Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Taran Five

Studying Behavioral Responses to Bioluminescence in Zooplankton

Master's thesis in Ocean Resources
Supervisor: Sanna Majaneva
Co-supervisor: Martta Viljanen, Stephen Grant & Geir Johnsen

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// Abstract

As the marine light climate undergoes changes, it may significantly impact biodiversity and interactions between organisms. This shift is particularly relevant in marine environments where bioluminescence, the natural production of light by organisms, plays a crucial role. This phenomenon is found among various marine species including fish, jellyfish, and crustaceans, especially in the deep ocean or polar regions where the amount of atmospheric light is minimal. The biochemical process that creates bioluminescence involves the enzyme luciferase and the light-producing molecule luciferin, which can differ between species. The emission of light in the blue-green spectrum is most common among marine life, as these wavelengths travel further in seawater. Bioluminescence is important for communication, predator-prey interactions and it influences the energy flow within marine food webs. Particularly in the Arctic and Nordic winters, bioluminescence becomes a vital source of light. However, our knowledge on its impact on interactions within marine ecosystems is highly limited and new information on behavioral responses to bioluminescence will enhance the understanding of the role bioluminescence plays in marine ecosystems.

Behavioral experiments using artificial light to simulate bioluminescent flashes on different zooplankton species were conducted in Trondheimsfjorden, Norway during March and June 2023. Zooplankton were collected with plankton nets and individuals from the genus *Calanus* and *Metridia* were chosen for the experiments based on their availability, robustness in the experiments and ecological relevance. The experiments used LED lights to replicate the light characteristics of bioluminescent emissions from three different taxa, *Metridia*, *Clytia* and *Beroe*. This study aimed to explore the visual responses of the zooplankton to different light stimuli and thus seeing how both bioluminescent and non-bioluminescent species respond to said light stimuli. The setup for these experiments included an aquarium equipped with adjustable lighting, a camera and infrared lights to allow for recording in the dark. The behaviors recorded, such as swimming speeds and movement towards or away from the light source, were analyzed using AI assisted tracking in a software called SLEAP.

This study found that *Metridia* spp. showed a higher baseline activity compared to *Calanus* spp. However, despite various light experiments, neither taxa showed any significant response to light stimuli, under any of the intensities tested. This suggests that under the experimental conditions used, light does not significantly influence the measured behaviors of these zooplankton species. Understanding the dynamics and interactions between different zooplankton species is important as it improves our understanding of how zooplankton adapt to changes in the marine light environment which is essential for predicting how marine ecosystems respond to environmental changes globally and helps shape our conservation efforts.

Sammendrag

Endringer i det marine lysklimaet kan ha betydelige konsekvenser for biodiversitet og samspillet mellom marine organismer. Denne endringen er spesielt relevant i marine miljøer der bioluminescens, biologisk produsert lys, spiller en avgjørende rolle. Fenomenet er observert i en rekke marine arter som fisk, maneter og krepsdyr, og er spesielt utbredt i dyphavet og polare regioner hvor mengden atmosfærisk lys er minimal. Den biokjemiske prosessen bak bioluminescens involverer enzymet luciferase og det lysproduserende molekylet luciferin, som kan variere mellom arter. Lysutsendelsen forekommer som oftest i den blågrønne delen av lysspekteret, som er ideelt i marine miljøer da disse bølgelengdene reiser lengst i sjøvann. Bioluminescens er viktig for kommunikasjon, predator-bytte-interaksjoner og energioverføringen i marine næringsnett. Spesielt i de arktiske og nordiske vintrene blir bioluminescens en økologisk viktig lyskilde. Likevel er vår kunnskap om dens innvirkning på samspillet i marine miljøer svært begrenset, og ny informasjon om innvirkning på atferd vil forbedre forståelsen av rollen bioluminescens spiller i marine økosystemer.

Atferdseksperimenter med bruk av kunstig lys for å etterligne bioluminescerende blink ble utført på ulike dyreplankton i Trondheimsfjorden, Norge, i mars og juni 2023. Dyreplankton ble samlet inn med planktonnett, og utvalgte individer fra slektene *Calanus* og *Metridia* ble valgt ut på grunn av deres tilgjengelighet, robusthet i eksperimentelle forhold og økologisk betydning. I eksperimentene ble LED-lys benyttet for å simulere bioluminescens fra tre forskjellige slekter, *Metridia*, *Clytia* og *Beroe*. Studien hadde som mål å undersøke dyreplanktons visuelle respons på lysstimuli og dermed observere reaksjonene til både bioluminescerende og ikke-bioluminescerende arter. Det eksperimentelle oppsettet inkluderte et akvarium utstyrt med justerbart lys, et kamera og infrarøde lys for videoopptak under mørke forhold. De registrerte responsene, som svømmehastighet og bevegelser mot eller fra lyskilden, ble analysert ved hjelp av kunstig intelligens (AI-assistert sporing) i programvaren SLEAP.

Studien viste at *Metridia* spp. har et høyere bevegelsesgrunnivå sammenlignet med *Calanus* spp. Likevel reagerte ingen av de undersøkte dyreplanktonslektene signifikant på lysstimuli, uavhengig av intensiteten som ble testet. Dette tyder på at under de gitte eksperimentelle forholdene har lys ingen betydelig innvirkning på atferden til disse dyreplanktonslektene. Forståelsen av dynamikken og samspillet mellom ulike dyreplankton er viktig, da dette øker vår kunnskap om hvordan dyreplankton tilpasser seg til endringer i det marine lysmiljøet. Dette er avgjørende for å kunne forutsi hvordan marine økosystemer vil respondere på globale miljøendringer, noe som igjen er viktig for å kunne effektivt konservere og ta vare på vår økologiske balanse.

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1 Introduction

1.1 Changes in marine biodiversity

The expression "Triple Planetary Crisis" effectively explains how the three challenges, climate change, biodiversity loss and pollution, collectively constitutes a significant threat to the health of our ecosystems (Passarelli et al., 2021). Climate change, mainly caused by human activities such as the burning of fossil fuels and deforestation, causes extreme weather and increased ocean temperatures amongst others (Passarelli et al., 2021). This can contribute to changes in the distribution of organisms and eventually lead to biodiversity loss and thereby has the potential to disrupt the ecosystems (Passarelli et al., 2021). Biodiversity loss, caused by for example habitat destruction, overexploitation and competition caused by introduction of non-native species, reduces the ecosystem's functionality and damages its resilience, potentially also leading to reduced ability to provide ecosystem services (Ayyad, 2003). Pollution, including chemical pollution and plastic debris as well as sound and light pollution, further degrades the ecosystems by contaminating water bodies, degrading soil quality, and causing respiratory problems and reproductive disorders, amongst others (Weldeslassie et al., 2018). Pollution therefore also contributes to biodiversity loss and climate change, adding to the triple planetary crisis (Schmidt et al., 2024).

The biodiversity of our oceans is a crucial part of our planet's health and sustainability (Talukder et al., 2022). Biodiversity refers to the variability of life, and can be seen in different levels; ecosystems, genetics and species, where the latter is also known as taxonomic diversity (Swenson, 2011). Sometimes the range of roles and interactions of the organisms, as well as their interactions with the environment, also known as functional biodiversity, is included (Swenson, 2011). Functional biodiversity refers to the variety of ecological roles and interactions performed by different individuals and species, including for example nutrient cycling and primary production (Loreau et al., 2001). Different species may have different ecological functions and they can therefore contribute with varying responses to the environmental stressors, allowing ecosystems to maintain important processes even when the conditions are changing (Loreau et al., 2001).

Marine biodiversity plays an important role in maintaining the stability and resilience of ocean ecosystems against different stressors (Bernhardt & Leslie, 2013). For example, having many different species in an ecosystem helps ensure that the important ecological processes are present and can keep going, even when the environment is changing (Hughes et al., 2005). This stability is important to keep marine ecosystems healthy and productive. Biodiversity also ensures that they can provide essential ecosystem services like food, jobs and protection from natural disasters, as well as functioning as CO_2 storage (Sandifer & Sutton-Grier, 2014). Yet, the impact of the triple planetary crisis has become a growing concern in marine ecosystems. For instance, due to climate change, the global average temperatures continue to rise, and the oceans experience significant changes that affects marine species and ecosystems (Prakash, 2021), as the diversity, distribution and migration patterns of marine species can be altered (Beaugrand et al., 2002). For example, in the North Atlantic - Arctic interface, due to temperature changes, some species are moving towards the pole to find colder waters (Beaugrand et al., 2002), while others are struggling to adapt to the changing temperature (Ingvaldsen et al., 2021). This kind of

changes in biodiversity and species distribution can impact the interactions between the different species and lead to imbalances in marine ecosystems (Brierley & Kingsford, 2009). Thus, understanding the ocean's biodiversity is essential for effective conservation, mitigation and management (McQuatters-Gollop et al., 2019). Generally, by identifying diversity, potential indicators and key species as well as recognizing important and vulnerable habitats, correct management strategies and regulations can be implemented in order to protect the marine ecosystems and to ensure sustainability of the oceans resources (Foley et al., 2010). However, assessing the overall health of the ocean requires knowledge of indicators such as species present, habitat use, environment quality and ecosystem functioning at the location (Foley et al., 2010; Rombouts et al., 2013). This is important as the same species can behave differently in different areas, thus varying in their sensitivity to change, as found by Lindström & Nilsson (1988) who found that there was a correlation between the water properties and the light sensitivity of the opossum shrimp *Mysis relicta*. With robust evaluation of these indicators, the current state of our oceans can be looked at and areas where conservation and management efforts are needed can be identified to ensure the areas health and sustainability (Foley et al., 2010; Rombouts et al., 2013).

As an example, the Kunming Declaration, a global commitment from the 15th Convention on Biological Diversity (CBD), is aimed to protect nature (cbd.int, n.d.). It includes the 30x30 goal, which is aiming to conserve 30% of all land and oceans by 2030 and to mitigate biodiversity loss, maintain the ecosystems services, and strengthen the ecosystems resilience to issues. However, to achieve this 30x30 goal, it is important to be able to identify areas in need of protection for effective conservation and management. To be able to identify such areas and ecosystems, knowledge of the species within the ecosystem, including their distribution, abundance, ecological roles and interactions is needed (Kremen, 2005).

1.2 Changing light climate

Together with the changing temperatures, the light climate is undergoing significant changes, greatly impacting the ecosystems (Doney et al., 2012), especially in the Arctic and Nordic regions with increased precipitation and ice thinning, leading to increased light penetration of the ocean (Castellani et al., 2022). In addition, there is light pollution caused by artificial lighting being emitted from unnatural sources, such as street lights, offshore rigs and boats, amongst others (Davies et al., 2014). Despite increasing research and its relevance as a current hot topic, our understanding of light pollution's impact on marine ecosystems remains limited (Candolin, 2024; Linares Arroyo et al., 2024).

Light pollution can have detrimental effects on marine ecosystems as it can disrupt the natural “day-night” cycles that are important for many marine organisms' behaviors, such as feeding, reproduction and predator-prey interaction (Ganguly & Candolin, 2023). For instance, light pollution can significantly impact different zooplankton species, key organisms in marine ecosystems, by disrupting their diel vertical migration (DVM) (Marangoni et al., 2022). DVM is when the zooplankton are moving closer to the surface at night to feed on phytoplankton, and then moving to deeper waters during the day, or vice versa, in order to avoid predators that rely on visuals to feed on the zooplankton (Brierley, 2014; Marangoni et al., 2022), see figure 1 for a visual explanation.

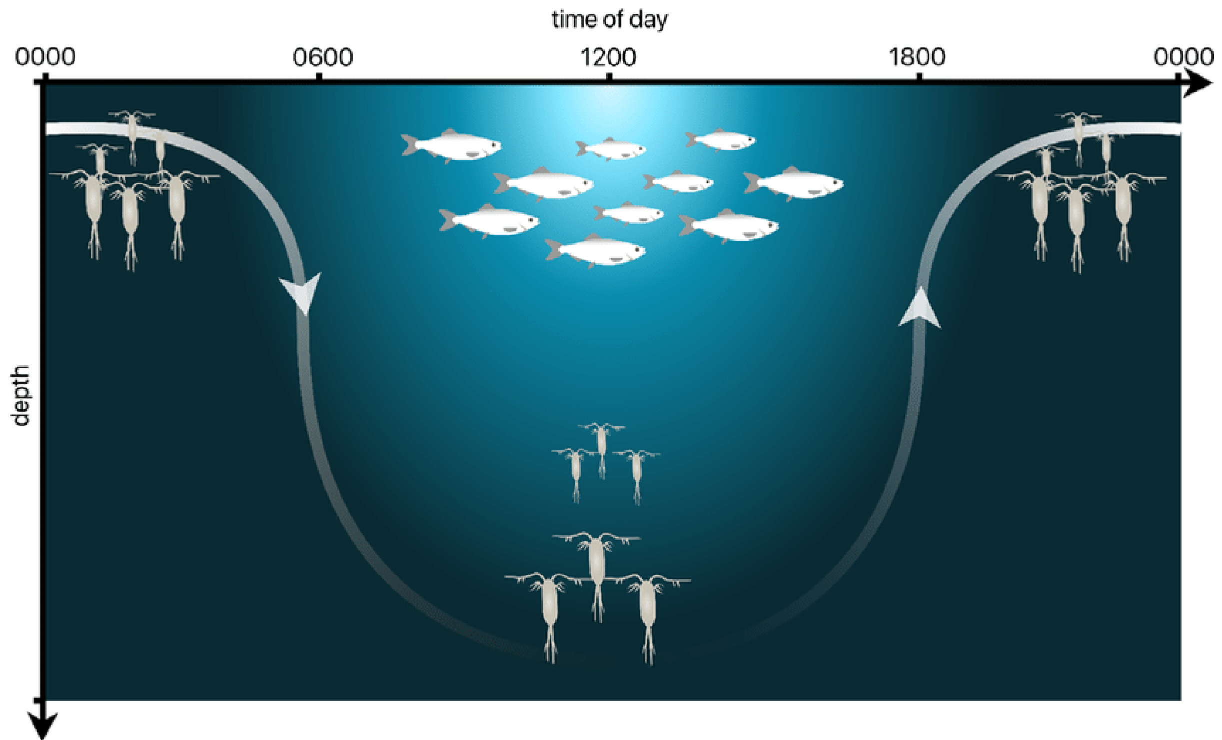

Figure 1: Visual explanation of the diel vertical migration (DVM) of zooplankton, showing how they move closer to the surface during the night to feed and descend during the day to avoid visual predators (Bandara et al., 2021).

Increased "Artificial Light At Night" (ALAN) can change the DVM pattern, as it may prevent the certain zooplankton species and life stages from moving towards the surface to feed (Marangoni et al., 2022). This is due to some species being negatively or positively phototactic, a term referring to the movement of an organism in response to light, either toward the light source or away from it (Marangoni et al., 2022). The changes in DVM patterns could disrupt their feeding behaviors and potentially lead to reduced energy intake, which in turn could affect their growth, reproduction and survival (Moore et al., 2000). Additionally, light pollution may alter the composition of zooplankton communities, by potentially favoring species that are more tolerant to light changes over those that are not (Sanders et al., 2021).

1.3 Bioluminescence

Light pollution may also affect the bioluminescence present in the water column and thereby impact the interactions of bioluminescent organisms (Perkin et al., 2011). Bioluminescence is the production of light from living organisms, found both in water and on land, most commonly found in marine ecosystems (Francis et al., 2016; Widder, 2010). Bioluminescence occurs in organisms such as fish, cephalopods, jellyfish, crustaceans etc., especially in the deep-sea, where sunlight is scarce or not existing. Marine bioluminescent organisms known to most people are the dinoflagellates, commonly called Morild ("sea-fire") in Norwegian, as they can be seen in the surface and shorelines, reacting to movements of the water (Tandberg, 2016). Organisms use bioluminescence for several reasons, for instance reproduction, predation, defense, counterillumination etc. (Haddock et al., 2010). Previous studies have explored the different functions of bioluminescence and created an overview of the different uses for bioluminescence, as seen in figure 2.

Some of the functions like "startle", "counterillumination" and "lure prey or attract host" are well studied in laboratories and in the field (Haddock et al., 2010). Whereas other functions like "sacrificial tag", "aposematism" and "stun or confuse prey" are observed only anecdotally, mainly on land or with little to no experimental evidence (Haddock et al., 2010).

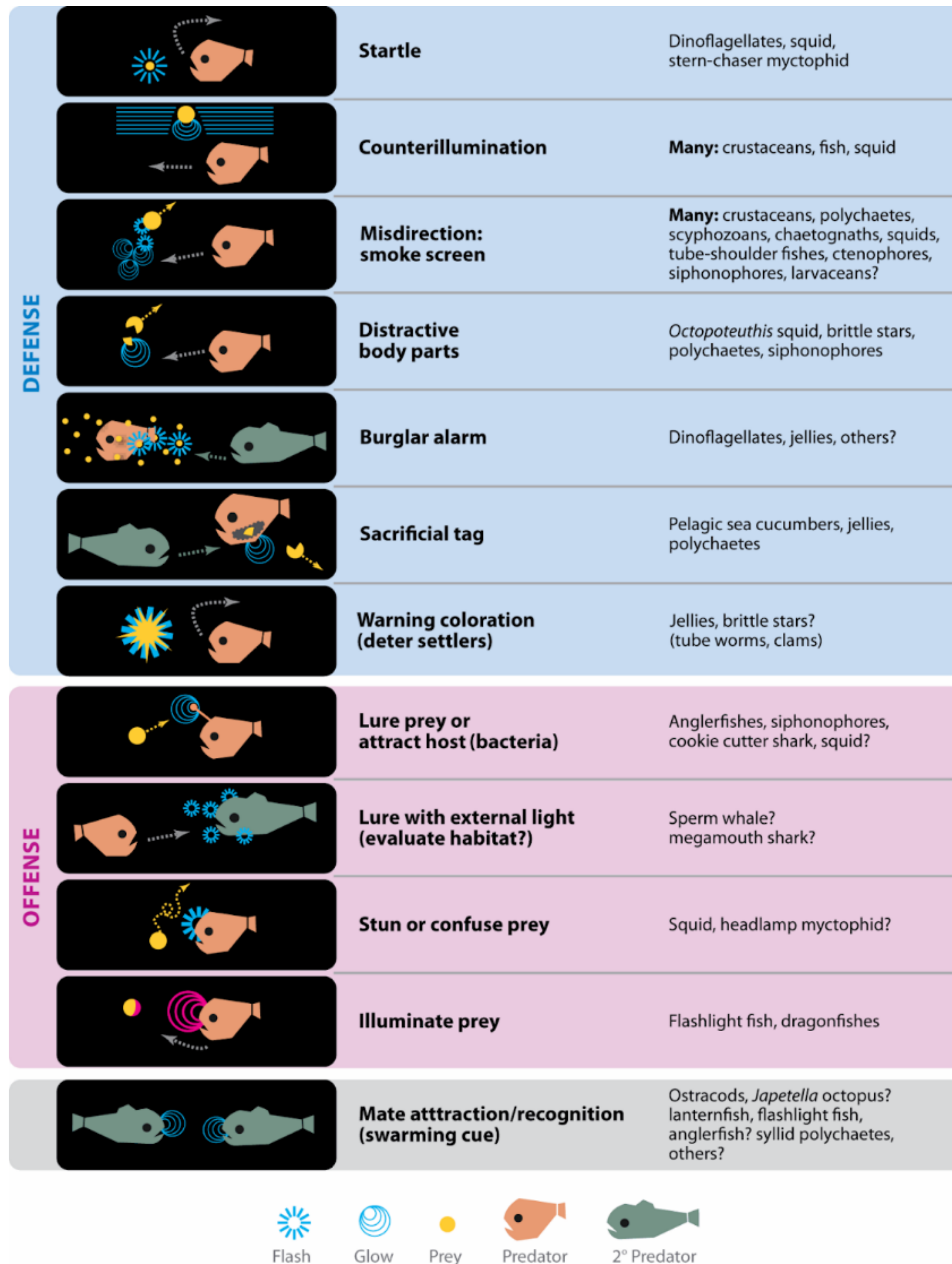

Figure 2: Illustration of the different uses for bioluminescence, where the blue area represents the different types of bioluminescence that can be used for defense, the pink area represents the uses for offense and the gray area represents intraspecific communication (Haddock et al., 2010). The organisms hypothesized intention can be seen in bold and some examples of organisms that display this behavior are listed in the far right.

Given its widespread distribution, with many of the marine organisms having bioluminescence capabilities, it is clearly an important form of communication in the sea (Martini & Haddock, 2017). Thus, bioluminescence can have important effects for example on predator-prey interactions. Bioluminescence only works as a signaling method if the recipient is able to see the bioluminescent signal. Bioluminescence is created by a chemical reaction involving an enzyme (luciferase) and a molecule emitting light (luciferin) (Francis et al., 2016). Luciferin and luciferase can vary from species to species and shows that bioluminescence has evolved independently several times (Haddock et al., 2010). Most marine animals, such as dinoflagellates and comb jellies, emit light in the blue-green spectrum around 440-530nm (Widder, 2010). This is because blue light travels farthest through seawater and the visual sensitivity of marine organisms are best on these wavelengths, with green light close behind (Widder, 2010). To understand the vision and visual capabilities of the organisms, behavioral experiments can be used. It is important to note that despite studies revealing complex functions of marine organisms' vision (Cronin et al., 2022), many visual functions are not well understood for many species (Schaefer, 2010). Observing these organisms in their natural habitats is often difficult due to the depths that these organisms live in, and laboratory studies may not accurately mimic the natural marine light environment (Loew & McFarland, 1990). Yet, knowledge is needed, as the increasing artificial light can mask or reduce the visibility of bioluminescent signals, making it harder for the organisms to communicate, find mates and avoid predators (Ganguly & Candolin, 2023). Simultaneously, other factors, such as climate change, may also have an impact on bioluminescence as warmer oceans have the potential to alter the abundance and composition of marine species (Doney et al., 2012).

1.4 Zooplankton in the northern regions

Organisms living in the Arctic and Nordic marine environment are adapted to extremely dim light conditions during the polar night, where bioluminescence becomes an important source of light to the environment (Cronin et al., 2016). They also show remarkable adaptations to the strong variations in light conditions, from the continuous daylight of the midnight sun to the complete darkness of the polar night (Berge et al., 2015). Zooplankton is crucial to the Arctic and Nordic marine ecosystems, as they are an important food source for higher trophic levels and plays an important role in nutrient and carbon cycling (Darnis et al., 2012). Their population dynamics offer great insights into environmental changes, making them key indicators of ecosystem health (Perry et al., 2004). Many of them are bioluminescent and the role of bioluminescence in interactions between these species is therefore of special interest (Cronin et al., 2016).

A review article by Ganguly & Candolin (2023) shows a significant gap in the research when it comes to the effects of light pollution on aquatic ecosystems, especially when comparing to the amount of attention given to terrestrial ecosystems. Additionally, the review shows that zooplankton has received the least attention compared to other taxa. Investigations of the behavioral responses to light pollution have also mainly focused on movement and attraction to artificial light, instead of social behaviors such as reproduction (Ganguly & Candolin, 2023). Studies of the effects of light pollution on aquatic organisms have mainly been conducted over short-term periods, either in laboratory settings or field experiments, giving little insight to the potential long-term behavioral changes and the consequences in regards to populations and ecosystems (Ganguly & Candolin, 2023).

An article by Båtnes et al. (2015) researches the light sensitivity of *Calanus* species during the polar night and explores how vertical migrations can be affected by the sun, moon or aurora borealis. Experiments using video recordings were conducted to examine the behavioral responses of *Calanus* to varying intensities and wavelengths of light. Their study showed that *Calanus* displayed great sensitivity to light and the potential to vertically migrate based on natural light cues. Similarly, the behavioral responses of oceanic zooplankton to artificial bioluminescence stimuli has been investigated in a study done by Buskey & Swift (1985). Their study focused on understanding how zooplankton species react to bioluminescence through laboratory experiments, mimicking the bioluminescent flashes of potential predators or prey. They found that some of the zooplankton (*Calanus finmarchicus*, *Metridia longa*, *Metridia lucens* and *Temora longicornis*) showed clear behavioral responses to bioluminescent flashes, such as change in swimming speed. The results indicate that certain zooplankton species react to bioluminescence and change their behavior accordingly. This is important for the understanding of the ecological roles of bioluminescence in marine ecosystems.

1.5 Aim

More knowledge on the role of bioluminescence among the Arctic and Nordic waters is needed, thus, this study aimed to assess the behavioral responses that co-occurring bioluminescent and non-bioluminescent zooplankton species have to bioluminescence. This was done by conducting behavioral experiments with abundant zooplankton taxa, *Calanus* spp. and *Metridia* spp., in Trondheimsfjorden, Norway. In these experiments, artificial lights were used to mimic bioluminescence. In preparation for the behavior experiments, bioluminescence intensity and wavelength emitted by bioluminescent species was determined. In addition, determining what bioluminescent and non-bioluminescent zooplankton can see, is the first step to evaluate the importance of a bioluminescent signal. Therefore, experiments determining their visual capacities were conducted.

The aim of this study was to conduct three experiments for each species, using two types of light stimuli; 10 seconds light exposure and mimic of bioluminescent flashes. This study aimed to observe their behavior before, during, and after these light exposures. Additionally, the aim was to compare the behaviors between species and to look for changes in total movement, movement in the x-axis, and top speed before and after exposure to each type of light stimulus for each species.

The project is based on two main assumptions with the first one being that *bioluminescence emitted from bioluminescent species can be measured in intensity and wavelength*. Second assumption being that *bioluminescence can be recreated by artificial light when we know the duration, intensity and wavelength*.

2 Methods

2.1 Study sites

This study was conducted in Trondheim, Norway. All zooplankton used in this study were collected from the pier outside Trondheim Biologiske Stasjon (TBS) (Figure 3) in March and June in 2023. TBS is located by Trondheimsfjorden, just outside the city center of Trondheim at 63°N and 10°E (Kuklinski & Barnes, 2008). Trondheimsfjorden is a typical Norwegian fjord with a shallow threshold, or "sill", at its mouth, which can restrict water exchange between the fjord and the open sea, an average depth of 165 meters (Faust et al., 2014) and tidal amplitudes, typically around 1.2 to 2.5 meters (Kuklinski & Barnes, 2008). Seasonal variations play a significant role to the marine ecosystem in these waters (Bluhm & Gradinger, 2008). For example, the amount of light available is the main driver for the spring blooms and the zooplankton will start to become more active as the amount of light and temperature increases (Winder & Sommer, 2012). Similarly, the abundance of zooplankton slowly declines during the summer months, only to increase in early fall due to increased amounts of nutrients due to upwelling (Gislason & Astthorsson, 1998), whereas during the winter, zooplankton abundance will be lower due to the limited food and light availability (Jensen, 2019).

Figure 3: Map showing the study location of Trondheim Biologiske Stasjon (TBS) (Google Maps, n.d.). (A) Shows TBS in relation to Scandinavia and Europe, whereas (B) shows TBS in relation to Trondheimsfjorden.

2.2 Study organisms

The zooplankton community in Trondheimsfjorden is dominated by copepods, with biomass peaks around spring/early summer and mid-fall (Arff and Tokle, 2023). As the zooplankton community composition varies during seasons, the organisms used for this study were chosen based on the following criteria: (A) accessibility, (B) suitability for the experiments by being easy and robust to sample and handle and (C) importance in the ecosystem. These criteria led the following taxa to be chosen for this study: *Calanus* spp. and *Metridia* spp. (hereafter referred to as *Calanus* and *Metridia*). In these Nordic waters, several species of *Calanus* and *Metridia* can be found, such as *C. helgolandicus*, *C. hyperboreus*, *C. finmarchicus* and *C. glacialis* (Croquet et al., 2017) for the genus *Calanus* as well as *M. longa*, *M. lucens* and *M. pacifica* (Beaugrand et al., 2002; Bucklin et al., 1995) for the genus *Metridia*. However, the species level identification of these organisms is known to

be difficult (Choquet et al., 2018; Gabrielsen et al., 2012; Lindeque et al., 2004). For example, a study done by Croquet et al. (2017) showed that the common species identification method, using number of legs, urosome and prosome segments to identify the life stage and then use measurement of the prosome length to find the species, was not a reliable method to differentiate between the *Calanus* species found in the Norwegian fjords. As the species level identification for both genus has proven to be difficult, the identification was kept to genus level.

The species within the genus *Calanus* are non-bioluminescent copepods that play a significant role in marine ecosystems, particularly in the Nordic waters along the Norwegian fjords and coast. They have a complex life cycle that is highly adapted to the seasonal dynamics of cold-water regions (Broms et al., 2009). These copepods have adapted to allow for exploitation of the seasonal abundance of food by accumulating lipid reserves, which are crucial for surviving the harsh winter months when food is scarce (Heath et al., 2004). *Metridia* is a bioluminescent zooplankton taxa and is adapted to varying environmental conditions, especially being opportunistic feeders (Grønvik & Hopkins, 1984). As part of the copepod community, both taxa are key components of marine ecosystems in Nordic waters, being the link between primary producers and higher trophic levels (Valdés et al., 2004).

2.3 Sampling

All specimens were collected from the pier outside TBS (Figure 3), using a custom-made zooplankton net with a mesh size of 200μm and a diameter of 55cm and a non-filtering cod-end. Samples were collected by doing vertical net tows, approximately 3-5 meters from the surface.

Seawater used in the experiments were also collected from the pier outside TBS using a Niskin water sampler, from around 3-5 meters depth at the same time points as the specimens for the experiments were collected. The water was kept in a temperature controlled room with ambient temperature and for the experiments it was filtered using a 60 micron sieve.

2.4 Behavioral experiments

Behavioral experiments were conducted in March and June of 2023 with specimens from the genus *Calanus* and the genus *Metridia*. The experimental setup, which was consistent across all experiments, is described in detail below.

2.4.1 Sorting experimental specimens

In order to avoid damaging the specimen' light receptors, the individuals used in the experiments were sorted under red light. This was done in a dark room using a red headlamp and sorting by hand with a large plastic pipet. The specimens were pre-identified based on illustrations from Castellani & Edwards (2017). However, the easiest identification characters was that specimens of the genus *Metridia* move faster/"jumps", is more hunchback, has a triangle shape and a longer urosome segment than other copepods during the sampling periods. *Calanus* is larger, has two long reddish antennas (Nielsen et al., 2014), a more oval shape, a shorter urosome segment and moves slower. However,

to maintain the good health of the experimental individuals, only quick pre-identification was conducted prior to the experiments. More detailed identification was conducted after the experiments (see section 2.5). The pre-sorted zooplankton were kept in separate 0.5L containers (10 individuals in each) for about 1-2 days allowing acclimation after sorting until used in experiments.

2.4.2 Experimental setup

The experimental setup was modified after Miljeteig et al. (2014) and consisted of a table, an aquarium, IR lamps, a video camera, black fabric, an LED connected to a filter wheel and an Arduino, amongst other things. To ensure optimal conditions a temperature controlled cold room was used to conduct the experiments. The temperature in the lab was set to +4°C to mimic the ambient temperature in the ocean. Having a closed room to perform the experiments also ensured no disturbances such as sound, movement and especially no light disturbances.

The setup (Figure 5) was built inside the cold room and consisted of several components including an aquarium (10cm x 40cm x 10cm) and a table with a cut out hole just slightly smaller than the aquarium. Underneath the table, in order to allow for illumination from below, four IR (infrared) lamps (IR30, SmartProdukter Norge AS, emission peak at 850 nm) with additional IR-filters (Kodak Wratten Infrared filters, #87C, Edmund Optics Ltd, York, UK; 0% transmission up to ~790 nm) were used. To record the experiments, a video camera (Sony Handycam HDR-XR550) using NightShot mode was placed over the aquarium. The light source was an LED attached to a filter wheel with an integrated chamber lined with aluminum foil and a diffuser plate (with and without cut-out holes to mimic the size of the bioluminescent organisms) to create a more even light stimulus. Additional elements to the experimental setup were an Arduino and all necessary power cords. Even though the lights in the room were turned off, black cardboard and black fabric was used around the whole setup to properly ensure that no light disturbed the experiments. To control the filter wheel, a computer program called WheelTool was used and a cable went from the filter wheel in the cold room to a computer in the room next door. The filter could then be changed without having to disturb the experiment. By changing the filter, the intensity of the light from the LED also changes, as the filters can block out the light and this will therefore determine how much light goes through.

Similar to the study by Miljeteig et al. (2014), the experiments monitored the movement of the zooplankton in response to light stimuli in a horizontal plane. This was to remove the effects of buoyancy and gravity, as this setup focused on researching their active and directional responses to light.

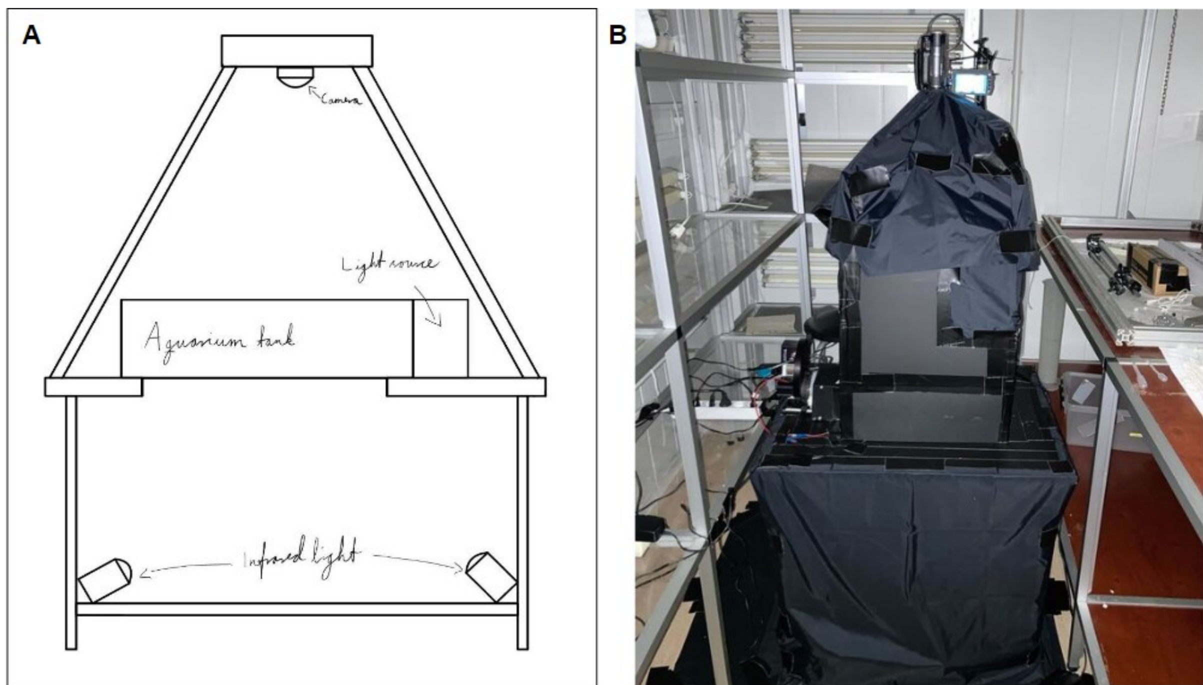

Figure 4: Overview of the behavioral experimental setup based on Miljeteig et al. (2014). (A) Drawing of the experimental setup (light source on the right). (B) Picture of the experimental setup in the cold room used to conduct the experiments (light source on the left).

2.4.3 Experiments conducted

There were 24 experiments conducted in total. Two different types of experiments were conducted on both *Calanus* and *Metridia*; one to map the general sensitivity and reaction to light (called "10 seconds (10s)" experiments), and one to measure the behavioral responses to artificially simulated bioluminescence flashes of *Metridia*, *Clytia* spp. and *Beroe* spp. (hereafter referred to as Calanus and Metridia) (called "mimic" experiments). For the 10s experiments 10 seconds were chosen as that has previously been successfully used in mapping light reactions in *Calanus* (Viljanen et al., in prep). Whereas *Metridia*, *Clytia* and *Beroe* were chosen for the mimic experiments due to them being abundant bioluminescent taxa in the community and co-occurring with both taxa used in this study. Mimic experiments on *Calanus* were conducted in March and *Metridia* in June, and the 10s experiments on both *Calanus* and *Metridia* were conducted in June. A list of experiments conducted can be seen in table 1. Due to time constraints, data was only analyzed from 12 of the 24 experiments. For the mimic experiments *Metridia* was chosen for data analysis, in order to be able to compare both intraspecific and interspecific communication.

Table 1: List of the experiments conducted in both March and June. Each experiment type is replicated three times, separated by a gray background. The thicker line separates the March and June experiments. Experiment nr. marked with * indicates those used in further analyses.

Experiment nr.	Month	Date	Taxa	Experiment type	Wavelength	LED used
1	March	22.03.24	*Calanus*	*Clytia* mimic	500nm	Cyan
2	March	22.03.24	*Calanus*	*Clytia* mimic	500nm	Cyan
3	March	22.03.24	*Calanus*	*Clytia* mimic	500nm	Cyan
4	March	23.03.24	*Calanus*	*Beroe* mimic	480nm	White + filter
5	March	23.03.24	*Calanus*	*Beroe* mimic	480nm	White + filter
6	March	23.03.24	*Calanus*	*Beroe* mimic	480nm	White + filter
7*	March	24.03.23	*Calanus*	*Metridia* mimic	480nm	White + filter
8*	March	24.03.23	*Calanus*	*Metridia* mimic	480nm	White + filter
9*	March	24.03.23	*Calanus*	*Metridia* mimic	480nm	White + filter
1	June	15.06.24	*Metridia*	*Beroe* mimic	480nm	White + filter
2	June	15.06.24	*Metridia*	*Beroe* mimic	480nm	White + filter
3	June	15.06.24	*Metridia*	*Beroe* mimic	480nm	White + filter
4*	June	15.06.24	*Metridia*	*Metridia* mimic	480nm	White + filter
5*	June	16.06.24	*Metridia*	*Metridia* mimic	480nm	White + filter
6*	June	16.06.24	*Metridia*	*Metridia* mimic	480nm	White + filter
7	June	16.06.24	*Metridia*	*Clytia* mimic	500nm	Cyan
8	June	16.06.24	*Metridia*	*Clytia* mimic	500nm	Cyan
9	June	16.06.24	*Metridia*	*Clytia* mimic	500nm	Cyan
10*	June	20.06.24	*Metridia*	10s	N/A	White
11*	June	20.06.24	*Metridia*	10s	N/A	White
12*	June	20.06.24	*Metridia*	10s	N/A	White
13*	June	20.06.24	*Calanus*	10s	N/A	White
14*	June	20.06.24	*Calanus*	10s	N/A	White
15*	June	20.06.24	*Calanus*	10s	N/A	White

2.4.4 Running the experiment

For the experiments, 500mL of filtered seawater was used for each experiment and the water was added to the middle part of the aquarium along with 10 previously collected and sorted individuals. Before each experiment it was ensured that the IR lamps, LED, Arduino and camera were plugged into their powersource and that the aquarium was properly covered to protect against unwanted light. The camera was then started manually and a stopwatch on an iPhone was started simultaneously, in order to keep track of the schedule of when to change filters and when the experiment was done. The filters were changed manually every 6th minute, whereas the stimulus lights were emitted automatically by the Arduino. Each stimulus light was repeated three times per intensity in increasing intensity series, every 2nd minute. An overview of the schedule can be seen in table A1 in appendix A. After each experiment, the experimental specimens were taken out and preserved in >70% ethanol for further morphological and molecular species identification. The aquariums were emptied and rinsed before new seawater was added along with 10 new individuals for the next experiment.

2.4.5 Light stimuli

The Arduino had different programs for the different experiments (code available on request). For the 10s experiments the Arduino was programmed to send out 10 seconds of light every 2nd minute. Whereas for the mimic experiments the Arduino was programmed to create a flash every 2nd minute, mimicking the exact bioluminescent flash either Metridia, Beroe or Clytia created in nature based on Krohn-Pettersen (2023) (Figure 5). The mimic experiments using bioluminescent flashes from Beroe and Clytia were not analyzed due to time constraints. Similarly, only the 2nd light stimulus per intensity was used for the 10s experiments due to time constraints.

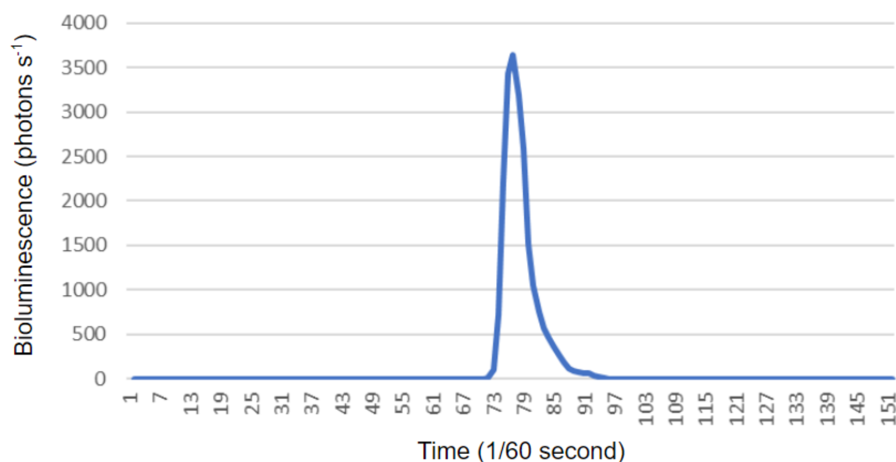

Figure 5: Visualization of the kinetics of bioluminescent flash produced by *Metridia*, which was used to generate the *Metridia* mimic stimulus. Bioluminescence in (photons s^{-1}) on the y-axis and time in (1/60 seconds) on the x-axis. Adopted from Krohn-Pettersen (2023).

For the 10s experiments, a white LED was used. Whereas for the mimic experiments, a bandpass filter (Filter int. 480nm 10nm FWHM 25mm or filter int. 505nm 10nm FWHM 25mm, Edmund Optics) was inserted in front of the white LED in order to mimic the wavelength composition of *Metridia* and *Beroe*. For the mimic experiments mimicking *Clytia*, a cyan LED was used to achieve the correct wavelength. The intensity of the light

from the LED varies depending on the filters chosen using the filter wheel. This was done by using neutral density filters at roughly 1 OD (optical density) intervals similar as in Miljeteig et al. (2014) and Båtnes et al. (2015). Filters 1 to 11 were used in the experiments, where filter 1 (and 11) blocked all light from passing through, whereas filters 2-10 gradually let more and more light through. For the analysis filter 6-10 was decided sufficient for the 10s experiments, whereas only filter 10 was chosen for the mimic experiments, as filter 1-5 allows no to very little light to go through and were therefore decided against, in order to save time.

The stimulus lights were measured using a QE Pro spectrometer with a 1µm fiber optic by Ocean Optics, both with and without a cosine corrector. Due to technical issues with the measurements, only the highest intensity measurement is reported here. The highest intensity value (filter 10), integrated between 400nm to 700nm was; 5.83 $\mu W/cm^2$ for the white LED with the bandpass filter, and 5.25 $\mu W/cm^2$ for the cyan LED. Due to technical problems there were not obtained any measurements from just the white LED without the bandpass filter. The optical density filters used (filters 1-10) reduced the intensity by a factor of 10 relative to the previous strength filter as in figure 1 in Miljeteig et al. (2014). Compared to Miljeteig et al. (2014) the light measurement was different, potentially due to the type of lens used and positioning of the aperture between the LED and the measurement fiber. Additionally, the intensity was measured closest to the LED, whereas Miljeteig et al. (2014) measured the farthest away from their LED. Thus, straightforward comparison of the measurements are not possible, due to differences in measuring techniques and in the setup.

2.5 Morphological and molecular species identification

As the specimens were sorted and pre-identified quickly based on their habitus and movements (as described in 2.4.1) before the experiments, in order to limit their light exposure, there was a risk for misidentification. A stereomicroscope (Leica) was therefore used to identify the EtOH preserved *Calanus* and *Metridia* individuals used in the experiments to species level. For *Calanus*, a fixed length table by Broms et al. (2009) to distinguish the individuals between *Calanus finmarchicus* and *Calanus glacialis* was used. As misidentification can still occur (Gabrielsen et al., 2012), and no similar identification key exists for *Metridia*, molecular species identification was therefore attempted in order to secure robust identification of the experimental specimens.

This was done by extracting the DNA using a modified Chelex rapid boiling procedure (Granhag et al., 2012). Around 0.5mg of body tissue was pipetted into a 1.5mL Eppendorf tube, where the lid was left open for 12 hours in order for the EtOH to evaporate. For the Chelex DNA extraction, 0.5mM EDTA and 30µL of 6% Chelex® 100 resin (BioRad) in 50mM Tris with a pH of 8.0 was added to the Eppendorf tube. The mixture was heated to 98°C for 10 minutes, before centrifuging for 10 minutes at 4°C, with 15000 RPM. The DNA supernatant was transferred to a new 1.5mL Eppendorf tube and frozen at -20°C. The polymerase chain reaction (PCR) was then used to amplify a specific target region. The mitochondrial encoded cytochrome c oxidase I (mtCOI) was chosen and universal Folmer primers LCO1490 and HC02198 (Folmer et al., 1994) were used. A PCR mix of 20µL containing: 1µL Folmer LCO1490, 1µL Folmer HCO2198, 4µL Phire® reaction buffer, 0.4µL dNTP, 0.6µL DMSO, 11.6µL nuclease free water, 0.4µL Phire® Hot Start DNA polymerase, and 1µL specimen DNA template was made. The mix was then pipetted into PCR strips that

were placed in a PCR machine (SimpliAmp Thermal Cycler, Applied Biosystems by Life Technologies). Different PCR cycles were used, for instance: 98°C – 5 min, (98°C – 8 sec, 57°C – 10 sec, 72°C – 1 min) x 35, 72°C – 5 min, 4°C – 10 min.

As it was later seen that Chelex extraction method did not provide sufficient results (<20% success rate), Qiagen DNeasy Blood & Tissue Kit was used for DNA extraction according to the manufacturer's protocol (QIAGEN Group, 2016). The same primers and PCR cycles were used.

As this still did not give any successful PCR products and DNA content measurements with NanoDrop indicated no DNA for all extracted samples, a new DNA extraction with Chelex was conducted for *Calanus*. Due to time constraints, *Calanus finmarchicus* species-specific 16S gene primers (Tarrant et al., 2008) were used with known *C. finmarchicus* positive controls. Gel electrophoresis was used to confirm if there was *C. finmarchicus* DNA present in the PCR product. An 1.5% agarose gel was made and placed in a 1/50x TAE buffer in the electrophoresis cage and the PCR product was sampled into the gel. Successful *C. finmarchicus* products showed a clear band on the gel when placed under UV light.

2.6 Data analysis

The videos recorded during the experiments were analyzed using a computer program called SLEAP (Social Leap Estimates Animal Poses). It is an open-source software package designed for tracking and analyzing the behavior of animals in videos (Gall et al., 2022). It utilizes artificial intelligence (AI) techniques to track the movement and poses of several individual animals in the same video, making this especially useful as doing the same tracking manually would take a tremendous amount of time. However, due to the small size of specimens and the quality of the videos, the poses were not possible to track. Thus this study focused only on the movement of the specimens in regards to total movement, movement in the x-axis and top speed.

2.6.1 Creating a training package

To use SLEAP it is first necessary to create a training package. This was done by using pre-trained models to predict the locations of the individuals and just correcting the wrong predictions. Three training packages were made for the analyses, one for *Metridia*, one for *Calanus* for the experiments conducted in June and one for *Calanus* for the experiments conducted in March. To do this a pre-cropped and frame rate adjusted (12.5 fps for *Calanus* and 25 fps for *Metridia*) video was imported to SLEAP and a sample of 20 suggested frames for labeling using the "stride" as the "Sampling method" was generated. Specimens were then predicted on these frames using pre-trained models from Viljanen et al. (in prep), ensuring a "multi-animal top-down" as the "Training/Inference Pipeline Type" and "none" for the "Tracker (cross-frame Identity) Method". After running the inference, all 20 frames were manually inspected and corrected for different mistakes like missing instances or registered instances that were not actually an animal. A training job package was created, again ensuring "multi-animal top-down" as the "Training/Inference Pipeline Type, "head" as the "Anchor Part" and using default training parameters, apart from changing "Rotation Min Angle" and "Rotation Max Angle" to -180 and 180 degrees to enable full rotation, and the "Max Stride" to "8" to adjust the receptive field to correspond with the size of the

zooplankton. The training job package was exported and the actual training run on an external server

2.6.2 Selecting the time windows

After developing the training package and creating the new models, the tracking of the experimental specimens could begin. Before the tracking was done, the desired time window to analyze was calculated as both the video recording and the stimulus protocol run by the Arduino were started manually, which caused some uncertainty of the timing of the light stimuli in the videos. This was done by checking what time the first light stimuli was emitted and calculating the start and end points of each analyzing window in seconds, and then converting the time into frames.

For the 10s experiments, it was chosen to analyze the whole 10 seconds of the light stimuli (stimulus), and also 10 seconds before (pre) and after (post) the light stimuli (Figure 6A). As for the mimic experiments the light stimuli were less than one second, and the stimuli were therefore included into the pre and post for the analysis (Figure 6B).

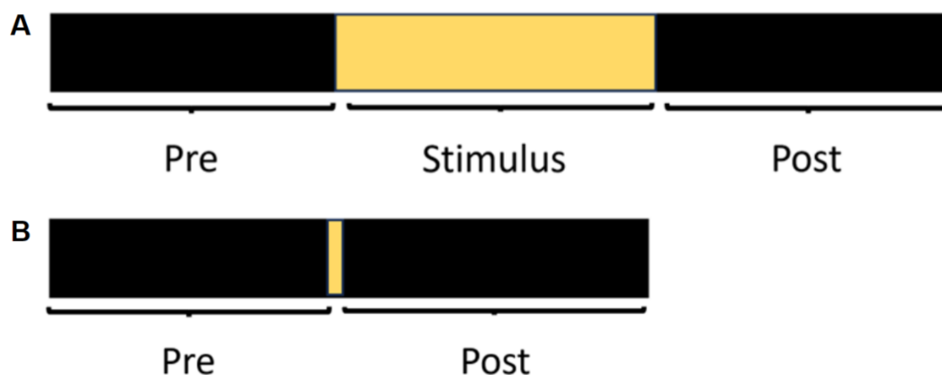

Figure 6: Visualization of the experimental stimuli. (A) Visual explanation of the time windows chosen for the 10s experiments and (B) visual explanation of the time windows chosen for the mimic experiments.

2.6.3 Generating frame chunks to analyze

To start the actual tracking, frame chunks were generated for each "pre"/"stimulus"/"post" time window for each filter chosen (filter 6-10) for the 10s experiments and for each "pre"/"post" for filter 10 for the mimic experiments (12 experiments in total). This was done by adding a pre cropped and frame rate adjusted video (12.5 fps for *Calanus* and 25 fps for *Metridia*) into SLEAP. Next step was choosing "frame chunk" as "Method" and selecting the calculated frame range for each desired part of each video.

2.6.4 Predicting and proofreading tracks

The frame chunks were then opened in SLEAP and inferences were run using the previously trained models appropriate for the experiment being analyzed, and ensuring a "*multi-animal top-down*" as the Training/Inference Pipeline Type "*flow*" for the Tracker (cross-frame Identity) Method, and checking the box for "Connect Single Track Breaks". The "Trail Length" was set to 250 to see the tracks across frames for the whole frame chunk.

The whole frame chunk was visually inspected for different mistakes like for example missing instances, swapped track identities or registered instances that were not actually an animal. For wrongly registered instances, the whole track was deleted, and for the missing instances they were given a new track. Other mistakes like swapped track identities were corrected and a final check was done to ensure the correct number of tracks. The following features of the tracks were extracted by a Python script in Jupyter notebook, available in GitHub (https://github.com/marttavi/Five_MSc_2024). The features chosen were total movement, movement in the x-axis (x translation) and top speed. Total movement was chosen as this explains the general swimming activity, whereas top speed is interesting as the copepods often perform fast leaps as an escape reaction. Additionally, movement in the x-axis (x translation) shows whether the individuals move towards or away from the light source.

2.6.5 Creating threshold for the total movement

The median of the baseline movement of each taxa was calculated in order to do thresholding for the total movement data (Table 2). The thresholding was done to remove tracks belonging to dust or dead individuals, in order to explore how the individuals who were actually moving were reacting to the light stimuli. Individuals moving less than the baseline median for each taxa were removed from the data set, see table 3 for an overview of the individuals removed for each experiment type, filter and stage (pre, stimulus and post).

Table 2: The calculated median of the pre (before light onset) for the total movement of both *Calanus* and *Metridia* for all the filters.

Taxa	Median
Calanus	2.989884
Metridia	33.77295

Table 3: The number of individuals removed from the total for each filter and stage for both *Calanus* and *Metridia*.

Taxa	Type	Filter	Stage	Individuals in total	Individuals removed
Calanus	10s	F6	Pre	30	18
Calanus	10s	F6	Stimulus	30	12
Calanus	10s	F6	Post	30	14
Calanus	10s	F7	Pre	22	13
Calanus	10s	F7	Stimulus	28	8
Calanus	10s	F7	Post	28	11
Calanus	10s	F8	Pre	24	12
Calanus	10s	F8	Stimulus	24	14

Calanus	10s	F8	Post	24	9
Calanus	10s	F9	Pre	24	10
Calanus	10s	F9	Stimulus	24	10
Calanus	10s	F9	Post	24	7
Calanus	10s	F10	Pre	23	11
Calanus	10s	F10	Stimulus	23	8
Calanus	10s	F10	Post	23	11
Metridia	10s	F6	Pre	37	16
Metridia	10s	F6	Stimulus	38	19
Metridia	10s	F6	Post	39	16
Metridia	10s	F7	Pre	28	16
Metridia	10s	F7	Stimulus	28	15
Metridia	10s	F7	Post	28	13
Metridia	10s	F8	Pre	28	14
Metridia	10s	F8	Stimulus	29	15
Metridia	10s	F8	Post	31	18
Metridia	10s	F9	Pre	29	16
Metridia	10s	F9	Stimulus	27	16
Metridia	10s	F9	Post	27	14
Metridia	10s	F10	Pre	30	14
Metridia	10s	F10	Stimulus	33	15
Metridia	10s	F10	Post	31	15
Calanus	Mimic	F10	Pre	120	13
Calanus	Mimic	F10	Post	122	14
Metridia	Mimic	F10	Pre	138	60
Metridia	Mimic	F10	Post	138	63

2.7 Statistical analysis

The parameters extracted by the python code (total movement, x translation and top speed) were analyzed using the software R (code available on request). The normality of the data was checked using Shapiro-Wilks test. The Shapiro-Wilks test showed that even though the W-values were close to 1 and thus indicating normal distribution, the p-values were mostly below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution (see table B1, B2, B3, B4 and B5 in appendix B). Kruskal-Wallis test was therefore chosen to compare the different experiments with each other. Also using R, ggplot was used to plot the figures (code available on request). For each 10s experiments the pre and stimulus were compared to get the reaction to light onset, and stimulus and post were compared to get the reaction to light offset. Whereas for the mimic experiments the pre and post were compared as the stimulus were very quick and therefore impossible to separate from the pre and post. This was done for the total movement, x translation and top speed.

3 Results

In total, 24 successful experiments were conducted, but due to time constraints 12 experiments were chosen for further data analysis, presenting both *Calanus* and *Metridia* and the two different types of experiments (10s and mimic).

3.1 Species identification

The pre-identification allowed only to identify the specimens to genus level; *Calanus* spp. and *Metridia* spp. The more detailed species identification allowed 37 individuals to be identified morphologically (microscopy) and/or molecularly (PCR) (Table 4 and table C1 in appendix C), whereas 90 were disintegrated partially during the preservation (e.g, missing the legs of the urosome segments) and the remaining individuals were not attempted to be identified due to time constraints and unsuccessful molecular identification protocols. From the 37 that were attempted to be identified, 8 were identified as *C. glacialis* by morphological identification. The molecular species identification has low success rate, as only 13% we successful with the Chelex DNA extraction and universal mtCOI primers, and 0% with the Qiagen DNA extraction kit and universal mtCOI primers, Also, NanoDrop measurements indicated that the DNA extraction had failed. In the third round of Chelex extraction, this time using species-specific *C. finmarchicus* primers, 9 out of 30 were identified as *C. finmarchicus.*

Table 4: Results from morphological and molecular species identification. Some individuals had contradicting results between the morphological and molecular identification. Whereas others only had one of the two identification methods conducted. N/D means that the type of identification was not attempted. For the molecular identification *Calanus* spp. means that the PCR product was negative, meaning that either the PCR failed or that the individual is another *Calanus* species than *C.finmarchicus*. A more detailed table, showing the results from the different PCR attempts can be seen in table C1 in appendix C.

Exp.	Taxa	ID	Month	Lifestage	Length (mm)	Species (morphological)	Species (molecular)
13	*Calanus*	1	June	CV	3.76	*C. glacialis*	*C. finmarchicus*
13	*Calanus*	2	June	CV	3.4	*C. glacialis*	*C. finmarchicus*
13	*Calanus*	3	June	CV	3.48	*C. glacialis*	*C. finmarchicus*
13	*Calanus*	4	June	CV	3.16	*C. glacialis*	*C. finmarchicus*
13	*Calanus*	5	June	CV	3.28	*C. glacialis*	*Calanus* spp.
13	*Calanus*	6	June	CV	3.28	*C. glacialis*	N/D
13	*Calanus*	7	June	CV	3.52	*C. glacialis*	N/D
13	*Calanus*	8	June	CV	3.52	*C. glacialis*	N/D
3	*Calanus*	1	March	N/D	2.85	N/D	*C. finmarchicus*
3	*Calanus*	2	March	N/D	2.64	N/D	*Calanus* spp.

3	*Calanus*	3	March	N/D	2.34	N/D	*Calanus* spp.
3	*Calanus*	4	March	N/D	2.7	N/D	*Calanus* spp.
5	*Calanus*	1	March	N/D	2.7	N/D	*Calanus* spp.
5	*Calanus*	2	March	N/D	2.04	N/D	*C. finmarchicus*
5	*Calanus*	3	March	N/D	2.37	N/D	*Calanus* spp.
5	*Calanus*	4	March	N/D	N/D	N/D	*Calanus* spp.
7	*Calanus*	1	March	N/D	3	N/D	*Calanus* spp.
7	*Calanus*	2	March	N/D	2.76	N/D	*Calanus* spp.
7	*Calanus*	3	March	N/D	2.55	N/D	*Calanus* spp.
7	*Calanus*	4	March	N/D	2.94	N/D	*Calanus* spp.
7	*Calanus*	5	March	N/D	N/D	N/D	*Calanus* spp.
7	*Calanus*	6	March	N/D	N/D	N/D	*Calanus* spp.
7	*Calanus*	7	March	N/D	N/D	N/D	*Calanus* spp.
14	*Calanus*	1	June	N/D	N/D	N/D	*Calanus* spp.
14	*Calanus*	2	June	N/D	N/D	N/D	*Calanus* spp.
14	*Calanus*	3	June	N/D	N/D	N/D	*Calanus* spp.
14	*Calanus*	4	June	N/D	N/D	N/D	*Calanus* spp.
14	*Calanus*	5	June	N/D	N/D	N/D	*C. finmarchicus*
15	*Calanus*	1	June	N/D	N/D	N/D	*C. finmarchicus*
15	*Calanus*	2	June	N/D	N/D	N/D	*Calanus* spp.
15	*Calanus*	3	June	N/D	N/D	N/D	*C. finmarchicus*
15	*Calanus*	4	June	N/D	N/D	N/D	*C. finmarchicus*
15	*Calanus*	5	June	N/D	N/D	N/D	*C. finmarchicus*
2	*Metridia*	1	June	N/D	N/D	N/D	*Calanus* spp.
6	*Metridia*	1	June	N/D	N/D	N/D	*Calanus* spp.
7	*Metridia*	1	June	N/D	N/D	N/D	*Calanus* spp.
12	*Metridia*	1	June	N/D	N/D	N/D	*Calanus* spp.

3.2 Baseline movement

The baseline for the total movement of both *Calanus* and *Metridia* in the 10 second time window before each light onset (pre) were similar for all the filters as seen in figure 7 and 8. There were no statistical differences between the baseline movement between the filters within the genus for the 10s experiments, and the experimental protocol did therefore not affect the baseline (Table 5). The combined baseline for the total movement before light onset for the 10s experiments were therefore compared to the mimic experiments of each genus, and also between the two genus (Table 5).

There was a statistical difference between the baseline movement of *Calanus* and *Metridia*, as *Calanus* had a lower baseline than *Metridia*, meaning that they generally move less, but with more outliers. It is important to note that there is also a significant difference between the 10s and mimic experiments of *Calanus*, but not for *Metridia* (Table 5 and Figure 9).

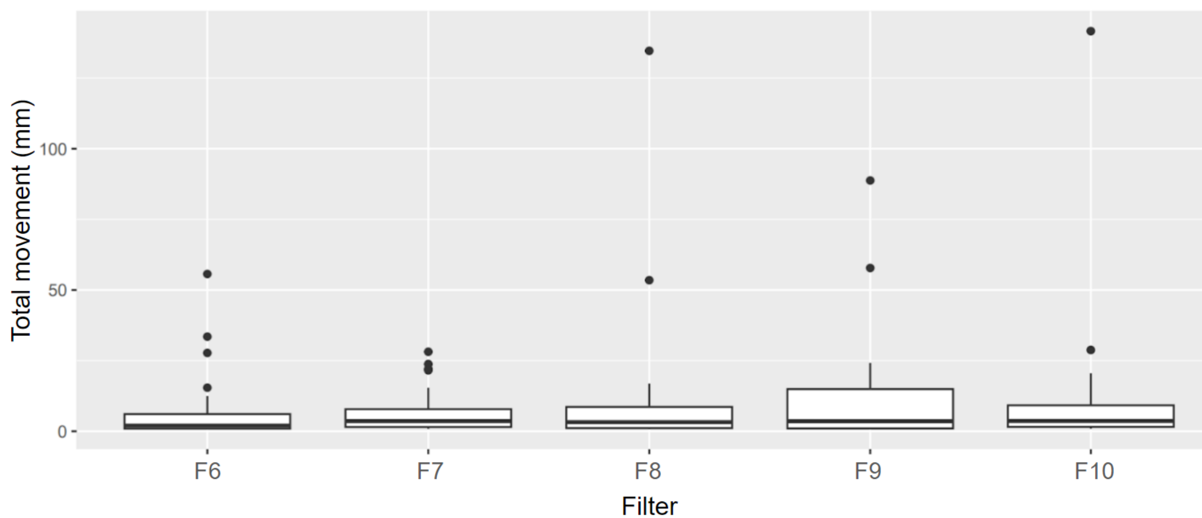

Figure 7: Box plot illustrating the distribution of the baseline movement of *Calanus* before light onset (pre), for the different filters for the 10s experiments, measured in millimeters, showing that the baseline movement remains similar throughout the experiment. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

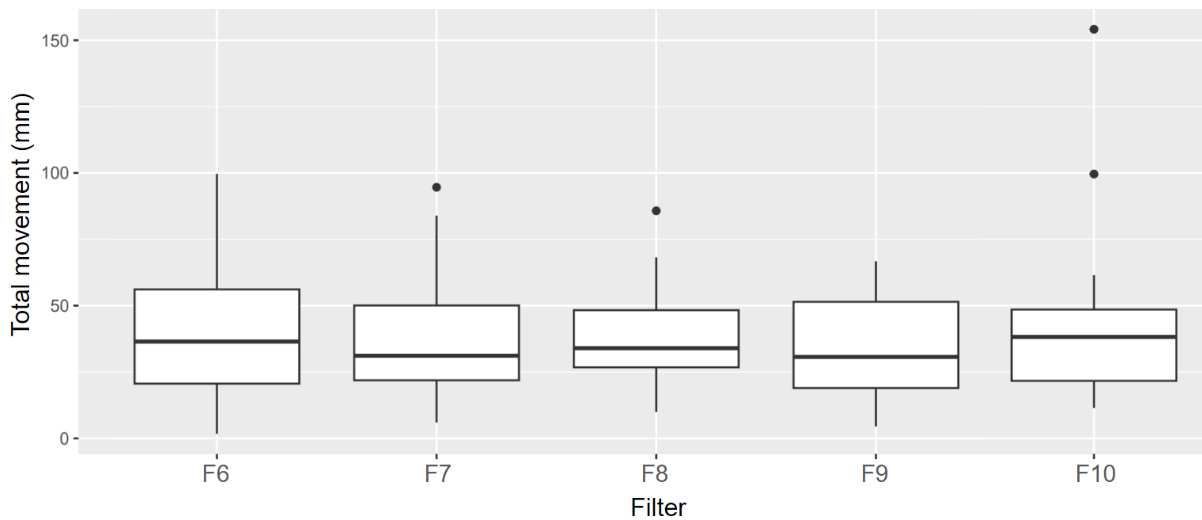

Figure 8: Box plot illustrating the distribution of the baseline movement of *Metridia* before light onset (pre), for the different filters for the 10s experiments, measured in millimeters, showing that the baseline movement remains similar throughout the experiment. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

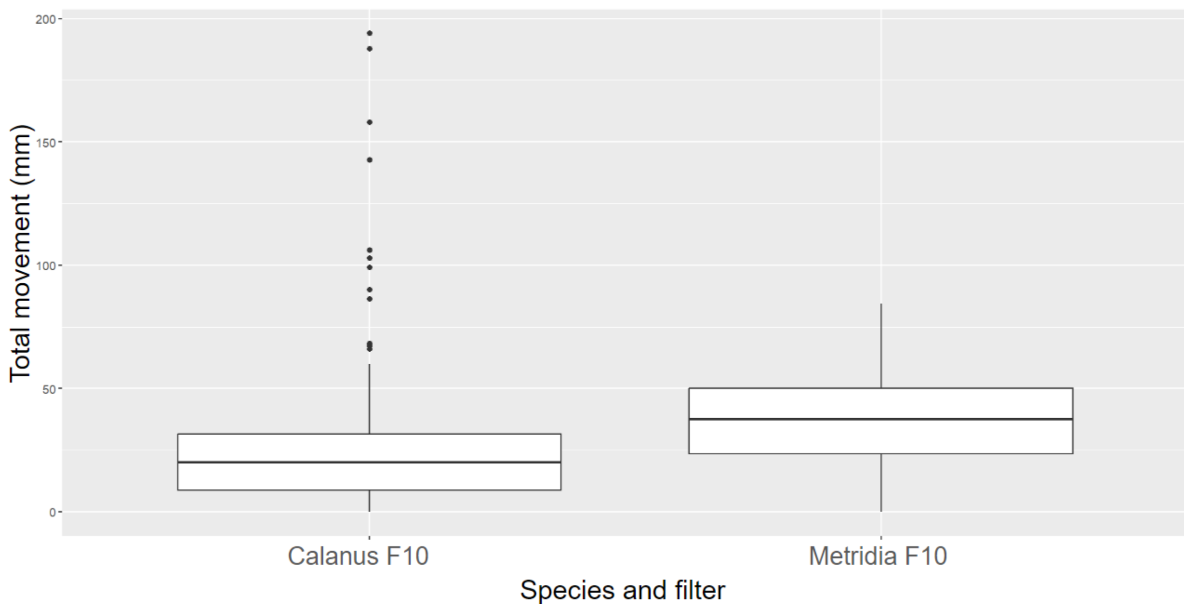

Figure 9: Box plot illustrating the distribution of the baseline movement of *Calanus* and *Metridia* before light onset (pre), for filter 10 for the mimic experiments, measured in millimeters. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

Table 5: Results from Kruskal-Wallis tests for the baseline movement of *Calanus* and *Metridia*, including chi-squared values, degrees of freedom (df), and p-values. The p-values below 0.05 (highlighted in green) show a statistically significant difference in the data, whereas the p-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	chi-squared	df	p-value
Calanus	10s	2.1	4	0.71
Metridia	10s	0.86	4	0.93
Calanus	Mimic & 10s	61	5	<0.01
Metridia	Mimic & 10s	0.94	5	0.97
Calanus & *Metridia*	10s	144	9	<0.01

3.3 Total movement

Total movement of *Calanus* and *Metridia* were observed during the 10s and the mimic experiments, and this was the case for all the filters and all the stages (before, during and after light onset). As visualized in the box plots (Figure 10, 11 and 12) there is no clear difference in the total movement between the different stages (before, during and after light onset) for either *Calanus* or *Metridia* for any of the filters. This was confirmed by the Kruskal-Wallis tests as seen in table 6, 7 and 8, where no statistically significant differences were found within each filter during light onset (Table 6 and 8) or light offset (Table 7 and 8) for any of the experiments.

For *Calanus* there seems to be a lot of variation, with many outliers, especially during filters F8-F10 (Figure 10). Whereas for *Metridia* there is a peak in variation during filter F8, but also during filters F9 and F10 (Figure 11). There is a higher variation for *Calanus* than for *Metridia*, but the range of movement is generally larger for *Metridia* as visualized by the longer whiskers and the 25th and 75th percentile further apart than for *Calanus* (Figure 10 and 11).

Even though there were no statistical differences, there seems to be a slight trend visible in figure 10 and 11. There appears to be less variation during the pre than during the stimulus and post for 10s experiments for *Calanus* (Figure 10). Whereas for *Metridia* there seems to be less variation during the stimulus than during the pre and post for the 10s experiments (Figure 11).

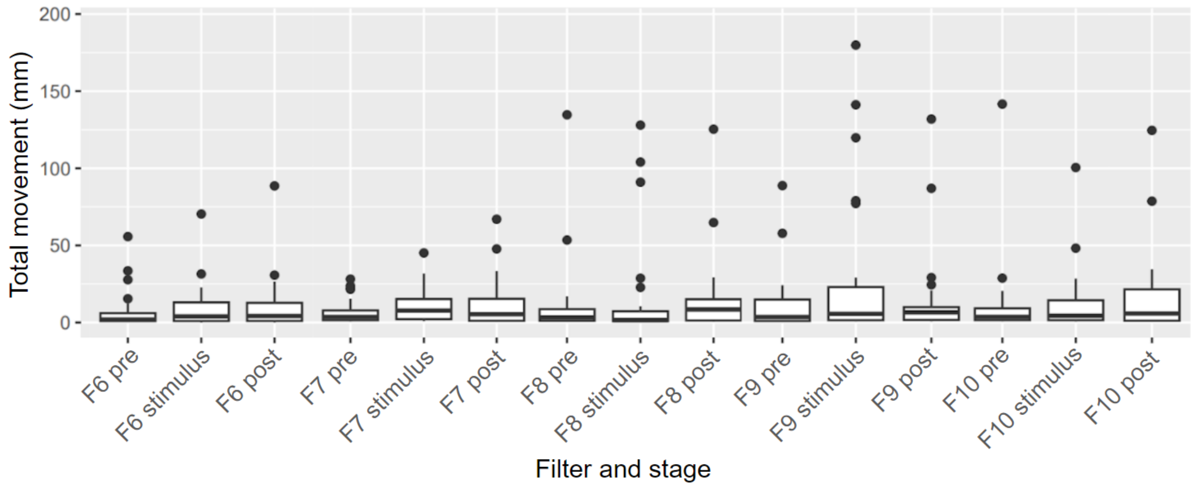

Figure 10: Box plot showing the total movement of all stages (pre, stimulus and post) and all filters for *Calanus*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

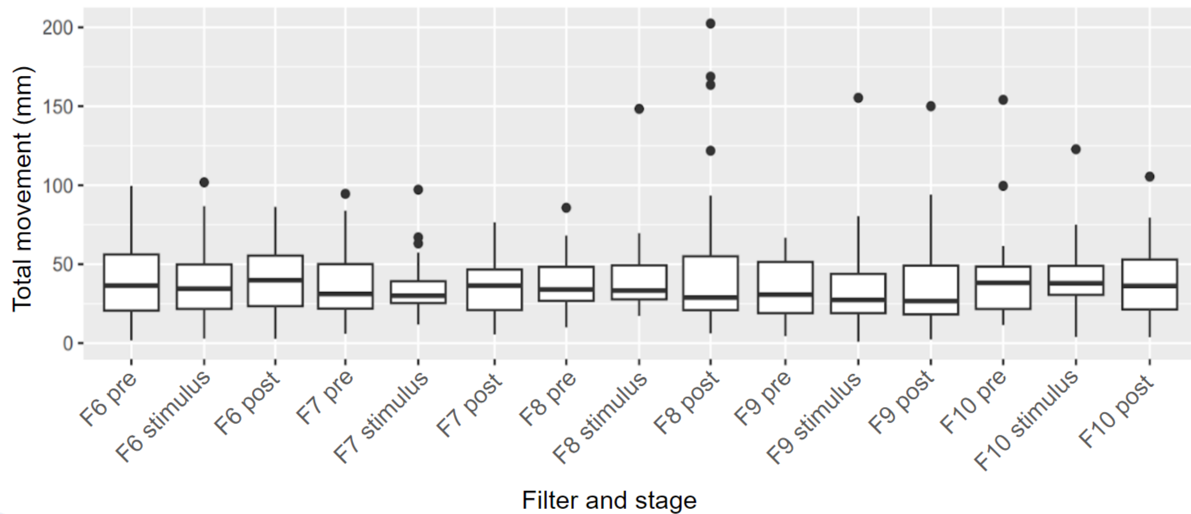

Figure 11: Box plot showing the total movement of all stages (pre, stimulus and post) and all filters for *Metridia*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

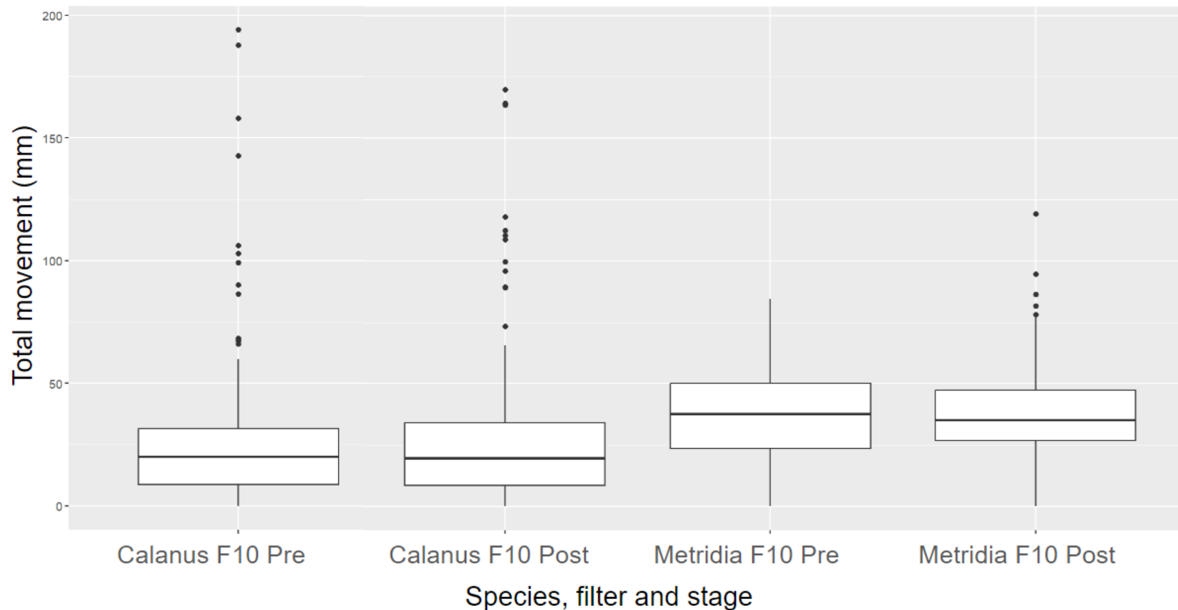

Figure 12: Box plot showing the total movement of both stages (pre and post) and filter 10 for *Calanus* and *Metridia*, measured in millimeters, in the mimic experiments. Pre and post refers to the stages of the experiment, meaning before and after light onset. F10 is referring to the filter used, where F10 provides the highest light intensity for these experiments. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

Table 6: Results from Kruskal-Wallis tests for the total movement as a response to light onset (pre compared to stimulus) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Pre & stimulus	0.81	1	0.37
Calanus	10s	F7	Pre & stimulus	2.0	1	0.16
Calanus	10s	F8	Pre & stimulus	0.52	1	0.47
Calanus	10s	F9	Pre & stimulus	0.82	1	0.36
Calanus	10s	F10	Pre & stimulus	0.22	1	0.64
Metridia	10s	F6	Pre & stimulus	<0.01	1	0.93
Metridia	10s	F7	Pre & stimulus	0.053	1	0.82
Metridia	10s	F8	Pre & stimulus	0.060	1	0.81
Metridia	10s	F9	Pre & stimulus	0.096	1	0.76
Metridia	10s	F10	Pre & stimulus	0.11	1	0.74

Table 7: Results from Kruskal-Wallis tests for the total movement as a response to light offset (stimulus compared to post) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Stimulus & post	<0.01	1	0.97
Calanus	10s	F7	Stimulus & post	0.23	1	0.64
Calanus	10s	F8	Stimulus & post	1.9	1	0.17
Calanus	10s	F9	Stimulus & post	0.14	1	0.71
Calanus	10s	F10	Stimulus & post	<0.01	1	0.94
Metridia	10s	F6	Stimulus & post	0.30	1	0.58
Metridia	10s	F7	Stimulus & post	<0.01	1	0.97
Metridia	10s	F8	Stimulus & post	0.15	1	0.70
Metridia	10s	F9	Stimulus & post	<0.05	1	0.86
Metridia	10s	F10	Stimulus & post	0.25	1	0.62

Table 8: Results from Kruskal-Wallis tests for the total movement as a response to light stimulus (pre compared to post) for *Calanus* and *Metridia* for filter F10, during the mimic experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	Mimic	F10	Pre & post	<0.05	1	0.91
Metridia	Mimic	F10	Pre & post	0.22	1	0.64

3.4 Movement in the x-axis (x translation)

Movement in the x-axis (x translation) of *Calanus* and *Metridia* were observed during the 10s and the mimic experiments, and this was the case for all the filters and all the stages (before, during and after light onset). As visualized in the box plots (Figure 13, 14 and 15) there seems to be no clear pattern in the movement in the x-axis between the different stages (before, during and after light onset) for either *Calanus* or *Metridia* for any of the filters. This was confirmed by the Kruskal-Wallis tests as seen in table 9, 10 and 11, where no statistically significant differences were found within each filter during light onset (Table 9 and 11) or light offset (Table 10 and 11) for any of the experiments. Except for filter F9 for *Metridia* in the 10s experiments during light offset (stimulus to post), where the p-value was under 0.05. As there was only one of the tests that showed a significant difference, there is no trend and it is most likely a coincidence. In this case (filter F9) they are moving

away from the light, and should therefore do the same for F10, as F10 has a higher intensity than F9. But as visualized in figure 14 they are moving towards the light instead.

For *Calanus* there seems to be some variation, with many outliers, especially during filters F8-F10 (Figure 13). Whereas for *Metridia* there is a lot of variation during all filters (Figure 14). There is a lower variation for *Calanus* than for *Metridia*, and the range of movement is generally larger for *Metridia* with the longer whiskers and the 25th and 75th percentile further apart than for *Calanus* (Figure 13 and 14). Overall there are a lot of outliers and no clear patterns for either *Calanus* or *Metridia*.

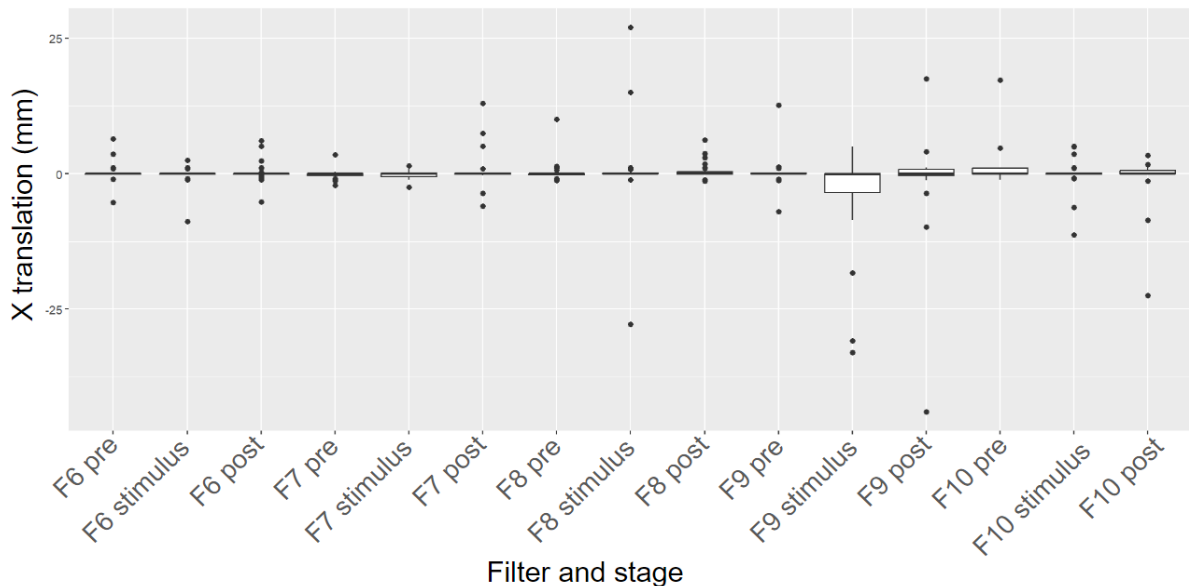

Figure 13: Box plot showing the movement in the x-axis (x translation) of all stages (pre, stimulus and post) and all filters for *Calanus*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

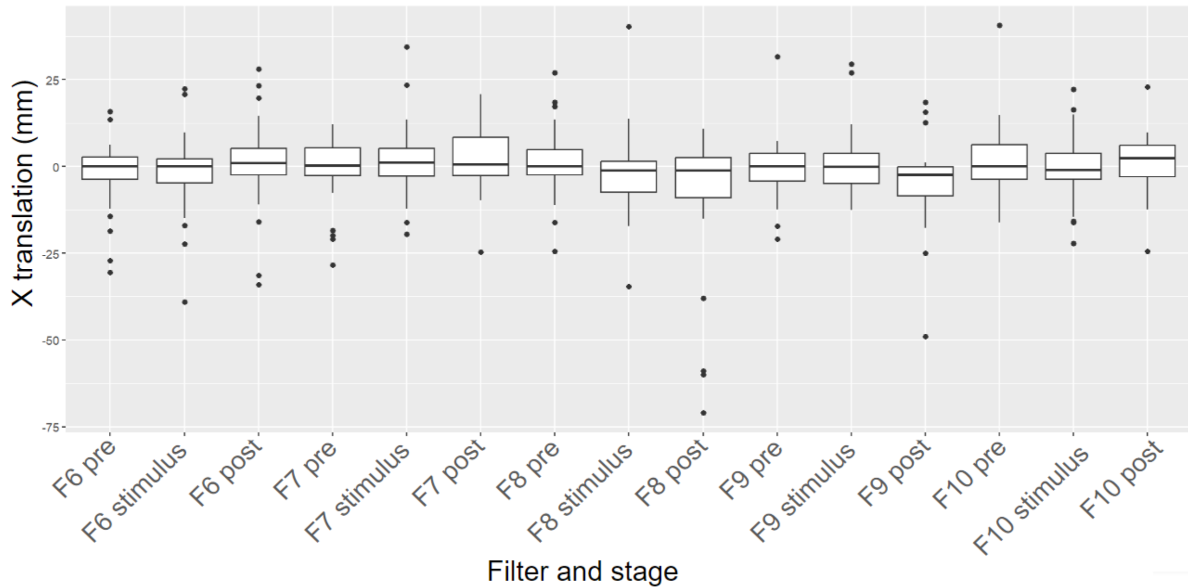

Figure 14: Box plot showing the movement in the x-axis (x translation) of all stages (pre, stimulus and post) and all filters for *Metridia*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

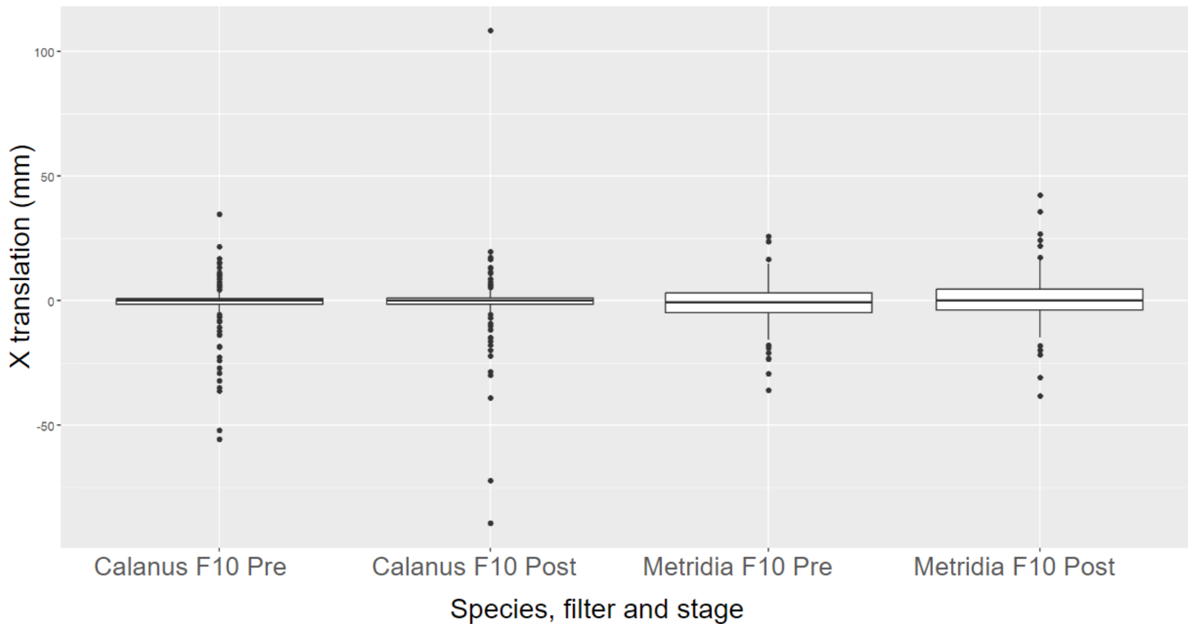

Figure 15: Box plot showing the movement in the x-axis (x translation) of both stages (pre and post) and filter 10 for *Calanus* and *Metridia*, measured in millimeters, in the mimic experiments. Pre and post refers to the stages of the experiment, meaning before and after light onset. F10 is referring to the filter used, where F10 provides the highest light intensity for these experiments. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

Table 9: Results from Kruskal-Wallis tests for the movement in the x-axis (x translation) as a response to light onset (pre compared to stimulus) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Pre & stimulus	0.071	1	0.79
Calanus	10s	F7	Pre & stimulus	0.73	1	0.39
Calanus	10s	F8	Pre & stimulus	0.19	1	0.67
Calanus	10s	F9	Pre & stimulus	1.9	1	0.17
Calanus	10s	F10	Pre & stimulus	0.79	1	0.37
Metridia	10s	F6	Pre & stimulus	<0.01	1	0.94
Metridia	10s	F7	Pre & stimulus	<0.05	1	0.88
Metridia	10s	F8	Pre & stimulus	1.6	1	0.20
Metridia	10s	F9	Pre & stimulus	<0.05	1	0.89
Metridia	10s	F10	Pre & stimulus	0.16	1	0.69

Table 10: Results from Kruskal-Wallis tests for the movement in the x-axis (x translation) as a response to light offset (stimulus compared to post) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. The p-value below 0.05 (highlighted in green) shows a statistically significant difference in the data, whereas the p-values above 0.05 shows no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Stimulus & post	<0.01	1	0.95
Calanus	10s	F7	Stimulus & post	0.23	1	0.64
Calanus	10s	F8	Stimulus & post	<0.05	1	0.90
Calanus	10s	F9	Stimulus & post	1.4	1	0.24
Calanus	10s	F10	Stimulus & post	0.17	1	0.68
Metridia	10s	F6	Stimulus & post	0.88	1	0.35
Metridia	10s	F7	Stimulus & post	0.14	1	0.71
Metridia	10s	F8	Stimulus & post	0.056	1	0.81
Metridia	10s	F9	Stimulus & post	4.4	1	<0.05
Metridia	10s	F10	Stimulus & post	1.1	1	0.30

Table 11: Results from Kruskal-Wallis tests for the movement in the x-axis (x translation) as a response to light stimulus (pre compared to post) for *Calanus* and *Metridia* for filter F10, during the mimic experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	Mimic	F10	Pre & post	1.2	1	0.28
Metridia	Mimic	F10	Pre & post	2.4	1	0.12

3.5 Top speed

Top speed of *Calanus* and *Metridia* were observed during the 10s and the mimic experiments, and this was the case for all the filters and all the stages (before, during and after light onset). As visualized in the box plots (Figure 16, 17 and 18) there seems to be no clear pattern in the movement in the x-axis between the different stages (before, during and after light onset) for either *Calanus* or *Metridia* for any of the filters. This was confirmed by the Kruskal-Wallis tests as seen in table 12, 13 and 14, where no statistically significant differences were found within each filter during light onset (Table 12 and 14) or light offset (Table 13 and 14) for any of the experiments.

For *Calanus* there seems to be some variation, with many outliers, especially during all filters (Figure 16). Whereas for *Metridia* there is also some variation during all filters, but

also a greater difference in distance from the 25th and 75th percentile (Figure 17). There is a more even variation for *Calanus* than for *Metridia*, but the general top speed is slightly larger for *Metridia* than for *Calanus* (Figure 16 and 17). Overall there are a lot of outliers and no clear patterns for either *Calanus* or *Metridia*.

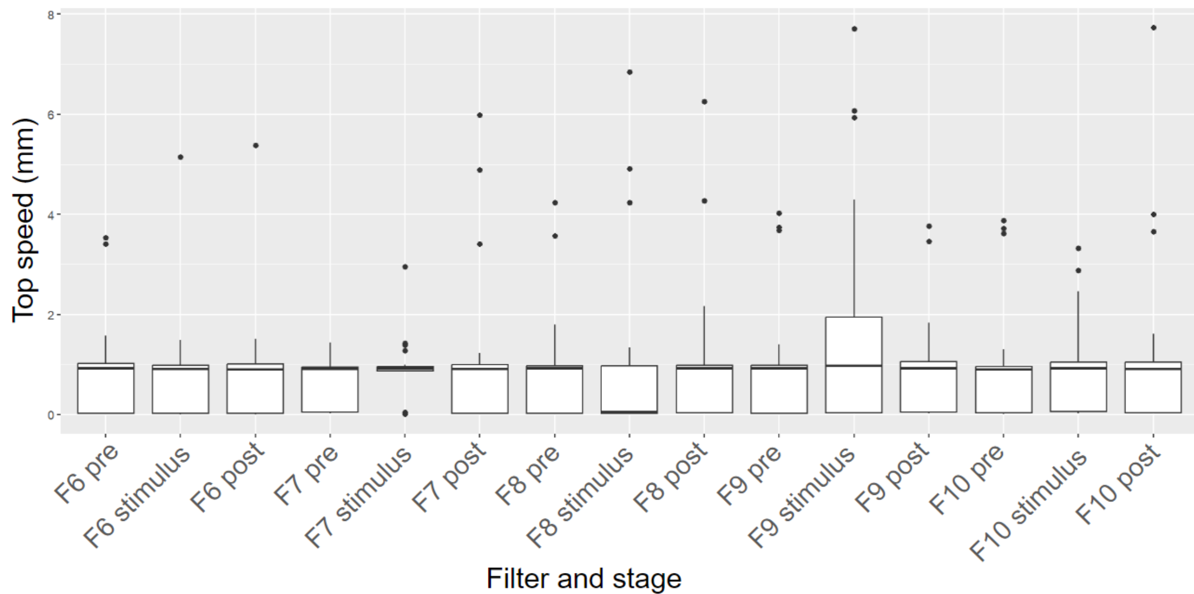

Figure 16: Box plot showing the top speed of all stages (pre, stimulus and post) and all filters for *Calanus*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

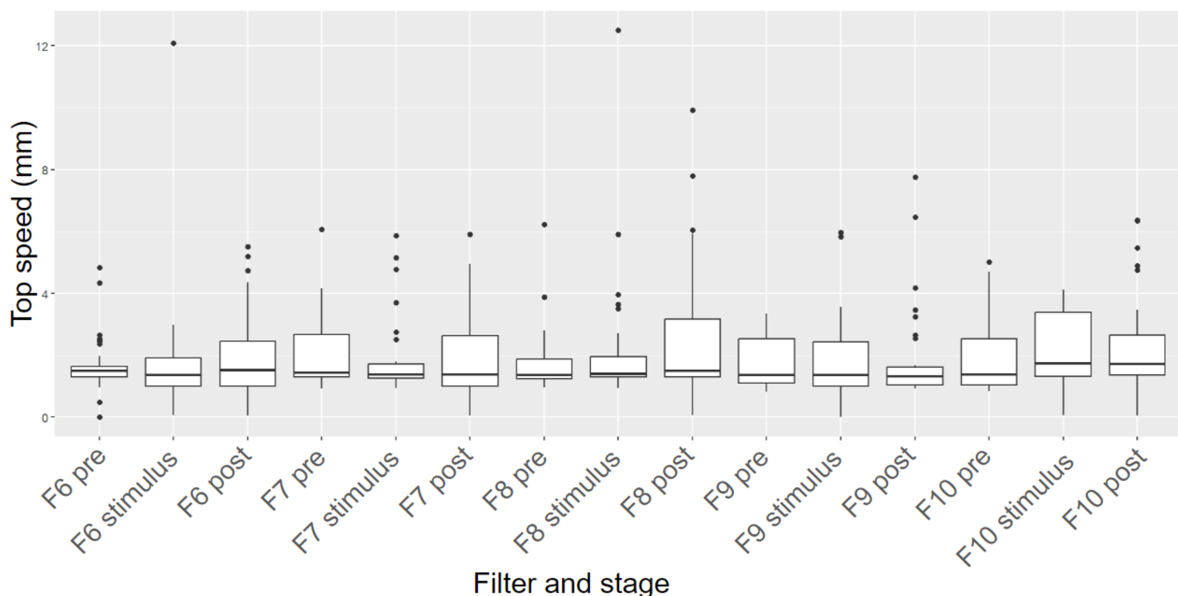

Figure 17: Box plot showing the top speed of all stages (pre, stimulus and post) and all filters for *Metridia*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

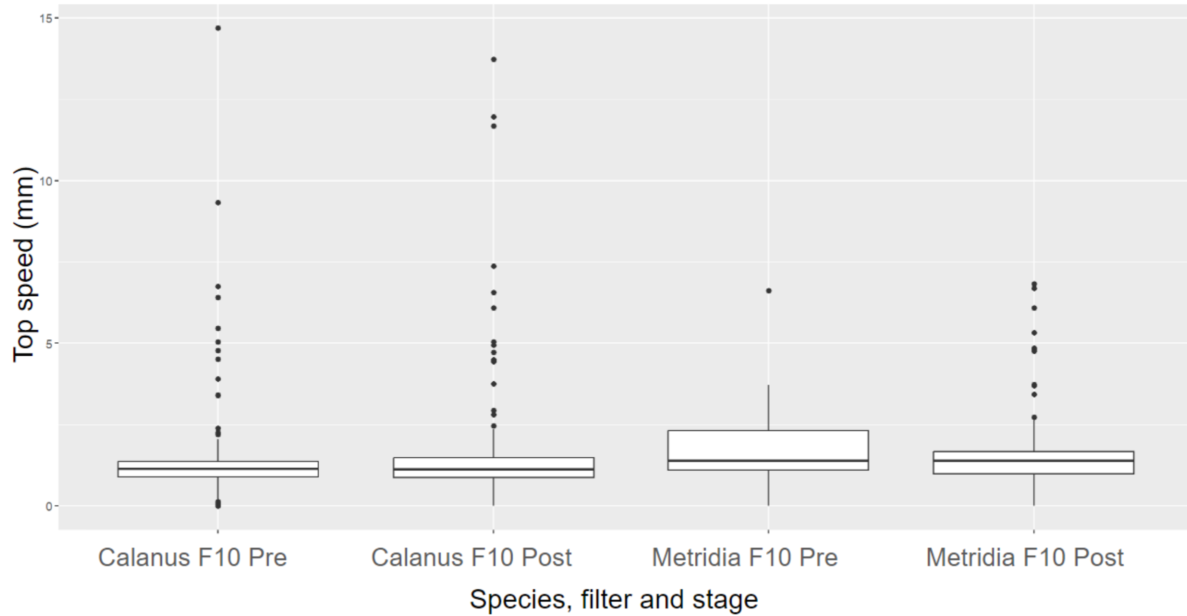

Figure 18: Box plot showing top speed of both stages (pre and post) and filter 10 for *Calanus* and *Metridia*, measured in millimeters, in the mimic experiments. Pre and post refers to the stages of the experiment, meaning before and after light onset. F10 is referring to the filter used, where F10 provides the highest light intensity for these experiments. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

Table 12: Results from Kruskal-Wallis tests for the movement in the top speed as a response to light onset (pre compared to stimulus) for Calanus and Metridia for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Pre & stimulus	<0.01	1	0.94
Calanus	10s	F7	Pre & stimulus	0.52	1	0.47
Calanus	10s	F8	Pre & stimulus	0.36	1	0.55
Calanus	10s	F9	Pre & stimulus	2.3	1	0.13
Calanus	10s	F10	Pre & stimulus	0.31	1	0.58
Metridia	10s	F6	Pre & stimulus	0.47	1	0.49
Metridia	10s	F7	Pre & stimulus	0.26	1	0.61
Metridia	10s	F8	Pre & stimulus	0.35	1	0.56
Metridia	10s	F9	Pre & stimulus	0.17	1	0.68
Metridia	10s	F10	Pre & stimulus	0.69	1	0.41

Table 13: Results from Kruskal-Wallis tests for the movement in the top speed as a response to light offset (stimulus compared to post) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Stimulus & post	<0.01	1	0.95
Calanus	10s	F7	Stimulus & post	<0.05	1	0.87
Calanus	10s	F8	Stimulus & post	0.81	1	0.37
Calanus	10s	F9	Stimulus & post	1.6	1	0.20
Calanus	10s	F10	Stimulus & post	0.20	1	0.65
Metridia	10s	F6	Stimulus & post	0.43	1	0.51
Metridia	10s	F7	Stimulus & post	<0.01	1	0.99
Metridia	10s	F8	Stimulus & post	0.43	1	0.51
Metridia	10s	F9	Stimulus & post	0.11	1	0.74
Metridia	10s	F10	Stimulus & post	<0.01	1	0.96

Table 14: Results from Kruskal-Wallis tests for the movement in the top speed as a response to light stimulus (pre compared to post) for *Calanus* and *Metridia* for filter F10, during the mimic experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	Mimic	F10	Pre & post	<0.05	1	0.84
Metridia	Mimic	F10	Pre & post	0.84	1	0.36

3.6 Total movement after thresholding

Total movement after thresholding of *Calanus* and *Metridia* were observed during the 10s and the mimic experiments, and this was the case for all the filters and all the stages (before, during and after light onset). As visualized in the box plots (Figure 19, 20 and 21) there seems to be no clear difference in the total movement between the different stages (before, during and after light onset) for either *Calanus* or *Metridia* for any of the filters. This was confirmed by the Kruskal-Wallis tests as seen in table 15, 16 and 17, where no statistically significant differences were found within each filter during light onset (Table 15 and 17) or light offset (Table 16 and 17) for any of the experiments.

For *Calanus* there seems to be some variation during all the filters, but especially during stimulus (during light onset) for filter F8 and F9 (Figure 19). Whereas for *Metridia* there is also some variation especially during post (after light offset) for filter F8 (Figure 20). There

is a lower variation for *Calanus* than for *Metridia*, and the range of movement is generally larger for *Metridia* as visualized by the longer whiskers and the 25th and 75th percentile further apart than for *Calanus* (Figure 19 and 20).

Even though there were no statistical differences, there seems to be a slight trend visible in figure 19 and 20. It appears to be a higher movement during the stimulus and post than during the pre for 10s experiments for *Calanus* (Figure 19). Whereas for *Metridia* there seems to be a lower movement during the stimulus than during the pre and post for the 10s experiments (Figure 20). Table 16 shows that there is a difference, but it is not statistically different.

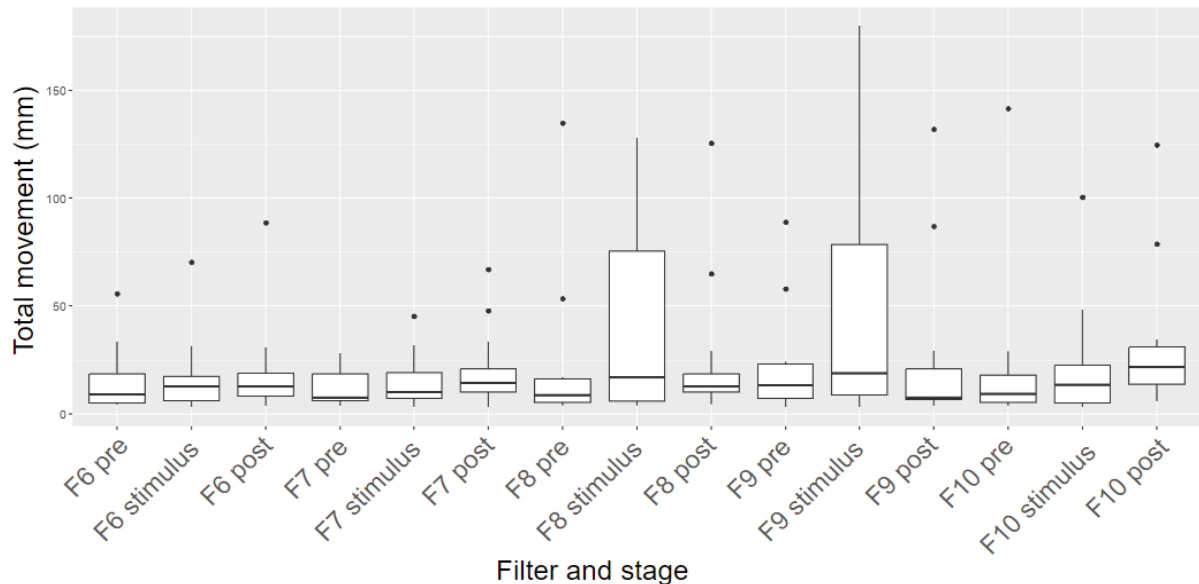

Figure 19: Box plot showing the total movement after thresholding of all stages (pre, stimulus and post) and all filters for *Calanus*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

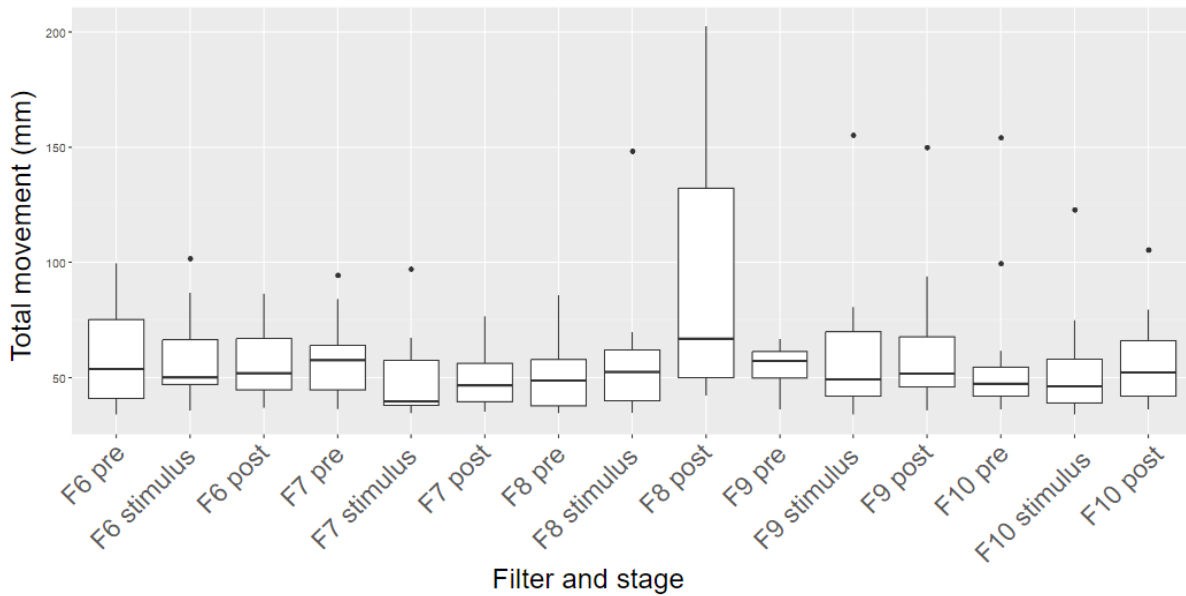

Figure 20: Box plot showing the total movement after thresholding of all stages (pre, stimulus and post) and all filters for *Metridia*, measured in millimeters, in the 10s experiments. Pre, stimulus and post refers to the stages of the experiment, meaning before, during and after light onset. F6-F10 is referring to the filters used, with F6 providing a lower light intensity than F10. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

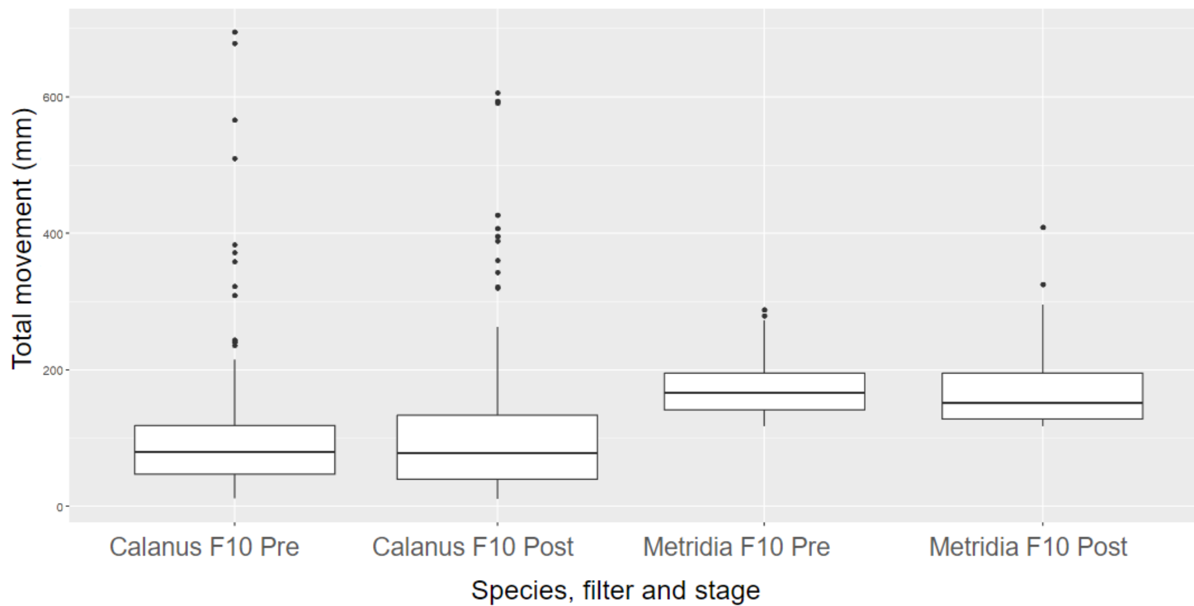

Figure 21: Box plot showing total movement after thresholding of both stages (pre and post) and filter 10 for *Calanus* and *Metridia*, measured in millimeters, in the mimic experiments. Pre and post refers to the stages of the experiment, meaning before and after light onset. F10 is referring to the filter used, where F10 provides the highest light intensity for these experiments. The boxes show the 25th (bottom) and 75th (top) percentile of the data, while the horizontal line inside the boxes shows the median value (50th percentile) of the data. The vertical whiskers show the range of the data points that are not unusually high or low, whereas any points beyond the whiskers are shown as dots and are considered outliers.

Table 15: Results from Kruskal-Wallis tests for the total movement after thresholding as a response to light onset (pre compared to stimulus) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Pre & stimulus	0	1	1
Calanus	10s	F7	Pre & stimulus	0.36	1	0.55
Calanus	10s	F8	Pre & stimulus	0.63	1	0.43
Calanus	10s	F9	Pre & stimulus	1.0	1	0.31
Calanus	10s	F10	Pre & stimulus	<0.05	1	0.88
Metridia	10s	F6	Pre & stimulus	0.097	1	0.76
Metridia	10s	F7	Pre & stimulus	1.4	1	0.23
Metridia	10s	F8	Pre & stimulus	0.68	1	0.41
Metridia	10s	F9	Pre & stimulus	<0.05	1	0.89
Metridia	10s	F10	Pre & stimulus	0.12	1	0.73

Table 16: Results from Kruskal-Wallis tests for the total movement after thresholding as a response to light offset (stimulus compared to post) for *Calanus* and *Metridia* for all filters (F6-F10) separately, during the 10s experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	10s	F6	Stimulus & post	0.27	1	0.61
Calanus	10s	F7	Stimulus & post	0.73	1	0.39
Calanus	10s	F8	Stimulus & post	0	1	1
Calanus	10s	F9	Stimulus & post	2.9	1	0.088
Calanus	10s	F10	Stimulus & post	2.8	1	0.097
Metridia	10s	F6	Stimulus & post	<0.01	1	0.93
Metridia	10s	F7	Stimulus & post	0.12	1	0.73
Metridia	10s	F8	Stimulus & post	3.4	1	0.064
Metridia	10s	F9	Stimulus & post	0.19	1	0.66
Metridia	10s	F10	Stimulus & post	0.48	1	0.49

Table 17: Results from Kruskal-Wallis tests for the total movement after thresholding as a response to light stimulus (pre compared to post) for *Calanus* and *Metridia* for filter F10, during the mimic experiments, including chi-squared values, degrees of freedom (df), and p-values. P-values above 0.05 show no statistically significant difference in the data. Higher chi-squared values suggest notable differences within the data, and df shows the number of groups minus one.

Taxa	Type	Filter	Stage	chi-squared	df	p-value
Calanus	Mimic	F10	Pre & post	1.6	1	0.21
Metridia	Mimic	F10	Pre & post	<0.01	1	0.10

4 Discussion

The behavioral reactions to the onset and offset of both continuous 10 seconds light stimuli (10s) and simulated bioluminescence (mimic) were examined for the two taxa of zooplankton. The 10 seconds light stimuli was used in order to determine the sensitivity limits of vision for the zooplankton taxa used, and to see how they react to light. This could also be relevant when thinking about light pollution and changing marine light climate. In these experiments, it was found that, on a general basis, *Metridia* was more active and moved more compared to *Calanus* (Figure 7 and 8). Interestingly, neither *Metridia* nor *Calanus* showed any noticeable reaction to the artificially simulated lights, including both the 10s and mimic experiments. These findings suggest a lack of sensitivity or responsiveness of these zooplankton taxa to the light stimuli used in these experiments. Additionally, the statistical analysis showed no significant differences between the 10 second light exposure and the bioluminescence mimic experiments, except for the baseline swimming activity (baseline total movement) for *Calanus*, suggesting no noticeable behavioral responses in either case. The difference in baseline swimming activity for *Calanus* in the 10s and mimic experiments can be due to the difference in season, where the mimic experiments were conducted in March and the 10s experiments were conducted in June. For the *Metridia*, both mimic and 10s experiments were conducted in June, possibly explaining the similar swimming activity. Overall, the findings show the complexity of zooplankton behavior and the challenges in studying their responses to environmental stimuli. Further research, using improved experimental methods, could reveal more about how zooplankton move and react to light in their natural habitats. Additionally, this study shows the importance of detailed planning of the methodology in order to achieve reliable results.

As the experiments conducted did not yield significant reactions to the light stimuli, in contradiction to earlier studies by Buskey & Swift (1985) and Båtnes et al. (2015) among others, it raises questions about this study's methods. Here, the aim was to mimic natural light conditions to study zooplankton behavior, but it appears that the methods used did not provide the expected responses from the zooplankton taxa *Metridia* and *Calanus*. Earlier studies, especially Buskey & Swift (1985) and Båtnes et al. (2015) who conducted similar experiments, yielded statistically significant reactions to the light stimulus used. This suggests a need to re-evaluate and potentially further improve the experimental design and consider other approaches that might better reflect the actual behaviors of these zooplankton in future studies.

4.1 Behavioral responses compared to literature

The findings regarding the higher activity of *Metridia* compared to *Calanus* are consistent with common observations from previous field studies (Majaneva, personal communication). Metridia is known for its "jumping" behavior, and despite that the speed can be fluctuating, the peak speeds of escape jumps may be up to 1000 body lengths per second (Kiørboe, 2011). Previous studies done by Hirche (1987), Hardy & Bainbridge (1954) and Enright (1977) investigated the swimming speed of species belonging to the genus *Calanus* and *Metridia* in relation to temperature effects on respiration and diel vertical migration, especially on upward migration. It is therefore important to note that these studies measured swimming speed under specific experimental conditions tailored

to their research questions. Hirche (1987) compared their findings with the ones from Hardy & Bainbridge (1954) and Enright (1977) and found that the average swimming speed varied between the different studies, but not significantly between the two zooplankton species *Calanus finmarchicus* and *Metridia pacifica*. In contrast, this study found that the baseline movement varied between the two taxa, albeit not identified to species. This difference therefore means that while previous studies provide valuable insights into some specific aspects of zooplankton movement, they are not completely comparable to this study.

Earlier studies have shown clear behavioral responses to simulated bioluminescence for both zooplankton species *Calanus finmarchicus* and *Metridia longa* (Buskey & Swift, 1985). In contrast, the experiments from this study did not provide such results for the individuals from the same genus *Calanus* and *Metridia*. Despite using similar methodologies to simulate bioluminescence, this study observed no significant behavioral responses from these zooplankton taxa. This difference might be due to small variations in experimental setups, the physical condition or life stages of the individuals used, or differences in environmental conditions during the experiments. These factors highlight the challenges involved in replicating biological experiments in different laboratories or with organisms taken from different places at different times. To mitigate these issues, it is important to provide clear protocols of the conducted experiments in order for others to be able to properly replicate or alter them, and to be able to compare results.

4.2 Possible factors behind the differences between studies

4.2.1 Stimulus duration and species identification

Small differences in the experimental setup can make a difference. The study by Buskey & Swift (1985) stated that they used an electronic shutter and therefore could not replicate the intensity pattern of true bioluminescence. They used a constant intensity flash of two different durations (0.06 seconds to simulate dinoflagellates, and 0.6 seconds to simulate copepods) instead. In contrast to Buskey & Swift (1985), Miljeteig et al. (2014) used a constant intensity light of 10 minutes for each intensity. Whereas this study opted for two different durations being 10 seconds constant intensity flash for different intensities and *Metridia* mimic using a programmed Arduino for different intensities. The variations in results could therefore be due to the difference in light exposure time for the zooplankton used. The use of the Arduino provided a more accurate stimuli as the change in intensity could be done during the short flash, and this would therefore be encouraged in future studies exploring the responses to bioluminescence.

As previous studies showed responses to light stimuli in shorter range (0.06-0.6 seconds) and longer range (10 minutes), yet this study did not with the in-between range (10 seconds). Perhaps this means that the zooplankton reacts to durations they are used to, like bioluminescent flashes and longer intervals due to DVM. And therefore the in between durations did not provide any responses as they are not representative to real life scenarios. Alternatively, this might indicate that not all kinds of artificial light affects the behavior of these zooplankton taxa. However, unpublished results from Viljanen et al. (in prep) shows that *Calanus glacialis* reacts to 10 seconds of light stimuli. Thus it is important to note that there are discrepancies in the species identification (Table 4) performed in this study, as well as other studies, and the exact species used are therefore uncertain. It is

likely that the individuals used in this study were mostly *Calanus finmarchicus*, as this species is far more common in the region compared to other *Calanus* species (Choquet et al., 2017). Viljanen et al. (in prep) also found out that this species was less likely to react to the 10 second light stimuli, which highlights the importance of correct species identification, as it is known that different stages and species can react differently to light stimuli.

4.2.2 Stimulus intensity

Stimulus light intensities are essential when comparing the results of different studies. As mentioned in section 2.4.5, the comparison between the light intensities used in this study and the study by Miljeteig et al. (2014) were difficult due to differences in measuring techniques and in the setup. Still, as the experimental setup in this study most likely provided the zooplankton with less light, since there was a diffuser plate in the front of the stimulus light and only small holes for the light, which could be an important factor to consider when looking at the difference in results between these two studies.

4.2.3 Seasonal effects

Other limitations to this study include the seasonal variation in the zooplankton collection. The seasonal variation in the zooplankton collection comes as a result of logistical issues due to the short time frame of this study, and experiments were therefore conducted both in March and June. It is possible that the behavior of *Calanus* and *Metridia* varies between the given months, affecting the outcome of this study. Future research should try to perform all experiments in the same season, to avoid issues regarding seasonal variation. Båtnes et al. (2015) conducted their experiments using zooplankton found in Svalbard during the polar night (when the ambient light is much dimmer), whereas this study was conducted in Trondheimsfjorden, during spring/summer. Increased intensities, in addition to zooplankton used to darker light conditions, could explain why the experiments done by Båtnes et al. (2015) got clear responses, whereas this study did not.

4.2.4 Acclimation to study conditions

The studies of both Båtnes et al. (2015) and Miljeteig et al. (2014) acclimated the individuals in the aquarium for each experiment for one hour, whereas this study opted for an acclimation time of 10 minutes in the aquarium for the individuals. Shorter acclimation time will increase the efficiency of the experiment, and therefore allowing more to be conducted in the limited time frame provided for this study. Still, this could affect the outcome of the study by not allowing the individuals sufficient time to adapt to the new conditions present in the experimental setup, potentially leaving the individuals stressed during the experiment, and thus not capturing their natural behavior. Ensuring enough acclimation time is therefore important for the reliability of the results.

All the individuals collected for this study were kept in buckets or separate containers (10 individuals in each) for a maximum of two weeks prior to the experiments they were used in. It was noticed that some of the individuals were appearing a bit weak during the experiments, potentially due to being kept in containers over a longer period of time and also by being handled when sorting. As the sorting was done in red light, the amount of time the zooplankton spent in a little petri dish were longer than if they were sorted in

normal light. In the future there should therefore be optimized sorting and reduced time in containers in order to maintain their fitness and thereby ensure more representative behavioral responses.

4.2.5 Sample size

Some limitations of the experimental design is the small sample size and few replicates, due to little time available, which may have affected the statistical power of the analyses. Future research should consider using a higher number of replicates. With that said, Miljeteig et al. (2014) had 250-300 individuals in total (50-60 per experiment), which was a much greater number than this study used (10 per experiment). Still, it is important to note that the zooplankton responded with increased movement when accidentally bumping into each other (Hirche, 1987). Keeping the number of individuals in the aquarium tank for each experiment low is therefore important, to avoid false results caused by overcrowding in the aquarium tank.

4.3 The importance of adequate video quality

Finally, one significant limitation encountered in this study was the poor quality of the video recordings that were used for tracking the movements of the individual zooplankton using SEAP. This was also an issue that could have been solved by using a better camera. The poor video quality made it harder for the AI program to accurately track the individuals and some movement may have been lost. Additionally, manual tracking was sometimes necessary due to the poor quality, and the combination of AI and human tracking was suboptimal for the data collection, as it was difficult to accurately copy the tracking method of the AI program. Examples of this can be seen in figure 22, where the AI program used a more zigzag like pattern, whereas the human tracking used straighter lines.

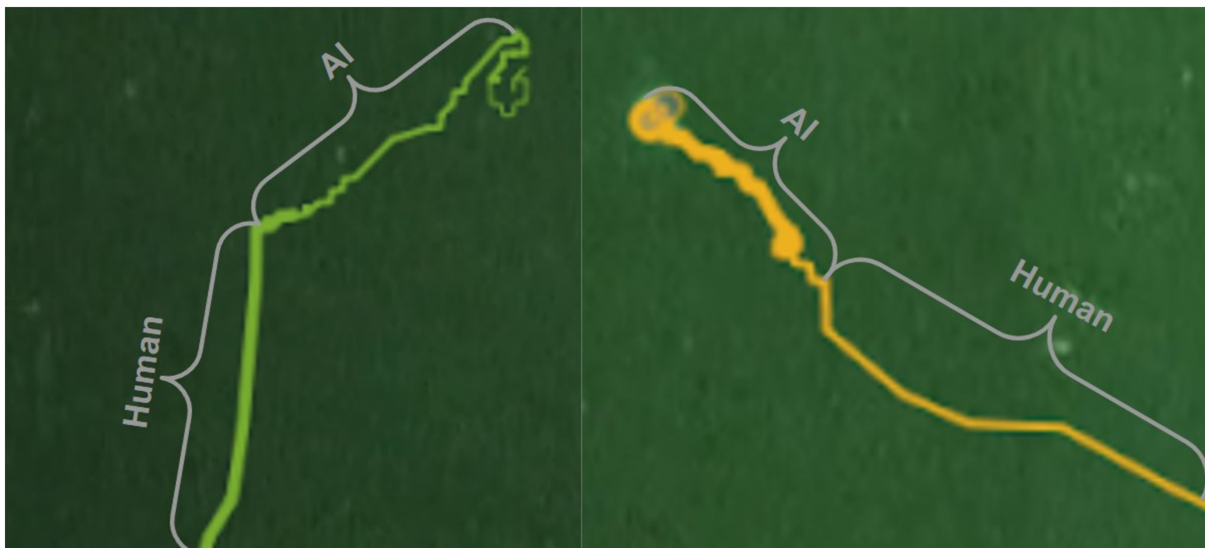

Figure 22: Visual explanation of the difference in AI vs human tracking of the individuals movement. The top parts of both tracks show a zigzag pattern created by the AI, whereas the straight lines are from the manual tracking done by a human.

This issue shows the importance of using high-quality video capture to ensure reliable tracking data, as well as saving time by reducing the amount of manual work needing to

be done. Future studies should prioritize proper recording equipment to ensure best results when using AI tracking programs.

4.4 Future perspectives

Future studies could investigate the impacts of increased artificial light on zooplankton communities, as this is important for understanding the long-term consequences of artificial light on zooplankton and how this influences marine ecosystems. Enhancing experimental protocols and addressing issues like the misalignment of the LED lighting in relation to the zooplankton placement in the experimental setup will ensure better data accuracy. Additionally, including the use of a variety of bioluminescent zooplankton mimics, in addition to *Metridia*, could reveal species-specific responses and improve our understanding of interspecies interactions. Finally, ensuring accurate species identification is essential, as precise research outcomes depend heavily on clear taxonomic distinctions. Together, these improvements in the research will provide deeper insights into the ecological dynamics shaped by artificial and natural lighting in marine environments.

5 Conclusion

This study investigated the behavioral responses of zooplankton, particularly *Calanus* and *Metridia*, to artificial light under controlled experimental conditions. The results revealed that *Metridia* showed a higher baseline activity compared to *Calanus*, yet neither species showed significant reactions to either the 10 seconds or the *Metridia* mimic light stimuli. These findings suggest that the specific artificial light stimuli used in the experiments did not significantly influence the behaviors of these zooplankton species. And this lack of response implies the potential resilience of marine zooplankton to changes in light conditions or the potential limitations in the experimental design. However, the consistent activity difference between the two species highlights the importance of considering species-specific traits when assessing the ecological impacts of environmental changes.

Further research is needed to refine the experimental setups and extend the range of light conditions tested, in order to better understand the complex interactions between marine zooplankton and their changing light environments. Expanding these studies could provide deeper insights into how light pollution affects marine ecosystems and thereby enhance conservation and management strategies. This thesis contributes to the field of marine ecology by pointing out important areas for future investigation and showing that, under certain experimental conditions, zooplankton may not exhibit noticeable behavioral reactions to changes in light, in contrast with findings from other studies where distinct responses were observed.

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Appendix A

Table A1: Schedule, in minutes, for stimulus lights and changing of the filters. The filters were changed manually every 6th minute, whereas the stimulus lights were emitted automatically by the Arduino. Each stimulus light was repeated three times per intensity in increasing intensity series, every 2nd minute.

Time (minutes)	Filter
0	Camera on
9	Manually changing to F2
10	Arduino on
10	F2 1st stimulus
12	F2 2nd stimulus
14	F2 3rd stimulus
15	Manually changing to F3
16	F3 1st stimulus
18	F3 2nd stimulus
20	F3 3rd stimulus
21	Manually changing to F4
22	F4 1st stimulus
24	F4 2nd stimulus
26	F4 3rd stimulus
27	Manually changing to F5
28	F5 1st stimulus
30	F5 2nd stimulus
32	F5 3rd stimulus
33	Manually changing to F6
34	F6 1st stimulus
36	F6 2nd stimulus
38	F6 3rd stimulus
39	Manually changing to F7

40	F7 1st stimulus
42	F7 2nd stimulus
44	F7 3rd stimulus
45	Manually changing to F8
46	F8 1st stimulus
48	F8 2nd stimulus
50	F8 3rd stimulus
51	Manually changing to F9
52	F9 1st stimulus
54	F9 2nd stimulus
56	F9 3rd stimulus
57	Manually changing to F10
58	F10 1st stimulus
60	F10 2nd stimulus
62	F10 3rd stimulus
63	Manually changing to F6
64	F6 1st stimulus
66	F6 2nd stimulus
68	F6 3rd stimulus
69	Manually changing to F11
70	F11 1st stimulus
72	F11 2nd stimulus
74	F11 3rd stimulus
75	Arduino off
76	Camera off

Appendix B

Table B1: W and p-values for Shapiro-Wilks test for the baseline movement of *Calanus* and *Metridia*. The test shows that the W-values are close to 1 indicating normal distribution, but the p-values are below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution.

Taxa	Type	Filter	Stage	W-value	p-value
Calanus	10s	All	Pre	0.45	<0.01
Metridia	10s	All	Pre	0.92	<0.01
Calanus	Mimic & 10s	All	Pre	0.61	<0.01
Metridia	Mimic & 10s	All	Pre	0.96	<0.01
Calanus & *Metridia*	10s	All	Pre	0.84	<0.01

Table B2: W and p-values for Shapiro-Wilks test for the total movement of *Calanus* and *Metridia*. The test shows that the W-values are close to 1 indicating normal distribution, but the p-values are mostly below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution.

Taxa	Type	Filter	Stage	W-value	p-value
Calanus	10s	F6	Pre	0.57	<0.01
Calanus	10s	F7	Pre	0.76	<0.01
Calanus	10s	F8	Pre	0.42	<0.01
Calanus	10s	F9	Pre	0.62	<0.01
Calanus	10s	F10	Pre	0.39	<0.01
Calanus	10s	F6	Stimulus	0.64	<0.01
Calanus	10s	F7	Stimulus	0.81	<0.01
Calanus	10s	F8	Stimulus	0.53	<0.01
Calanus	10s	F9	Stimulus	0.64	<0.01
Calanus	10s	F10	Stimulus	0.60	<0.01
Calanus	10s	F6	Post	0.58	<0.01
Calanus	10s	F7	Post	0.73	<0.01
Calanus	10s	F8	Post	0.55	<0.01
Calanus	10s	F9	Post	0.64	<0.01
Calanus	10s	F10	Post	0.61	<0.01
Calanus	Mimic	F10	Pre	0.68	<0.01
Calanus	Mimic	F10	Post	0.73	<0.01

Metridia	10s	F6	Pre	0.95	0.072
Metridia	10s	F7	Pre	0.92	<0.05
Metridia	10s	F8	Pre	0.95	0.15
Metridia	10s	F9	Pre	0.92	<0.05
Metridia	10s	F10	Pre	0.75	<0.01
Metridia	10s	F6	Stimulus	0.95	0.069
Metridia	10s	F7	Stimulus	0.87	<0.01
Metridia	10s	F8	Stimulus	0.73	<0.01
Metridia	10s	F9	Stimulus	0.76	<0.01
Metridia	10s	F10	Stimulus	0.90	<0.01
Metridia	10s	F6	Post	0.98	0.58
Metridia	10s	F7	Post	0.97	0.60
Metridia	10s	F8	Post	0.75	<0.01
Metridia	10s	F9	Post	0.76	<0.01
Metridia	10s	F10	Post	0.94	0.065
Metridia	Mimic	F10	Pre	0.98	0.10
Metridia	Mimic	F10	Post	0.96	<0.01

Table B3: W and p-values for Shapiro-Wilks test for the x translation of *Calanus* and *Metridia*. The test shows that the W-values are close to 1 indicating normal distribution, but the p-values are mostly below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution.

Taxa	Type	Filter	Stage	W-value	p-value
Calanus	10s	F6	Pre	0.65	<0.01
Calanus	10s	F7	Pre	0.72	<0.01
Calanus	10s	F8	Pre	0.44	<0.01
Calanus	10s	F9	Pre	0.52	<0.01
Calanus	10s	F10	Pre	0.44	<0.01
Calanus	10s	F6	Stimulus	0.48	<0.01
Calanus	10s	F7	Stimulus	0.92	<0.05
Calanus	10s	F8	Stimulus	0.55	<0.01

Calanus	10s	F9	Stimulus	0.61	<0.01
Calanus	10s	F10	Stimulus	0.70	<0.01
Calanus	10s	F6	Post	0.69	<0.01
Calanus	10s	F7	Post	0.61	<0.01
Calanus	10s	F8	Post	0.69	<0.01
Calanus	10s	F9	Post	0.61	<0.01
Calanus	10s	F10	Post	0.46	<0.01
Calanus	Mimic	F10	Pre	0.75	<0.01
Calanus	Mimic	F10	Post	0.57	<0.01
Metridia	10s	F6	Pre	0.87	<0.01
Metridia	10s	F7	Pre	0.86	<0.01
Metridia	10s	F8	Pre	0.96	0.36
Metridia	10s	F9	Pre	0.87	<0.01
Metridia	10s	F10	Pre	0.85	<0.01
Metridia	10s	F6	Stimulus	0.87	<0.01
Metridia	10s	F7	Stimulus	0.91	<0.05
Metridia	10s	F8	Stimulus	0.88	<0.01
Metridia	10s	F9	Stimulus	0.87	<0.01
Metridia	10s	F10	Stimulus	0.96	0.26
Metridia	10s	F6	Post	0.91	<0.01
Metridia	10s	F7	Post	0.96	0.35
Metridia	10s	F8	Post	0.69	<0.01
Metridia	10s	F9	Post	0.87	<0.01
Metridia	10s	F10	Post	0.94	0.068
Metridia	Mimic	F10	Pre	0.94	<0.01
Metridia	Mimic	F10	Post	0.92	<0.01

Table B4: W and p-values for Shapiro-Wilks test for the top speed of *Calanus* and *Metridia*. The test shows that the W-values are close to 1 indicating normal distribution, but the p-values are below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution.

Taxa	Type	Filter	Stage	W-value	p-value
Calanus	10s	F6	Pre	0.74	<0.01
Calanus	10s	F7	Pre	0.78	<0.01
Calanus	10s	F8	Pre	0.72	<0.01
Calanus	10s	F9	Pre	0.72	<0.01
Calanus	10s	F10	Pre	0.70	<0.01
Calanus	10s	F6	Stimulus	0.63	<0.01
Calanus	10s	F7	Stimulus	0.76	<0.01
Calanus	10s	F8	Stimulus	0.61	<0.01
Calanus	10s	F9	Stimulus	0.75	<0.01
Calanus	10s	F10	Stimulus	0.83	<0.01
Calanus	10s	F6	Post	0.61	<0.01
Calanus	10s	F7	Post	0.63	<0.01
Calanus	10s	F8	Post	0.64	<0.01
Calanus	10s	F9	Post	0.75	<0.01
Calanus	10s	F10	Post	0.64	<0.01
Calanus	Mimic	F10	Pre	0.56	<0.01
Calanus	Mimic	F10	Post	0.56	<0.01
Metridia	10s	F6	Pre	0.79	<0.01
Metridia	10s	F7	Pre	0.81	<0.01
Metridia	10s	F8	Pre	0.67	<0.01
Metridia	10s	F9	Pre	0.86	<0.01
Metridia	10s	F10	Pre	0.80	<0.01
Metridia	10s	F6	Stimulus	0.45	<0.01
Metridia	10s	F7	Stimulus	0.67	<0.01
Metridia	10s	F8	Stimulus	0.53	<0.01
Metridia	10s	F9	Stimulus	0.78	<0.01

Metridia	10s	F10	Stimulus	0.91	<0.05
Metridia	10s	F6	Post	0.86	<0.01
Metridia	10s	F7	Post	0.82	<0.01
Metridia	10s	F8	Post	0.76	<0.01
Metridia	10s	F9	Post	0.78	<0.01
Metridia	10s	F10	Post	0.86	<0.01
Metridia	Mimic	F10	Pre	0.91	<0.01
Metridia	Mimic	F10	Post	0.79	<0.01

Table B5: W and p-values for Shapiro-Wilks test for the total movement after thresholding of *Calanus* and *Metridia*. The test shows that the W-values are close to 1 indicating normal distribution, but the p-values are mostly below the threshold of 0.05 and therefore rejects the hypothesis of normal distribution.

Taxa	Type	Filter	Stage	W-value	p-value
Calanus	10s	F6	Pre	0.76	<0.01
Calanus	10s	F7	Pre	0.85	<0.05
Calanus	10s	F8	Pre	0.55	<0.01
Calanus	10s	F9	Pre	0.72	<0.01
Calanus	10s	F10	Pre	0.50	<0.01
Calanus	10s	F6	Stimulus	0.68	<0.01
Calanus	10s	F7	Stimulus	0.84	<0.01
Calanus	10s	F8	Stimulus	0.76	<0.01
Calanus	10s	F9	Stimulus	0.79	<0.01
Calanus	10s	F10	Stimulus	0.67	<0.01
Calanus	10s	F6	Post	0.63	<0.01
Calanus	10s	F7	Post	0.81	<0.01
Calanus	10s	F8	Post	0.59	<0.01
Calanus	10s	F9	Post	0.79	<0.01
Calanus	10s	F10	Post	0.72	<0.01
Calanus	Mimic	F10	Pre	0.67	<0.01
Calanus	Mimic	F10	Post	0.72	<0.01

Metridia	10s	F6	Pre	0.90	<0.05
Metridia	10s	F7	Pre	0.91	0.23
Metridia	10s	F8	Pre	0.90	0.12
Metridia	10s	F9	Pre	0.91	0.19
Metridia	10s	F10	Pre	0.62	<0.01
Metridia	10s	F6	Stimulus	0.91	0.078
Metridia	10s	F7	Stimulus	0.81	<0.01
Metridia	10s	F8	Stimulus	0.68	<0.01
Metridia	10s	F9	Stimulus	0.74	<0.01
Metridia	10s	F10	Stimulus	0.75	<0.01
Metridia	10s	F6	Post	0.93	0.12
Metridia	10s	F7	Post	0.91	0.12
Metridia	10s	F8	Post	0.83	<0.05
Metridia	10s	F9	Post	0.74	<0.01
Metridia	10s	F10	Post	0.89	<0.05
Metridia	Mimic	F10	Pre	0.91	<0.01
Metridia	Mimic	F10	Post	0.83	<0.01

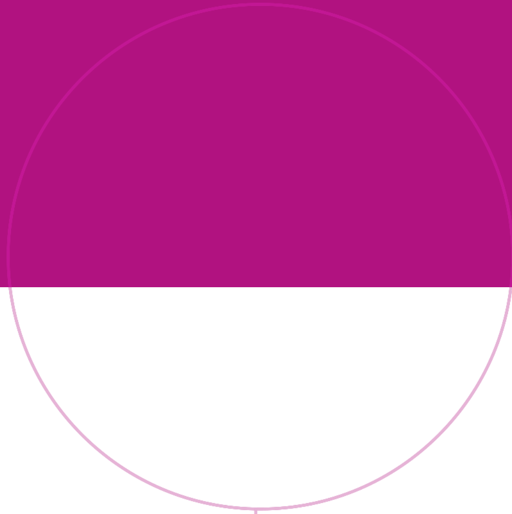
Appendix C

Table C1: Results from morphological and molecular species identification. Some individuals had contradicting results between the morphological and molecular identification. But it is important to note that some individuals had one of the two identification methods conducted. The 1st and 2nd PCR was run using Chelex with universal mtCOI primers targeting the common barcoding region. Whereas the 3rd PCR was run with Qiagen extraction using the same primers as the 1st and 2nd PCR. And the 4th PCR was run with Chelex using *C. finmarchicus* specific primers targeting the 16S region. Other individuals used in the experiments were not attempted to be identified.

Experiment	Taxa	ID	Month	Species (microscopy)	Species (1st PCR, Chelex)	Species (2nd PCR, Chelex)	Species (3rd PCR, Qiagen)	Species (4th PCR, Chelex)
13	*Calanus*	1	June	*C. glacialis*	-	-	-	*C. finmarchicus*
13	*Calanus*	2	June	*C. glacialis*	N/D	N/D	-	*C. finmarchicus*
13	*Calanus*	3	June	*C. glacialis*	N/D	N/D	-	*C. finmarchicus*
13	*Calanus*	4	June	*C. glacialis*	N/D	N/D	-	*C. finmarchicus*
13	*Calanus*	5	June	*C. glacialis*	N/D	N/D	-	-
13	*Calanus*	6	June	*C. glacialis*	N/D	N/D	N/D	N/D
13	*Calanus*	7	June	*C. glacialis*	N/D	N/D	N/D	N/D
13	*Calanus*	8	June	*C. glacialis*	N/D	N/D	N/D	N/D
3	*Calanus*	1	March	N/D	*C. finmarchicus*	N/D	-	-
3	*Calanus*	2	March	N/D	N/D	N/D	-	-
3	*Calanus*	3	March	N/D	N/D	N/D	-	-
3	*Calanus*	4	March	N/D	N/D	N/D	-	-
5	*Calanus*	1	March	N/D	-	N/D	-	-

5	*Calanus*	2	March	N/D	-	*C. finmarchicus*	-	-
5	*Calanus*	3	March	N/D	N/D	N/D	-	-
5	*Calanus*	4	March	N/D	N/D	N/D	-	-
7	*Calanus*	1	March	N/D	-	N/D	-	-
7	*Calanus*	2	March	N/D	-	-	-	-
7	*Calanus*	3	March	N/D	N/D	N/D	-	-
7	*Calanus*	4	March	N/D	N/D	N/D	-	-
7	*Calanus*	5	March	N/D	N/D	N/D	-	-
7	*Calanus*	6	March	N/D	N/D	N/D	-	-
7	*Calanus*	7	March	N/D	N/D	N/D	-	-
14	*Calanus*	1	June	N/D	N/D	N/D	-	-
14	*Calanus*	2	June	N/D	N/D	N/D	-	-
14	*Calanus*	3	June	N/D	N/D	N/D	-	-
14	*Calanus*	4	June	N/D	N/D	N/D	-	-
14	*Calanus*	5	June	N/D	N/D	N/D	-	*C. finmarchicus*
15	*Calanus*	1	June	N/D	N/D	N/D	-	*C. finmarchicus*
15	*Calanus*	2	June	N/D	N/D	N/D	-	-
15	*Calanus*	3	June	N/D	N/D	N/D	-	*C. finmarchicus*
15	*Calanus*	4	June	N/D	N/D	N/D	-	*C. finmarchicus*
15	*Calanus*	5	June	N/D	N/D	N/D	-	*C. finmarchicus*
2	*Metridia*	1	June	N/D	-	N/D	N/D	N/D

6	*Metridia*	1	June	N/D	-	N/D	N/D	N/D
7	*Metridia*	1	June	N/D	-	-	N/D	N/D
12	*Metridia*	1	June	N/D	-	-	N/D	N/D



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