolution of a single gene was shown, we believe this number of generations should be sufficient. We fully acknowledge and stress the fact that this is not reality. It is however sufficient to prove that our model works and that evolution happens.

Modeling tools

Fortran (Formula Translation)

Fortran is a widely used programming language in the natural sciences, including biology. Fortran’s proficiency in handling numerical calculations and array operations makes it suitable for modeling natural events and data analysis. The language provides a broad spectrum of mathematical functions and supports advanced operations, enabling biologists to develop complex models that can capture the dynamics of biological systems.

SVN (Subversion)

SVN is a version control system used in software development. With SVN, developers can work simultaneously on the same code without conflicts, as the system tracks and manages the merging of changes. It allows users to check out a working copy of the project, make modifications, and then commit those changes back to the repository. SVN maintains a comprehensive record of all changes, making it easy to roll back to previous versions if needed.

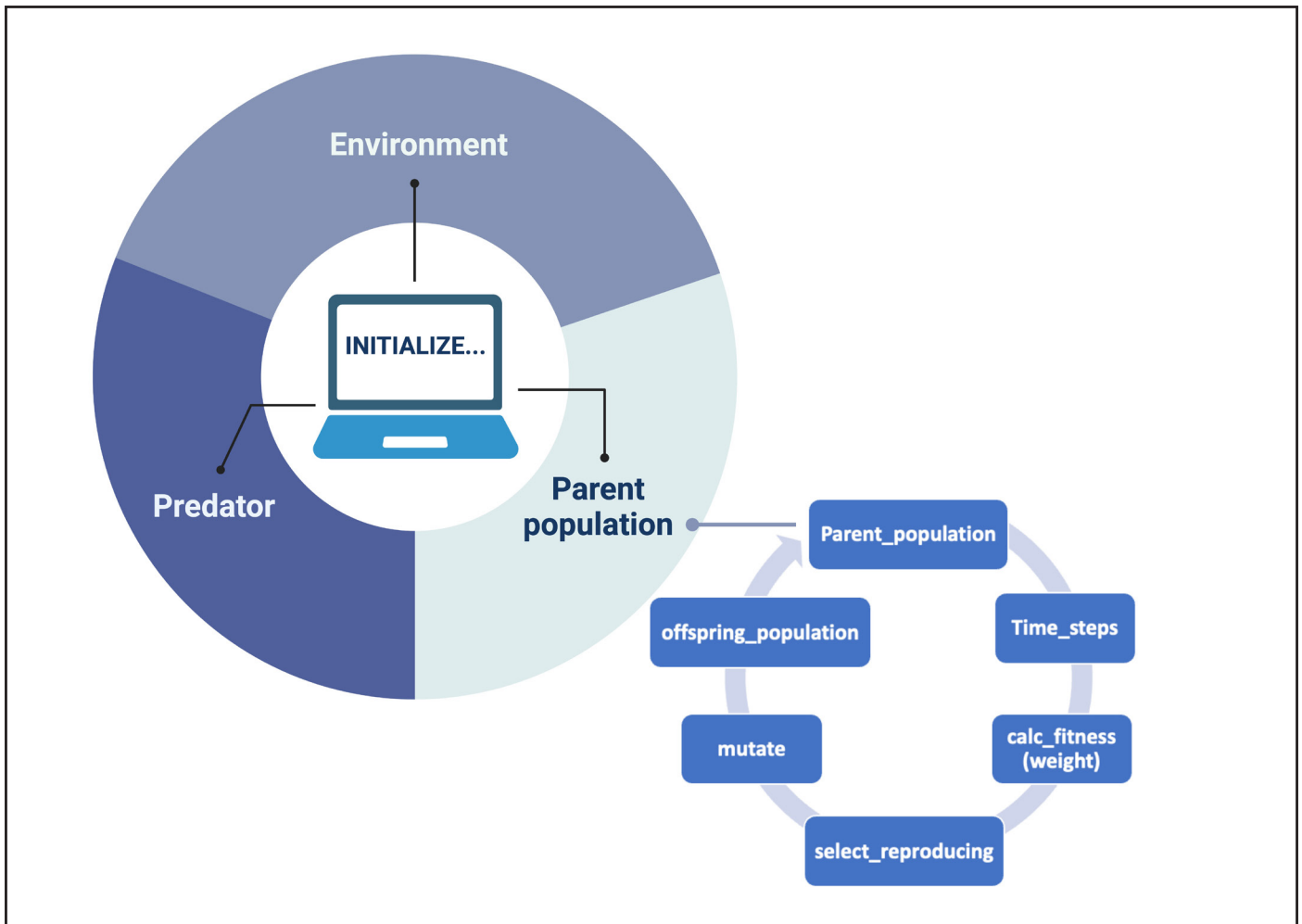


Figure 2. How the model works. For each Initialized simulation our objects (the parent population of birds, the predator and the environment interact). For the birds that show the greatest fitness (measured only by “best mass”), a selection undergoes mating and creates the offspring generation. To incorporate genetic diversity, we also select a certain number of birds that are less fit than optimal and introduce mutation of genome in each generation.

Visual Studio and Visual Studio Code

Visual Studio is a widely used integrated development environment (IDE) created by Microsoft. It provides a comprehensive set of tools and features for building a variety of software applications, including desktop, web, mobile, and cloud-based applications. Visual Studio Code is a versatile and lightweight source code editor that offers a wide range of features and customization options. Its ease of use, extensive extension ecosystem, built-in Git integration, debugging support, and productivity-enhancing tools make it a popular choice among developers for various programming languages and platforms.

The Code

The code in its entirety can be found and examined at GitHub: <https://github.com/Kaw-Han/songbird-evo-model-FORTRAN>. The model code can be examined, tested, run, and furthered under the restrictions of the assigned trademark.

Results & discussion

Our findings indicate that evolution occurs rapidly in our model (Figure 3). The phenotypic expression associated with the gene in our digital songbirds is characterized by moderately cautious behavior during their search for food. This suggests that a moderate level of fear serves as a beneficial trait for increasing fitness, measured by the songbirds' gain in mass. Figure 4a further supports this result. The population of songbirds experiences a significant decline in the first five generations, followed by a subsequent rise and stabilization. This pattern indicates the presence of selection, with the gene favored by the environment quickly dominating the population. Additionally, the graph in Figure 4c depicting the average mass of our birds contributes to our findings. It shows that the rapid growth observed in the first five generations is not sustainable; suggesting that the less fearful songbirds, which gained mass due to bold behavior, were not favored by the environment.

This point is further supported by comparing this graph to the graph in Figure 4d, which depicts the mass gain of

the fittest birds. Unlike the initial increase seen in the first five generations, the graph shows a steady, linear growth rate. This indicates that the most fit songbirds are those expressing the phenotype of moderate fear (Figure 3) from the beginning of our simulation. Furthermore, the dominance of the gene is demonstrated by the standard deviation of mass in Figure 4b. As the songbirds adapt their foraging strategies through selection, the gain in mass becomes almost uniform across the population after five generations.

Another important finding in our model is the expression of a single gene through multiple phenotypes. When designing the digital birds with a single gene, we observed variations in the expression of this gene across the population. This plasticity, though constrained by the gene, was evident in our initial population. As time progressed in our model, evolution occurred, and the phenotypic expression we referred to as “moderate fear” remained the main phenotype. This finding is further supported by both Figures 3 and 4, which demonstrate that the fittest birds were those expressing this phenotype from the outset of our simulations.

Through our findings, we present a demonstration of how heuristics (Hutchinson & Gigerenzer, 2005) come into play. The digital birds in our study exhibited responses to predators or hunger based on the information they sensed from their environment while operating within the constraints imposed by their proximate genetic architecture. Notably, we observed variations in the responses of individuals, suggesting that the rules of thumb guiding their behavior may have limitations but are not entirely genetically predetermined. Expanding on this line of thinking, we can question how the concept of the phenotypic gambit (Grafen, 1984, 1991) explains this phenomenon. Although the gene is expressed initially in different ways, the scope of the diversity in phenotypes where still genetically limited. The phenotypic gambit does not consider that the proximate mechanisms sometimes constrain the expression of adaptive behavior, as pointed out by Fawcett et al. (2013) and is therefore sometimes wrong. The numerical findings we present add to the arguments against the simplicity of the gambit and give us cause to further investigate this phenomenon and continue questioning the benefit of the gambit’s exclusion of genetics.

While our model provides only a partial explanation, it offers valuable insights into the findings of Allen et al. (2022). In their study, the songbirds exhibited not only a significant decline in fitness but also a decrease in population size. It is worth noting that the intervention implemented in their experiment was discontinued after five generations. Interestingly, our model demonstrated a similar timeframe before the beneficial phenotype became dominant. On one hand, the fact that the model mirrored real-life events reinforces the validity and utility of the

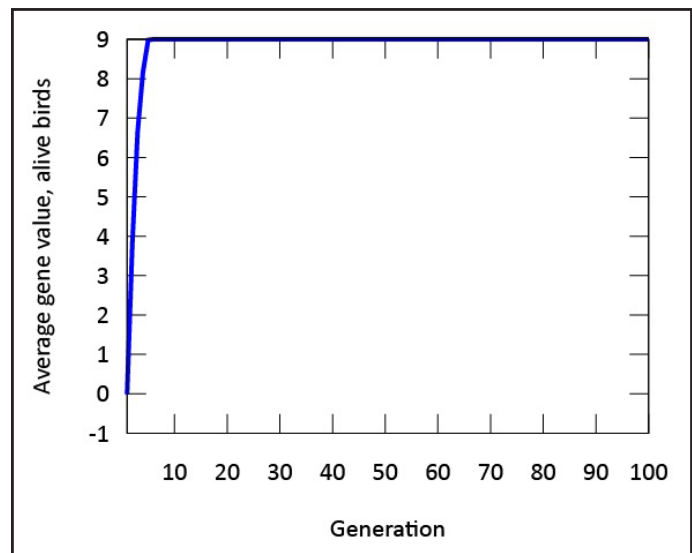


Figure 3. The genetic value “9” is evolutionary stable after approximately five generations. The phenotype expressed by this gene value is associated with moderate amounts of fear.

models. On the other hand, it underscores the growing significance of modeling interventions in nature as initial steps toward comprehending population dynamics and the effects of factors like predation in novel environments.

It is important to acknowledge the immediate issues raised by this thought experiment. Firstly, the ethical implications of prolonging or conducting similar experiments as Allen and co-authors (2022) on free-living populations would not align with the standards of conduct in biological science, as recognized by the ASAB Ethical Committee/ABS Animal Care Committee (2023). Secondly, adhering to animal welfare guidelines prompts further research inquiries, such as investigating the impact of invasive urbanization on wildlife inhabiting forest areas.

This underscores the growing importance of utilizing computational biology to develop models that aid in predicting likely scenarios. While models heavily rely on data obtained from field studies, they offer possibilities to explore nuances, address knowledge gaps, and even challenge established theories without sacrificing lives or causing harm to nature. In our model, we could simply design the neural capacity of the songbirds, their habitat, their physiological needs, and their psychology. By applying what we already *know*, we can provide information valuable for future studies. The need for such an application of our method, is best expressed by the 3Rs (ASAB Ethical Committee/ABS Animal Care Committee, 2023; Sneddon et al., 2017): *Replacement, reduction and refinement*. Through models and simulations, we can *replace* real animals with digital ones. We are then able to *reduce* the number of animals affected. Finally, we can *refine* future studies by using predictive modeling to suggest ideal points of research that affects animal life in the future.

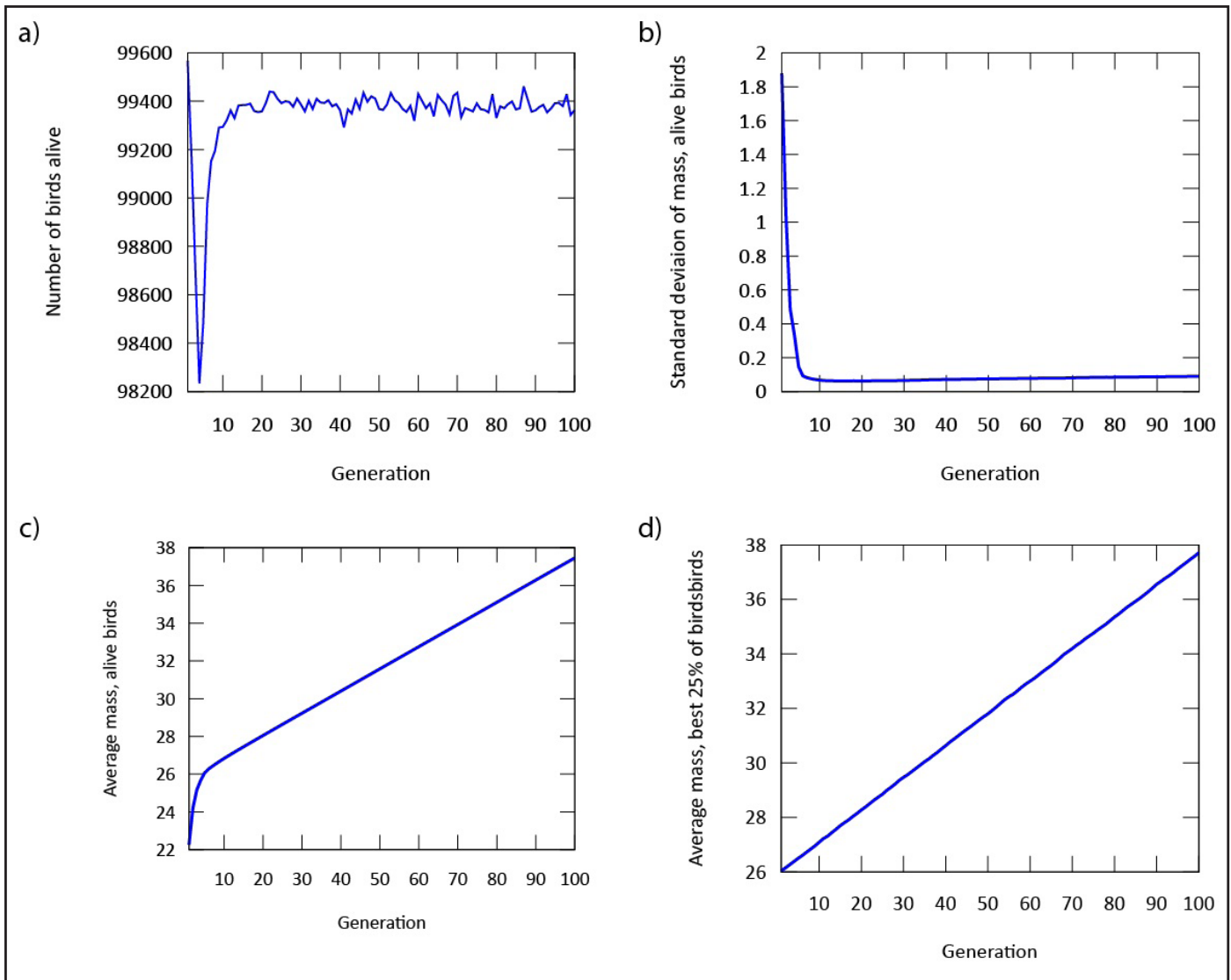


Figure 4. a) The population declines, but quickly regains its numbers after approximately five generations. b) The standard deviation of mass in our population is stabilized after approximately five generations. c) The average mass of all digital songbirds rises significantly throughout the simulation. d) The average mass of the birds with the highest fitness rises linearly and significantly throughout the simulation.

Expanding the code

We fully acknowledge the simplicity of the model. The fitness of our birds is measured solely by their gain in mass. Although this represents successful strategies, more modules, subroutines, and functions could be interesting to include. Alternate strategies for survival and reproduction are commonly seen in nature. This would provide an interesting addition to our model. We could introduce more genes, more environmental factors such as seasonal changes or migration into the population.

In the times of environmental change, we also suggest introducing objects that represent installations placed in nature by humans. Windmills are such an object and are by many considered as intrusive to nature. Data collected from the existing sites containing windmills, can be utilized to create a valuable addition to our model, and subsequently to stake-holders on all sides of the matter.

Summary

In this article, we utilized an individual-based model (IBM) to investigate the proximate mechanisms underlying the response patterns exhibited by songbirds, as studied by Allen et al. (2022). While our understanding of the «whole picture» remains incomplete, our model offers valuable insights into how fear-based behaviors contribute to evolution. Firstly, our model demonstrated the occurrence of evolution. Our results revealed that the digital songbirds experienced an increase in body mass, leading to a higher probability of survival and reproduction. We deduced that the fittest individuals possessed phenotypes that provided advantages right from the start of our simulations. The remaining population adopted similar phenotypes within a mere few generations. Specifically, the most advantageous phenotype expressed a moderate level of fear during foraging. Secondly, our model demonstrated that multiple phenotypes could originate from the same genotype. This

finding is particularly intriguing when compared to the concept known as “the phenotypic gambit” (Grafen, 1984, 1991). Although our model, like the gambit and previous models, simplifies for the sake of optimization, our findings suggest that proximate mechanisms warrant further attention from behavioral ecologists who favor the gambit’s premises in their research. Finally, we propose the need for future development of our model in order to introduce more true-to-nature complexity, and therefore increase the value and precision of our simulations.

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Leaf warblers (*Phylloscopus*)
Photo: Maria Ulvang

A high-magnification micrograph showing a dense population of Chroococcales cyanobacteria. The organisms are primarily oval-shaped, with many containing a prominent, dark brown or reddish-brown central body (heterocyst or akinete) surrounded by a lighter, greenish-yellow cytoplasm. They are distributed across the entire field of view, with some appearing in small clusters and others as single cells. The background is a pale, slightly textured blue-grey.

About the authors

Chrooccales
Photo: Noemi Segura



Hannah L.A. Gaustad, Oda Bellika Kjæmpenes, Paulina Pokusa

We are a group of students that have finished our bachelor's in molecular biology spring 2022. We have Oda who is 24 years old and Paulina and Hannah who are 22 years old. This review was written during a course at the University of Bergen called "Innovation in industrial biotechnology" (MOL232). The class broadened our horizons, and we became highly interested in microalgae and how they might impact future industries. We hope that our work has inspired you and made you aware of microalgal applications.



Sara Rodrigues de Miranda

If you had told me before I started my bachelor that I would be the crazy bug person, I would have laughed in your face. Hindsight is 20/20 and entomology has nevertheless become a subject near and dear in my heart. The work that goes into the taxonomy, the puzzle of finding out what species we're dealing and constantly learning about new aspects of a family of insects is a fundamental part of why I enjoy what I enjoy. I chose to take BIO299 because I had a prior taste for species identification and wanted to further explore practical lab experience with a larger assignment. During my work in BIO299, I unexpectedly fell head-over-heels in love with the hoverfly family, and it has, and will likely remain my passion for quite some time.

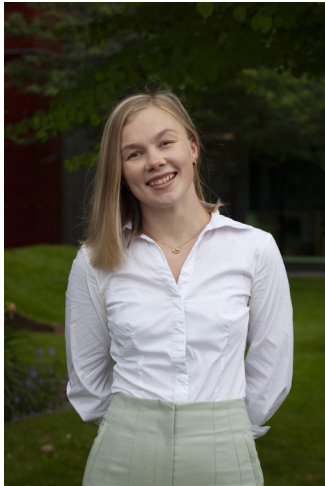


Ingrid Vaksvik

I am a 38-year young lady from a small village in Sunnmøre, Western Norway. My greatest passion in life are insects, and they have fascinated me for as long as I can remember. It was therefore a natural choice to take a master's degree in biology, with a priority on insects. In addition, I am also interested in plants, sustainability, nature management and agriculture – something I have learned more about during my studies, but which I also got to use in my contribution to Bikuben. My text is a revised article that was prepared in connection with the topic "BIO299 - Research Practice." The experience from the course as well as the writing process with through Bikuben has given me a taste for academic writing, laboratory work and fieldwork. This type of holistic work methodology is something I want to combine with a future job in terrestrial biology. My aim with the study and not least with this article is to pay more attention to the bizarre but important insects that surround us wherever we go, but which we often take for granted. They are perhaps best known for giving us beautiful flowers, honey, strawberries, apple, almonds and many other species of berries, fruits, and vegetables. But insects are more than just pollinators; They are nature's own waste collectors and can be indicators of the health of an ecosystem. In addition, they are important for birds and fish - something that humans also benefit from. Not least, the insects entertain us with a mysterious world full of crazy and ingenious inventions.

The structures between the biotic and the abiotic; air, light, water, soil, plants, insects, and other animals are complex and vulnerable at the same time. If one

step on the ladder of life changes, it can have fatal consequences for subsequent steps, and so on. The world will in many ways be a poorer place without the insects. We need increased knowledge and commitment to protect these creatures – both among decision-makers and the general population. My article is an attempt to raise this awareness and I hope my contribution might make a difference.



Nora Solheim

If you had told me a few years ago that I would not only enjoy working at the lab but also be enthusiastic about working with fruit flies, I would have been incredulous! This study was a part of the BIO299 Research Practice in Biology course at UiB during the third year of my teaching degree. The experience was truly eye-opening and gave me a first-hand glimpse of what it's like to work as a biologist and discover how fruit flies can provide valuable insights into cancer research. Currently, I am pursuing my master's project with great enthusiasm, looking forward to re-joining the lab.

I am incredibly grateful to my supervisor, Fergal O'Farrell, who has provided me with this opportunity. In terms of both my academic and professional goals, this experience has been a turning point, and I am eager to see where it takes me next.



Anette Aune, Kristian R. Fjeld, Joseph C. Lawrence and Adam Mortensen

Hi! We are the “Fjord Bros”. A group of MSc marine biology students, but more importantly - a group of friends. The Fjord Bros consists of Anette Aune (ecology and ethology girl), Joe Lawrence (genetics guy), Kristian Fjeld (mesopelagic fan), and Adam Mortensen (fisheries man).

Our group was formed by random assignment during the BIO325 “Ocean Science” course in 2022, and as fate would have it, through our shared passion and curiosity for the oceans - especially the mesopelagic fjord ecosystems, we developed a strong bond and quickly chose to name ourselves the Fjord Bros.

Our paper all started with a couple of very simple questions. How does the number of light organs (photophores) change as the mesopelagic fish “pearlsides” (*Maurolicus muelleri*) grows? And why do some individuals have pink light organs, as opposed to green? To answer this question, we spent a week aboard G.O. Sars in Masfjorden and Fensfjorden, working long shifts at the wet lab sampling hundreds of pearlsides caught by trawling.

Back on land, countless hours and late evenings were spent counting each individual fish in a microscope. Through thick and thin, we stood together, determined to find the answers. As a result, through all this hard work, you are now able to read our paper which we are very proud to present. We hope you enjoy it.

During our writing process, we had regular meetups at K1/K2, to feast on the waffles served by biORAKEL. We would like to extend our gratitude to the people working there, ensuring that we had a great environment to meet and work in.



Hanif Kawousi

Hello and thank you for looking at my paper. My name is Hanif Kawousi and I am currently working on my master's thesis at the Department of Biological Sciences at the University of Bergen. I believe some of the most interesting questions one could ask are within the field of evolutionary ecology. To answer these questions I am working on developing models that can simplify complex systems in real-life nature into something we can measure, handle and research, without compromising the systems' integrity itself.

I would like to take the opportunity to thank my supervisors and co-authors: Sergey Budaev and Jarl Giske, both at Theoretical Ecology Group at UiB. I would also like to thank my wife, Jenny, for all her continuous love and support.



Willow ptarmigan (*Lagopus lagopus*), Østerbo.
Photo: Thomas Alexander Bondevik

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As an author

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As a reviewer

As a peer reviewer, you will be sent various manuscripts that must be peer-reviewed within the journal's guidelines. If you have experience in specific subject areas, you will be sent the manuscripts that best suit your expertise.

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Guidelines for authors

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Bikuben welcomes both single author- and group contributions.

Who can submit

For submitting a contribution, you must be or have recently been enrolled as a student at the Department of Biological Sciences, University of Bergen.

How to submit

All contributions must be sent to Bikuben via the submission form at <https://bikuben.org/contribute/>.

What are the contribution criteria

1. The contributions must be submitted in English or in Norwegian.
2. Use a standard font (e.g. Times New Roman), size 12, spacing 1.5.
3. All authors must have agreed to submit the contribution to Bikuben Student Journal.
4. The corresponding author is defined as the one who has submitted the contribution via the submission form, and represents the whole group of authors, if applicable.

Deadline for submitting a contribution

We accept contributions at any time of the year, and publish these online if and when they are ready. We also aim to publish a summary volume for each study year, where the deadline will be announced on our webpage and social media channels early in the spring semester.





UNIVERSITY OF BERGEN
Department of Biological Sciences