Master's thesis

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

Gitte Krohn-Pettersen

Blinks in the dark - Detecting and characterizing flash kinetics of bioluminesscence for *in situ* taxa recognition of zooplankton

Master's thesis in Ocean Resources
Supervisor: Sanna Majaneva
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Abstract

With the biodiversity crisis the Earth is facing, where the pressure on biodiversity increases and the impact of human actions on natural ecosystem are growing, the need for holistic biodiversity assessments and monitoring programs are urgently needed. Zooplankton is a highly diverse group with many taxa that can be considered as indicators of the anthropogenic changes. However, today, there is lack of knowledge on certain zooplankton groups, such as the gelatinous zooplankton. These organisms still stay misunderstood as the sampling, preservation and identification methods commonly used for marine zooplankton have not been suitable for these fragile species. A great portion of these gelatinous zooplankton are bioluminescent, which is a ubiquitous phenomenon in the world's oceans. The emission of bioluminescence differs among organisms, where some emit single flashes, continuous glow or repetitive pulse pattern and is often dependent on the function the species utilize the light for. For example, some organisms use it as 1) defense; to evade or deter predators, 2) offense; to obtain prey and/or 3) intraspecific communication. Earlier studies have suggested that the light emitted from bioluminescent have a species-specific bioluminescent fingerprint. However, there is a lack of comprehensive research on species-specific bioluminescence flash kinetics (change of bioluminescence intensity over time during a flash) today. This study aims to contribute to filling this knowledge gap by identifying the species-specific bioluminescent fingerprint and by evaluating the possibility for using bioluminescence flash kinetics as an identification method for bioluminescent gelatinous zooplankton and other marine taxa.

Samples used in the study were collected in Hopavågen, a landlocked bay on the coast of Trøndelag in Mid-Norway and from different fjords in the Svalbard region in autumn 2022. The areas were chosen to retrieve samples from different latitudinal gradients representing different light climates to see if the bioluminescent fingerprint is constant. In total 382 individuals from taxa across five phyla were collected and identified to the lowest taxonomic level as possible. Laboratory experiments were conducted with an Underwater Bioluminescence Assessment Tool (UBAT) to create a taxa-specific library of bioluminescent taxa by analysing the shape, length and brightness of the bioluminescence flash. In addition, *in situ* measurements with UBAT were done in Hopavågen following a full tidal cycle and the results were analysed to see if it would be possible to identify the species present based on the information from the taxa-specific library. The results of the study showed both intraspecific and interspecific variation of the flash kinetics extracted from laboratory experiments. There is an indication that the intraspecific variation is likely due to background signals, damage on individuals or other reasons that were not found in the study. However, the interspecific variation might be because of taxa emitting light based on their functions, which should make it possible to distinguish between taxa. The results from the *in situ* measurements were not as expected, with high activity of bioluminescence of dinoflagellates, making it hard to isolate flashes from zooplankton. From this study it is clear that this is an identification method that needs more development, and it would be ideal to combine flash kinetics with other techniques, such as genetic tools and imaging, for correct species identification.

Sammendrag

Med biodiversitetkrisen jorden står overfor, hvor presset på biologisk mangfold øker og effekter av menneskelige handlinger på de naturlige økosystemene vokser, er det et presserende behov for overvåkningsprogrammer og helhetlige vurderinger av biologisk mangfold. Dyreplankton er en svært mangfoldig gruppe med mange taksoner som kan betraktes som indikatorer på de menneskeskapte endringene, hvor det i dag er mangel på kunnskap om visse dyreplanktongrupper, som for eksempel gelatinøse dyreplankton. Dette er skjøre arter som ikke har egnet seg til prøvetakings-, konserverings- og identifiseringsmetodene som vanligvis brukes for marint dyreplankton. En stor andel av taksoner under gelatinøse dyreplankton er bioluminescerende, som er et allestedsnærværende fenomen i verdenshavene. Emisjonen av bioluminescens er forskjellig mellom organismer, hvor noen avgir enkelte lysglimt, mens andre har kontinuerlig glød eller repeterende pulsmønster. Bioluminescens er ofte avhengig av hvilken funksjon arten bruker det til, som for eksempel 1) forsvar; for å unngå eller avskrekke predatorer, 2) predasjon; for å lokke til seg byttedyr og/eller 3) intraspesifikk kommunikasjon. Tidligere studier har antydet at lyset som avgis fra bioluminescerende organismer er et artsspesifikt bioluminescerende fingeravtrykk, men det er i dag mangel på detaljert forskning på området. Denne studien tar sikte på å bidra til å fylle dette kunnskapsgapet ved å identifisere det artsspesifikke bioluminescens-fingeravtrykket, samt å evaluere muligheten for å bruke bioluminescens-lyskinetikk (endringen av bioluminescens intensitet over tid i et lys) som identifikasjonsmetode for gelatinøse dyreplankton og andre marine taksoner.

Organismer ble samlet inn fra Hopavågen, en bukt på kysten av Trøndelag i Midt-Norge, og fra ulike fjorder i Svalbard-regionen høsten 2022. Områdene ble valgt for å bruke organismer fra ulike breddegradienter som representerer ulike lysklima for å se om det bioluminescerende fingeravtrykket er konstant. Totalt ble 382 individer fra taksoner på tvers av fem rekker samlet og identifisert til lavest mulig taksonomiske nivå. Laboratorieeksperiment ble utført med en sensor kalt Underwater Bioluminescence Assessment Tool (UBAT) for å lage et takson-spesifikt bibliotek av bioluminescerende organismer, samt analysere formen, lengden og lysstyrken til den avgitte bioluminescens-lyskinetikk. I tillegg ble det gjort *in situ* målinger med UBAT i Hopavågen gjennom en runde med flo og fjære og resultatene ble analysert for å se om det ville være mulig å identifisere artene som var til stede basert på informasjonen fra det takson-spesifikke biblioteket. Resultatene av studien viste både intraspesifikk og interspesifikk variasjon av bioluminescens-lyskinetikken fra laboratorieeksperimentene. Det er en indikasjon på at den intraspesifikke variasjonen sannsynligvis skyldes bakgrunnssignaler, skade på individer eller andre årsaker som ikke ble funnet i studien. Den interspesifikke variasjonen kan komme av at takson sender ut lys basert på deres funksjoner, noe som skal gjør det mulig å skille mellom taksoner. Resultatene fra *in situ*-målingene viste at det var høy aktivitet av bioluminescens, noe som gjorde det vanskelig å isolere lys fra ulike dyreplankton-taksoner. Fra denne studien er det klart at dette er en identifiseringmetode som trenger mer utvikling, og det vil være ideelt å kombinere lyskinetikk med andre teknikker, som molekylære metoder og avbildning, for korrekt artsidentifikasjon.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

AUV	Autonomous Underwater Vehicle
BL	Bioluminescence
BL_20%	20% of the maximum intensity of measured BL
BL_max	Maximum intensity of measured BL
BL_mean	Average of measured BL
CBD	Convention on Biological Diversity
CTD	Conductivity, Temperature and Depth
DNA	Deoxyribonucleic acid
DT	Decay Time
GFP	Green Fluorescent Protein
IPBES	Intergovernmental Platform on Biodiversity and Ecosystem Services
NAC	North Atlantic Current
NAO	North Atlantic Oscillation
NCC	Norwegian Coastal Current
NTNU	Norwegian University of Science and Technology
PMT	Photo Multiplier Tube
RNA	Ribonucleic acid
ROV	Remotely Operated Vehicle
RT	Rise Time
Sigma_max	Cumulative sum from first value until maximum BL
T_decay	Time between last point over BL_20% to last point of threshold a.
T_high	Time between first and last value over BL_20%
T_max	Time from the first value until maximum BL
TT	Total flash Time
UBAT	Underwater Bioluminescence Assessment Tool
WP	Work Package

1 Introduction

1.1 Biodiversity

Human population has since 1998 increased with two billion (United Nations, no date a) and simultaneously to the growth, populations of wildlife have rapidly declined (IPBES, 2019). Today, we are facing a crisis of biodiversity loss that has had worldwide attention for decades but still been put under a lot of pressure (Singh, 2002, IPBES, 2019). Biodiversity, also known as biological diversity, is the variety of life on Earth (Chivian & Bernstein, 2010). The term biodiversity often refers to the number of species, but it can also be referred to as genetic diversity or diversity in habitats and niches in an area (Ratikainen, 2021). It can be said that biodiversity represents the very foundation of human existence (Heywood, 1995). Not obtaining biodiversity can create damage on a healthy ecosystem, and in worst case scenario push it into destruction (Isbell et al., 2014). Ecosystems are these complex networks of interdependent relationships within every living organism (Ratikainen & Semb-Johansson, 2020), and is often referred to as natural capital since it provides the Earth with these "ecosystem services" that humans and other living species can't live without. Therefore, an ecosystem needs to be kept healthy for the Earth to obtain a web of life which cleans our waters, purifies our air, maintains our soil and regulates the climate (Chivian & Bernstein, 2010).

Setting a number on biodiversity and how many species there are on earth have showed itself to be difficult. Scientists developing formulas to estimate biodiversity where numbers range from 3 million to a 100 million species in total (Erwin, 1982; United Nations Environment Programme, 1995; May, 2010;), is a sign showing how hard it is. One of the most widely cited figures of an estimate of biodiversity is set by Mora et al. (2011), where they estimated eukaryotic species alone at around 8.7 million species. As of 2022, there were 2.16 million identified species (IUCN Red List, 2020). With these numbers, it indicates that there are still more than 75% unidentified species on Earth and with the incomplete taxonomic knowledge, attempts to protect biodiversity impedes (Wilson, 2017). Modern extinction rates appear to be exceptionally high and still increasing (Ceballos, 2015), with the state of biodiversity showing negative trends and pressure on biodiversity showing increasing trends (Butchart et al., 2010).

Today, it has been identified five main direct drivers for biodiversity loss that make our nature disappear rapidly; changes in land and the use of sea, climate change, overexploitation, pollution and invasive alien species (IPBES, 2019). These are drivers that are visible in humans' everyday lives. Cities have more concrete blocks than green space, species are being put at risk of extinction right in front of our eyes and wilderness is disappearing. Global wildlife population fell by 60% only in the last four decades as a result of human activity (World Wildlife Fund, 2018) as well as large parts of the Earth's surface has been altered (IPBES, 2019), our nature has been squeezed into a small corner. As stated by the European Commission (2020) for the "EU Biodiversity Strategy for 2030": **"we need nature in our lives".**

1.2 Monitoring programs

With the growing impact on natural ecosystems from human activities, it is required development of programs for biodiversity assessment and monitoring (Pereira & Cooper, 2006; Pereira et al., 2010). Earlier, it has been said that monitoring programs have suffered from three main constraints, with incomplete taxonomic and spatial coverage, lack of compatibility between data because of differences in collection methods and insufficient integration at different scales (Pereira & Cooper, 2006, references within). The development of a monitoring program today would ideally need collecting data over large spatial extents within a short time and with the highest possible quality. However, monitoring over such an extent is limited for accurately measure diversity (Chiarucci et al., 2011). The ocean needs to be protected, to obtain the relationship with the sea and all its resources it gives the human kind, with Marine Protected Areas (MPAs), which are areas governments has placed limits on human activity (Laffoley et al., 2019). Large-scale biodiversity monitoring efforts have been set (Navarro et al., 2017), such as the agreement of the Aichi Biodiversity Targets by the Convention on Biological Diversity (CBD)(Conventional Biological Diversity, 2020), the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES)(Larigauderie & Mooney, 2010) and the sustainable Development Goals of the UN Agenda 2030 (Resolution 70/1)(United Nations, no date b). At the same time, the ability to assess biodiversity change needs to be improved drastically. For the goals to be efficient in these international efforts, which are often approached from a data-centric perspective (Kühl et al., 2020), comprehensive monitoring programs that are robust need to be in place (Tittensor et al., 2014). In short terms, a more effective approach for such a large-scale monitoring of biodiversity is urgently needed (Kühl et al., 2020). Then the real question is, how can one start monitoring biodiversity change when there is not enough knowledge of species diversity?

1.3 Lack of taxonomic knowledge at species level

1.3.1 Species identification

There are still species on Earth that are yet to be identified and described, and misidentifications still occurring (Costa et al., 2015). Scientists have been asking "how many?" and "how much?" before even knowing "who?" and "how?" (Haddock, 2004). Ideally, a universal identification method should be put in place. This way, data can be collected and shared across country boarders (Hillis, 1987, Friedheim, 2016). For a universal method like this to be accepted, there has to be thought about multiple components as 1) available information, 2) feasibility, 3) costs and 4) practicability (Johansen, 2019).

Morphological recognition has been the traditionally most common method for taxonomic identification, distinguishing between phenotypic characteristics of individual organisms (Hillis, 1987, Friedheim, 2016, Herbert & Gregory, 2005). Even though the method is old (Herbert & Gregory, 2005), it has some downfalls to it. Most of the species described are nearly impossible to see with the naked eye (Savolainen et al., 2005), making one dependent on for example a microscope. It is a time-consuming process that often requires trained personnel (Friedheim, 2016) and lacks data for quantifying rare species. As well as it is a subject for misidentification, because of the difficulty to distinguish between close or

identical species (Danovaro et al., 2016), where taxonomists redefine species and rename characteristics (Hillis, 1987, Condon et al., 2012).

1.3.2 Zooplankton

Zooplankton are all forms of aquatic animals that move freely in the water masses. They are not able to carry out horizontal movements against flow of waters, but move vertically, significantly stimulated by changes in the light conditions (Jonsgård & Sømme, 2022). There are two main groups of zooplankton, holoplankton and meroplankton. Holoplankton (permanent plankton) are organisms that are pelagic throughout their whole life cycle, while meroplankton are both benthic and pelagic in their life cycles (Jonsgård & Sømme, 2022). Zooplankton in the size range of 0.2-20 mm are called mesozooplankton and microzooplankton that range from 20-200 μm. These animals are the link between phytoplankton and fish in the food chain (Hays et al., 2005). Among zooplankton, there are species that are morphologically indistinguishable, these are called cryptic species (Sáez & Lozano, 2005, Bucklin et al., 2021). Cryptic species are so similar to each other that it can be impossible to distinguish them from each other without the help of molecular data (Korshunova et al., 2019). Additionally, rare species that infrequently are identified, may have characteristics that are less known to the normal taxonomist and some species end up as unidentified. Gelatinous zooplankton are groups of taxa that are often simplified and therefore lack proper identification (Condon et al., 2012).

1.3.3 Gelatinous Zooplankton

Gelatinous zooplankton are transparent and delicate planktonic species which regulates their buoyancy with mesoglea-like internal tissues (Raskoff et al., 2003). The taxonomic groups that are included in this generic term are Medusae, Siphonophorae, Chaetognatha, Ctenophora, Pteropoda, Hydrozoa, Salpida, Appendicularia and others (Hamner et al., 1975; Haddock, 2004). The gelatinous zooplankton term is used to avoid the taxonomic baggage that can come with it, compared to other names such as 'coelenterata', which has been considered inaccurate in the realness of taxonomic context (Haddock, 2004). The species within this term are often simplified and misunderstood, since their body designs and the planktonic lifestyle have such a basic similarity, which puts them all into a single catch-all category (Condon et al., 2012). Gelatinous zooplankton are widely distributed in all oceans, throughout water columns in large numbers (Madin & Harbison, 2001). However, the gelatinous zooplankton are the least understood planktonic group (Raskoff et al., 2003) partly because of their fragility and the difficult species to sample, affecting possible collection of time series of growth within the groups (Hay, 2006). The reported blooming of jellyfish can have deleterious consequences (Richardson et al., 2009, references within), and not monitoring gelatinous zooplankton will keep us ignorant to if their populations will increase, decrease or stay the same in the future, which will lead to lack of knowledge on how gelatinous species will influence ecosystem processes, human activities and economies (Condon et al., 2012).

1.4 How to monitor gelatinous zooplankton species

The taxonomic groups within gelatinous zooplankton are as mentioned, often very fragile. Collection of these species needs to have minimized handling to prevent damage, which often precludes collection with trawls and nets (Raskoff et al., 2003). Many of these species are accessible by surface collection, from a boat and by snorkeling. Specimens can be collected with the use of containers, glass or plastic, or buckets in bigger cases. Some specimens are more robust than others and can handle small plankton nets with fine mesh without too much damage on their bodies. For collection of specimens deeper than the surface, where snorkeling and diving might not be the option, plankton nets can be effective if they are pulled up gently (seen by eye). In situations where neither nets nor trawls would be optimal, remotely operated vehicles (ROVs) can be used to collect from meso- and bathypelagic depths (Robison et al., 2017). For identification of species that are morphologically indistinguishable, molecular identification methods can be possible (Haddock, 2004; Friedheim, 2016). This includes the examinations of DNA, RNA and proteins, which provides information about the present and past of organisms (Haddock, 2004). To identify at species level, DNA barcoding is often used, where a short genetic marker from an organism's DNA is extracted (Munaut et al., 2011). DNA metabarcoding, which is the method of many taxa simultaneously being identified within the same sample (Aylagas et al., 2018), is now also being more and more used in the assessments of ecosystems, because of its cost-efficient and less time-consuming method of identification compared to morphology (Van der Loos & Nijland, 2020). However, the DNA barcoding is limited in the sense that it relies on barcode reference libraries for the identification of the sequences, and it cannot always distinguish between closely related species (cryptic species) (Parmentier et al., 2013). Given the restrictions of existing methodology, new approaches are needed. It has been suggested that bioluminescence can be used to identify species, because of their possibly species-specific characteristics of flash kinetics (Johnsen et al., 2014) as well as characterizing different bioluminescent communities (Moline et al., 2013).

1.5 Bioluminescence

1.5.1 What is bioluminescence?

Bioluminescence is a ubiquitous phenomenon in the world's ocean, from the deepest point where no sunlight penetrates to the top layer of the surface (Haddock et al., 2010; Wilson & Hastings, 2013). It is the emission of visible light from living organisms and is the result of a natural chemical reaction. For many marine animals, biologically generated light is their primary visual stimulus rather than atmospheric light (Haddock et al., 2010).

Bioluminescence does not require absorption of sunlight or other electromagnetic radiation to emit light compared to fluorescence and phosphorescence (Kahlke & Umbers, 2016). Production of bioluminescence happens through the oxidation of the molecule luciferin and an enzyme, either luciferase or a photoprotein. The chemical reaction between these two creates light, generated as a result of energy released (Haddock et al., 2010; Wilson & Hastings, 2013; Kahlke & Umbers, 2016). Luciferin is the light-emitting compound, luciferase is the enzyme that triggers oxidation of luciferin and photoprotein is a catalyzing enzyme that emit light with itself, luciferin and oxygen bound together (Haddock, 2010).

There are four luciferins that are responsible for most light production in the ocean, bacterial, dinoflagellate, cypridina and coelenterazine, however, there are most likely more light-emitting reactions undiscovered (Haddock, 2010, see Fig. 3). Luciferases and photoprotein are on the other hand unique. Even though one is familiar with their chemical reaction, their evolutionary origins still remain mysterious. Haddock et al. (2010) estimated that bioluminescence has evolved a minimum of 40 times, and most likely more than 50 times among extant organisms (See fig. 1 in Haddock et al. 2010). With their evolution, it seems that it is important for the organisms to have the ability to produce light, and that its evolution must be relatively easy.

Bioluminescence observed by humans, have been in most cases induced to emit light by a physical disturbance. However, bioluminescence in natural context is controlled by chemical and neurological mechanisms, where species can turn their photophores on and off, regulate the intensity, color and control their angular distribution of light (Haddock et al., 2010).

1.5.2 Functions of Bioluminescence

Bioluminescence serves several functions, and some serve multiple functions for a single organism. Marine bioluminescence is greatly used for defense, offense, and intraspecific communication. Defense is often used to startle predators with a bright flash evoked at close range (Haddock et al., 2010). Species like Copepoda, Siphonophorae and Ctenophora creates a smoke screen, a cloud of flash that can make it difficult for the predator to locate their prey (Haddock & Case, 1999). Others apply a sacrificial tag from their body to a predator, where lost tissue can light for some time after (Herring & Widder, 2004) on their surface and even within a predator. This way, the first predator will then again attract secondary predators and become a target themselves (Haddock et al., 2010). Therefore, consuming bioluminescent prey can be risky.

Offense is often used as a way to lure prey using glowing light (Haddock et al., 2010). A great example on this is the anglerfish (Lophiiformes), which uses bacteria to produce a long glow to attract prey, controlling it by altering the conditions in the light organ where bacteria are cultured (Freed et al., 2019). Species that are nonluminous sometimes actively use bioluminescence in their surroundings to attract their prey (Haddock et al., 2010). They trigger light production in other luminescent species around them, attracting prey that would be interested in the luminescent species themselves, making them vulnerable to the predator.

As well as defense and offense, bioluminescence is used by species for intraspecific communication. This is a type of communication that is less known in the sea compared to bioluminescence in terrestrial. To attract a mate, species-specific spatial or temporal pattern of light can be emitted (Widder, 2010), where at least one individual emits the light, and the one other individual detects it. In most cases where organisms can emit light, they can also perceive it (Morin, 1983). The energetics of bioluminescence makes it possible to communicate over large distances and with great conditions, a flash can be seen from hundreds of meters away (Warrant & Locket, 2004). During dark hours, bioluminescence is often the only light source available for vision for the mesopelagic environment (Turner et al., 2009)

1.5.3 Taxonomic distribution of bioluminescence

Marine bioluminescence is found in most major marine phyla, ranging from bacteria to fish (Widder, 2010). The phyla Ctenophora have the highest proportion of bioluminescent species within, where the planktonic genera have over 90% species that are known to produce light (Haddock et al., 2010). Bacteria are also known to be bioluminescent and are quite common in the ocean. They are easily cultured and often found in marine fish and squid species (Haddock et al., 2010). To be luminous, the bacteria have to grow to high cell density (Waters & Bassler, 2005) before it glows continuously in the presence of oxygen. These properties are specific for bacteria (Haddock et al., 2010). Dinoflagellates are the most common bioluminescent organism, next to fireflies. The famous "bioluminescent bays" found in Puerto Rico and Jamacia are produced by dinoflagellates. Other phyla where bioluminescence is found are Cnidarians, where both benthic and planktonic species emit light (Haddock et al., 2010), Crustaceans, where Copepoda are one of the most abundant bioluminescent groups (Widder et al., 1999) and fish, where most of the groups use bacterial symbionts for light production (Haddock et al., 2010). There are also bioluminescent Annelids, Molluscs, Echinoderms and Tunicates.

1.5.4 Variable properties of bioluminescence

The emission of light differs among bioluminescent organisms. Some emit continuous glow while others emit single flashes of light or repetitive pulse patterns, which are often species-specific. The light emission is often dependent on what kind of function each species utilize (Morin, 1983). Some species startle their predator with a bright short flash, other create smoke screen with a longer glow (see section 1.5.2).

Bioluminescent light is emitted in wavelength from 400 to 720 nm, from violet into near infrared (Kahlke & Umbers, 2016). Most of the spectral properties is constrained to blue-green wavelengths (Turner et al., 2005; Haddock et al., 2010). The wavelengths of the light seem to shift based on habitat, where violet and blue (420-500 nm) dominates in the deep sea, blue-green (460-520 nm) in more shallow waters and green-yellow (520-580 nm) on land (Kahlke & Umbers, 2016). There are many factors that play a role in the color of the bioluminescent light, such as the green fluorescent protein (GFP) act as a secondary emitter and influence the emitted color in some bioluminescent systems to produce green light (Chalfie, 1995).

1.5.5 How is bioluminescence measured?

Bioluminescence can be measured differently, but often it involves mechanical stimulation that leads to the emission of light. Mechanical stimulation does not need to be complicated; one can simply stir around a volume of water with bioluminescent organisms within and measure the emission of light with a photo multiplier tube (PMT). Another approach is to use a low-light camera to image/record the bioluminescence while something is stimulating the organisms (Watson & Zielinski, 2013). The spectrum of the bioluminescence can be measured by a spectrometer, measuring the lights wavelength (Latz et al., 1988).

A common measuring tool for bioluminescence potential and flash kinetics is a bathyphotometer that mechanically stimulates the bioluminescent organisms before it enters a detection chamber with a PMT that detects the light. Thus, a bathyphotometer

does not measure the actual bioluminescence in the environment, but the total light output that would be produced by mechanically stimulating the luminescent organisms in a prescribed volume of seawater. Bathyphotometers come in different forms and goes back in time (Seliger et al., 1969, Lapota & Loose, 1984, Davis et al., 2005). Flash kinetics are the measured change of bioluminescence intensity over time during a flash, which describes bioluminescence capacity and characteristics of a bioluminescent organism (Chen et al., 2023). There have been conducted research where a bathyphotometer have been integrated onto an autonomous underwater vehicle (AUV), to determine the concentrations and community of bioluminescence organisms in the Arctic (Berge et al., 2012; Moline et al., 2013). Newer studies on measured bioluminescence have conducted the research with an Underwater Bioluminescence Assessment Tool (UBAT), which goes under the category bathyphotometer (Johnsen et al., 2014; Cronin et al., 2016).

1.5.6 Bioluminescence as a potential tool for species identification

Since bioluminescent emission differ between taxa, studies have tried to distinguish between communities of zooplankton and phytoplankton as well as distinguish taxa from each other. Earlier research on the topic, concludes that flash kinetics from bioluminescence have a strong potential for *in situ* taxa recognition of zooplankton (Johnsen et al., 2014) and other bioluminescent species (Nealson et al., 1986; Xue et al., 2020). The idea of a possible bioluminescent fingerprint has triggered the interest to see if there are possibilities to identify species from their emitted light. The study by Johnsen et al. (2014) is the pilot to include also gelatinous zooplankton to bioluminescence flash kinetics approach, but the study fails to fulfill some central knowledge gaps. There are few individuals used within each species without information about how many collected and individuals emitting light. The species used in the study are not closely related, and therefore hard to compare up towards each other. It does not take into consideration that there might be differences between locations among species and seasons, does not address intraspecific variation and does not mention the effect of the mechanical stimulation of the species. These are gaps that need to be studied more carefully in order to create a functional tool since light emission as seen in nature may not be shown the same way when conducting laboratory stimulation (Haddock et al., 2010).

1.6 Study aim

The thesis is a part of a bigger research project that looks into how changes in the light climate affects the marine zooplankton. The project is called Light as a Cue for Life in Arctic and Northern Seas, known as LightLife. It is funded by the Research Council of Norway and expands from 2021 till 2024. LightLife is divided into three working packages; WP 1: "Species dynamic and role of light climate on photo-biological response in the Arctic", WP 2: "The functional light regime and acclimation capacities of visual systems in key species with a latitudinal perspective" and WP 3: "Bioluminescence (BL) as contributing factor to underwater light environment in the Arctic", which are all supporting each other both theoretically and methodically. This thesis is a part of work package 3 (WP 3). It concentrates on bioluminescence as a contributor of biological produced light and the role of bioluminescent species in ecological interactions in Arctic and Northern waters. Lastly, WP3 looks into developing a remote tool for species recognition within bioluminescent zooplankton, using their bioluminescent fingerprint.

The aim of the thesis is to conduct taxa-specific laboratory experiments as well as *in situ* measurements of bioluminescent zooplankton using a bathyphotometer. The goal is to create a library of bioluminescent fingerprint based on the flash kinetics and to evaluate the potential of these fingerprints for *in situ* zooplankton recognition.

2 Materials and methods

2.1 Study sites

For this thesis, different sampling sites in the Norwegian Sea and the Barents Sea, were studied. The physical conditions of both seas are governed by local conditions and inflow of the Atlantic water flowing through the Faroe-Shetland Channel. The North Atlantic Current (NAC) splits up into two main branches, creating two separate ecosystems. One branch breaks south-eastward in the North Sea and the other one in the Norwegian Sea (Hamre, 1994) (Fig. 2.1). The flow of the NAC is determined by local atmospheric conditions, linked to a larger atmospheric feature as the North Atlantic Oscillation (NAO) (Ingvaldsen & Loeng, 2009). The NAC is characterized by high temperature and high salinity and often mixed with northern cold polar water along coastal areas. The combination of the current and winds create a milder climate in Northern Europe as it transports more tropical water to northern latitudes than any other currents (Knudsen, 2023). Along the Norwegian coast, the Norwegian Coastal Current (NCC) is the most important transport artery (Institute of Marine Research, 2021). The current originates from the Baltic Sea, bringing relatively fresh water northwards because of low-salinity water from the North Sea and fresh water from Norwegian rivers (Sætre, 2007). On its way north, it gradually increases in salinity as more Atlantic water is mixed in (Institute of Marine Research, 2021). The NCC is deep and narrow during winter and wide and shallow during summer (Wassmann et al., 2000). On the outside of the Norwegian Coastal Current, the Norwegian Atlantic Current carries Atlantic water northwards to the Barents Sea (Ingvaldsen & Loeng, 2009). The current splits in Northern Norway, creating a northward and eastward branch. The northward branch dispatches a branch of Atlantic Water in south-west of Spitsbergen, before continuing along the coast of West Spitsbergen while the eastward branch enters the Barents Sea (Ingvaldsen & Loeng, 2009).

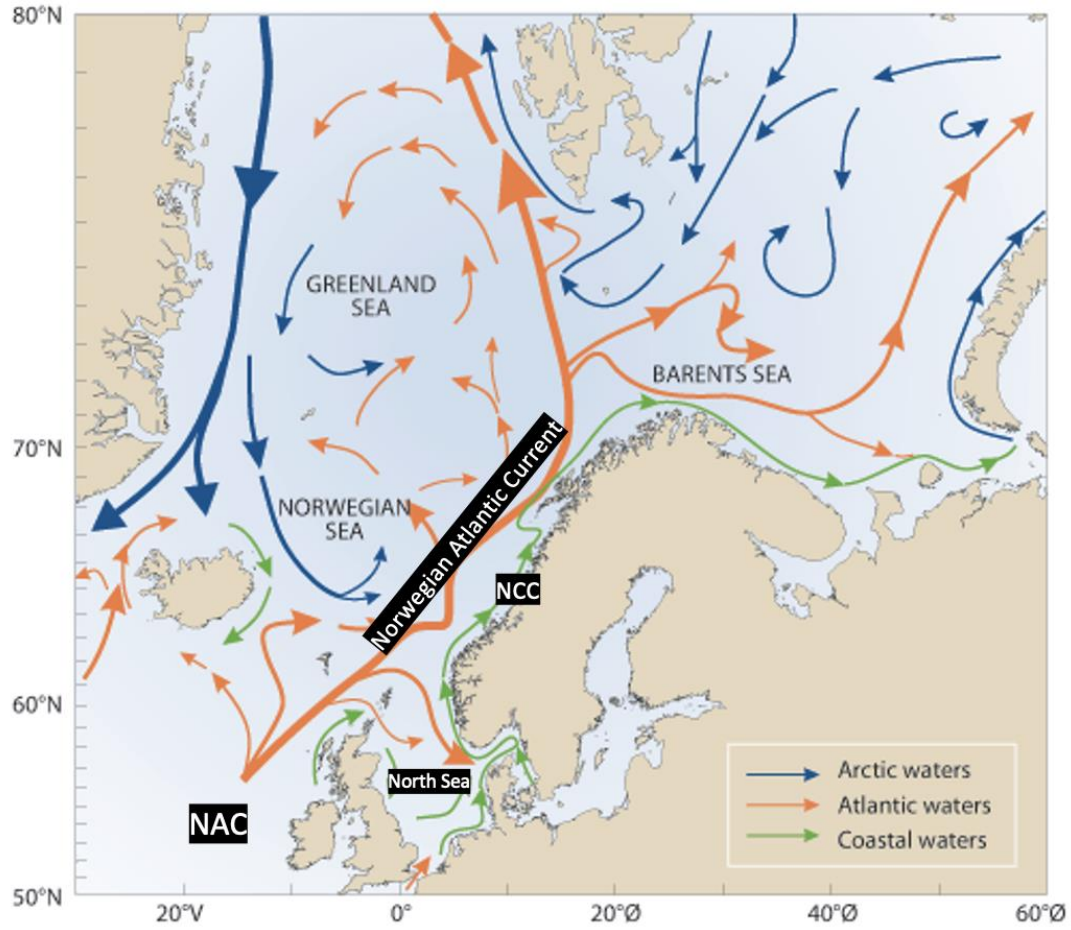


Figure 2.1 *Map of current systems nearby Norway, NAC: The North Atlantic Current, NCC: The Norwegian Coastal Current. Source: Institute of Marine Research, Norway.*

2.2 Location specifics

All the sampling conducted was carried out in coastal areas of Norwegian Sea and Barents Sea (Fig. 2.2), where seasonal variation of hydrographic conditions varies greatly (Wassmann, 2000). Sampling carried out on board G.O. SARS took place in fjord systems in the Barents Sea and in the Western part of Spitsbergen; Isfjorden, Billefjorden, Storfjorden and Kongsfjorden. These are subpolar fjords where the water masses are affected by multiple dynamic components such as large amounts of meltwater during a short season, shallow fjords and water exchange (Svendsen et al., 2000; Howe et al., 2010). Water exchange processes in fjords are characterized by depth zones; surface layer, the intermediate layer and the deep layer. First layer, the surface layer, transports the freshwater out from the fjords. The intermediate layer has the greatest transport of water, which is governed by differences in the pressure between fjord and coast. The deep layer contains the basin water, which is often stagnant water and only exchanged occasionally with coastal water (Aure et al., 2007).

Sampling was also done in Hopavågen, which is a landlocked bay in Orkland, on the coast of Trøndelag in mid-Norway. The bay is around 370 000 m^2 with a maximum depth at 31 m, where around 1/3 of Hopavågen has a depth exceeding 25 m (Marion, 1996). It has a narrow and shallow channel, called Straumen that connects Hopavågen to the main fjord.

Straumen secures water exchange in the bay, mainly through tidal action. Since Straumen is such a shallow sill, tidal movements are delayed with a smaller tidal range within the bay compared to the main fjord (Marion, 1996). Hopavågen is restricted to a few small streams of freshwater runoff surrounding it which is insignificant compared to the daily inflow of sea water through Straumen (Marion, 1996).

Figure 2.2 *The different sampling sites from this study, including Hopavågen, Kongsfjorden, Isfjorden, Billefjorden and Storfjorden. Map from: Kartverket, norgeskart.no*

2.3 Instrumentation

2.3.1 Underwater Bioluminescence Assessment Tool

The Underwater Bioluminescence Assessment Tool (UBAT) from Sea-Bird Scientific was used in this study to measure flash kinetics from different taxa emitting light. The UBAT is a sensor that is designed to mechanically stimulate and measure flash kinetics and bioluminescence potential (Fig. 2.2). It consists of a water inlet and outlet, and its impeller ~~to~~ creates flow and pumps water to the detection chamber and induces bioluminescence. Within the detection chamber, a photo multiplier tube (PMT) is set to count bioluminescence as photons s^{-1} (Orrico et al., 2009; Johnsen et al., 2014). Because of its size and weight, the sensor can easily be deployed on multiple platforms such as Autonomous Underwater Vehicles (AUVs), on moorings over long-term (Orrico et al., 2009) or in a simple frame as done in this project.

The software, BLINC (WET Labs), was used during laboratory experiments for validations and recordings. During *in situ* measurements, the DH4 software was used to control sampling and data was also extracted from it.

Figure 2.3 *The UBAT separate, clearly showing the detection chamber where bioluminescence is emitted. Both impellers are out but would normally be put in the middle part of the UBAT underneath the detection chamber. Photos: Gitte Krohn-Pettersen.*

2.3.2 CTD

In this study, the CTD was used to measure different parameters (with focus on temperature, depth and fluorescence) in the water during *in situ* deployments. The CTD profiler used was the Model SD204. It measures, calculates and records sea water conductivity, temperature, salinity, depth (pressure), water density and sound velocity. In addition, other sensors can be added (SAIV AS, no date), in this case fluorescence and dissolved oxygen ere added. The software, SD200W, was used to program the CTD before *in situ* deployments and retrieving data.

2.4 Sampling

Sampling during fieldworks was conducted over a period of time on different locations. It was mainly done *in situ* collection of zooplankton by using different zooplankton nets, both vertical and horizontal from vessels. Within Hopavågen and Ny-Ålesund, the specimens were visible enough and quite shallow in the water masses to be manually collected into plastic containers.

During fieldwork in Sletvik, a self-made zooplankton net (opening 55 cm with mesh size ca. 400 μm) (Fig. 2.3b) was used. The net was submerged vertical mostly, from a rowing boat and a small motorboat. The vertical net tows were set with a rope between 5 to 50 meter depths. Horizontal tows were conducted from the boats as well, while rowing. The net was let out on approximal 10 meter length and were then dragged by the boat while handholding the rope. Samples were then poured into containers and put in a dark cooling room before sorting. The cooling room had a temperature between 14-15°C, imitating the temperature of the water in Hopavågen.

At G.O. Sars, the sampling was conducted with a bongo- (Opening 2 x 0.2827 m^2, with mesh sixe 1 x 60 μm and 1x 180 μm) and a multinet (opening 0.25 m^2, with mesh size 180 μm) at the depth of 175 to 279m (Husson et al., 2023). These were deployed by the crew on the vessel into the sea (Fig. 2.3a). After deployment, the samples were then transferred to containers and put in a dark cooling storage with the temperature around 3°C. The temperature in the sea was around 0-1 °C.

During Polar Night 2023 in January, some specimens were sampled around Ny-Ålesund in Kongsfjorden. It was done manually as in Hopavågen, when collecting zooplankton in containers in the shallow waters.

Figure 2.4 a. *Multinet used on board G.O. SARS for sampling zooplankton, picture taken before deployment. Photo: Gitte Krohn-Pettersen* ***b****. Vertical zooplankton tows from boat during field work in Hopavågen. Photo: Thea Svendsen*

2.5 Laboratory experiments with taxa-specific bioluminescence

Before conducting the laboratory experiment, sorting was necessary. Sorting out specimen by taxa was done with the help of a transparent aquarium, light table, and with light underneath (Fig. 2.4). The specimens were collected out of the aquarium with a pipette or a spoon, dependent on their size and how fragile they were. They were then put in small containers one by one and placed back into climate chamber to recover. Laboratory experiments with pre-sorted species, both known and unknown to be bioluminescent, were conducted over a period of time on different locations. Taxa used in the study was morphologically identified to lowest taxonomic level possible.

Validation measurements for the UBAT were completed each day when conducting laboratory experiment. It was done through the BLINC software, in a calibration window. The validation was done to compare previous measurements and see if the device has had any changes throughout the use of it, compared to the factory calibration values.

For the laboratory experiments with taxa-specific bioluminescence, a 33 L rectangle aquarium was filled up with filtered (125 μm) sea water. The UBAT was then submerged into the container with a mesh (300 μm) placed at the outlet. These pre-sorted living specimens were fed gently one by one from a container to the inlet of the UBAT with the help of a funnel. The mesh was used as a collector of the specimens that went through the UBAT, to remove the need for changing water in the container between each individual. To avoid interference from earlier taxa during measurements, new filtered seawater was filled in the container and the mesh was cleaned and placed back at the outlet prior to next measuring. Despite filtering the seawater, the water still contained a lot of phytoplankton that in between interfered with measurements.

During the laboratory experiment, the wet lab was completely dark, and a black fabric was used to hide the container with the UBAT from light sources that could interfere with the measurements. At G.O. Sars, it was polar night and no disturbance from other light sources than the light in the room itself, which made it possible to work without black fabric.

Figure 2.5 *Showing the set up for sorting on board G.O. SARS. It was easier to have the room dark with minimal light from light sources above during sorting. Photo: Gitte Krohn-Pettersen*

2.6 *In situ* measurements of bioluminescence

During *in situ* measurements of bioluminescence, a frame consisting of the UBAT, CTD, a logger and a battery (Fig. 2.5a) was deployed just below the surface during low tide in Hopavågen at Sletvik. Most of the deployments were done during nighttime (Fig. 2.5b), where it followed a tidal cycle of 12 hours, from low tide at the afternoon to low tide in the morning. This way one wouldn't risk the water to be lower than the frame itself. The frame was deployed at two different spots in Hopavågen, one almost direct into the current, known as Straumen, where the water masses went in and out of Hopavågen. The other placement was closer to the station with less current.

The DH4 logger was used to control the *in situ* sampling protocols, programmed with a delay (5 s), pre-flush (10 s), warm up (30 s) and five minutes for a sampling period within a ten minute sample interval. The logger was plugged in after the frame was set at a desired place. In total, there were seven runs with the frame, five nighttime, one daytime and one run with both (Table 2.1).

During each deployment, samples with a zooplankton net were taken at the start of the deployment, in the middle and by the end of it. These samples were studied to get a clue on what might go through the UBAT during deployments. All deployments where either on full moon, new moon, or close to it. During times like these, high tides are extra high and low tides are very low (Sælen & Weber, 2023).

Table 2.1 *Information about the conducted in situ deployments in Hopavågen. *Low voltage cut off in battery power – uncertain of end time.*

Deployment name	Date	Day nighttime	Time started	Time ended	Other sensors
Run.000	10.09.22	Daytime	14:15	17:30	CTD
Run.001	10.09.22	Nighttime	20:20	09:20	CTD
Run.002	11.09.22	Nighttime	21:00	09:15	CTD
Run.003	12.09.22	Both	15:50	09:30*	CTD
Run.005	14.09.22	Nighttime	22:00	09:15	CTD
Run.007	25.10.22	Nighttime	21:30	09:30	CTD, ECO triplet, mspec
Run.008	26.10.22	Nighttime	21:20	09:30	CTD, ECO triplet, mspec

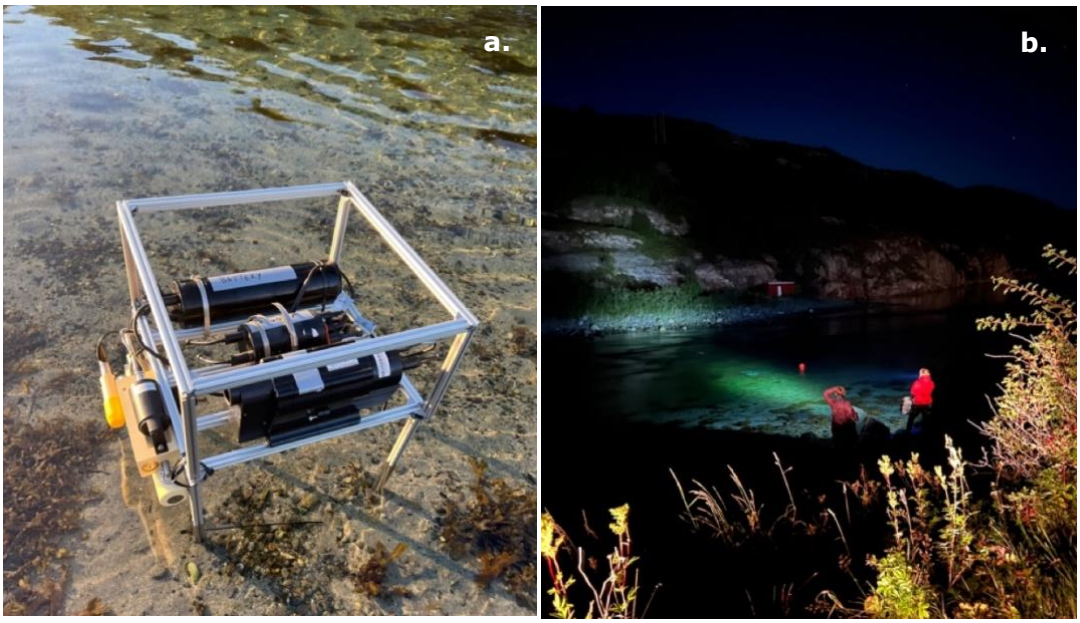


Figure 2.6 a. *frame for in situ deployment with the UBAT, the DH4 logger, external battery and the CTD.* ***b.*** *frame deployed in water during night.*

2.7 Data analyses

2.7.1 Analysing of laboratory experiments with taxa-specific bioluminescence

Taxa-specific bioluminescence data collected by the sensor UBAT was analyzed using an in-house developed code for MATLAB (Appendix A). The code consisted of different parameters inspired by Johnsen et al. (2014) to extract bioluminescence flash kinetic peaks (Fig. 2.7a). Two thresholds, a and b, were set (Fig 2.7b). Threshold a was the threshold used to calculate the peak, it defined where the peak started and ended. Threshold b defined a value below which a signal was not considered as a flash. Threshold a was set at 10*1.46e7 and threshold b was set at 100*1.46e7 to avoid any emitted light from phytoplankton being extracted (Appendix B). An exception was for measurements with *Metridia longa*, where one measurement was extracted from threshold 90*1.46e7. It was done to have an extra extraction from *M. longa*. 1.46e7 were the calibration coefficient.

During the analysing in MATLAB, two files per measurement were created. One containing a long continuous measurement of the extracted peak and one with values of all parameters of the flash kinetics: BL_max, the maximum intensity of the measured bioluminescence; BL_20%, 20% of the BL_max; BL_mean, average of the bioluminescence; Sigma_max, cumulative sum from the first value until BL_max; T_max, time from the first value until the BL_max; T_high, time between first and last value over BL_20%; T_decay, time between last point over BL_20% to last point of threshold a. BL_20% was not included further in results, since it showed the exact same trend as BL_max.

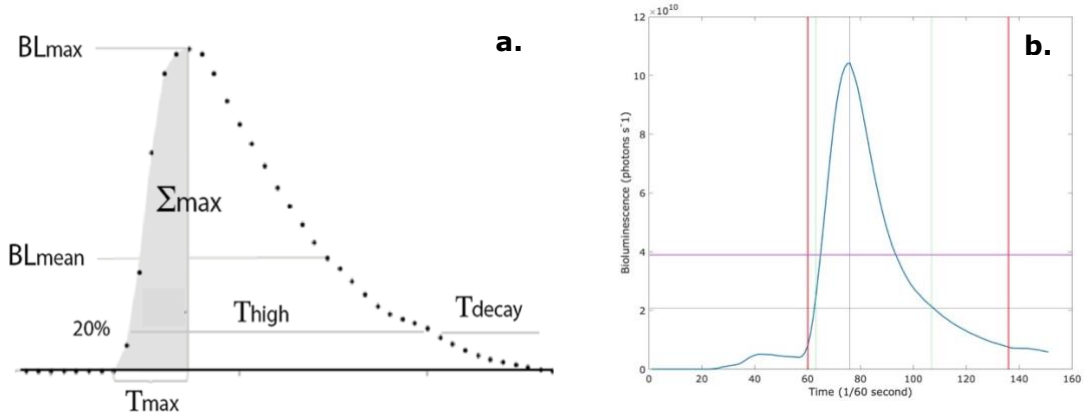


Figure 2.7 a. *Presentation of the bioluminescence parameters extracting values from flash kinetics during analysing. BL_max, the maximum intensity of the measured bioluminescence; BL_20%, 20% of the BL_max; BL_mean, average of the bioluminescence; Sigma_max, cumulative sum from the first value until BL_max; T_max, time from the first value until the BL_max; T_high, time between first and last value over BL_20%; T_decay, time between last point over BL_20% to last point of threshold a. BL_20% was not included further in results, since it showed the exact same trend as BL_max. Source: Johnsen et al., 2014* ***b.*** *Illustration of a single flash extracted with visible thresholds. Threshold a (red line) defines where the peak start and end. Threshold b (purple) defines a value below which a signal is not considered as a flash.*

2.7.2 Analysing of in situ data

Bioluminescence flash kinetics *in situ* data collected by the UBAT and retrieved from the DH4 logger were analysed with in-house developed code for MATLAB. The code consisted of the same parameters as the taxa-specific data (BL_max, BL_20%, BL_mean, Sigma_max, T_max, T_high & T_decay; Appendix C). In line with earlier studies, the *in situ* datasets had high activity of background signals, such as bioluminescent dinoflagellates (Messie et al., 2019) which make it hard to extract single flashes of target organisms. Therefore, a new parameter was added into the code that splits the complete dataset into upper and lower parts based on the bioluminescence intensity to remove the background signals. In other words, a baseline was implemented to discard bioluminescent signals under a set value inspired by Messie et al. (2019). The threshold values (a and b) and the baseline were different for each *in situ* measurement because of the high differences in the bioluminescent activity and intensity in the datasets. In this study, *Beroe* spp., *Clytia gracilis, Bolinopsis infundibulum, Metridia longa* and *Nanomia cara* were used as target organisms and their taxa-specific mean (high and low values of the mean; Appendix C) from parameters (T_max and T_high) based on the laboratory experiments (see methods 2.7.1) were added into the code. This way, the code extracted all peaks fulfilling the criteria separately for each of the six taxa.

The CTD data obtained from *in situ* deployments was retrieved from the SD200W software and plotted in Excel.

2.7.3 Statistical analysis

All statistical analyses were performed using the statistical software PAST (Paleontological Statistics), version 4.11 Mac, with the significance level of 0.05. All the data was non-parametric and did not assume normal distribution. All parameters (see section 2.7.1) were statistically tested for equal medians in the Kruskal-Wallis and Mann-Whitney pairwise post-hoc in the several-sample tests.

3 Results

3.1 Sampling

Sampling during field work gave rich diversity of marine animals with taxonomic levels being different. Five phyla were used in this study (Table 3.1). The 27 collected individuals within the phylum Annelida, were all *Tomopteris* spp. Arthropoda had the most collected individuals in total, where Copepoda *Metridia longa* was the dominant species with 107 individuals collected. 21 of them were collected in Hopavågen and the rest were collected around Svalbard in Isfjorden, Billefjorden and Storfjorden. Also, within the phylum Arthropoda, Euphausiacea spp. had individuals collected both in Hopavågen (n=3) and in Kongsfjorden (n=4). Chaetognatha were abundant in Hopavågen and had all its individuals collected there, they were also not identified down to lower taxonomic level. Cnidaria has a high number of individuals within, where all of them were Hydrozoa. *Clytia gracilis* had the highest abundance in the phylum, with 30 collected individuals and only two *Eutonina* sp. were collected. The order Siphonophorae had the most diversity, with 13 individuals not further identified and seven *Nanomia Cara*. Ctenophora was the second most dominant phyla, where most of the individuals were collected in Hopavågen and some individuals in Kongsfjorden. *Beroe* spp. had the highest number of specimens, with 51 individuals, where only one individual was collected in Kongsfjorden and the rest in Hopavågen. *Bolinopsis infundibulum* were collected mostly in Hopavågen, with one individual from Kongsfjorden. The *Euplokamis* cf. *dunlapae* had only one collected individual, which was in Kongsfjorden where *Mertensia ovum* was also collected with its eight individuals.

Table 3.1 *Overview of phylum collected during field work on all sampling sites.*

Phylum	Collected	Hopavågen	Svalbard			
			Isfjorden	Billefjorden	Storfjorden	Kongsfjorden
Annelida	27	x				x
Arthropoda	155	x	x	x	x	x
Chaetognatha	20	x				x
Cnidaria	67	x				x
Ctenophora	113	x				x
Total	**382**					

3.2 Laboratory experiment with taxa-specific bioluminescence

Out of 382 individuals sorted (Table 3.1), 276 of them were believed to be bioluminescent and 120 individuals emitted light that was above threshold b (threshold b, see section 2.7.1) (Table 3.2).

Table 3.2 *Taxa that emitted light in the UBAT after being sorted and the extracted peaks that came out after analyzing. *The table is only showing the total sorted individuals that emitted light. Ctenophora* Mertensia ovum *were run in the UBAT as a mock community.*

Taxa	Collected*	Extracted peaks
Beroe spp.	51	45
Bolinopsis infundibulum	23	16
Clytia gracilis	30	26
Euplokamis cf. *dunlapae*	1	1
Eutonina sp.	2	2
Euphausiacea spp.	7	1
*Mertensia ovum**	8*	7*
Metridia longa	107	7
Nanomia cara	7	7
Siphonophorae spp.	13	6
Tomopteris sp.	27	2
Total	**276***	**120**

From all the zooplankton collected and which emitted light in the UBAT after being mechanically triggered, taxa that can go under the generic term gelatinous zooplankton were the dominant ones. (Table 3.2). Running through the UBAT did not trigger bioluminescence in eight of the taxa sampled in this study. These were in the class Hydrozoa: *Aglantha digitale, Leuckartiara* sp. and *Rathkea octopunctata,* in the phylum Ctenophora: *Pleurobrachia pileus,* Amphipoda spp. and in the class Copepoda: *Calanus* sp. and *Anomalocera* sp.

Beroe spp., *Bolinopsis infundibulum*., *Clytia gracilis*, *Metridia longa*, *Nanomia cara* & Siphonophorae spp. were the most dominant taxa that emitted light above threshold b (see section 2.7.1) (Table 3.2). The graphs present the flash kinetics of the individuals used in the study (Fig. 3.1). There were a few high intensity light emitted by *Beroe* spp., making the less intense flashes barely visible in graphs (Fig. 3.1a). One of the peaks of the high intensity flashes also differs in shape with multiple peaks compared to the other ones within the genus. *B. infundibulum* had one high intensity flash emitted as well as several other individual flashes that had multiple peaks (Fig. 3.1b). *M. longa* had only seven extracted peaks from 107 individuals collected (Table 3.2). The few peaks extracted showed a fast flash kinetic, in both high and low intensity flashes (see section 2.7.1) (Fig. 3.1c). The individuals of *C. gracilis* (Hydrozoa) had a high abundance of collected individuals with a high number of emitted light (Table 3.2). They showed great variation in intensity of flashes with several individuals having fast flash kinetics and a few producing multiple peaks (Fig. 3.1d). *N. cara* had seven collected individuals, where all of them emitted light (Table 3.2). The flash kinetics of these seven *N. cara* were very similar to each other with some diversity in intensity of the flashes (Fig. 3.1e). The shape of *N. cara* showed to have both a smooth rise and decay. The individuals in Siphonophorae spp.

produced a fast flash kinetic with a slower decay with the exception of one individual having a more jagged decay (Fig. 3.1f).

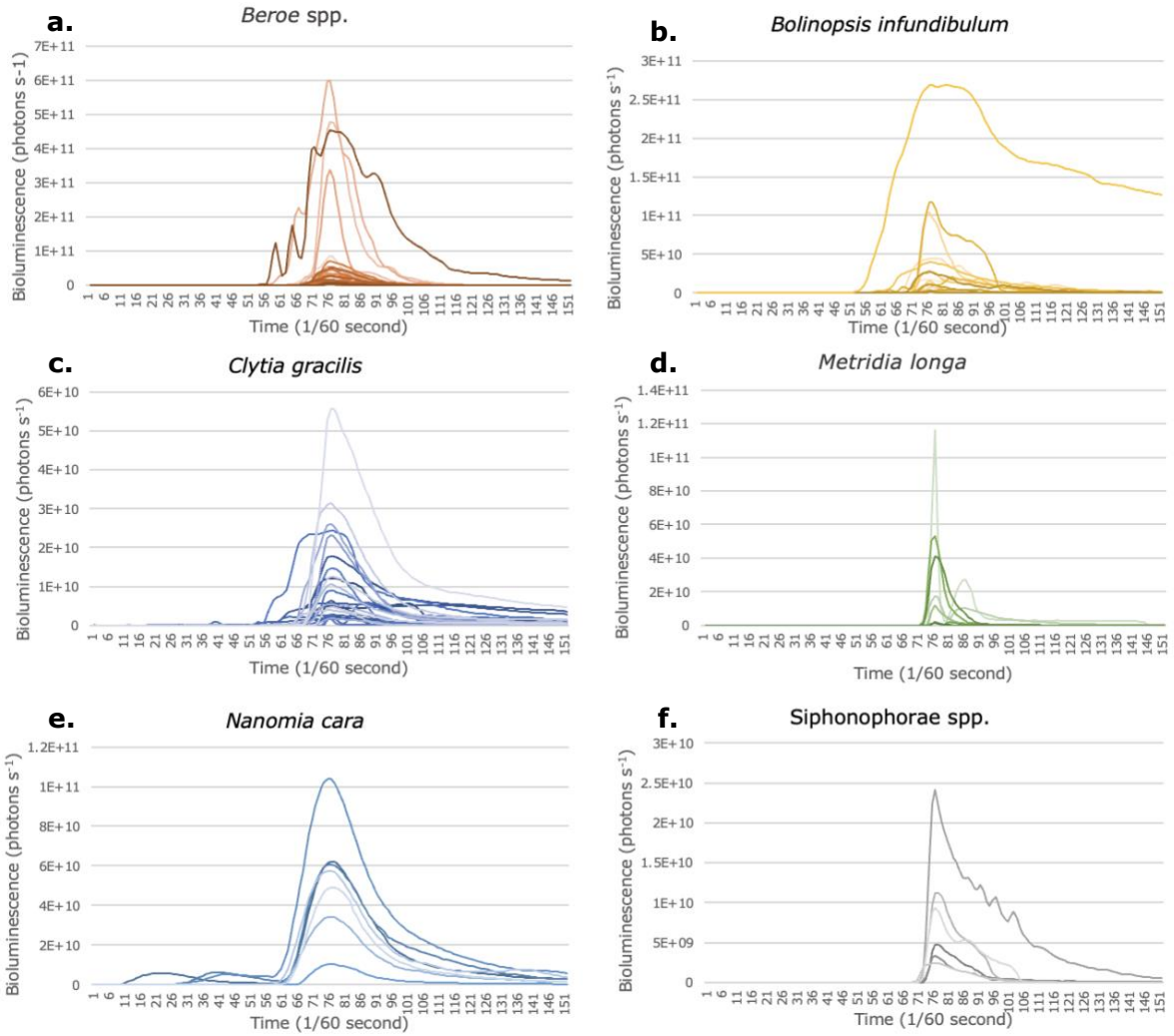


Figure 3.1 *Flash kinetics of the six most abundant taxa which have been extracted from laboratory measurements to create a taxa-specific library.* ***a****.* Beroe *spp. showing its flask kinetics from all 45 individuals who had extracted peaks* ***b****.* Bolinopsis infundibulum *flash kinetics from the 16 extracted peaks* ***c.*** *the 26 extracted peaks in flash kinetics from* Clytia gracilis ***d****.* Metridia longa *with its seven extracted peaks* ***e****.* Nanomia cara *with its seven extracted peaks, showing its flash kinetics* ***f****. the six extracted peaks from the order Siphonophorae spp. and their flash kinetics.*

Mean and standard deviation for extracted flash kinetic parameters were calculated per taxa (Table 3.3). The selected individuals were used because of similarities between the flash kinetics to get the most precise kinetic characteristics. For example, all individual *N. cara* (Fig. 3.1e) had a very consistent shape of their flashes compared to *M. longa* (Fig. 3.1c) and *B. infundibulum* (Fig. 3.1b) that had some individuals not showing any similarities to the majority of the flashes within the taxon. There was a lot of variations in flash kinetic parameter values between the six most dominant taxa (Table 3.3).

Table 3.3 *Extracted parameters for flash kinetics of the bioluminescent taxa from laboratory experiment. The values are the mean and standard deviation from each taxon. *The number of extracted peaks in the table does not represent how many individuals in total emitted light. These are individual measurements that showed little to no disturbance and therefore are more representative in the flash kinetics characteristics (Appendix D)*

Taxa	Extracted peaks*	BL_max	BL_mean	Sigma_max	T_max	T_high	T_decay
Unit		$X10^9$ photons s^{-1}	$X10^9$ photons s^{-1}	$X10^9$ photons s^{-1}	Seconds	Seconds	Seconds
Beroe spp.	33	44.87 +/- 98.79	6.08 +/- 1.23	161.23 +/- 3.35	0.19 +/- 0.22	0.27 +/- 0.09	0.31 +/- 0.26
Bolinopsis infundibulum	5	35.79 +/- 40.14	6.42 +/- 4.74	144.46 +/- 168.49	0.28 +/- 0.37	0.31 +/- 0.28	0.58 +/- 0.43
Clytia gracilis	14	15.86 +/- 14.66	3.34 +/- 4.49	76.77 +/- 70.07	0.15 +/- 0.05	0.57 +/- 0.27	0.61 +/- 0.34
Metridia longa	4	26.79 +/- 24.23	6.67 +/- 5.98	53.49 +/- 55.81	0.05 +/- 0.03	0.12 +/- 0.05	0.20 +/- 0.16
Nanomia cara	7	63.24 +/- 21.94	3.17 +/- 4.47	641.65 +/- 272.66	0.82 +/- 0.31	0.68 +/- 0.08	0.76 +/- 0.08
Siphonophorae spp.	6	9.21 +/- 8.10	1.28 +/- 1.53	22.41 +/- 18.62	0.07 +/- 0.02	0.39 +/- 0.10	0.35 +/- 0.27

3.2.1 Maximum intensity of bioluminescence (BL_max)

The mean BL_max values ranged from 9 X 10^9 to 63 X 10^9 photons s^{-1}, with Siphonophorae spp. having the lowest mean at 9.21 X 10^9 photons s^{-1} and *N. cara* having the highest mean at 63.24 X 10^9 photons s^{-1} (Table 3.3). The median of the measured BL_max of *N. cara* was the highest compared to the other taxa (Fig. 3.2), and the pairwise comparison stated that *N. cara* was significantly different from most taxa, whereas *B. infundibulum* was the only one with no significant difference to *N. cara*. (Table 3.4). *Beroe* spp. had a high standard deviation at 98.79 X 10^9 photons s^{-1} (Table 3.3), because of two outliers (Appendix E) and a high standard error (Fig. 3.2).

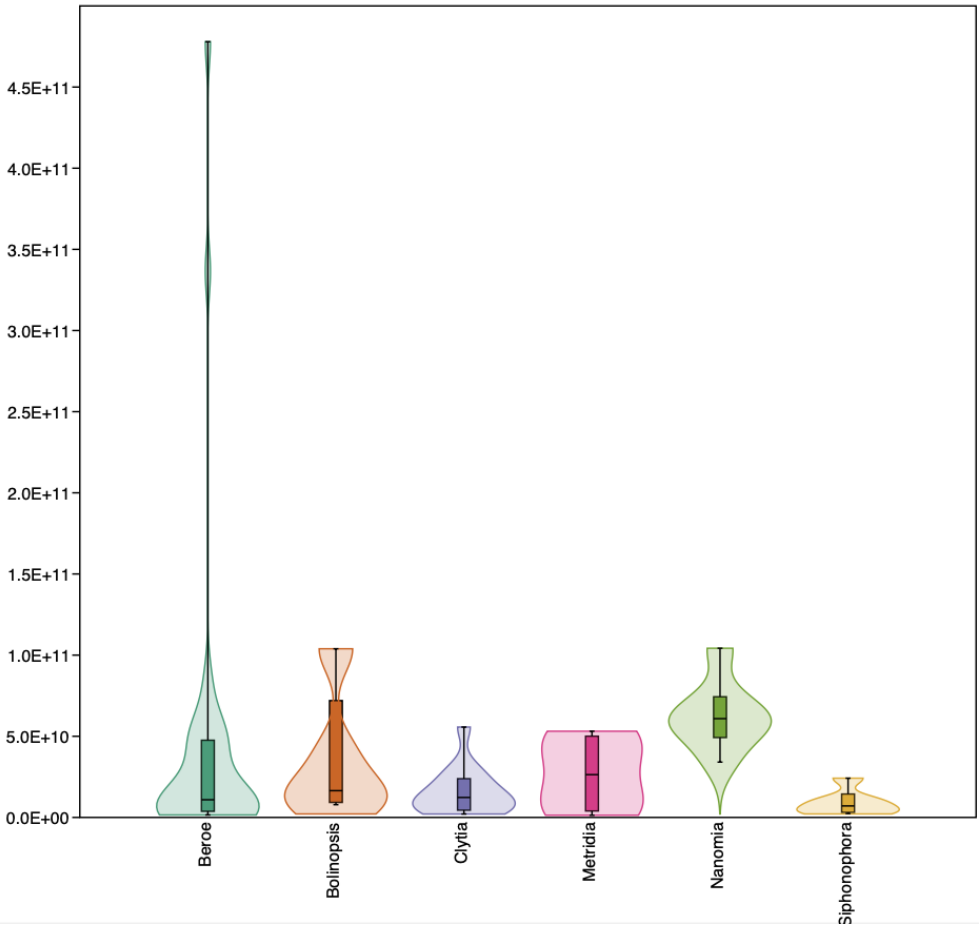


Figure 3.2 *Maximum intensity of measured bioluminescence (BL_max) from the six most dominant taxa, showing the median of the BL_max from different taxa and the standard error. Taxa in the figure is* Beroe *spp.,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp.,* where N. cara *is showing ~~a~~ significantly higher median than the others and* Beroe *spp. having two clear outliers.*

Table 3.4 *Pairwise difference in the maximum intensity of measured bioluminescence (BL_max) of the target organisms based on Pairwise Mann-Whitney-Tests. Significant difference (p<0.05) between the organisms is marked in pink.*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,3645	0,8433	0,9805	0,003936	0,4027
Bolinopsis infundibulum	0,3645		0,287	0,9025	0,1044	0,1207
Clytia gracilis	0,8433	0,287		0,7101	0,0005222	0,3429
Metridia longa	0,9805	0,9025	0,7101		0,04722	0,3374
Nanomia Cara	0,003936	0,1044	0,0005222	0,04722		0,003405
Siphonophorae spp.	0,4027	0,1207	0,3429	0,3374	0,003405	

3.2.2 Average bioluminescence intensity (BL_mean)

The mean BL_mean values for the different taxa did not differ much from each other. Siphonophorae spp. was the one that stood out, with the lowest mean at 1.28 X 10^9 photons s^{-1}, while *M. longa* had the highest mean and standard deviation at 6.67 X 10^9 photons s^{-1} and 5.98 X 10^9 photons s^{-1} (Table 3.3). There was no significant difference between the taxa medians in the pairwise comparison (Table 3.5). *Beroe* spp. were the conspicuous taxa within the BL_mean, with one outliner and a high standard error (Fig. 3.3).

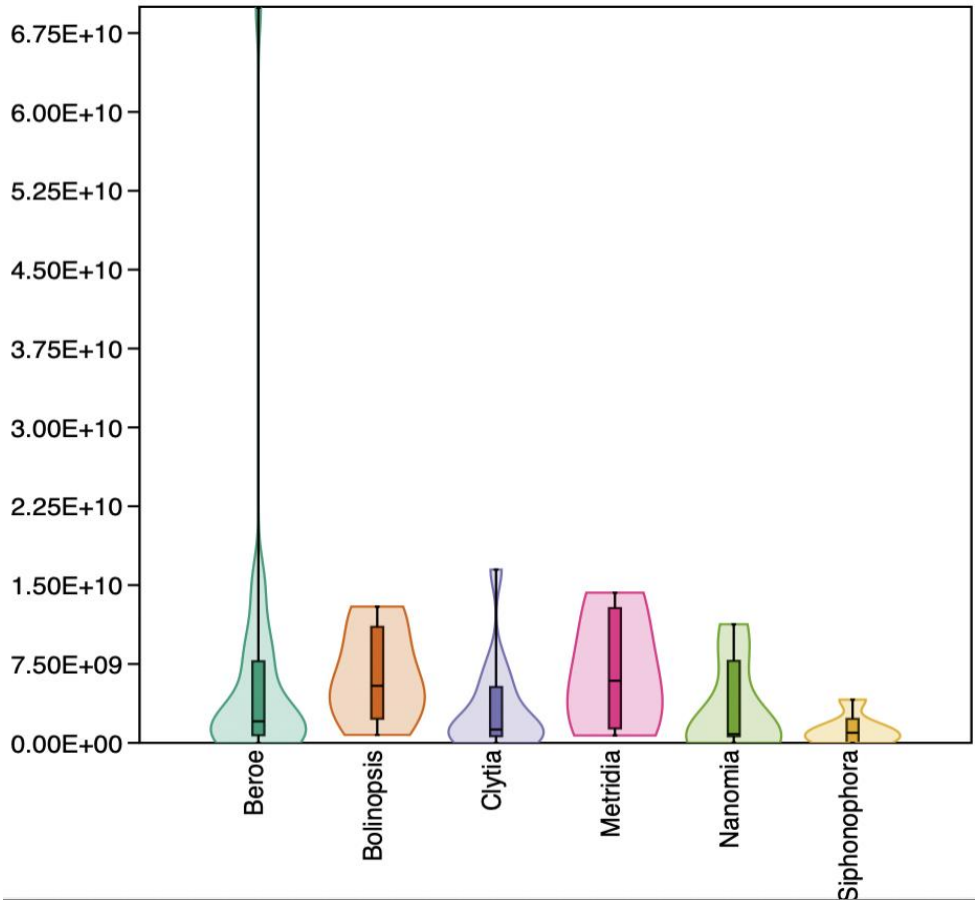


Figure 3.3 *Average of the bioluminescence (BL_mean) from the six most dominant taxa, showing the median of the BL_mean from different taxa and the standard error. Taxa in the figure is* Beroe *spp,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp.*

Table 3.5 *Pairwise difference in the average of the bioluminescence (BL_mean) of the target organisms (Pairwise Mann-Whitney-Tests, p<0.05).*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,2436	0,5687	0,392	0,4988	0,1147
Bolinopsis infundibulum	0,2436		0,1795	0,9025	0,2556	0,08214
Clytia gracilis	0,5687	0,1795		0,313	0,526	0,2648
Metridia longa	0,392	0,9025	0,313		0,2986	0,1645
Nanomia Cara	0,4988	0,2556	0,526	0,2986		0,6166
Siphonophorae spp.	0,1147	0,08214	0,2648	0,1645	0,6166	

3.2.3 Cumulative sum of bioluminescence until maximum intensity is reached (Sigma_max)

The conspicuous taxa regarding Sigma_max compared to other taxa was *N. cara* with its mean Sigma_max of 641.65 X 10^9 photons s^{-1}. The next highest values (641.65 X 10^9 photons s^{-1}) were found in *Beroe* spp. *N. cara* were also the taxa with the highest standard deviation at 272.66 X 10^9 photons s^{-1} (Table 3.3). Only the results of *N. cara* were significantly different in the pairwise comparison of the medians of the taxa (Table 3.6). the two outliers of *Beroe* spp. stood out in Sigma_max (Fig. 3.4) as well as they did in BL_max and BL_mean.

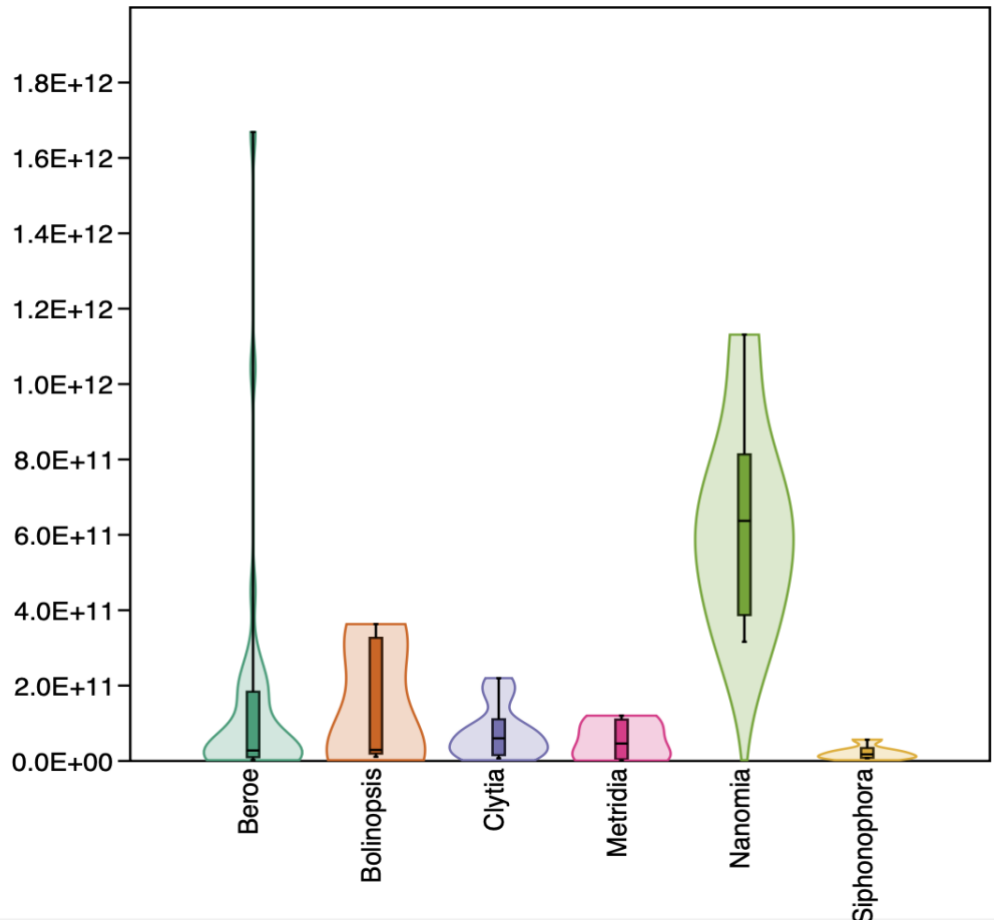


Figure 3.4 *Cumulative sum of bioluminescence until maximum intensity is reached (Sigma_max) from the six most dominant taxa, showing the median of the Sigma_max from different taxa and the standard error. Taxa in the figure is* Beroe *spp,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp.* N. cara *shows significantly higher median than the other taxa.*

Table 3.6 *Pairwise difference in cumulative sum of bioluminescence until maximum intensity is reached (Sigma_max) of the target organisms (Pairwise Mann-Whitney-Tests, p<0.05). Significant difference (p<0.05) between the organisms is marked in pink.*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,4119	0,7712	0,5091	0,0003715	0,2673
Bolinopsis infundibulum	0,4119		0,817	0,5403	0,009366	0,1207
Clytia gracilis	0,7712	0,817		0,5592	0,0002965	0,06349
Metridia longa	0,5091	0,5403	0,5592		0,01073	0,594
Nanomia Cara	0,0003715	0,009366	0,0002965	0,01073		0,003405
Siphonophorae spp.	0,2673	0,1207	0,06349	0,594	0,003405	

3.2.4 Time to reach maximum bioluminescence (T_max)

It took a lot longer time for the specimens of *N. cara* reach the maximum intensity of the flash compared to the other taxa (Fig. 5). These results were also significantly different in the pairwise comparison, except for with *B. infundibulum* (Table 3.7). *N. cara* used 0.82s to reach the maximum intensity, whereas *M. longa* used as little as 0.05s and Siphonophorae spp. used 0.07s, which indicates that their flash kinetics were fast. *B. infundibulum* had a lot of variation in the time it used to reach the BL_max compared to other species with mean time at 0.28s and standard deviation of 0.37s (Table 3.3).

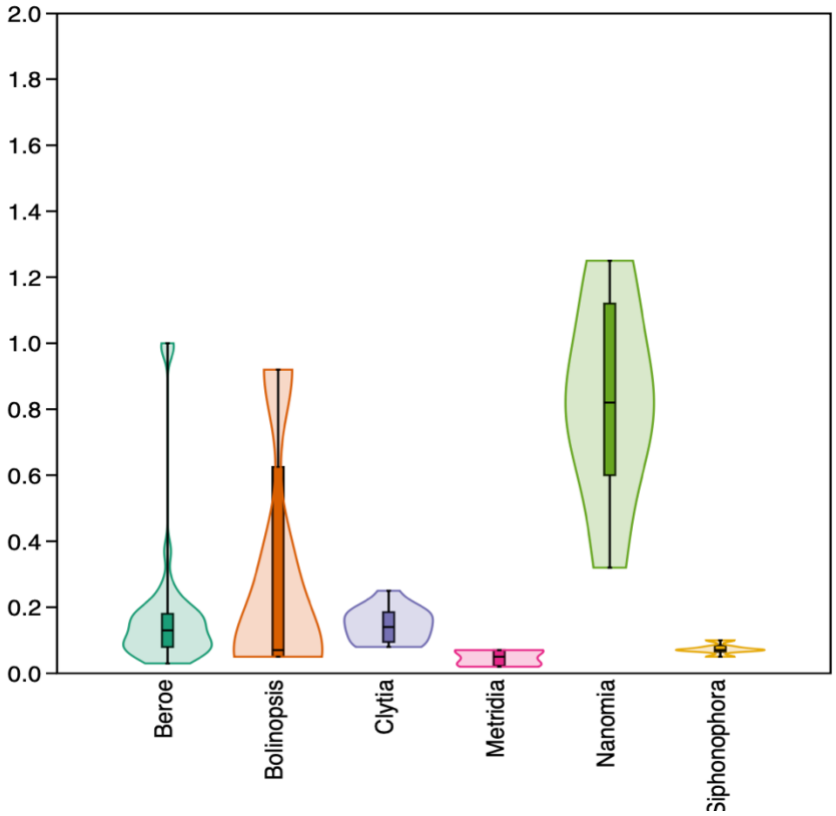


Figure 3.5 *Time to reach maximum bioluminescence (T_max) from the six most dominant taxa, showing the median of the T_max from different taxa and the standard error. Taxa in the figure is* Beroe *spp,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp.,* N. cara *shows a significantly higher median than most of the taxa and* M. longa *and Siphonophorae spp. have significantly lower medians.*

Table 3.7 *Pairwise difference in the time to reach maximum bioluminescence (T_max) of the target organisms based on Pairwise Mann-Whitney-Tests. Significant difference (p<0.05) between the organisms is marked in pink.*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,6648	0,49	0,007454	0,0002065	0,02211
Bolinopsis infundibulum	0,6648		0,5454	0,2602	0,07353	1
Clytia gracilis	0,49	0,5454		0,003312	0,0002833	0,002409
Metridia longa	0,007454	0,2602	0,003312		0,01056	0,1393
Nanomia Cara	0,0002065	0,07353	0,0002833	0,01056		0,003232
Siphonophorae spp.	0,02211	1	0,002409	0,1393	0,003232	

3.2.5 High intensity duration over 20% maximum bioluminescence (T_high)

Most taxa differed significantly from each other in the pairwise comparison (Table 3.8). *B. infundibulum* had significant difference only to *C. gracilis* while *C. gracilis* had a significant difference to all taxa except Siphonophorae spp. (Fig. 3.6). The T_high mean values ranged from 0.12s – 0.68s (Table 3.3) where *C. gracilis* and *B. infundibulum* had the highest standard deviation at 0.28s and 0.27s and the highest standard error (Fig. 3.6).

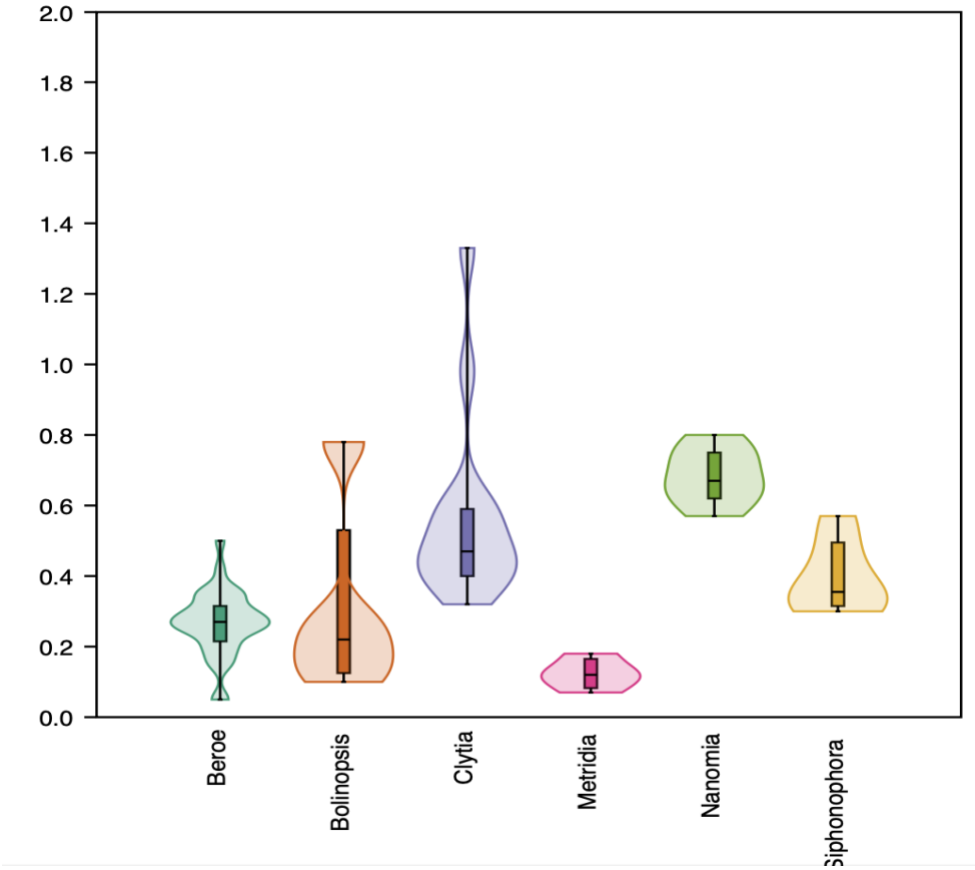


Figure 3.6 *Time between first and last value over 20% maximum bioluminescence (T_high) from the six most dominant taxa, showing the median of the T_high from different taxa and the standard error. Taxa in the figure is* Beroe *spp,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp., most of the taxa show significant differences between each other.*

Table 3.8 *Pairwise difference in the time between first and last value over 20% maximum bioluminescence (T_high) of the target organisms based on Pairwise Mann-Whitney-Tests. Significant difference (p<0.05) between the organisms is marked in pink.*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,4742	7,251E-07	0,007408	3,989E-05	0,006568
Bolinopsis infundibulum	0,4742		0,03699	0,1761	0,07404	0,1207
Clytia gracilis	7,251E-07	0,03699		0,003428	0,02057	0,06269
Metridia longa	0,007408	0,1761	0,003428		0,01056	0,1393
Nanomia Cara	3,989E-05	0,07404	0,02057	0,01056		0,004222
Siphonophorae spp.	0,006568	0,1207	0,06269	0,1393	0,004222	

3.2.6 Time from 20% maximum bioluminescence to last value (T_decay)

T_decay had variability among the values. There were no exceptionally high standard deviations, except for *B. infundibulum*, the mean decay value of which was 0.28s while the mean was 0.31s (Table 3.3). *N. cara* had the most consistent T_decay of all taxa (Fig. 3.7). However, there was a significant difference between several of the taxa in the pairwise comparison (Table 3.9). *B. infundibulum* was the only taxa that had no significant difference to the others and had the highest standard deviation at 0.43s (Table 3.3) and standard error (Fig. 3.7).

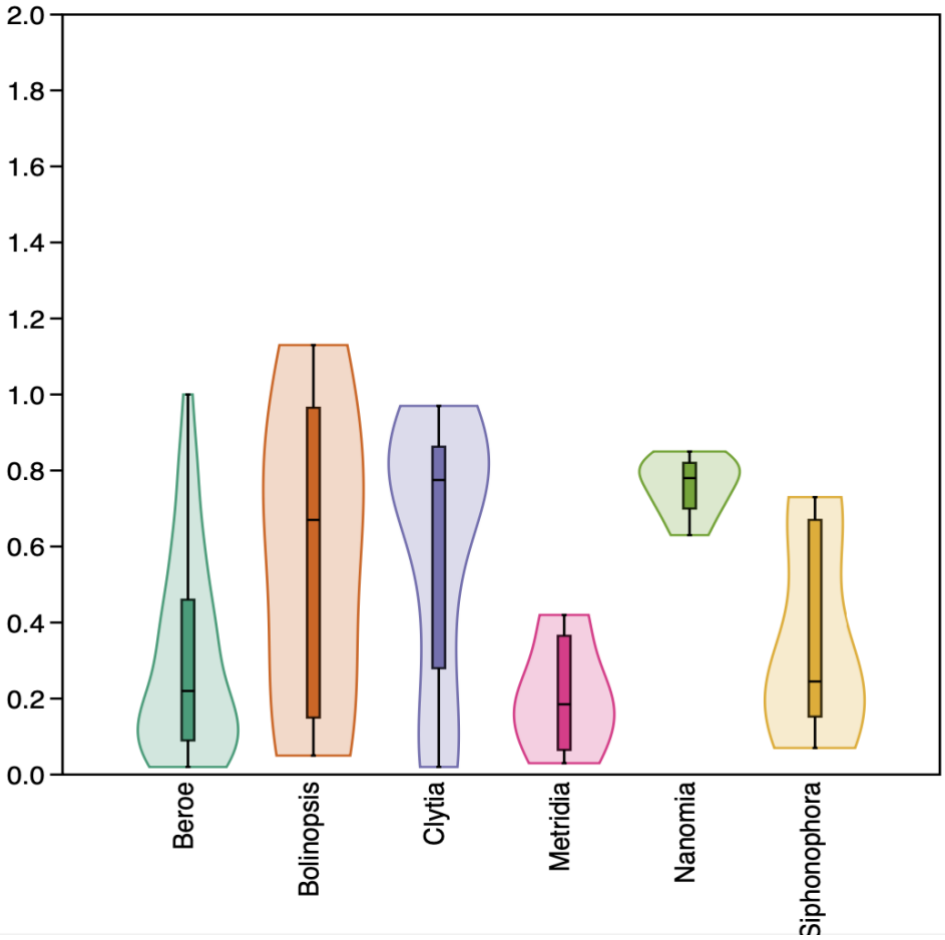


Figure 3.7 *Time between last point over 20% of maximum bioluminescence to last point over threshold a (T_decay) from the six most dominant taxa, showing the median of the T_decay from different taxa and the standard error. Taxa in the figure is* Beroe *spp.,* Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara *and Siphonophorae spp.*

Table 3.9 *Pairwise difference in the time between last point over 20% of maximum bioluminescence to last point over threshold a (T_decay) of the target organisms based on Pairwise Mann-Whitney-Tests. Significant difference (p<0.05) between the organisms is marked in pink.*

	Beroe spp.	*Bolinopsis infundibulum*	*Clytia gracilis*	*Metridia longa*	*Nanomia Cara*	Siphonophorae spp.
Beroe spp.		0,1874	0,01652	0,6245	0,0006283	0,5722
Bolinopsis infundibulum	0,1874		0,8893	0,1779	0,4641	0,4113
Clytia gracilis	0,01652	0,8893		0,07895	0,7361	0,0899
Metridia longa	0,6245	0,1779	0,07895		0,01073	0,3374
Nanomia Cara	0,0006283	0,4641	0,7361	0,01073		0,01502
Siphonophorae spp.	0,5722	0,4113	0,0899	0,3374	0,01502	

3.3 *In* situ measurements

This section presents the data collected during *in situ* deployments with the UBAT sensor and the CTD. The UBAT data is presented directly from MATLAB and shows the output from the program.

3.3.1 Flash kinetics from UBAT *in situ*

In most of the in situ measurements, several peaks matching the parameters derived from *B. infundibulum, Beroe* spp*., C. gracilis* and *M. longa* could be extracted. (Fig. 3.8). There were clearly a lot of multiple peaks extracted as well, and *M. longa* had an extremely high amount of them (Fig. 3.8d) compared to the other three. The multiple peaks were seen as unclassified peaks since they could not be recognized as a given taxa.

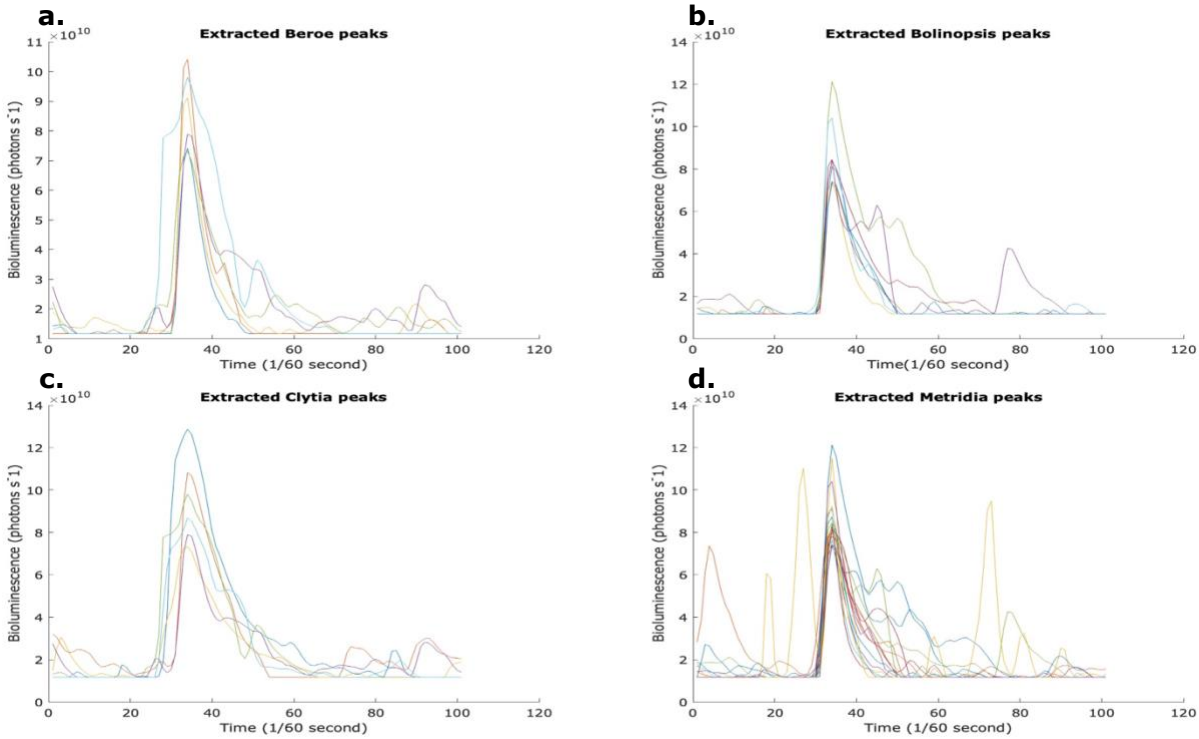


Figure 3.8 *Peaks extracted from* in situ *data matching the parameters for* Bolinopsis infundibulum, Beroe *spp.,* Clytia gracilis *and* Metridia longa. *Data from a nighttime deployment.*

The general intensity difference in all the *in situ* measurements made it hard to extract peaks within the same thresholds. The nighttime deployments (Table 2.1) had the highest intensity of extracted flashes (Fig. 3.8) compared to the daytime deployments, where the intensity of the extracted flashes was remarkably lower (Fig. 3.9). As a result, the daytime measurements had much lower thresholds and baseline, and many low-intensity peaks were extracted from these measurements. Like in the nighttime measurements, *M. longa* parameters extracted the most multiple peaks (Fig. 3.9d) from the daytime measurements. *N. cara* was the taxa that had the least amount of extracted peaks from the *in situ* data with zero extracted flash kinetics from all seven datasets, whereas *M. longa* had the most on all measurements.

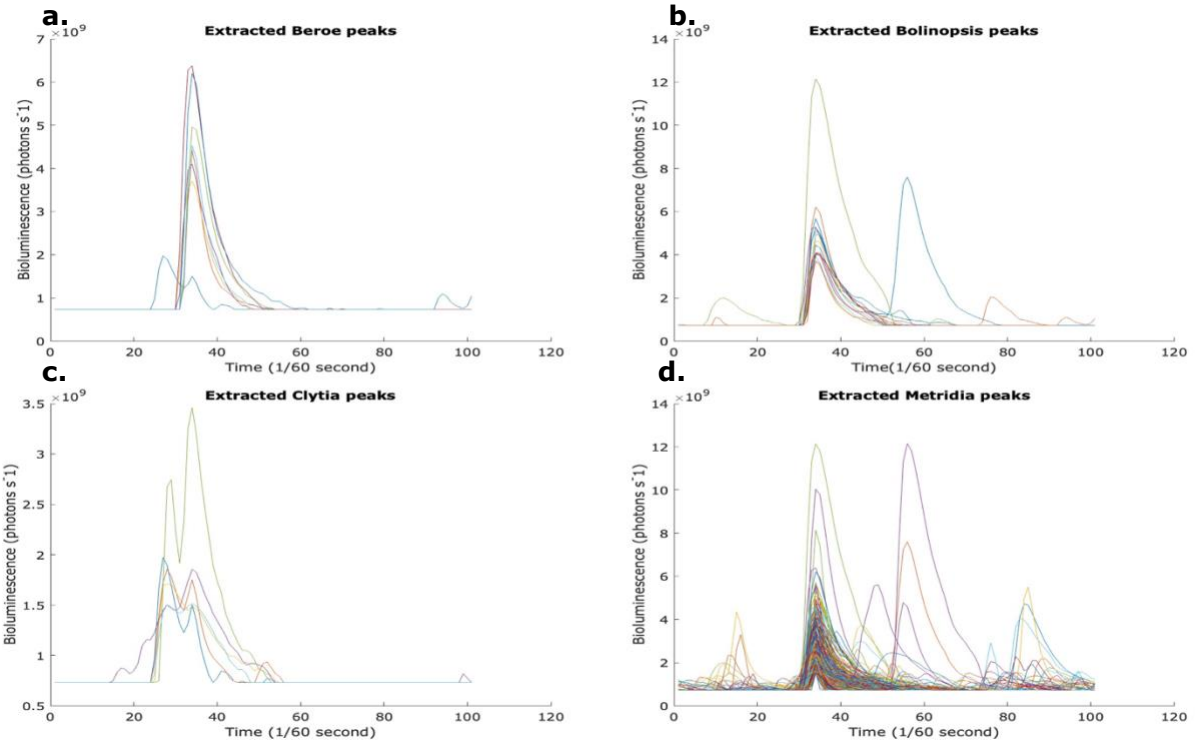


Figure 3.9 *Extracted peaks matching the parameters values for* Bolinopsis infundibulum*,* Beroe *spp.,* Clytia gracilis *and* Metridia longa *from* in situ *measurements during a daytime deployment.*

There were peaks extracted by the parameter values based on multiple taxa. Such as, parameter values by *Beroe* spp. and *C. gracilis* both extracted the same peak (Fig. 3.10). The two taxa showed no significant difference in their T_max in their pairwise comparison but greatly difference in their T_high.

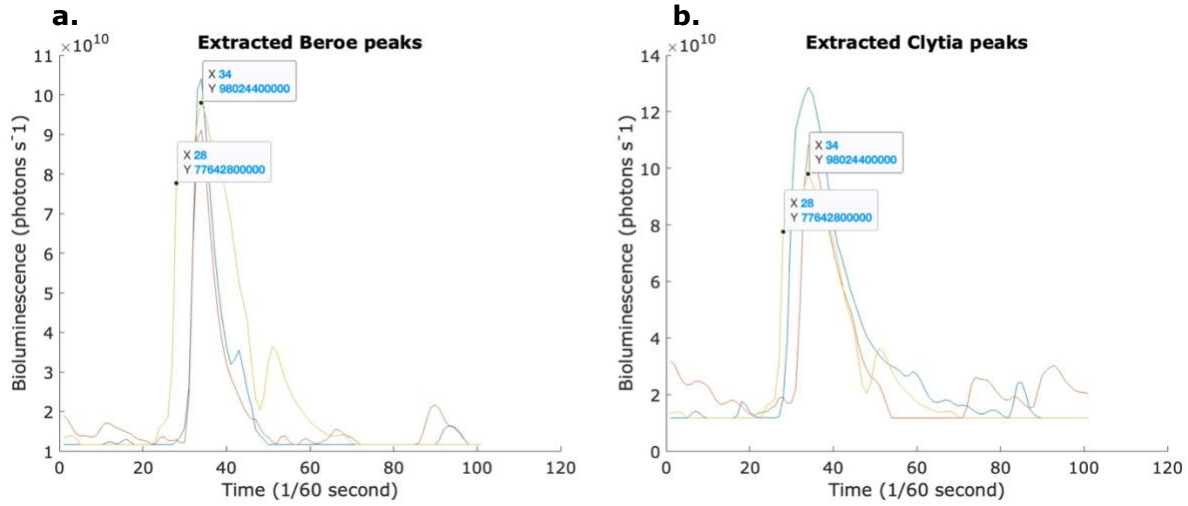


Figure 3.10 *Overlapping peaks extracted from* in situ *data matching taxa-specific parameter values of T_max and T_high.* ***a.*** *peaks extracted by using the values for* Beroe *spp.* ***b.*** *peaks extracted by values from* Clytia gracilis. *Pin-points show that exactly same peak has been extracted by both.*

Another peak extracted both by the parameter values based on *B. infundibulum* and *M. longa* (Fig. 3.11). The taxa did not have any significant difference in T_max and T_high from the pairwise comparison. The mean T_max values were 0.28s (*B. infundibulum*) and 0.05s (*M. longa*), while their T_high values were 0.31s (*B. infundibulum*) and 0.12s (*M. longa*) (Table 3.3).

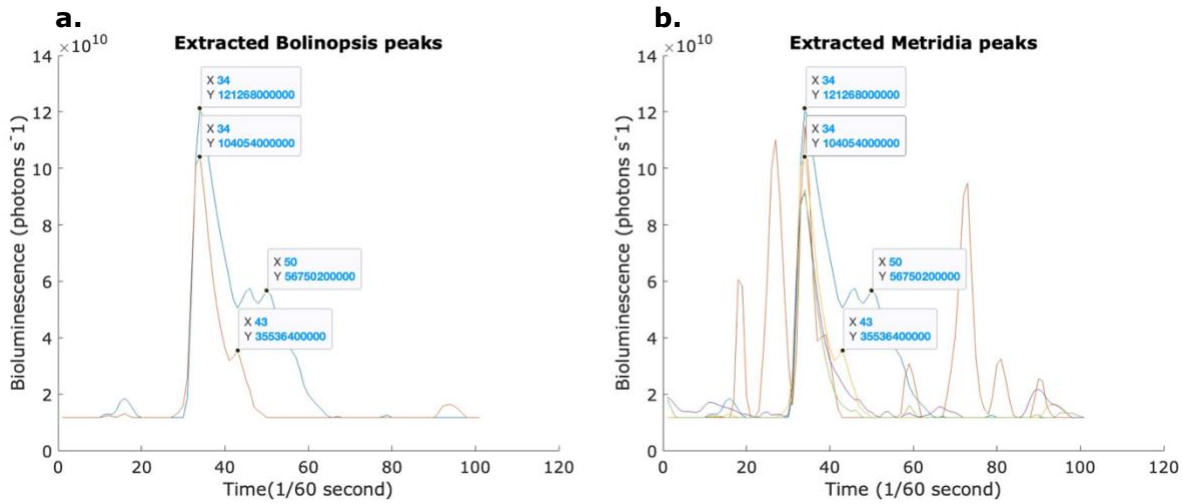


Figure 3.11 *Overlapping peaks extracted from* in situ *data matching taxa-specific parameter values of T_max and T_high.* ***a.*** *peaks extracted by using the values for* Bolinopsis infundibulum ***b.*** *peaks extracted by values from* Metridia longa. *Pin-points show that exactly same peak has been extracted by both.*

3.3.2 Net sampling during *in situ* deployments

The taxa looked for in net sampling was Ctenophora, which was found in nine of the samples with a various of numbers (Table 3.10). No other bioluminescent taxa were identified in the samples.

Table 3.10 *Overview of samples taken with zooplankton net during deployments and the amount of Ctenophora found in the samples. Sample 1,2 and 3 were collected on deployments with no UBAT data.*

Sample	Deployment	Time	Taxa	Counted
1	-	19:55	-	-
2	-	00:00	Ctenophora spp.	4
3	-	09:15	Ctenophora spp.	24
4	Run000	17:30	-	-
5	Run001	20:20	Ctenophora spp.	22
6	Run001	01:00	Ctenophora spp.	5
7	Run001	09:20	Ctenophora spp.	85
8	Run002	21:00	Ctenophora spp.	1
9	Run002	09:15	-	-
10	Run003	15:55	-	-
11	Run003	20:00	-	-
12	Run003	23:30	-	-
13	Run003	09:30	Ctenophora spp.	6
14	Run005	22:00	Ctenophora spp.	9
15	Run005	09:15	Ctenophora spp.	4

3.3.3 CTD

CTD profile (Fig. 3.12) was done during deployments along with the UBAT on the frame. This profile from Run002 (Table 2.1) shows a temperature vary between 13.7 °C and 13.1 °C (Fig. 3.12a), decreasing in temperature in the morning. The fluorescence decreased as well in the morning (Fig. 3.12b) and varied during the whole deployment from below 1 to 3. The depth clearly increased midway of the deployment (Fig. 3.12c), showing the tide during the whole deployment. The deployment had also high activity of bioluminescence (Fig. 3.12d), with a less intense activity in the start.

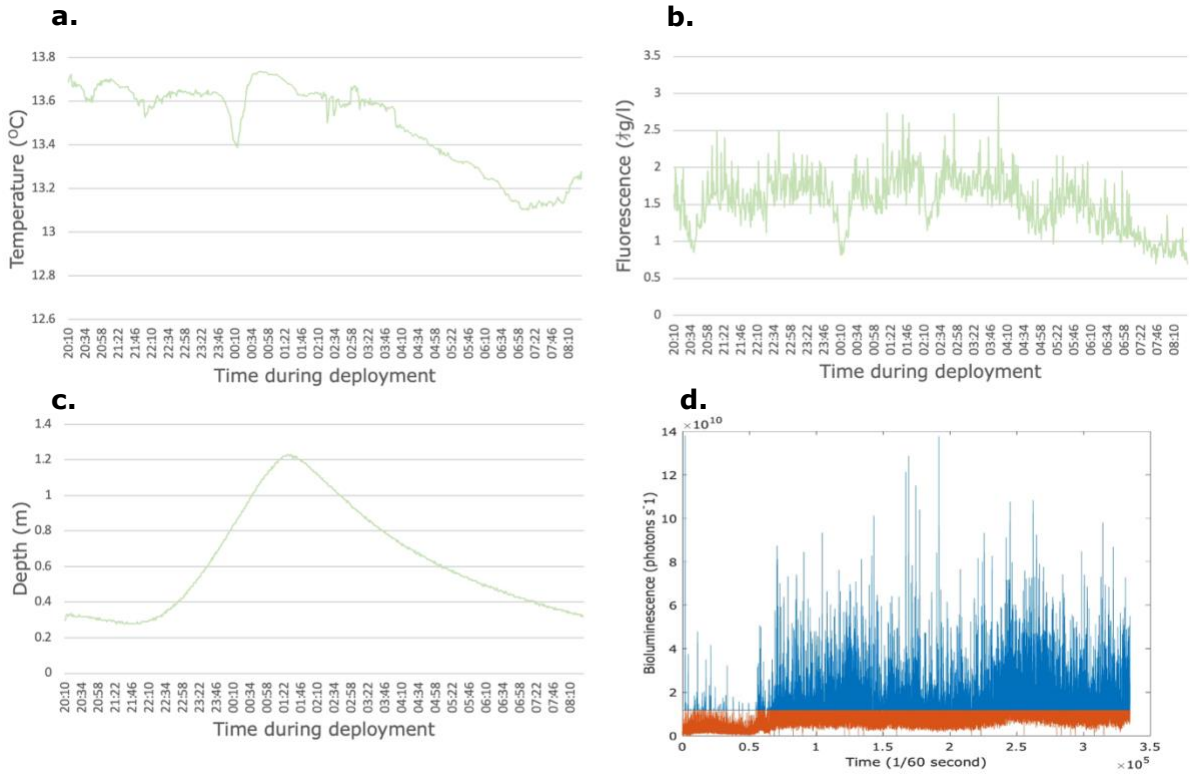


Figure 3.12 *CTD measurements taken during a nighttime deployment (run002) in Hopavågen.* ***a****. temperature* ***b****. fluorescence* ***c****. depth, clearly showing the high tide in the middle of deployment.* ***d****. UBAT data measured at the same time as the CTD. The red marks the split parameter (baseline) that was put into the MATLAB code, data presented directly from MATLAB.*

4 Discussion

Being able to estimate biodiversity and monitor its change is crucial for the future of healthy ecosystems. To do so, questions like "who?" and "how?" need to be asked before "how much?" and "how many?" (Haddock, 2004). Studies that aim to identify species are crucial for answering these questions. Simultaneously studies, such as this one, aiming to figure out how taxa that are currently left on the outside of current surveys and monitoring programs could be included and identified to the lowest taxonomic level as possible. As also in this study, many of the bioluminescent species in general are part of gelatinous zooplankton, which are otherwise neglected and misunderstood because of difficulties in the sampling, handling and preservations as well as species identification (Raskoff et al., 2013). There are some studies indicating the potential for using bioluminescent flash kinetics as a method for discriminating zooplankton species (Nealson et al., 1986; Johnsen et al., 2014; Moline et al., 2013). Therefore, using instruments that are able to detect their emitted light creates a chance to identify them by their flash, but these methods have remained limited when it comes to number of species tested or spatial and temporal coverage. This study, however, used 69 individuals representing six taxa (*Beroe spp., Bolinopsis infundibulum, Clytia gracilis, Metridia longa, Nanomia cara* and Siphonophorae spp.) to test if species-specific flash kinetics were distinguishable. Based on the results of this study, it is clear that the variation within taxa in flash kinetics and their ability to emit light from mechanical stimulation such as the pump impeller has been underestimated in earlier research. Over 150 individuals from this dataset belonging to species that were supposed to be bioluminescent did not emit light, and several individuals belonging to some taxa were not affected by the mechanical stimulation. However, the different taxa emitting light did in some cases show a unique pattern of flash kinetics compared to other taxa.

Be aware that this is a replication study of the work done in Johnsen et al. (2014), which was somewhat a pilot study of introducing bioluminescence flash kinetics approach on gelatinous zooplankton. In general, there is limited research done on this topic and even less on taxa's flash kinetic examined in this study.

4.1 Taxa-specific bioluminescence

Bioluminescence is found across most major phyla (Widder, 2010) and a minimum of five phyla contain bioluminescent species are present along the Norwegian coast and around the Svalbard region. These five phyla represented in this study presented minimum eleven taxa's flash kinetics that were put into a library with 120 measured individuals. Originally, the library created during laboratory experiment was planned to be on a species level, but with only morphological identification available, the classifications ended up being mixed level.

The flash kinetics of the six most dominant taxa in this study showed variation both within each group and between each other (Fig. 4.1, for variation between taxa, see Fig. 3.1). From the appearance of the shape of the flashes of individuals within a taxon, it was possible to separate some taxa from each other such as *M. longa* to *N. cara*. In addition,

species belonging to same order showed to emit significantly different flash kinetics compared to each other in this study. For example, *N. cara* belong to the order Siphonophorae and their flash kinetics showed a smooth rise with a slow decay. *N. cara* kinetics were clearly different than those of the Siphonophorae spp., which can contain several different species. Siphonophorae spp. had a faster flash kinetics with a bumpy decay. From these results, it is possible to say that there are differences that can potentially make it possible to distinguish within the order. However, it is important to note that some individuals emitted light that differed completely from the majority, which made it hard to know if there were disruptive background signals, damage to the individual, species emitting kind of different light than the rest or because of another unknown reason. The results of the flash kinetics from *C. gracilis* showed some flashes that looked abnormal compared to the majority. In other words, some individuals had multiple peaks and/or completely different start and endings to the flash when compared to the majority of individuals measured of *C. gracilis*. The species *C. gracilis* showed a lot of movements and tended to change its shape of body drastically when moving around and in some of the movements, *C. gracilis* increased its length (seen by eye) which could potentially have an effect on how the light emitted from the species when having such a different body shape compared to its circular shape. There is little research available on *C. gracilis* and its bioluminescence.

Furthermore, distinguishing between data from the same genus but different species would be optimal for differing between species with similar characteristics, such as the *Beroe*. For instance, there have been found both *Beroe cucumis* and *Beroe gracilis* in Trøndelag as well as *Beroe abyssicola* (Johnsen, 2019) and *Beroe 'norvegica'* (Johansson, 2018) in Svalbard. *B. cucumis* and *B. gracilis* are both bioluminescent (Haddock & Case, 1995) and are likely to be damaged during sampling where identifying down to species level are impossible by morphology (Johansen, 2019). In other words, it would be ideal to see if it is possible to distinguishing between *B. cucumis* and *B. abyssicola* by their flash kinetic when morphology is not an option. In this study, the measured *Beroe* spp. showed varying flash kinetics but with minor differences. This can be an indicator of situations such as different species but same genus emitting light, background signal disturbing the measurement or damaged individual. *B. cucumis* (Ctenophora) often prey on other gelatinous zooplankton that often are bioluminescent, such as *Mnemiopsis leidyi* (Galil & Gevili, 2013). Therefore, it can occur that a *B. cucumis* have recently preyed on another bioluminescent species that emit light together with *B. cucumis* during mechanical stimulation. To what extent this situation could be realistic remains open for discussion.

Most of the taxa had a relatively fast flash kinetic with a smoother decay (Fig. 4.1), except for *N. cara* that had a slower start of the flash. The dataset of *Beroe* spp. in this study showed a smooth shape with a reasonable fast flash kinetic on most of the measured individuals. In contrast, earlier research found that *B. cucumis* had a jagged curve decay (Johnsen et al., 2014). The individuals of the measured Beroe spp. in this study are identified to genus level and not to species level because of difficulties with morphological identification. From Johnsen et al. (2014), the identification method of species is not described, which makes the accuracy of the identification questionable. A new species has been described around Norway and Svalbard, which have been initially described incorrectly as *B.* cucumis but is actually another species named *B. 'norvegica'* in Johansson et al. (2018). Therefore, the likelihood that the *B. cucumis* measured in Johnsen et al. (2014) is actually a *B. 'norvegica',* is there. From the results in this study, *B. infundibulum* showed a bumpy curve decay, which can be an indicator of pulsating light production where the specimen gave out varying light intensity throughout the emission. It was also the only

taxa that emitted light in the funnel before being fed into the UBAT and also when it exited the outlet into the mesh, which might have affected the flash on the three measurements it occurred.

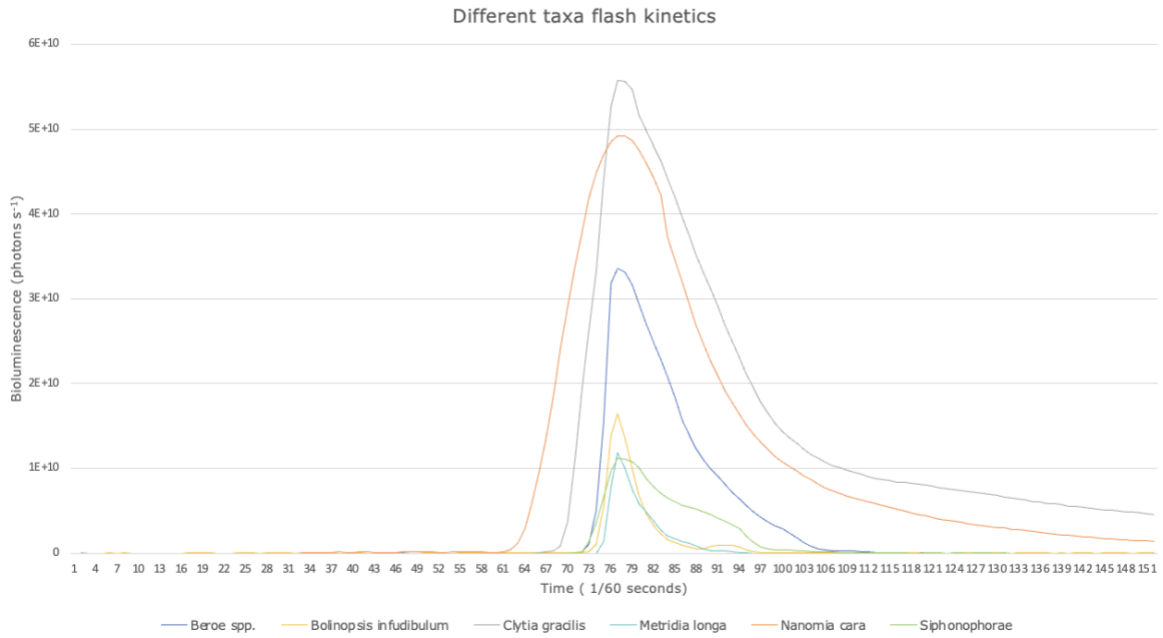


Figure 4.1 *The flash kinetics curves from the six dominant taxa in the study, representing the average curve of measured individuals.*

The intraspecific variation of the flash kinetic is likely due to, aforementioned, disruptive background signals from dinoflagellates, damaged individuals, or another reason not found in this study. The interspecific variation, on the other hand, might be because of taxa performing different bioluminescence functions, such as 1) evading or deterring predators, 2) obtaining prey or 3) intraspecific communication (Morin, 1983). For instance, *M. longa* produced most likely a delaying contact flash (Morin, 1983) to startle a potential predator with its fast and thin flash kinetic (from parameters T_max and T_high) and *N. cara*, which had a slow start and long flash kinetic, produced presumably a glow that can indicate several functions within defense, as misdirection, offense as lure prey and communication (Haddock et al., 2010). From these results, it indicates that it is possible to some extent figure out different functions used by targeted taxa. However, it is likely that in most datasets done on measuring bioluminescence flash kinetics and datasets to be created in the future will contain measurements with disturbances.

4.2 Power of the defined parameters

The results of the flash kinetic values were able to tell that the different parameters were quite affiliated with each other and the variation within a targeted taxa appeared to be high in some parameters, such as BL_max, Sigma_max and T_max while low in others such as T_high. The different max parameters (BL_max, Sigma_max and T_max) had the highest variations, where the standard deviation was high for the majority of taxa, which most likely had a correlation to the intensity of the flashes emitted among the individuals. *B. infundibulum* in this study is a great example of a taxon which had high flash kinetic value variation with standard deviations exceeding or being close to the mean in all

parameters. Great variation in the light emitted among the *B. infundibulum* and a small number of individuals (n=5) created a high standard deviation, and there might have been another outcome if more individuals would have been measured. The use of flash kinetic characteristics has shown to allow substantial discrimination in earlier studies and has proven to provide cleaner data about the organisms producing light than spectral data (Nealson et al., 1986). It is good to be aware that the parameter used in Nealson et al. (1986) were only Total flash time (TT), Rise time (RT) and Decay time (DT) compared to the parameters (BL_max, BL_mean, Sigma_max, T_max, T_high and T_decay) used in this study. From the results in Johnsen et al. (2014), where the parameters in this study were inspired from, it is believed that the parameters extracted from the flash kinetics show a substantial possibility to discriminate between species. However, there are some gaps in the study which can make the results questionable, such as the low number of individuals measured in laboratory experiment and not showing any intraspecific variation. In Johnsen et al. (2014), they have not shown variation among flash kinetics emitted from same species, which can affect the values of the flash kinetics and give misleading results. In this study, errors from the extracted flash kinetic values under the parameters occurred, such as BL_mean where values gotten were 0 in a few measurements. As a consequence, error in smaller datasets is expected to have a substantial effect on the result compared to taxa with more individuals within the datasets. For example, comparing *Beroe* spp. with 33 individuals measured towards *M. longa* four individuals, where both datasets had two errors would have more impact on the results for *M. longa* than *Beroe* spp.

If the recording of a measurement during laboratory experiment or in a setting where one manually starts the recording is stopped or the animal is expelled from the device before flash from organisms is finished, the parameter T_decay will not have any power and give the flash kinetic value a 0. The value will be at 0 because of the lack of an ending to the flash. In this study, this happened during a measurement of *Mertensia Ovum*, which had an abrupt ending to its flash. In all probability, this is a situation that will rarely occur as long as one is aware of it. All in all, this study shows that the defined parameters have more power together with larger datasets, but still need improvement to remove errors in analysing.

4.3 Comparing the achieved parameter values with previous studies

Comparing data collected with the flash kinetic characteristics in this study and data from the limited research available, showed a clear difference. In the article by Johnsen et al. (2014), some of the same species and genera were measured with the same parameters. For example, *M. longa* used in this study had a lot higher BL_max, BL_mean and Sigma_max than in Johnsen et al. (2014), while T_high and T_decay was similar and T_max a lot lower in this study. The collected specimens were all from the Arctic in the Barents Sea, with this study having species collected in November 2022 and Johnsen et al. (2014) in January 2013. It removes the suspicion of differences between environments but increases it between seasonal change. The sampling in November occurred at the start of the polar night, meaning that these species were exposed to light more recently than the ones collected in January, deep into the polar night. In Johnsen et al. (2014), they measured 12 *M. longa*, compared to only four in this study. It is thus unknown why the results of these datasets are so unsimilar, but the higher maxima of parameters in this study, show that the specimens emitted more light compared to the measured *M. longa* in

Johnsen et al. (2014). Freshly caught *M. longa* can have a reduced bioluminescence capacity because of the mechanical stimulation they have been put through during sampling and sorting and use up to ten hours to recover (Buskey & Stearns, 1991). If specimens of *M. longa* were not completely recovered, the bioluminescence capacity of the species might have been reduced during measurements. It is unknown if this is the case in these results.

The measured *B. cucumis* in Johnsen et al. (2014) had overall significantly higher characteristics in all parameters compared to the measured *Beroe* spp. in this study. There were only five individuals measured in Johnsen et al. (2014) and 33 individuals measured in this study. All of the 33 were collected in Hopavågen while the five in Johnsen et al. (2014) were collected in the Svalbard region (Kongsfjorden), indicating that there might be differences across environments. *B. cucumis* collected around Svalbard were found to be significantly bigger than along the Norwegian coast (Johnsen, G., pers. comm.), leading to issues running individuals through the UBAT without them being clogged. The mean size of *Beroe* spp. in this study measured, was at 11.06 mm. There were individuals of *Beroe* spp. measured in the study around the size of 50 mm, but they emitted light at such high intensity that the sensor got saturated. The biggest individuals, otherwise, were at 40 mm, which emitted an average flash intensity for *Beroe* spp. The individuals with the highest intensity flash emitted on the other hand were 9 mm in size where the next highest were as small as 4 mm. From these results, size does not matter but should be investigated further to see if it actually does with more data to compare and to see if it is just a coincidence or not. The dataset from Berge et al. (2012) was reanalyzed in Moline et al. (2013), where three main clusters of flashes representing three different groups of bioluminescent taxa were distinguished based on their maximum intensity of their flash, flash duration and rise time. From the results in Moline et al. (2013), they suggest the measuring of bioluminescent organisms with their technique to be an effective way to distinguish between different size classes within zooplankton community as well as define the dinoflagellates portion but need more testing to conclude. In other words, the results from this study contradict the results in Moline et al. (2013), however, there is a lack of data to state if size matters for the intensity of the flash or not in this study.

4.4 Identifying taxa from *in situ* data

Throughout all the seven *in situ* measurements, *M. longa* based parameters extracted the greatest number of flashes. In Johnsen et al. (2014), about 50% of total bioluminescent flashes in both their measurements matched to *M. longa*. These result give the impression of a species with high abundance and active emission of light. *M. longa* was the species that emitted the least amount of light in the laboratory measurements in this study, with only 10% of the collected individuals emitting light. The deployments of the *in situ* measurements were also done in Hopavågen, where only a few *M. longa* were collected and none of them emitted light in the laboratory experiments. This indicates that there were flashes which were not emitted by *M. longa*, but by a species which have similar flash kinetic characteristics. Another possibility is that *M. longa* were missed in the net samples collected in this study. On the contrary, *N. cara* had the most conspicuous and significantly different flash kinetics of the measured taxa but no flashes matching their kinetic parameters were extracted from the *in situ* data. Although all the individuals of this species in this study were collected in Kongsfjorden at Svalbard, they have been found in Trøndelag, specifically seen in the inner part of Trondheimsfjorden in Inderøy commune

(Artskart.artsdatabanken.no, 2023). From the observation of *N. cara* in Trøndelag, there is a likely chance of it being present in Hopavågen as well. Aforementioned, *in situ* measurements were conducted in Hopavågen, where there were no *N. cara* individuals collected throughout the study, which indicates that there were no *N. cara* in Hopavågen during the deployments or the species were missed in the sampling.

High bioluminescence activity during measurements made it hard to extract flashes without having disruptive background noise. A typical time series conducted in coastal waters might have high activity of light, where individual flashes cannot be isolated (Messie et al., 2019). Therefore, the *split* parameter defining a baseline was added to separate possible dinoflagellates constantly emitting light in the UBAT and potential zooplankton. This is a proxy calculation from Messié et al. (2019), where all the continuous background noise is generated by dinoflagellates and individual flashes by zooplankton. During this study, there were no measurements of known dinoflagellates, only background signal that is assumed to be dinoflagellates. Earlier research has suggested that a similar technique of research is an effective mean to separate between size classes within zooplankton community and dinoflagellates (Moline et al., 2013), which supports the proxy of the activity in the measurements.

During analysing of *in situ* data, flashes matching the kinetics of several taxa were extracted. However, same flashes were extracted by using the parameters for two different taxa. For example, using the parameters of *Beroe* spp. resulted in the same flashes being extracted as *C.* gracilis, and *B. infundibulum* parameters extracted the same flashes as *M. longa*. These results indicate that the flash kinetic values from the different parameters, T_max and T_high, added into the code, were too similar to each other and therefore extracted the same flashes. Also, it might be that there is a need for more parameters used to extract flash kinetics than only the two T_max and T_high. In addition, flashes with multiple peaks were still occurring. They were labeled as unclassified peaks since it most likely represented more than one individual - possibly belonging to different taxa - emitting light at the same time. Unfortunately, the majority of the flashes extracted from the *in situ* data had unclassified peaks, which made it difficult to match them with any taxa.

4.5 Limitations of the method

4.5.1 Determining the taxa specific signals

There were a few challenges during laboratory measurement throughout the study that need to be addressed. The measurements conducted in the laboratory of the Sletvik Field Station had seawater from pipes that came directly from Hopavågen. Despite this water being filtered with a sieve before pouring it into the experiment aquarium with the UBAT, there was still some disruptive background signal during test runs and the measurements of the individual taxa. Despite careful analysis of the data, it remained uncertain if the background noise had any effect on the results on some of the measurements, as there could have been some dinoflagellates or other phytoplankton going into the detection chamber and emitting light at the same time as the individual taxa that were fed to the UBAT. This kind of background noise can affect the reference measurements also in possible later studies and needs to be taken into account.

Another challenge was to actually get the specimens to emit light. Over 276 specimens were collected within different taxon known to be producing bioluminescence (Table 3.2). However, only 120 of them successfully emitted enough light above threshold b. In this study, *M. longa* was the species which had most individuals collected throughout the whole study but ended up having only 10% of the individuals emitting light. The hardest part with *M. longa* was to mechanically stimulate them to produce light in the UBAT. There can be several reasons for this but first and most importantly, light emission as seen in nature may not be shown the same way conducting laboratory stimulation (Haddock et al., 2010). In other words, it is possible that the stimulation *M.* longa is exposed to in nature differs from the mechanical stimulation in this study. On the other hand, it can be discussed that the specimens were too small and did not hit or became affected by the pump impeller that mechanically stimulated them during the experiment. For that reason, there were several tries with the *M. longa* where different stimulation forms were tested before it went into the UBAT with no luck, such as exposing it to freshwater at the moment it went in the inlet of the UBAT. Aforementioned, the bioluminescence capacity might also be reduced after mechanical stimulation (Buskey & Stearns, 1991) and without enough recovery, intensity of the flash might be affected and the reason for lack of emitted light from the *M. longa.*

A third problem was a challenge associated with the type of organisms used in the study. The Arthropoda individuals measured seemed to have more difficulties to emit light than gelatinous zooplankton from the mechanical stimulation. However, as the majority of the taxa were gelatinous zooplankton, which are often very fragile and get easily damaged (Hamner et al., 1975; Haddock, 2004; Condon et al., 2012). The likelihood of them being damaged from the pump impeller in the UBAT that are there for mechanical stimulation was high. In some cases, it can be discussed if the animal had tissue entering the detection chamber after being damaged by the pump impeller later than the animal itself and emitted light, that the flash kinetic from the light emitted from the animal might have been affected. Another challenge with these gelatinous zooplankton, are the sampling procedures. Some species within are more robust than others when it comes to handling, and some get destroyed along the way (seen by eye). It is uncertain if the damaged species emit light different than they would have in a "perfect" shape, which is something for future reference to test out if possible.

The UBAT is not created to measure big specimens and is therefore limitational for the size of organisms that can be measured. The inlet and the funnel through the UBAT are small in size and organisms over a certain size might clog it. However, there were no individuals measured during this study that clogged the UBAT. Clogging could happen during *in situ* deployments if the UBAT is to be deployed in an area with seaweed or other stuff that can clog it along the way. In addition, there is a limited range of light intensities the UBAT is able to measure. If the species produce more intense light than the UBAT can handle, the PMT will turn itself off as a self-defense and data will be saturated. It is impossible to know what kind of species went through the device based on that kind of data.

4.5.2 Applying the method for *in situ* data

The high activity of bioluminescence during *in situ* deployments were not excepted in this quantity, where datasets contained heaps of messy signals and extremely variable baseline activity. As a result, values on the thresholds a and b, as well as the split (baseline) needed to be adjusted manually for each *in situ* dataset when analysing. Aforementioned, there

were overlap in defined parameters between taxa, implying that there was low specificity. In addition, high variability in parameter values within taxa occurred, measured by the same device. It is uncertain if there would be higher variability with the use of different bathyphotometer during measurements.

4.6 Suitability of the method in biodiversity assessments

The end goal of a research on this level is to implement an identification method that can be used in both small- and large-scale operations together with other instruments or platforms to include species that are otherwise overlooked. The combination of a bathyphotometer, UBAT in this case, on an autonomous platforms with other sensors as an underwater imaging sensor (Picheral et al., 2021) would be optimal for assessing for example community of zooplankton and/or phytoplankton, as well as assess what kind of taxa that are in the area. Autonomous platforms can reach great depth, where a bathyphotometer can assess diversity among bioluminescent organisms in the deep sea with sensors like the underwater imaging sensor (UVP6) size ranging the organisms. For this to be realistic, the conditions need to be sufficient for *in situ* measurements as it has proven not to be in coastal waters (Messiè et al., 2019), as well as an already taxa-specific library created to have characteristics to proceed from. Examples on underwater platforms that are able to have sensors on it are Autonomous Underwater Vehicle (AUV) (Berge et al., 2012), Remotely Operated Vehicle (ROV) (ROV Aurora, no date) and an instrumentation rig that can be placed on the seabed (OceanLab Node 1: Subsea Facility, no date).

To assess seasonal variations within bioluminescent organisms, attaching the UBAT on a buoy deployed year around (OceanLab Observatory, no date) would hopefully create datasets of seasonal pattern and together with other sensors such as fluorescent, address the effect from for example atmospheric light on bioluminescence or how big of a contributor to "light in the dark" is the bioluminescent organisms and to see if there are differences with salinity and/or temperature.

4.7 Ways forward

The challenge during identification of individuals after sampling effected the taxa-specific library created during the study, where it ideally should have been species-specific. Further development of the library should restrict the identification level on species, to hopefully avoid any mixed light emitted. Aforementioned, identifying down to species level can be difficult with only morphology, however, including molecular ID as DNA barcoding in the process should make it possible. For instance, having a net or a sampler on the outlet of the UBAT and preserve individuals already run in the UBAT in ethanol to barcode afterwards. It will most likely be a more time consuming method, but also a lot more precise. In addition, creating a species-specific library from scratch only need to happen once, unless there are differences across environment in the same species. Ideally, the data already existing and future data should be stored in an open database.

If there are still intraspecific variation on species level, testing and comparing different conditions should be carried out to get reliable image of how constant light is emitted by bioluminescent organisms. Conditions like temperature, pH, seasons and locations might play a role in the emitted light by bioluminescent organisms. As well as light exposure

history, in the sense of how much light they have been exposed to before the measurement, handling history, as the sampling and sorting and the size, sex or stage of the specimens.

For the extraction of flash kinetics and its values in the different parameters, a large amount of individuals should be measured for each species used in the study. The likelihood of small errors happening during analyses are on this point of the research high. As a result, datasets containing few individuals will have higher standard error than other datasets with substantially higher number of individuals. If it was known that there would be this many differences within taxa's emitted light, more individuals would have been measured from the taxa that had high abundance in Hopavågen. Furthermore, it should be a higher number of individuals if it is possible, to get as many undisturbed measurements as possible. During this study, there were set a limit on 30 individuals that emitted light for the taxa when there was high abundance of a species in the samples, which limited the results to some extent. In addition, measure species across environments to see if the bioluminescent fingerprint is constant should also be done with high number of individuals for the same reasons as with analyses errors and to see if there is a trend and not just coincidence. As for the *in situ* data collection, experiments around deployments conducted simultaneously should be taken into account. As a result, it makes it possible to verify the actual species present around deployments.

To improve the *in* situ recognition of zooplankton, the current method for analysing should be considered developed, especially for the *in situ* where a dynamic baseline and more parameters should be included to the extraction of flashes or for instance, create species-specific templates for the whole flash based on the flash kinetics. Also, conducting deployments in areas where there is less activity from bioluminescent dinoflagellates that continuously emit light will probably create a dataset where isolating zooplankton flashes will be easier, such as deep sea areas and in the Arctic.

And lastly, to improve species identification, sampling, sorting and the use of flash kinetics will be needed more thorough work. Using flash kinetic and spectral characteristics together have allowed in earlier research discrimination and identification of species with a high degree of accuracy (Nealson et al., 1986). Combining these two techniques in future research can potentially make it easier to especially distinguish between species and hopefully identify them.

5 Conclusion

The goal of the study was to create a library of bioluminescent fingerprint based on the flash kinetics and to evaluate the potential of these fingerprints for *in situ* zooplankton recognition. In general, using flash kinetic from bioluminescent organisms as an identification method needs to be studied more but despite limitations, the UBAT can be a great tool to distinguish between some taxa. Measurements of single flashes in laboratory appeared to be sufficient to the extent of getting gelatinous zooplankton to emit light. The creation of a taxa-specific library was possible since there were 120 individuals emitting light during recordings. Taxa with over five measured individuals in this study except for *Nanomia cara* had something interfering with the flashes, but if one disregard the intraspecific variation on the flash kinetic in the different taxa, the interspecific variation showed possibility to distinguish between some taxa from their flash kinetics. The parameters in this study extracted values of differences between taxa but showed that there are many similarities between some taxa. As a result, extracting targeted taxa in larger datasets was hard with the use of flash kinetic values extracted from specific parameters. However, it was possible to isolate single flashes from *in situ* data and with some fine adjustments, extract flashes from targeted species should be possible.

Johnsen et al. (2014, p. 713) defined the result in their study as "bioluminescence flash kinetics of arctic zooplankton has clear species-specific characteristics that allow for *in situ* identification of species". In this study as of now, the method appears to be insufficient in terms of identifying species from *in situ* but as the approach becomes more adjusted and better defined, it will hopefully provide better understanding of possible diversity mapping of communities and species identifications. The questions "who?" and "how?" was thus not answered today, but hopefully will be in the future with new approaches to identification methods to limit the biodiversity crisis we are facing.

6 References

Aarnes, H. (2019) *Bioluminescens*. Available at: https://snl.no/bioluminescens (Accessed: 16.01.22)

Artskart.artsdatabanken.no 24.05.23. Discovered data for *Nanomia cara* from Norsk entomologisk forening, so2-bugs, 21064104

Aylagas, E., Borja, A., Muxika, I., & Rodriguez-Ezpeleta, N. (2018) Adapting metabarcoding-based benthic biomonitoring into routine marine ecological status assessment networks, *Ecological Indicators*, 95, pp. 194-202. doi: 10.1016/j.ecolind.2018.07.044

Berge, J., Båtnes, A. S., Johnsen, G., Blackwell, S. M., & Moline, M. A. (2012) Bioluminescence in the high Arctic during polar night, *Marine Biology,* 159, pp.231-237. doi: 10.1007/s00227-011-1798-0

Berge, J., Geoffroy, M., Daase, M., Cottier, F., Priou, P., Cohen, J.H., Johnsen, G., McKee, D., Kostakis, I., Renaud, P.E., Vogedes, D., Anderson, P., Last, K.S., & Gauthier, S. (2020) Artificial light during the polar night disrupts Arctic fish and zooplankton behavior down to 200 m depth, *Communications Biology*, 3(1). doi: 10.1038/s42003-020-0807-6

Bucklin, A., Peijnenburg, K. T. C. A., Kosobokova, K., & Machida, R. J. (2021) New insight into biodiversity, biogeography, ecology, and evolution of marine zooplankton based on molecular approaches, *ICES Journal of Marine Science*, 78(9) pp. 3282-3287. doi: 10.1093/icesjms/fsab198

Buskey, E. J. & Sterns, D. E. (1991) The effects of starvation on bioluminescence potential and egg release of the copepod Metridia longa, *Journal of Plankton Research*, 13(4), pp. 885-893. doi: 10.1093/plankt/13.4.885

Butchart, S. H. M., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J. P. W., Almond, R. E. A., Baillie, J. E. M., Bomhard, B., Brown, C., & Watson, R. (2010) Global Biodiversity: Indicators of Recent Declines, *Science*, 328(5982), pp. 1164-1168. doi: 10.1126/science.1187512

Ceballos, G., Ehrlich, P. R., Barnosky, A. D., García, A., Pringle, R. M., & Palmer, T. M. (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction, *Science advances*, 1(5), pp.1-5. doi: 10.1126/sciadv.1400253

Chalfie, M. (1995) Green fluorescent protein. *Photochemistry and Photobiology*, 62(4), pp. 651-656. doi: 10.1111/j.1751-1097.1995.tb08712.x

Chen, S., Gan, S., Hu, L., Bi, R. & Gao, Y. (2023) Effects of typical marine environmental factors on the bioluminescence intensity of individual Noctiluca scintillans, *Optics Express*, 31(8), pp. 12114-12127. doi: 10.1364/OE.485445

Chiarucci, A., Bacaro, G. & Scheiner, S. M. (2011) Old and new challenges in using species diversity for assessing biodiversity, *Biological science*, 366(1576), pp. 2426-2437. doi: 10.1098/rstb.2011.0065

Chivian, E., & Bernstein, A. (2010) *How our Health Depends on Biodiversity*. Center for Health and the Global Environment, Harvard Medical School. Available at: https://www.bu.edu/sph/files/2012/12/Chivian_and_Bernstein_2010_How_our_Health_Depends_on_Biodiversity.pdf (Accessed 09.03.23)

Condon, R., Graham, W. M., Robinson, K. L., Duarte, C. M., Pitt, K. A., Lucas, C. H.,

Haddock, S. H., Sutherland, K. R., Dawson, M. N., Decker, M. B., Mills, C. E., Purcell, J. E., Malej, A., Mianzan, H., Uye, S., Gelcich, S., & Madin, L. P. (2012). Questioning the rise of gelatinous zooplankton in the world's oceans, *Bioscience*, 62(2), pp. 160-169. doi: 10.1525/bio.2012.62.2.9

Convention on Biological Diversity (2020) *Aichi Biodiversity Targets*. Available at: https://www.cbd.int/sp/targets/ (Accessed: 29.03.23)

Costa, H., Foody, G. M., Jimenez, S., & Silva, L. (2015) Impacts of Species Misidentification on Species Distribution Modeling with Presence-Only Data, *ISPRS International Journal of Geo-Information*, 4, pp. 2496-2518. doi: 10.3390/ijgi4042496

Cronin, H. A., Cohen, J. H., Berge, J., Johnsen, G., & Moline, M. A. (2016) Bioluminescence as an ecological factor during high Arctic polar night, *Scientific Reports*, 6, 36374. doi: 10.1038/srep36374

Danovaro, R., Carugati, L., Berzano, M., Cahill, A. E., Carvalho, S., Chenuil, A., Corinaldesi, C., Cristina, S., David, R., Dell'Anno, A., Dzhembekova, N., Garces, E., Gasol, J. M., Goela, P., Feral, J., Ferrera, I., Forster, R. M., Kurekin, A. A., Rastelli, E., Marinova, V., Miller, P. I., Moncheva, S., Newton, A., Pearman, J. K., Pitois, S. G., Rene, A., Rodriguez-Ezpeleta, N., Saggiomo, V., Simis, S. G .H., Stefanova, K., Wilson, C., Lo Martire, M., Greco. S., Cochrane, S. K. J., Magoni, O., & Borja, A. (2016) Implementing and Innovating Marine Monitoring Approaches for Assessing Marine Environmental Status, *Frontiers in Marine Science*, 3(213), doi: 10.3389/fmars.2016.00213

Davies T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2014) The nature, extent, and ecological implications of marine light pollution, *Frontiers in Ecology and the Environment*, 12, pp. 347-355. doi: 10.1890/130281

Davis, J. W., Thosteson, E. D., Widder, E. A., & Frey C. L. (2005) Examination of Bioluminescent Excitation Response Using Empirical Orthogonal Function Analysis, *OCEANS 2005*, 1. doi: 10.1109/OCEANS.2005.1639863

Erwin, T. L. (1982) Tropical Forests: Their Richness in Coleoptera and Other Arthropod Species, *The Coleopterists Bulletin*, 36(1), pp. 74-75. Available at: http://www.jstor.org/stable/4007977 (Accessed 19.04.23)

European Commission (2020) *EU Biodiversity Strategy for 2030*. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:52020DC0380

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C., Elvidge, C. D., Baugh, K., Portnoc, B. A., Rybnikova, N. A. & Furgoni, R. (2016) The new world atlas of artificial night sky brightness, *Science Advances*, 2(6). doi: 10.1126/sciadv.1600377

Freed, L. L., Easson, C., Baker, L. J., Fenolio D., Sutton, T. T., Khan, Y., Blackwelder, P., Hendry T. A., & Lopez, J. L. (2019) Characterization of microbiome and bioluminescent symbionts across life stages of Ceratioid Anglerfishes of the Gulf of Mexico, *FEMS Microbiology Ecology*, 95(10). doi:10.1093/femsec/fiz146

Friedham, S. (2016) Comparison of Species Identification Methods DNA Barcoding versus Morphological Taxonomy, *Marine Ecology and Evolution*, 1, pp. 74-86. Available at: https://scholarspace.manoa.hawaii.edu/server/api/core/bitstreams/8cfe8674-d1aa-4965-ba74-79fbf3c1e2cd/content

Galil, B. S. & Gevili, R. (2013) A moveable feast: *Beroe cucumis sensu* Mayer, 1912 (Ctenophora; Beroida; Beroidae) preying on *Mnemiopsis leidy*i A. Agassiz, 1865. (Ctenophora; Lobata; Bolinopsidae) off the Mediterranean coast of Israel, *BioInvasions Records*, 2(3), pp. 191-194. doi: 10.3391/bir.2013.2.3.03

Haddock, S. H. D. (2004) A golden age of gelata: past and future research on planktonic

ctenophores and cnidarians, *Hydrobiologia*, 530, pp. 549-556. doi: 10.1007/s10750-004-2653-9

Haddock, S. H. D. & Case, J. (1999) Bioluminescence spectra of shallow and deep-sea gelatinous zooplankton: ctenophores, medusae and siphonophores, *Marine Biology*, 133, pp. 571-582. doi: 10.1007/s002270050497

Haddock, S. H. D. & Case, J. (1995) Not all Ctenophores Are Bioluminescent: Pleurobrachia, *Biological Bulletin*, 189(3), pp. 356-362. doi: 10.2307/1542153

Haddock, S. H. D., Moline, M. M., & Case, J. F. (2010) Bioluminescence in the Sea, *Annual Review of Marine Science*, 2, pp. 443-493. doi: 10.1146/annurev-marine-120308-081028

Hamner, W. M., Madin, L. P., Alldredge, A. L., Gilmer, R. W. & Hamner, P. P. (1975) Underwater observations of gelatinous zooplankton: Sampling problems, feeding biology, and behavior, *Limnology and Oceanography*, 20(6), pp. 907-917. doi: 10.4319/lo.1975.20.6.0907

Hamre, J. (1994) Biodiversity and exploitation of the main fish stocks in the Norwegian – Barents Sea ecosystem, *Biodiversity and conservation*, 3, pp. 473-492. doi: 10.1007/BF00115154

Hay, S. (2006) Marine Ecology: Gelatinous Bells May Ring Change in Marine Ecosystems, *Current Biology*, 16(17), pp. R679-R682. doi: 10.1016/j.cub.2006.08.010

Hays, G. C. (2003) A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migration, *Hydrobiologia*, 503, pp. 163-170. doi: 10.1023/B:HYDR.0000008476.23617.b0

Hays, G. C., Richardson, A. J. & Robinson, C. (2005) Climate change and marine Plankton, *Trends in Ecology & Evolution*, 20(6), pp. 337-344. doi: 10.1016/j.tree.2005.03.004

Herbert, P. D. N. & Gregory, T. R. (2005) The Promise of DNA Barcoding for Taxonomy, *Systematic Biology*, 54(5), pp. 852-859. doi: 10.1080/10635150500354886

Herring, P. J. (2000) Species abundance, sexual encounter and bioluminescent signalling in the deep sea, *Phil. Trans. R. Soc. Lond. B Biol. Sci.,* 355(1401), pp. 1273-1276. doi: 10.1098/rstb.2000.0682

Herring, P. J. (2007) Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea, *Journal of the Marine Biological Association of the United Kingdom*, 87(4), pp. 829-842. doi: 10.1017/S0025315407056433

Herring, P. J., & Widder, E. A. (2004) Bioluminescence of deep-sea coronate medusae (Cnidaria: Scyphozoa), *Marine Biology*, 146, pp. 39-51 doi: 10.1007/s00227-004-1430-7

Heywood, V. H. (1995) *Global Biodiversity Assessment*. United Nations Environment Programme. Cambridge University Press, Cambridge.

Hillis, D. M. (1987) Molecular versus morphological approaches to systematics, *Annual review of ecology and systematics*, 18, pp. 23-42. doi: 10.1146/annurev.es.18.110187.000323

Howe, J. A., Austin, W. E. N., Forwick, M., Paetzel, M., Harland, R., & Cage, A. G. (2010) Fjord systems and archives: a review, *Geological Society, London, Special Publications,* 344(1), pp. 5–15. doi: 10.1144/sp344.2

Husson, B., Eriksen, E., Jentoft, S., Boge, E., Borgå, K., Chierici, M., Christensen, J., Dupont, S., Fransson, A., Frøyen, E., Gonzalez Triginer, V., Hoff, S., Hylland, K., Jackson, M. C., Ji, W., Jones, E., Karlson, S., Krohn-Pettersen, G., Lemke, M., Lunde, A., Lutier, M., Macaulay, S., Majaneva, S., Marsden, L., Maurstad, M. F., Orr, J., Præbel, K., Rodes, N., Sandig, A., Sen, A., Stige, L. C., Søreide, J. E., Van Dinh, K., Waardal Heum, S., Wilhemsen, J. F. & Ingvaldsen, R. (2023) Polar cod connectivity

cruise 2022: Cruise report, *The Nansen Legacy Report Series,* 37. doi: 10.7557/nlrs.6944

IPBES (2019) Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Díaz, S., Settele, J., Brondízio E.S., Ngo, H. T., Guèze, M., Agard, J., Arneth, A., Balvanera, P., Brauman, K. A., Butchart, S. H. M., Chan, K. M. A., Garibaldi, L. A., K. Ichii, K., Liu, J., Subramanian, S. M., Midgley, G. F., Miloslavich, P., Molnár, Z., Obura, D., Pfaff, A., Polasky, S., Purvis, A., Razzaque, J., Reyers, B., Chowdhury, R., Shin, Y. J., Visseren-Hamakers, I. J., Willis, K. J., & C. N. Zayas (eds.). *IPBES secretariat*, Bonn, Germany. 56 pages. doi: 10.5281/zenodo.3553579

Ingvaldsen, R. & Loeng, H. (2009) Physical oceanography, in Sakshaug, E. (ed.) *Ecosystem Barents Sea*. Trondheim: Tapir Academic Press, pp. 33-64.

Institute of Marine Research (2021) *Topic: The ocean, coast and fjords*. Available at: https://www.hi.no/en/hi/temasider/ocean-and-coast/the-ocean,-coast-and-fjords (Accessed: 21.03.23)

Isbell, F., Tilman, D., Polasky, S., Loreau, M. (2014) The biodiversity-dependent ecosystem service debt, *Ecology Letters*, 18(2), pp. 119-134. doi: 10.1111/ele.12393

IUCN Red List (2020) *Summary Statistics*. Available at: https://www.iucnredlist.org/resources/summary-statistics (Accessed: 28.03.23)

Johansson, M. L., Shiganova, T. A., Ringvold, H., Stupnikova, A. N., Heath, D. D., & Maclsaac, H. J. (2018) Molecular Insight Into the Ctenophore Genus Beroe in Europa: New Species, Spreading Invaders, *Journal of Heredity*, 109(5), pp. 520-529. doi: 10.1093/jhered/esy026

Johnsen, E. (2019) *Ctenophore diversity along the Norwegian coast and Svalbard region*. Master's thesis. NTNU.

Johnsen, G., Candeloro, M., Berge, J. (2014) Glowing in the dark: Discriminating patterns of bioluminescence from different taxa during the Artic polar night, *Polar Biology*, 37(5), pp. 707-713. doi: 10.1007/s00300-014-1471-4

Jongsgård, Å. & Sømme, L. S. (2022) *Dyreplankton.* Available at: https://snl.no/dyreplankton (Accessed: 07.04.23)

Kahlke, T. & Umbers K. D. L. (2016) Quick Guide Bioluminescence. *Current Biology*, 26(8), pp. R313-R314 doi: 10.1016/j.cub.2016.01.007

Knudsen, C. (2023) *Den Nordatlantiske strømmen*. Available at: https://snl.no/Den_nordatlantiske_strømmen (Accessed: 21.03.23)

Korshunova, T., Picton, B, Furfaro, G., Mariottini, P., Pintes, M., Prkic, J., Fletcher, K., Malmberg, K., Lundin, K. & Martynov, A. (2019) Multilevel fine-scale diversity challenges the 'cryptic species' concept, *Scientific Reports*, 9(6732). doi: 10.1038/s41598-019-42297-5

Kühl, H. S., Bowler, D. E., Bösch, L., Bruelheide, H., Dauber, J., Eichenberg, D., Eisenhauer, N., Fernández, N., Guerra, C. A., Henle, K., Herbinger, I., Isaac, N. J. B., Jansen, F., König-Ries, B., Kühn, I., Nilsen, E. B., Pe'er, G., Richter, A., Schulte, R., Settele, R., van Dam, N. M., Voigt, M., Wägele, W. J., Wirth, C., & Bonn, A. (2020) Effective Biodiversity Monitoring Needs a Culture of Integration, *One Earth*, 3(4), pp. 462-474. doi: 10.1016/j.oneear.2020.09.010

Laffoley, D., Baxter, J. M., Day, J. C., Wenzel, L., Bueno, P., & Zischka, K. (2019) Marine Protected Areas, in Sheppard, C. (Ed.) World Seas: An Environmental Evaluation. Second edition, *Academic Press*, pp. 549-569. doi: 10.1016/B978-0-12-805052-1.00027-9

Lapota, D. & Loose, J. R. (1984) Observations of bioluminescence in marine plankton from the Sea of Cortez, *Journal of Experimental Marine Biology and Ecology*, 77, pp. 209-240. doi: 10.1016/0022-0981(84)90121-7

Larigauderie, A. & Mooney, H. A. (2010) The Intergovernmental science-policy Platform on Biodiversity and Ecosystem Services: moving a step closer to an IPCC-like mechanisms for biodiversity*, Current Opinion in Environmental Sustainability*, 2(1-2), pp. 9-14. doi: 10.1016/j.cousust.2010.02.006

Latz, M. I., Frank, T. M., & Case, J. F. (1988) Spectral composition of bioluminescence of epipelagic organisms from the Sargasso Sea, *Marine Biology*, 98, pp. 441-446. doi: 10.1007/BF00391120

Madin, L. P. & Harbison, G. R. (2001) Gelatinous Zooplankton, in Steele, J. H. (Ed.) *Encyclopedia of Ocean Sciences*. 1st edition. Academic Press, pp. 1120-1130. doi: 10.1006/rwos.2001.0198

Marion, P.V. (1996) Ecological studies in Hopavågen, a landlocked bay in Agdenes, Sør Trøndelag, Norway, *Gunneria,* 71, pp. 1-39. Available at: http://hdl.handle.net/11250/228116

May, R. M. (2010) Tropical Arthropod Species, More or Less? *Science*, 329(5987), pp. 41-42. doi: 10.1126/science.1191058

Messie, M., Shulman, I., Martini, S. & Haddock, S. H. D. (2019) Using fluorescence and bioluminescence sensors to characterize auto- and heterotrophic plankton communities, *Progress in Oceanography*, 171, pp. 76-92. doi: 10.1016/j.pocean.2018.12.010

Meteorologisk institutt (2018) *Den nord-atlantiske oscillasjonen*. Available at: https://snl.no/Den_nord-atlantiske_oscillasjonen (Accessed: 21.03.23)

Moline, M. A., Blackwell, S. M., Case, J. F., Haddock, S. H. D., Herren, C. M., Orrico, C., & Terril, E. (2009) Bioluminescence to Reveal Structure and Interaction of Coastal Planktonic Communities, *Deep Sea Research Part II Tropical Studies in Oceanography*, 56(3-5), pp. 232-245, doi: 10.1016/j.dsr2.2008.08.002

Moline, M. A., Berge, J., Johnsen, G., Båtnes, A. S., & Blackwell, S. (2013) Bioluminescence flash kinetics characterize pelagic community structure, in Båtnes, A. S. (Ed.) *Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night.* Doctoral thesis. NTNU.

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., Worm, B. (2011) How Many Species Are There on Earth and in the Ocean? *PLOS Biology*, 9(8), doi: 10.1371/journal.pbio.1001127

Morin, J. G., Cohen, A. C. (2010) It's All about Sex: Bioluminescent Courtship Displays, Morphological Variation and Sexual Selection in Two New Genera of Caribbean Ostracodes, *Journal of Crustacean Biology*, 30(1), pp. 56-67. doi: 10.1651/09-3170.1

Morin, J. G. (1983) Coastal Bioluminescence: Patterns and Functions, *Bulletin of Marine Science,* 33(4), pp. 787-817. Available at: https://www.ingentaconnect.com/content/umrsmas/bullmar/1983/00000033/00000004/art00001?crawler=true (Accessed: 18.04.23)

Munaut, F., Van Hove, F. & Moretti, A. (2011) Molecular identification of mycotoxigenic fungi in food and feed, in: De Saeger, S. (Ed.) *Determining Mycotoxins and Mycotoxigenic Fungi in Food and Feed.* Woodhead Publishing, Cambridge, UK, pp. 298-331. doi: 10.1533/9780857090973.4.298

Navarro, L. M., Fernandez, n., Guerra, C., Guralnick, R., Kissling, W. D., Londono, M. C., Muller-Karger, F., Turak, E., Balvanera, P., Costello, M. J., Delavaud, A., Serafy, G. E., Ferrier, S., Geijzendorffer, I., Geller, G. N., Jetz, W., Kim, E., Kim, H., Marin, C.

S., McGeoch, M. A., … & Pereira, H. M. (2017) Monitoring biodiversity change through effective global coordination, *Current Opinion in Environmental Sustainability*, 29, pp. 158-169. doi: 10.1016/j.cousust.2018.02.005

Nealson, K. H., Arneson, A. C. & Huber, M. E. (1986) Identification of marine organisms using kinetic and spectral properties of their bioluminescence, *Marine Biology*, 91, pp. 77-83. doi: 10.1007/BF00397573

OceanLab Node 1: Subsea Facility (no date) Available from: https://www.ntnu.edu/oceanlab/node1 (Accessed: 27.05.23)

OcenLab Observatory (no date) Available from: https://oceanlabobservatory.no (Accessed: 27.05.23)

Parmentier, I., Duminil, J., Kuzmina, M., Philippe, M., Thomas, D. W., Kenfack, D., Chuyong, G. B., Craus, C., & Hardy, O. J. (2013) How Effective Are DNA Barcodes in the Identification of African Rainforest Trees? *PLOS One*, 8(4), e54921. doi: 10.1371/journal.pone.0054921

Pereira, H. M. & Cooper, H. D. (2006) Towards the global monitoring of biodiversity change, *Trends in Ecology & Evolution*, 21(3), pp. 123-129. doi: 10.1016/j.tree.2005.10.015

Pereira, H. M., Leadley, P. W., Proenca, V., Alkemade, R., Scharlemann, J. P. W., Fernandez-Manjarres, J. F., Araujo, M. B., Balvanera, P., Biggs, R., Cheung, W. W. L., Chini, L., Cooper, H. D.., Gilman, E. L., Guenette, S., Hurtt, G. C., Huntington, H. P., Mace, G. M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R. J., Sumaila, U. R. & Walpole, M. (2010) Scenarios for Global Biodiversity in the 21st Century, *Science*, 330(6010), pp. 1496-1501. doi: 10.1126/science.1196624

Picheral, M., Catalano, C., Brousseau, D., Claustre, H., Coppola, L., Leymarie, E., Coindat, J., Dias, F., Fevre, S., Guidi, L., Irisson, J. O., Legendre, L., Lombard, F., Mortier, L., Penkerch, C., Rogge, A., Schmechtig, C., Thibault, S., Tixier, T., Waite, A., & Stemmann, S. (2021) The Underwater Vision Profiler 6: an imaging sensor of particle size spectra and plankton, for autonomous and cabled platforms, *Limnology and Oceanography Methods*, 20(2), pp. 115-129. doi: 10.1002/lom3.10475

Purvis, A. & Hector, A. (2000) Getting the measure of biodiversity, *Nature* 405, pp. 212-219. doi: 10.1038/35012221

Ratikainen, I. I. (2021) *Biologisk mangfold*. Available at: https://snl.no/biologisk_mangfold (Accessed: 15.03.23)

Ratikainen, I. I. & Semb-Johansson, A. (2020) *Økosystem*. Available at: https://snl.no/økosystem (Accessed: 18.03.23)

Raskoff, K. A., Sommer, F. A., Hamner, W. M. & Cross, K. M. (2003) Collection and Culture Techniques for Gelatinous Zooplankton, *The Biological Bulletin*, 204(1), pp. 68-80. doi: 10.2307/1543497

Richardson, A. J., Bakun, A., Hays, G. C., & Gibbson, M. J. (2009) The jellyfish joyride: causes, consequences and management responses to a more gelatinous future, *Trends in Ecology and Evolution*, 24(6), pp. 312-322. doi: 10.1016/j.tree.2009.01.010

Robison, B. J., Reisenbichler, K. R. & Sherlcok, R. E. (2017) The Coevolution of Midwater Research and ROV Technology at MBARI, *Oceanography*, 30(4), pp. 26-37 Available at: http://www.jstor.org/stable/26367623

ROV Aurora (no date) Available from: https://www.revocean.org/vessel/rov/ (Accessed: 27.05.23)

Sáez, A. G. & Lozano, E. (2005) Body doubles, *Nature*, 433(111). doi: 10.1038/433111a

SAIV AS (no date) *CTD profiler Model SD204*. Available at: https://saiv.no/sd204-ctd-profiler (Accessed: 25.03.23).

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., & Lane, R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding, *Philos Trans R Soc Lond B Biol Sci*, 360(1462), pp. 1805-1811. doi: 10.1098/rstb.2005.1730

Seliger H., Fastie, W., & McElroy, W. (1968) Towable photometer for rapid area mapping of concentrations of bioluminescent marine dinoflagellates, *Limnology and Oceanography*, 14, pp. 806-813. doi: 10.4319/lo.1969.14.5.0806

Singh, T. S. (2002) The biodiversity crisis: A multifaced review, *Current Science*, 82(6), pp. 638-647. Available at: https://www.jstor.org/stable/24106689

Svendsen H., Beszczynska-Møller A., Hagen J. O., Lefauconnier B., Tverberg V., Gerland S., Børre Ørbæk J., Bischof K., Papucci C., Zajaczkowski M., Azzolini R., Bruland O., & Wiencke C. (2002) The physical environment of Kongsfjorden–Krossfjorden, an Arctic fjord system in Svalbard, *Polar Research*, 21(1), 133-166. doi: 10.3402/polar.v2lil.6479

Sælen, O. H., & Weber, J. E. (2023) *tidevann*. Available at: https://snl.no/tidevann (Accessed: 02.05.23)

Sætre, R. (2007) Coast/fjord water exchange, Aure, J., Asplin, L. & Sætre, R (ed). *The Norwegian Coastal Current – Oceanography and Climate*. Tapir Academic Press, Trondheim, pp. 115-125.

Tittensor, D. P., Walpole, M., Hill, S. L. L., Boyce, D. G., Britten, G. L., Burgess, N. D., Butchartm S. H. M., Leadley, P. W., Regan E. C., … & Ye, Y. (2014) A mid-term analysis of progress toward international biodiversity targets, *Science*, 346(6206), pp. 241-244. doi: 10.1126/science.1257484

Turner, J. R., White, E. M., Collins, M. A., Partidge, J. C & Douglas, R. H. (2009) Vision in lanternfish (Myctophidae): Adaptions for viewing bioluminescence in the deep-sea, *Deep Sea Research Part 1: Oceanographic Research Papers*, 56(6), pp. 1003-1017. doi: 10.1016/j.dsr.2009.01.007

United Nations (no date a) *Population*. Available from: https://www.un.org/en/global-issues/population (Accessed: 19.05.23)

United Nations (no date b) *Transforming our world: the 2030 Agenda for Sustainable Development*. Available from: https://sdgs.un.org/2030agenda (Accessed 11.04.23)

United Nations Environment Programme (1995) *Global Biodiversity Assessment*. Available at: https://wedocs.unep.org/20.500.11822/29355 (Accessed: 15.03.23)

Van der Loos, L. M., & Nijland, R. (2020) Biases in bulk: DNA metabarcoding of marine communities and the methodology involved, *Molecular Ecology*, 30, pp. 3270-3288. doi: 10.1111/mec.15592

Warrant, E. J. & Locket, N. A. (2004) Vision in the deep sea, *Biological Reviews*, 79, pp. 671-712. doi: 10.1017/S1464793103006420

Wassmann, P., Reigstad, M., Øygarden, S., & Rey, F. (2000) Seasonal variation in hydrography, nutrients, and suspended biomass in a subarctic fjord: Applying hydrographic features and biological markers to trace water masses and circulation significant for phytoplankton production, *Sarsia*, 85(3), pp. 237-249. doi: 10.1080/00364827.2000.10414576

Waters, C. M. & Bassler, B. L. (2005) Quorum Sensing: Cell-to-Cell Communication in Bacteria. *Annual Review of Cell and Developmental Biology*, 21, pp. 319-346. doi: 10.1146/annurev.cellbio.21.012704.1310001

Watson, J. & Zielinski, O. (2013) Bioluminescence in the sea, Moline, M. A., Oliver, M. J., Orrico, C., Zanveld, R., & Shulman, I. (ed.) *Subsea Optics and Imaging*. Woodhead Publisher, pp. 134-179. doi: 10.1533/9780857093523.2.134

Widder, E. A. (2010) Bioluminescence in the Ocean: Origins of Biological, Chemical, and

Ecological Diversity. *Science*, 328(5979), pp. 704-708. doi: 10.1126/science.1174269

Widder, E. A., Johnsen, S., Bernstein, S. A., Case, J. F., & Neilson, D. J. (1999) Thin layers of bioluminescent copepods found at density discontinuities in the water column. *Marine Biology*, 134, pp. 429-437. doi: 10.1007/s002270051559

Willig, M. R., Kaufman, D. M., & Stevens, R. D. (2003) Latitudinal gradients of biodiversity: Pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics*, 34, 273–309. doi: 10.1146/annurev.ecolsys.34.012103.144032

Wilson, E. O. (2017) Biodiversity research requires more boots on the ground, *Nature Ecology & Evolution*, 1, pp. 1590-1591, doi: 10.1038/s41559-017-0360-y

Wilson, T. & Hastings, J. W. (2013) Bioluminescence: Living Lights, Lights for Living. Cambridge, Massachusetts: Harvard University Press

World Wildlife Fund (2018) *Living Planet Report 2018: Aiming higher.* Available from: https://www.wwf.org.uk/sites/default/files/2018-10/wwfintl_livingplanet_full.pdf (Accessed: 18.03.23)

Xue, C., Chen, S., & Zhang, T. (2020). Optical proxy for the abundance of red Noctiluca scintillans from bioluminescence flash kinetics in the Yellow Sea and Bohai Sea, *Optics Express*, 28(17), pp. 25618-25632. doi: 10.1364/OE.400257

7 Appendix

Appendix A – MATLAB code for single flash kinetics from laboratory measurements

```
% uBAT data processing file V1

% S. Grant

clc

clear

%%%%%%%% Change Directory to folder containing ONLY uBat files

dinfo = dir(fullfile("/Users/gittekrohn-pettersen/Desktop/sea water 5 min*"));

n = numel(dinfo);

%% You can ignore this part (Line 9 - 30), it is just matlab import parameters %%%

% Set up the Import Options and import the data

opts = delimitedTextImportOptions("NumVariables", 72);

% Specify range and delimiter

opts.DataLines = [1, Inf];

opts.Delimiter = ",";

% Specify column names and types

opts.VariableNames = ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10", "Var11", "Var12", "VarName13", "VarName14", "VarName15",
"VarName16", "VarName17", "VarName18", "VarName19", "VarName20", "VarName21",
"VarName22", "VarName23", "VarName24", "VarName25", "VarName26", "VarName27",
"VarName28", "VarName29", "VarName30", "VarName31", "VarName32", "VarName33",
"VarName34", "VarName35", "VarName36", "VarName37", "VarName38", "VarName39",
"VarName40", "VarName41", "VarName42", "VarName43", "VarName44", "VarName45",
"VarName46", "VarName47", "VarName48", "VarName49", "VarName50", "VarName51",
"VarName52", "VarName53", "VarName54", "VarName55", "VarName56", "VarName57",
"VarName58", "VarName59", "VarName60", "VarName61", "VarName62", "VarName63",
"VarName64", "VarName65", "VarName66", "VarName67", "VarName68", "VarName69",
"VarName70", "VarName71", "VarName72"];

opts.SelectedVariableNames = ["VarName13", "VarName14", "VarName15",
"VarName16", "VarName17", "VarName18", "VarName19", "VarName20", "VarName21",
"VarName22", "VarName23", "VarName24", "VarName25", "VarName26", "VarName27",
"VarName28", "VarName29", "VarName30", "VarName31", "VarName32", "VarName33",
"VarName34", "VarName35", "VarName36", "VarName37", "VarName38", "VarName39",
"VarName40", "VarName41", "VarName42", "VarName43", "VarName44", "VarName45",
```

```
"VarName46", "VarName47", "VarName48", "VarName49", "VarName50", "VarName51",
"VarName52", "VarName53", "VarName54", "VarName55", "VarName56", "VarName57",
"VarName58", "VarName59", "VarName60", "VarName61", "VarName62", "VarName63",
"VarName64", "VarName65", "VarName66", "VarName67", "VarName68", "VarName69",
"VarName70", "VarName71", "VarName72"];

opts.VariableTypes = ["string", "string", "string", "string", "string", "string", "string",
"string", "string", "string", "string", "string", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double"];

% Specify file level properties

opts.ExtraColumnsRule = "ignore";

opts.EmptyLineRule = "read";

% Specify variable properties

opts = setvaropts(opts, ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10", "Var11", "Var12"], "WhitespaceRule", "preserve");

opts = setvaropts(opts, ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10", "Var11", "Var12"], "EmptyFieldRule", "auto");

opts = setvaropts(opts, ["VarName13", "VarName14", "VarName15", "VarName16",
"VarName17", "VarName18", "VarName19", "VarName20", "VarName21", "VarName22",
"VarName23", "VarName24", "VarName25", "VarName26", "VarName27", "VarName28",
"VarName29", "VarName30", "VarName31", "VarName32", "VarName33", "VarName34",
"VarName35", "VarName36", "VarName37", "VarName38", "VarName39", "VarName40",
"VarName41", "VarName42", "VarName43", "VarName44", "VarName45", "VarName46",
"VarName47", "VarName48", "VarName49", "VarName50", "VarName51", "VarName52",
"VarName53", "VarName54", "VarName55", "VarName56", "VarName57", "VarName58",
"VarName59", "VarName60", "VarName61", "VarName62", "VarName63", "VarName64",
"VarName65", "VarName66", "VarName67", "VarName68", "VarName69", "VarName70",
"VarName71", "VarName72"], "ThousandsSeparator", ",");

% Set Threshold levels to extract plots, threshold B is the important one

% at the moment

calib_coef=1.46e7;

threshold_a=10*calib_coef;

threshold_b=20*calib_coef;

% This loop opens each file, combines the individual lines into a long

% continuous measurement, finds the peaks above threshold b, and saves the

% data in a csv file. Some metadata, peak magnitude, duration etc. is also
```

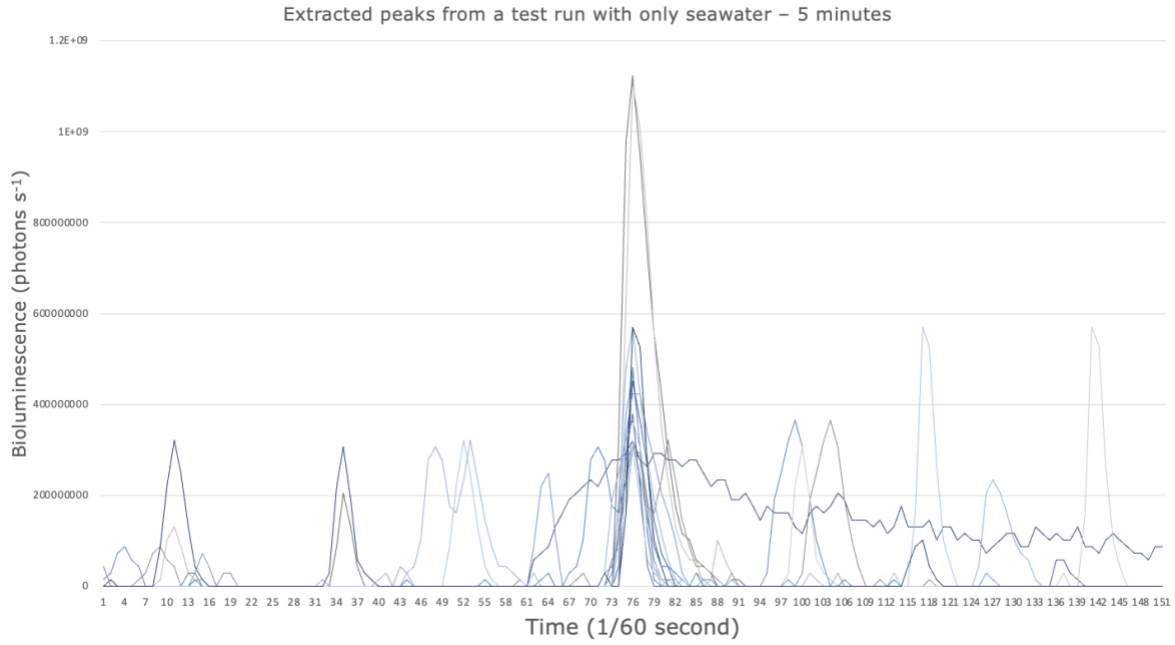
```
% saved in a metadata file for each peak extracted.
% If not peaks are above threshold_b the file is skipped.
for xx=1:n
    filename = fullfile(dinfo(xx).folder, dinfo(xx).name);
    ubat_data = readtable(filename, opts);
    % Linearize the ubat data, make 1 long data stream rather than 60 data
    % point chunks
    linear_ubat_data=reshape(table2array(ubat_data).',1,[]);
    linear_ubat_data=linear_ubat_data.*calib_coef;
    [pks,locs,wdt] = findpeaks(linear_ubat_data);
    threshold_b_index=find(pks>threshold_b&wdt>1);
    if isempty(threshold_b_index) == 1      %Skips files with no large enough peaks
        continue
    end
    peak_magnitude=pks(threshold_b_index);
    peak_position=locs(threshold_b_index);
    peak_duration=wdt(threshold_b_index)/60; % Peak duration is the peak width times
(1/60) seconds
    peak_magnitude_20=peak_magnitude.*0.2;
    % These are arrays of zeros to hold a variable in the next section.
    peaks=zeros(length(threshold_b_index),151);
    sigma_max=zeros(length(threshold_b_index),1);
    T_max=zeros(length(threshold_b_index),1);
    T_high=zeros(length(threshold_b_index),1);
    T_decay=zeros(length(threshold_b_index),1);
    start_points=zeros(length(threshold_b_index),151);
    T_high_points=zeros(length(threshold_b_index),151);
    % This for loop saves each peak as a line in variable peaks, the if
    % statement takes care of peaks at the begining and end of the long
    % data stream
    for ii=1:length(peak_position)
        if peak_position(ii)>=length(linear_ubat_data)-80
```

```
            peaks(ii,:)=padarray(linear_ubat_data(peak_position(ii)-
75:length(linear_ubat_data))',150-(length(linear_ubat_data)-(peak_position(ii)-
75)),'post');
            peak_point(ii)=76;
        elseif peak_position(ii)<=80
            peaks(ii,:)=padarray(linear_ubat_data(1:peak_position(ii)+75)',151-
(peak_position(ii)+75),'post');
            peak_point(ii)=peak_position(ii);
        else
            peaks(ii,:)=linear_ubat_data(peak_position(ii)-75:peak_position(ii)+75);
            peak_point(ii)=76;
        end
    % Sigma max is an integral of area below the curve from when the peak
    % rises to when it is at its maximum, this needs to be a bit better
    % defined but is ok for now.
    % Average magnitude is the average magnitude of the peak taken at the
    % full width half maximum (FWHM)
        %start_points=findchangepts(peaks(ii,:),"Statistic","rms",'MaxNumChanges',10);
        start_points = find(peaks(ii,:)>threshold_a);     %Finds points with values over the
lower threshold
        start_point(ii,1:length(start_points))=start_points;
        %[peak_mag,peak_point]=max(peaks(ii,:));     %Finds the location of the peak
        sigma_max(ii)=trapz(peaks(ii,start_points(1):peak_point));   %Adds up all values
from the first value over threshold a until the peak
        T_max(ii)=(peak_point(ii)-start_points(1))/60;   %Find the time between the first
value over threshold a until the peak


        T_high_points = find(peaks(ii,:)>peak_magnitude_20(ii));  %Finds points over 20%
of the peak magnitude
        T_high_point(ii,1:length(T_high_points))=T_high_points;
        T_high(ii)=(T_high_points(end)-T_high_points(1))/60;     %Finds the time between
the first and last point over the peak magnitude
        T_decay(ii)=(start_points(end)-T_high_points(end))/60;  %Find the time between
the last point over 20% and the last point over threshold a
average_magnitude(ii)=mean(peaks(start_points(1):start_points(end)));     %Finds the
average of all points over threshold a
```

```
    end
    %%%%% Uncomment this to see some plots of the peaks extracted for each
    %%%%% file.
    size_peaks=size(peaks);
    for ii=1:size_peaks(1)
        figure
        plot(peaks(ii,:),'LineWidth',1.5)
        xlabel('Sample Number (60Hz sampling rate)')
        ylabel('Digital Counts')
        hold on
        high_point=T_high_point(ii,:);
        high_point(high_point==0) = [];
        starts_point=start_point(ii,:);
        starts_point(starts_point==0) = [];
        xline(high_point(1),'g')
        xline(starts_point(1),'r')
        xline(high_point(end),'g')
        xline(starts_point(end),'r')
        xline(peak_point(ii),'b')
        yline(peak_magnitude_20)
        yline(average_magnitude)
    end
%%%%% This saves the data in a csv for each input file. The peaks are in one
%%%%% file and the metadata (Magnitude, 20% magnitude, duration, average magnitude,
and sigma max)


    savename = sprintf('Extracted_peaks_%s.csv',dinfo(xx).name);
    savename2 = sprintf('Extracted_peaks_metadata_%s.csv',dinfo(xx).name);
    meta                                                                    =
cat(2,peak_magnitude',peak_magnitude_20',average_magnitude',sigma_max,T_max,T_
high,T_decay);
    meta=array2table(meta);
```

```
    meta.Properties.VariableNames(1:7)                                    =
{'BL_max','BL_20%','BL_mean','Sigma_max','T_max','T_high','T_decay'};

    writematrix(peaks,savename)

    writetable(meta,savename2)

    clear average_magnitude

end
```

Appendix B – Peaks extracted from test runs with the UBAT containing only sea water



The figure proves the point of why it is needed a threshold b that defines a value below which a signal was not considered as a flash to avoid disruptive background signals in measurements. This test run were conducted at the laboratory of Sletvik field station.

Appendix C – MATLAB code for analysing *in situ* data

```
% uBAT in Situ Data (Excel Format) processing file V1.2

% S. Grant

clc

clear

%%%%%%%% Change Directory to folder containing ONLY uBat files

dinfo = dir(fullfile("/Users/gittekrohn-pettersen/Desktop/in situ/raw_run_21.002*.xlsx"));

n = numel(dinfo);

%% Set up the Import Options and import the data

opts = spreadsheetImportOptions("NumVariables", 70);

% Specify sheet and range

opts.Sheet = "raw_run_21";

opts.DataRange = "A2:BR5585";

% Specify column names and types

opts.VariableNames = ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10", "VarName11", "VarName12", "VarName13", "VarName14",
"VarName15", "VarName16", "VarName17", "VarName18", "VarName19", "VarName20",
"VarName21", "VarName22", "VarName23", "VarName24", "VarName25", "VarName26",
"VarName27", "VarName28", "VarName29", "VarName30", "VarName31", "VarName32",
"VarName33", "VarName34", "VarName35", "VarName36", "VarName37", "VarName38",
"VarName39", "VarName40", "VarName41", "VarName42", "VarName43", "VarName44",
"VarName45", "VarName46", "VarName47", "VarName48", "VarName49", "VarName50",
"VarName51", "VarName52", "VarName53", "VarName54", "VarName55", "VarName56",
"VarName57", "VarName58", "VarName59", "VarName60", "VarName61", "VarName62",
"VarName63", "VarName64", "VarName65", "VarName66", "VarName67", "VarName68",
"VarName69", "VarName70"];

opts.SelectedVariableNames = ["VarName11", "VarName12", "VarName13",
"VarName14", "VarName15", "VarName16", "VarName17", "VarName18", "VarName19",
"VarName20", "VarName21", "VarName22", "VarName23", "VarName24", "VarName25",
"VarName26", "VarName27", "VarName28", "VarName29", "VarName30", "VarName31",
"VarName32", "VarName33", "VarName34", "VarName35", "VarName36", "VarName37",
"VarName38", "VarName39", "VarName40", "VarName41", "VarName42", "VarName43",
"VarName44", "VarName45", "VarName46", "VarName47", "VarName48", "VarName49",
"VarName50", "VarName51", "VarName52", "VarName53", "VarName54", "VarName55",
"VarName56", "VarName57", "VarName58", "VarName59", "VarName60", "VarName61",
"VarName62", "VarName63", "VarName64", "VarName65", "VarName66", "VarName67",
"VarName68", "VarName69", "VarName70"];

opts.VariableTypes = ["char", "char", "char", "char", "char", "char", "char", "char", "char",
"char", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
```

```
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double", "double", "double",
"double", "double", "double", "double", "double", "double", "double"];

% Specify variable properties

opts = setvaropts(opts, ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10"], "WhitespaceRule", "preserve");

opts = setvaropts(opts, ["Var1", "Var2", "Var3", "Var4", "Var5", "Var6", "Var7", "Var8",
"Var9", "Var10"], "EmptyFieldRule", "auto");

% Import the data

ubat_data = readtable("/Users/gittekrohn-pettersen/Desktop/in situ/raw_run_21.002
excel.xlsx", opts, "UseExcel", false);

%% Clear temporary variables

clear opts

%%%% Set Parameters Here%%%%

% Split is the level at which the linear data is split into upper and lower

% parts

calib_coef=1.46e7;

split=800*calib_coef;

% Threshold levels to extract plots, threshold A is used for time calculations

% threshold B is for filtering which peaks to extract

threshold_a=1200*calib_coef;

threshold_b=5000*calib_coef;

% peak_size is the size of the extracted peak vector

% front and rear_window are the buffer zones at each end of the data

peak_size=101;

front_window=80;

rear_window=80;

% Search parameters for classification of species

%Beroe

beroe_T_max_hi=0.20;          beroe_T_max_lo=0.16;          beroe_T_high_hi=0.30;
beroe_T_high_lo=0.24;

%Clytia

clytia_T_max_hi=0.18;         clytia_T_max_lo=0.12;         clytia_T_high_hi=0.61;
clytia_T_high_lo=0.56;
```

```
%Metridia

metridia_T_max_hi=0.09;       metridia_T_max_lo=0.03;       metridia_T_high_hi=0.14;
metridia_T_high_lo=0.08;

%nanomia

nanomia_T_max_hi=0.83;       nanomia_T_max_lo=0.78;       nanomia_T_high_hi=0.70;
nanomia_T_high_lo=0.65;

%bolinopsis

bolinopsis_T_max_hi=0.30;    bolinopsis_T_max_lo=0.27;    bolinopsis_T_high_hi=0.33;
bolinopsis_T_high_lo=0.26;

% This loop opens each file, combines the individual lines into a long

% continuous measurement, finds the peaks above threshold b, and saves the

% data in a csv file. Some metadata, peak magnitude, duration etc. is also

% saved in a metadata file for each peak extracted.

% If not peaks are above threshold_b the file is skipped.

for xx=1

    % Linearize the ubat data, make 1 long data stream rather than 60 data

    % point chunks

    linear_ubat_data=reshape(table2array(ubat_data).',1,[]);

    linear_ubat_data=linear_ubat_data.*calib_coef;

    linear_ubat_data_lower=linear_ubat_data;

    linear_ubat_data_upper=linear_ubat_data;

    for x=1:length(linear_ubat_data)

        if linear_ubat_data(x)<split

            linear_ubat_data_upper(x)=split;

        elseif linear_ubat_data(x)>split

            linear_ubat_data_lower(x)=split;

        end

    end

    plot(linear_ubat_data_upper)

    hold on

    plot(linear_ubat_data_lower)

    xlabel('Time (1/60 second)')

    ylabel('Bioluminescence (photons s^-1)')
```

```
    [pks,locs,wdt] = findpeaks(linear_ubat_data_upper);
    threshold_b_index=find(pks>threshold_b&wdt>1);
    if isempty(threshold_b_index) == 1      %Skips files with no large enough peaks
        continue
    end
    peak_magnitude=pks(threshold_b_index);
    peak_position=locs(threshold_b_index);
    peak_duration=wdt(threshold_b_index)/60; % Peak duration is the peak width times (1/60) seconds
    peak_magnitude_20=peak_magnitude.*0.2;
    % These are arrays of zeros to hold a variable in the next section.
    peaks=zeros(length(threshold_b_index),peak_size);
    sigma_max=zeros(length(threshold_b_index),1);
    T_max=zeros(length(threshold_b_index),1);
    peak_point=zeros(length(threshold_b_index),1);
    T_high=zeros(length(threshold_b_index),1);
    average_magnitude=zeros(length(threshold_b_index),1);
    T_decay=zeros(length(threshold_b_index),1);
    start_points=zeros(length(threshold_b_index),peak_size);
    T_high_points=zeros(length(threshold_b_index),peak_size);
    % This for loop saves each peak as a line in variable peaks, the if
    % statement takes care of peaks at the begining and end of the long
    % data stream
    for ii=1:length(peak_position)
        if peak_position(ii)>=length(linear_ubat_data_upper)-rear_window
            %continue
            peaks(ii,:)=zeros(1,peak_size);
            %peaks(ii,:)=padarray(linear_ubat_data(peak_position(ii)-(round((peak_size-1)/3)):length(linear_ubat_data))',(peak_size-1)-(length(linear_ubat_data)-(peak_position(ii)-(round((peak_size-1)/1.5)))),'post');
            %peak_point(ii)=round(peak_size*0.3);
        elseif peak_position(ii)<=front_window
            peaks(ii,:)=zeros(1,peak_size);
```

```
            %continue
%peaks(ii,:)=padarray(linear_ubat_data(1:peak_position(ii)+(round((peak_size-
1)/1.5)))',peak_size-(peak_position(ii)+(round((peak_size-1)/3))),'post');
            %peak_point(ii)=peak_position(ii)-30;
        else
            peaks(ii,:)=linear_ubat_data_upper(peak_position(ii)-(round((peak_size-
1)/3)):peak_position(ii)+(round((peak_size-1)/1.5)));
            peak_point(ii)=round(((peak_size-1)/3)+1);
        end
    % Sigma max is an integral of area below the curve from when the peak
    % rises to when it is at its maximum, this needs to be a bit better
    % defined but is ok for now.
    % Average magnitude is the average magnitude of the peak taken at the
    % full width half maximum (FWHM)
     %start_points=findchangepts(peaks(ii,:),"Statistic","rms",'MaxNumChanges',10);
        start_point_a = peaks(ii,20:50) > threshold_a;
        start_point_a=padarray(start_point_a',19,'pre');
        start_point_a=padarray(start_point_a,51,'post');
        start_points(ii,:)=start_point_a;
        start_points_start=strfind(start_points(ii,:),[0 1]);
        start_start(ii)=start_points_start(1);
        start_points_end=strfind(start_points(ii,:),[1 0]);
        start_end(ii)=start_points_end(1);
        if isempty(start_points)
            sigma_max(ii)=0;
            T_max(ii)=0;
            T_high(ii)=0;
            T_decay(ii)=0;
            average_magnitude(ii)=0;
        else
            sigma_max(ii)=trapz(peaks(ii,start_start(ii)+1:peak_point));      %Adds up all
values from the first value over threshold a until the peak
            T_max(ii)=(peak_point(ii)-start_start(ii)+1)/60;   %Find the time between the
first value over threshold a until the peak
```

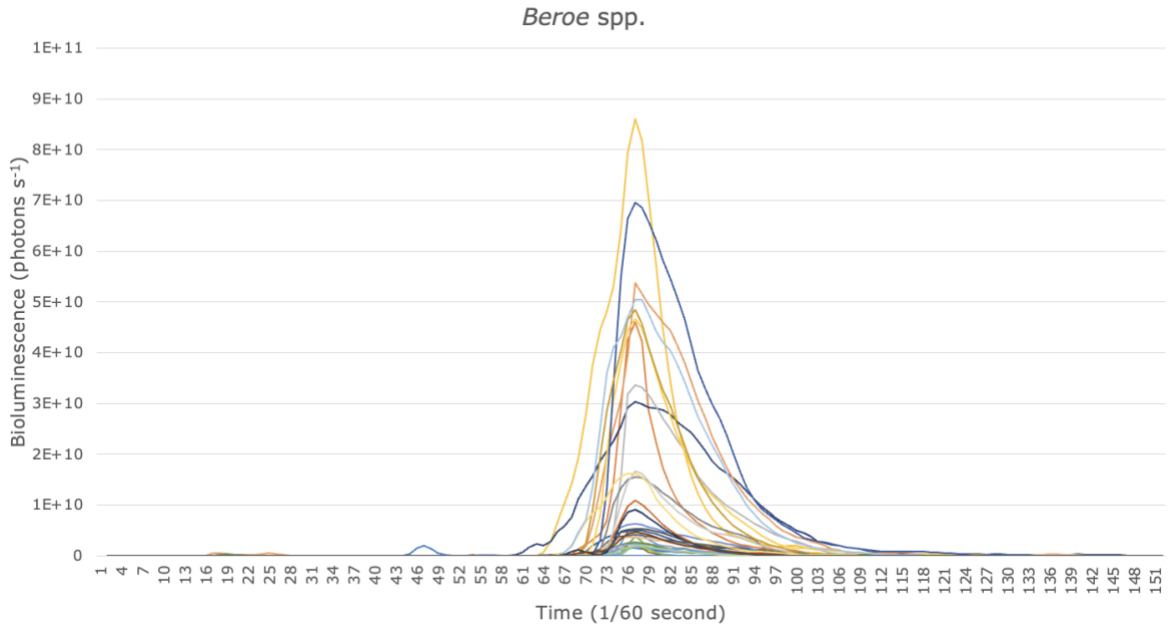
```
        T_high_point_a = peaks(ii,20:50)>peak_magnitude_20(ii);  %Finds points over 20% of the peak magnitude
        T_high_point_a=padarray(T_high_point_a',19,'pre');
        T_high_point_a=padarray(T_high_point_a,51,'post');
        T_high_points(ii,:)=T_high_point_a;



        T_high_points_start=strfind(T_high_points(ii,:),[0 1]);
        T_high_start(ii)=T_high_points_start(1);
        T_high_points_end=strfind(T_high_points(ii,:),[1 0]);
        T_high_end(ii)=T_high_points_end(1);
        T_high(ii)=((T_high_end(ii)+1)-(T_high_start(ii)+1))/60;       %Finds the time between the first and last point over the peak magnitude
        T_decay(ii)=((peak_point(ii)+1)-(T_high_end(ii)+1))/60;      %Find the time between the last point over 20% and the last point over threshold a
        average_magnitude(ii)=mean(peaks(start_start(ii):start_end(ii)));    %Finds the average of all points over threshold a
    end
  end
  %%%%% Uncomment this to see some plots of the peaks extracted for each
  %%%%% file.
%%%% This saves the data in a csv for each input file. The peaks are in one
%%%% file and the metadata (Magnitude, 20% magnitude, duration, average magnitude, and sigma max)
%
%    savename = sprintf('Extracted_peaks_%s.csv',dinfo(xx).name);
%    savename2 = sprintf('Extracted_peaks_metadata_%s.csv',dinfo(xx).name);
%                                                              meta              = cat(2,peak_magnitude',peak_magnitude_20',average_magnitude',sigma_max,T_max,T_high,T_decay);
%    meta=array2table(meta);
%                                          meta.Properties.VariableNames(1:7)          = {'BL_max','BL_20%','BL_mean','Sigma_max','T_max','T_high','T_decay'};
%    writematrix(peaks,savename)
```

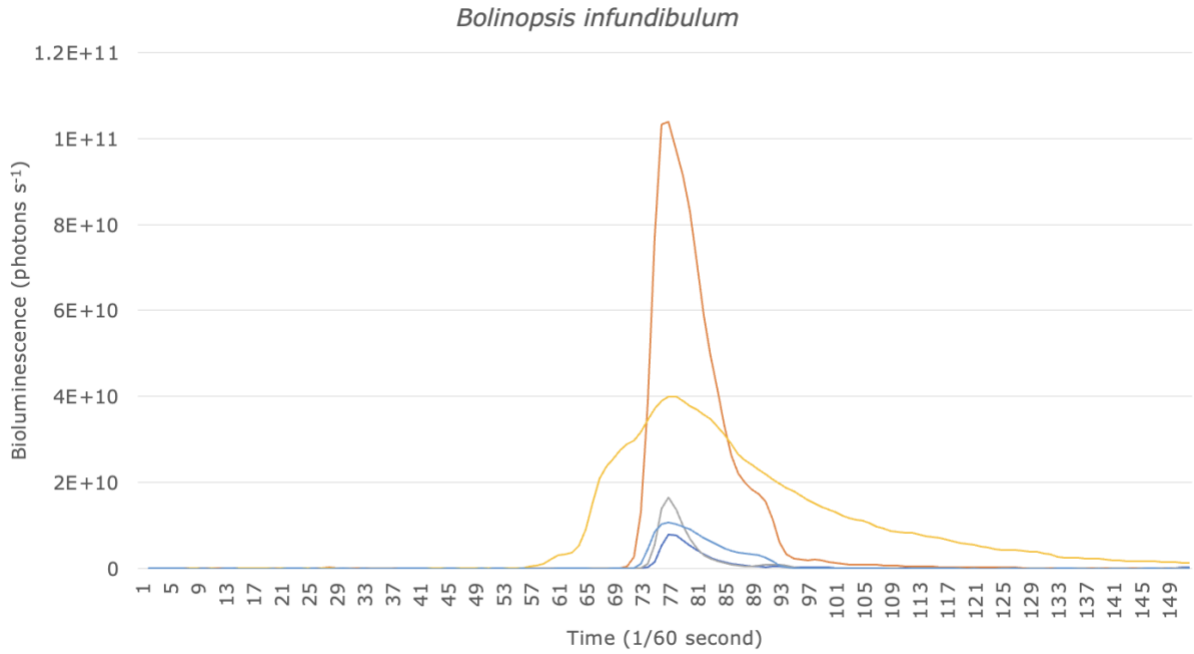
```
%     writetable(meta,savename2)
%
%     clear average_magnitude
end
savename = sprintf('Extracted_peaks_%s.csv',dinfo(xx).name);
savename2 = sprintf('Extracted_peaks_metadata_%s.csv',dinfo(xx).name);
meta                                                                    =
cat(2,peak_magnitude',peak_magnitude_20',average_magnitude,sigma_max,T_max,T_h
igh,T_decay);
meta=array2table(meta);
meta.Properties.VariableNames(1:7)                                      =
{'BL_max','BL_20%','BL_mean','Sigma_max','T_max','T_high','T_decay'};
meta(~meta.T_max,:) = [];
writematrix(peaks,savename)
writetable(meta,savename2)
beroe=find(or((meta.T_max>beroe_T_max_lo&meta.T_max<beroe_T_max_hi),(meta.T_
high>beroe_T_high_lo&meta.T_high<beroe_T_high_hi)))
clytia=find(or((meta.T_max>clytia_T_max_lo&meta.T_max<clytia_T_max_hi),(meta.T_
high>clytia_T_high_lo&meta.T_high<clytia_T_high_hi)))
metridia=find(or((meta.T_max>metridia_T_max_lo&meta.T_max<metridia_T_max_hi),(
meta.T_high>metridia_T_high_lo&meta.T_high<metridia_T_high_hi)))
nanomia=find(or((meta.T_max>nanomia_T_max_lo&meta.T_max<nanomia_T_max_hi),
(meta.T_high>nanomia_T_high_lo&meta.T_high<nanomia_T_high_hi)))
bolinopsis=find(or((meta.T_max>bolinopsis_T_max_lo&meta.T_max<bolinopsis_T_max_
hi),(meta.T_high>bolinopsis_T_high_lo&meta.T_high<bolinopsis_T_high_hi)))
   size_peaks=size(peaks);
%
%   for ii=1:length(peak_position)
%       figure
 %      plot(peaks((ii),:),'LineWidth',1.5)
  %     xlabel('Time (1/60 second)')
   %    ylabel('Bioluminescence (photons s^-1)')
    %   hold on
%       high_point=T_high_point(ii,:);
%       high_point(high_point==0) = [];
```

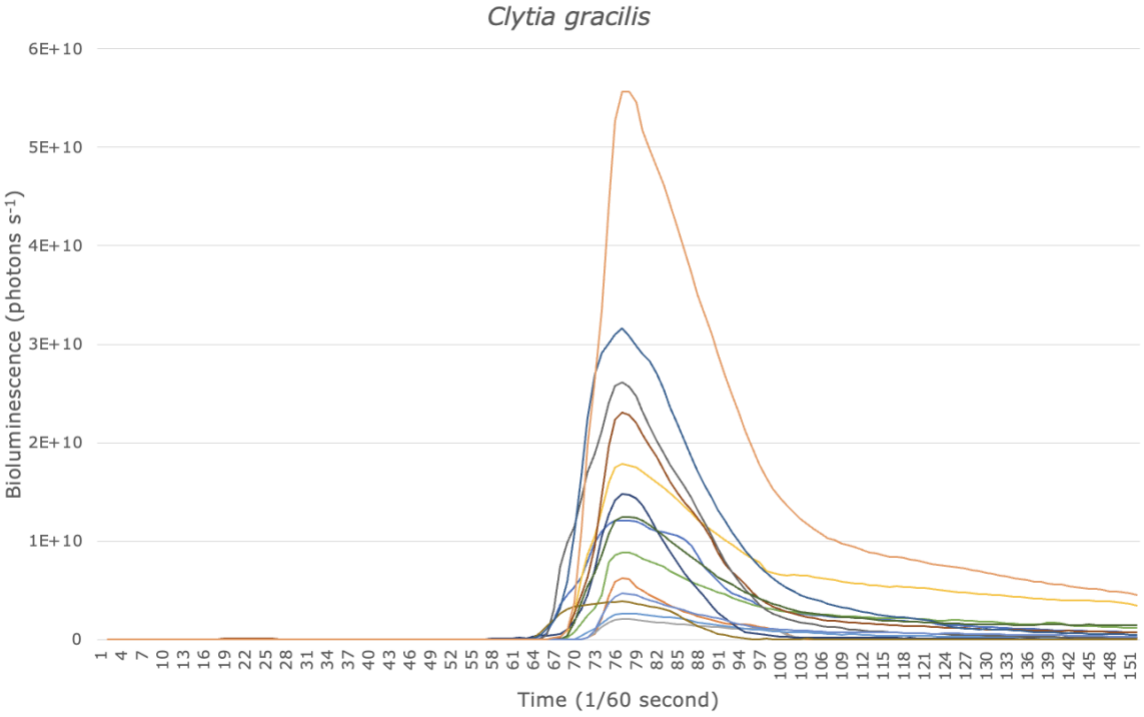
```
%         starts_point=start_point(ii,:);
%         starts_point(starts_point==0) = [];
%        xline(T_high_start(ii),'g')
%        xline(start_start(ii),'r')
%        xline(T_high_end(ii),'g')
%        xline(start_end(ii),'r')
%         xline(peak_point(ii),'b')
%        yline(peak_magnitude_20(ii))
%        yline(average_magnitude(ii))
%    end
figure
for x_beroe=1:length(beroe)
hold on
plot(peaks(beroe(x_beroe),:))
title('Extracted Beroe peaks')
xlabel('Time (1/60 second)')
ylabel('Bioluminescence (photons s^-1)')
end
figure
for x_clytia=1:length(clytia)
hold on
plot(peaks(clytia(x_clytia),:))
title('Extracted Clytia peaks')
xlabel('Time (1/60 second)')
ylabel('Bioluminescence (photons s^-1)')
end
figure
for x_metridia=1:length(metridia)
hold on
plot(peaks(metridia(x_metridia),:))
title('Extracted Metridia peaks')
xlabel('Time (1/60 second)')
```

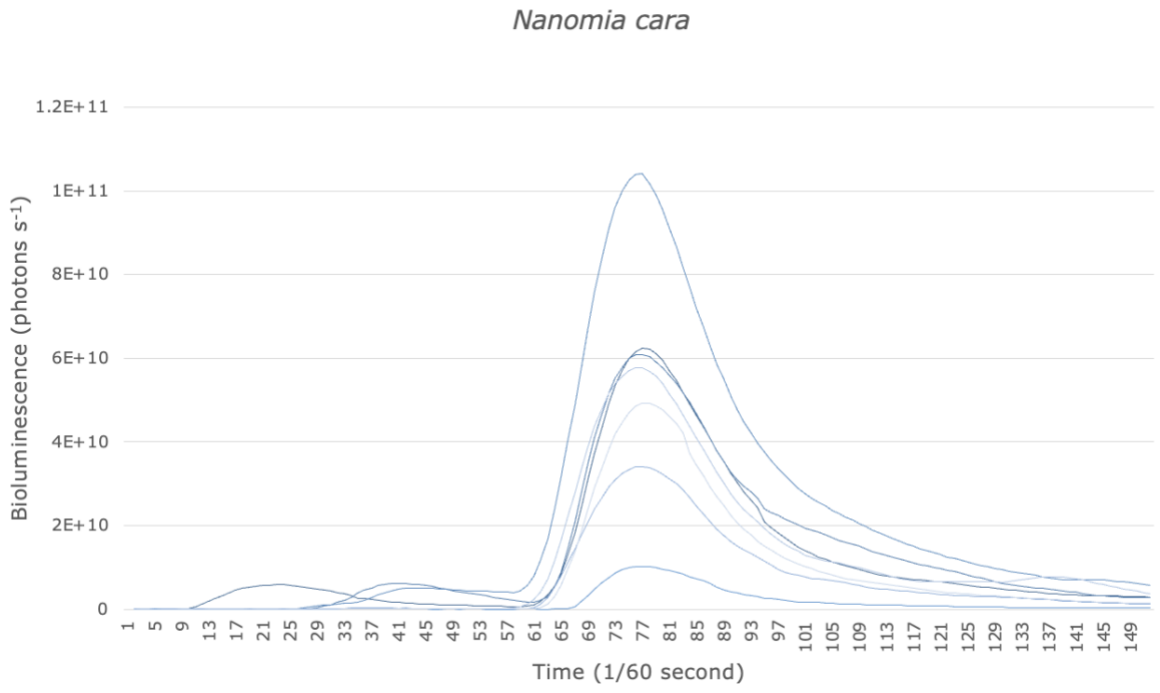
```
ylabel('Bioluminescence (photons s^-1)')
end
figure
for x_nanomia=1:length(nanomia)
hold on
plot(peaks(nanomia(x_nanomia),:))
title('Extracted Nanomia peaks')
xlabel('Time(1/60 second)')
ylabel('Bioluminescence (photons s^-1)')
end
figure
for x_bolinopsis=1:length(bolinopsis)
hold on
plot (peaks(bolinopsis(x_bolinopsis),:))
title('Extracted Bolinopsis peaks')
xlabel('Time(1/60 second)')
ylabel('Bioluminescence (photons s^-1)')
end
linear_data_lower_mm = movmean(linear_ubat_data_lower,1000);
figure
plot(linear_ubat_data_lower)
hold on
plot(linear_data_lower_mm)
xlabel('Time (1/60 second)')
ylabel('Bioluminescence (photons s^-1)')
```

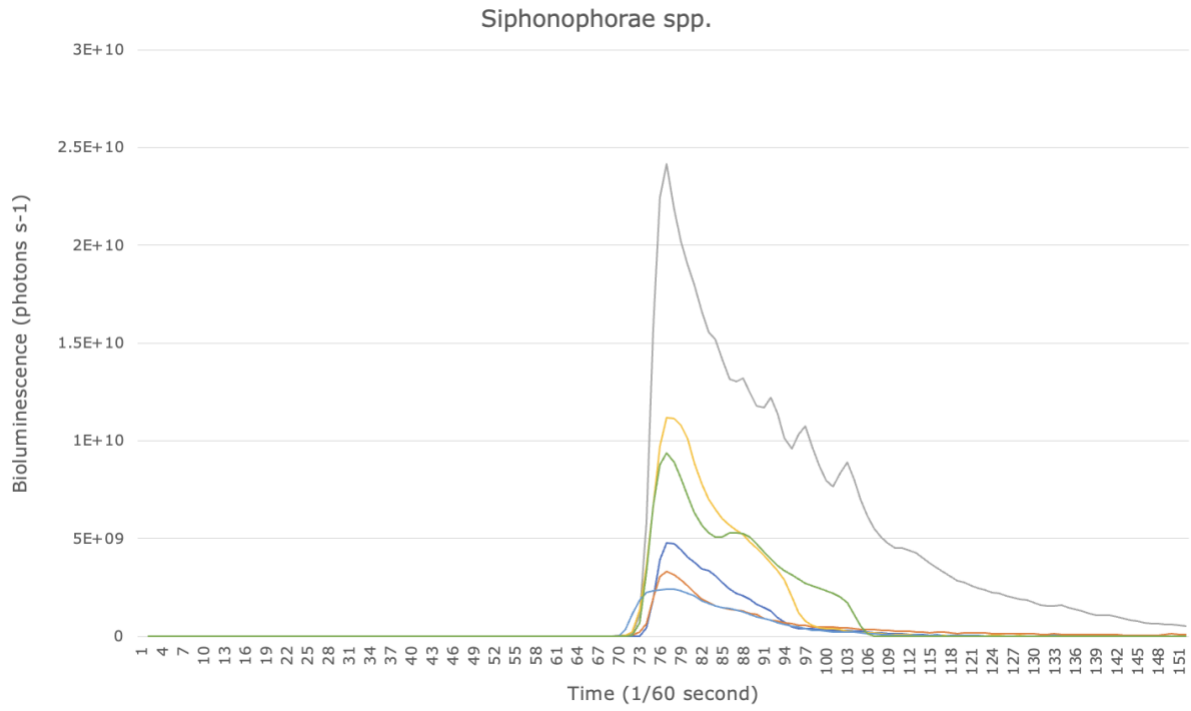
Appendix D – Flash kinetics extracted of the different taxa used in the study

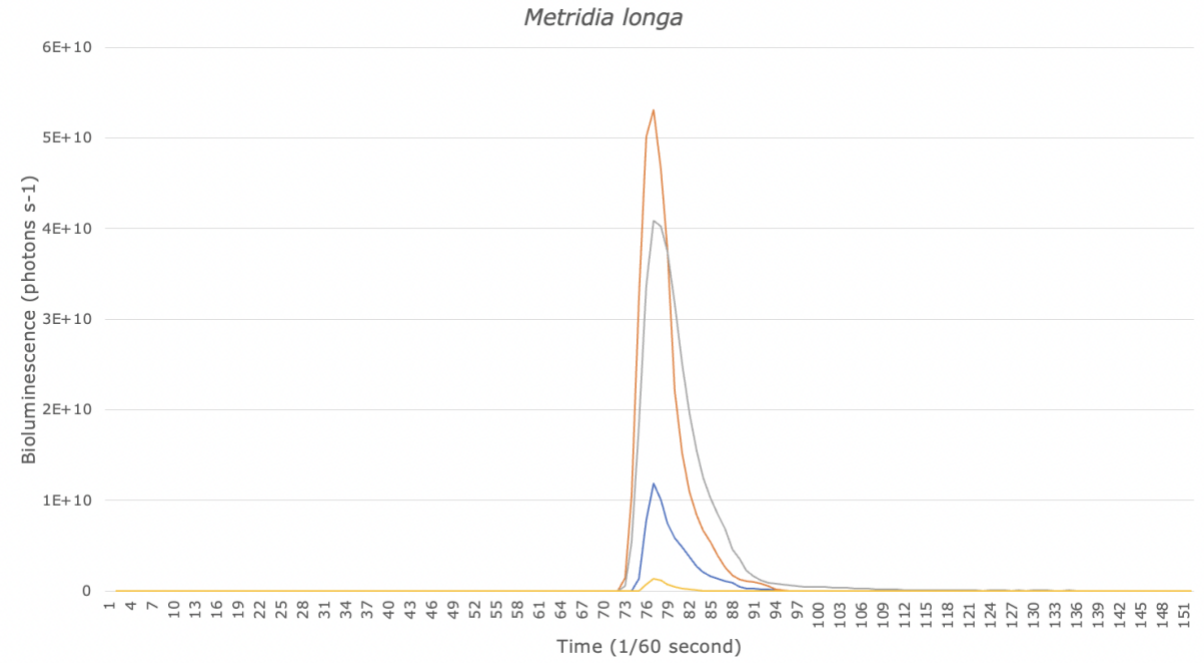




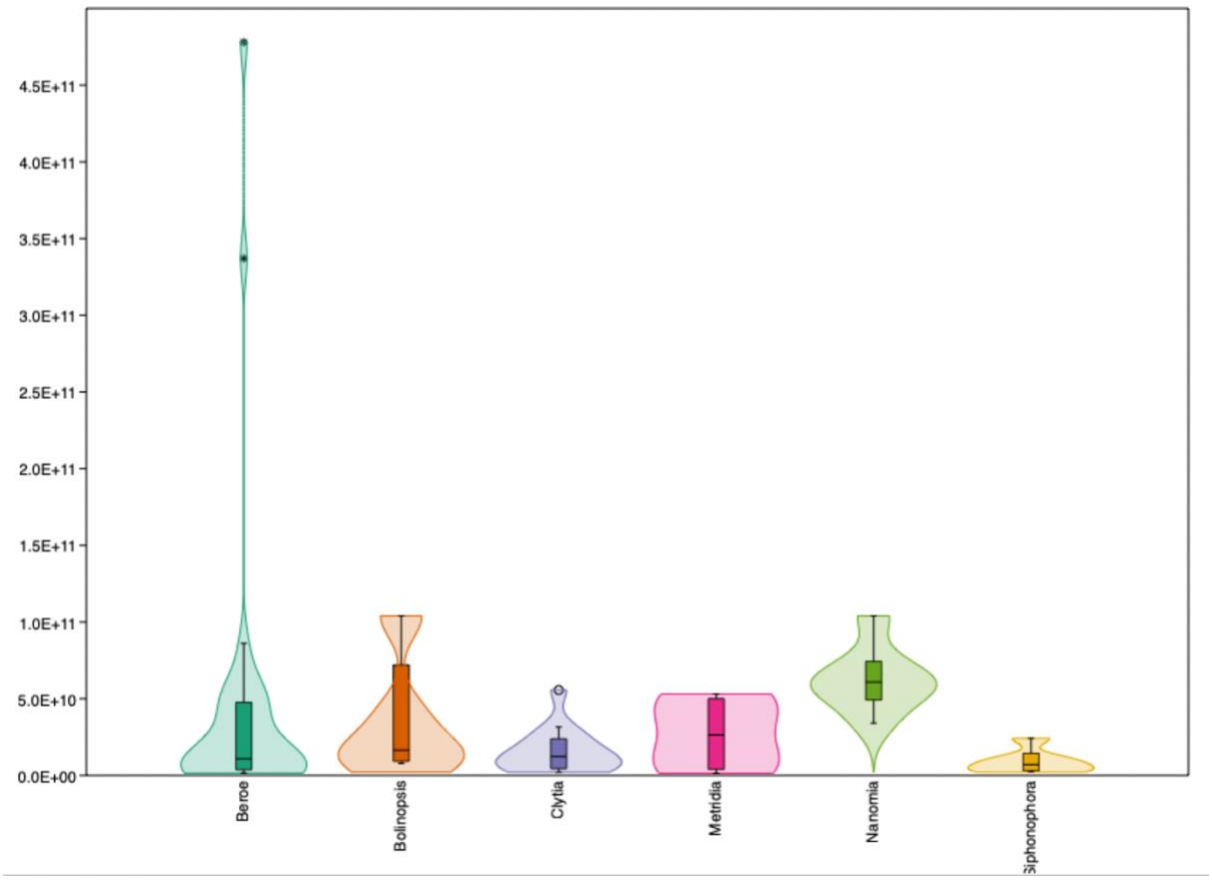

Clytia gracilis
Bioluminescence (photons s-1)
6E+10
5E+10
4E+10
3E+10
2E+10
1E+10
0
Time (1/60 second)


Nanomia cara
Bioluminescence (photons s-1)
1.2E+11
1E+11
8E+10
6E+10
4E+10
2E+10
0
Time (1/60 second)


Siphonophorae spp.
Bioluminescence (photons s-1)
3E+10
2.5E+10
2E+10
1.5E+10
1E+10
5E+09
0
Time (1/60 second)


Metridia longa
Bioluminescence (photons s-1)
6E+10
5E+10
4E+10
3E+10
2E+10
1E+10
0
Time (1/60 second)

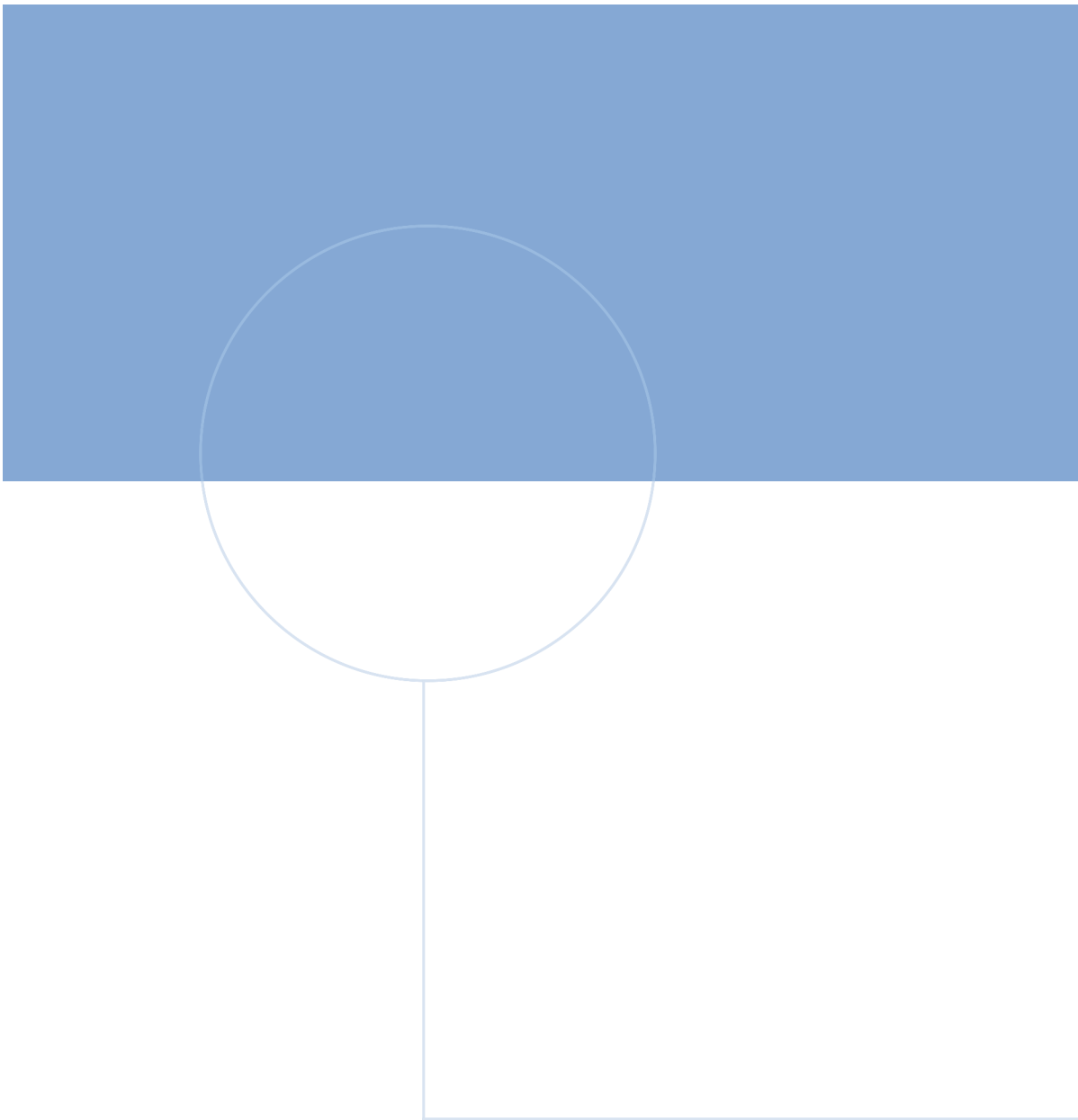
Appendix E – Outliers from *Beroe* spp.



Appendix F- Species reviewed through the study

DATA log	*Scientific Name*	kingdom	phylum	class	order	Family	*genus*	Occurrence in Norway	Author
	Mertensia ovum	Animalia	Ctenophora	Tentaculata	Cydippida	Mertensiidae	*Mertensia*	Yes	Fabricius, 1780
	Beroe cucumis	Animalia	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	Yes	Fabricius, 1780
	Bolinopsis infundibulum	Animalia	Ctenophora	Tentaculata	Lobata	Belinopsidae	*Bolinopsis*	Yes	O.F. Müller, 1776
	Pleurobrachia pileus	Animalia	Ctenophora	Tentaculata	Cydippida	Pleurobrachiidae	*Pleurobrachia*	Yes	O.F. Müller, 1776
	Beroe gracilis	Animalia	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	Yes	Künne, 1939
	Beroe ovata	Animalia	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	The Great Belt	Bruguière, 1789
	Aulacocteba acuminata	Animalia	Ctenophora	Tentaculata	Cydippida	Aulacoctenidae	*Aulacoctena*	no	Mortensen, 1932
	Bathocyroe fosteri	Animalia	Ctenophora	Tentaculata	Lobata	Bathocyroidae	*Bathyocyroe*	no	Madin & Harbison, 1978
	Bathyctena chuni	Animalia	Ctenophora	Tentaculata	Cydippida	Bathocyroidae	*Bathyctena*	no	Moser, 1909
	Beroe abyssicola	Animalia	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	Yes	Mortensen, 1927
	Beroe forskalii	Animalia	Ctenophora	Nuda	Beroida	Beroidae	*Beroe*	no	Milne Edwards, 1841
	Bolinopsis Vitrea	Animalia	Ctenophora	Tentaculata	Lobata	Bolinopsidae	*Bolinopsis*	no	L. Agssiz, 1860
	Cestum veneris	Animalia	Ctenophora	Tentaculata	Cestida	cestidae	*Cestum*	no	Lesueur, 1813
	Charistephane fugiens	Animalia	Ctenophora	Tentaculata	Cydippida	Mertensiidae	*Charistephane*	no	Chun, 1879
	Deiopea kaloktenota	Animalia	Ctenophora	Tentaculata	Lobata	Eurhamphaeidae	*Deiopea*	no	Chun, 1879
	Euplikomais stationis	Animalia	Ctenophora	Tentaculata	Cydippida	Euplokamidae	*Euplokamis*	no	Chun, 1879
	Euplikamis dunlapae	Animalia	Ctenophora	Tentaculata	Cydippida	Euplokamidae	*Euplokamis*	Yes	Mills, 1987
	Eurhamphaea vexiligera	Animalia	Ctenophora	Tentaculata	Lobata	Eurhamphaeidae	*Eurhamphaea*	No	Gegenbaur, 1856
	Haeckelia beehleri	Animalia	Ctenophora	Tentaculata	Cydippida	Haeckeliidae	*Haeckelia*	No	Mayer, 1912
	Haeckelia rubra	Animalia	Ctenophora	Tentaculata	Cydippida	Haeckeliidae	*Haeckelia*	No	Kôlliker, 1853
	Lampea lactea	Animalia	Ctenophora	Tentaculata	Cydippida	Lampeidae	*Lampea*	No	Mayer, 1912
	Lampea pancerina	Animalia	Ctenophora	Tentaculata	Cydippida	Lampeidae	*Lampea*	No	Chun, 1879
	Leucothea multicornis	Animalia	Ctenophora	Tentaculata	Lobata	Leucotheidae	*Leucothea*	No	Quoy & Gaimard, 1824
	Leucothea pulchra	Animalia	Ctenophora	Tentaculata	Lobata	Leucotheidae	*Leucothea*	No	Matsumoto, 1988
	Ocyrpsis maculata	Animalia	Ctenophora	Tentaculata	Lobata	Ocyropsidae	*Ocyropsis*	No	Rang, 1827
	Thalassocalyce inconstans	Animalia	Ctenophora	Tentaculata	Thalassocalycida	Thalassocalycdiae	*Thalassocalyce*	No	Madin & Harbison, 1978
	Velamen parallelum	Animalia	Ctenophora	Tentaculata	Cestida	cestidae	*Velamen*	No	Folm 1869
	Mnemiopsis leidyi	Animalia	Ctenophora	Tentaculata	Lobata	Belinopsidae	*Mnemiopsis*	Yes	A. Agassiz, 1865

	Metridia Longa	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Metridia*	Yes	Lubbock, 1854
	Metridia lucens	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Metridia*	Yes	Boeck, 1856
	Oncaea borealis	Animalia	Arthropoda	Copepoda	Cyclopoida	Incaeidae	*Triconia*	Yes	Sars G.O., 1918
	Meganyctiphanes norvegica	Animalia	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	*Meganyctiphanes*	Yes	M. Sars, 1857
	Thysanoessa longicaudata	Animalia	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	*Thysanoessa*	Yes	Krøyer, 1846
	Thysanoessa inermis	Animalia	Arthropoda	Malacostraca	Euphausiacea	Euphausiidae	*Thysanoessa*	Yes	Krøyer, 1846
	Lucicutia flavicornis	Animalia	Arthropoda	Copepoda	Calanoida	Lucicutiidae	*Lucicutia*	Yes	Claus, 1863
	Lucicutia grandis	Animalia	Arthropoda	Copepoda	Calanoida	Lucicutiidae	*Lucicutia*	Lucicutia - yes	Giesbrecht, 1895
	Pleuromamma abdominalis	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Pleuromamma*	Yes	Lubbock, 1856
	Pleuromamma gracilis	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Pleuromamma*	Yes	Claus, 1863
	Pleuromamma robusta	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Pleuromamma*	Yes	Dahl F., 1893
	Heterohabdus norvegicus	Animalia	Arthropoda	Copepoda	Calanoida	Heterorhabdidae	*Heterorhabdus*	Yes	Bock, 1872
	Diastylis lucifera	Animalia	Arthropoda	Malacostraca	Cumacea	Diastylidae	*Diastylis*	Yes	Krøyer, 1837
	Pleuromamma xiphias	Animalia	Arthropoda	Copepoda	Calanoida	Metridinidae	*Pleuromamma*	North of Scotland	Giesbrecht, 1889
	Candacia norvegica	Animalia	Arthropoda	Copepoda	Calanoida	Candaciidae	*Candacia*	Yes	Boeck, 1856
	Candacia armata	Animalia	Arthropoda	Copepoda	Calanoida	Candaciidae	*Candacia*	Yes	Boeck, 1872
	Candacia elongata	Animalia	Arthropoda	Hexanauplia	Calanoida	Candaciidae	*Candacia*	Yes	Bock, 1872
	Euchirella rostrata	Animalia	Arthropoda	Hexanauplia	Calanoida	Aetideidae	*Euchirella*	Yes	Claus, 1866
		Animalia	Annelida	Polychaeta	Phyllodocida	Tomopteridae	*Tomopteris*	Yes	Eschscholtz, 1825
	Periphylla Periphylla	Animalia	Cnidaria	Scyphocoa	Coronatae	Periphyllidae	*Periphylla*	Yes	Péron & Lesueur, 1810
	Atolla parva	Animalia	Cnidaria	Scyphocoa	Coronatae	Atollidae	*Atolla*	Atolla - yes	Russel, 1958
	Atolla vanhoeffeni	Animalia	Cnidaria	Scyphocoa	Coronatae	Atollidae	*Atolla*	Atolla - yes	Russel, 1957
	Atolla wyvillei	Animalia	Cnidaria	Scyphocoa	Coronatae	Atollidae	*Atolla*	Atolla - yes	Haeckel, 1880
	Nausithoe globifera	Animalia	Cnidaria	Scyphocoa	Coronatae	Nausithoidae	*Nausithoe*	No	Broch, 1913
	Nausithoe atlantica	Animalia	Cnidaria	Scyphocoa	Coronatae	Nausithoidae	*Nausithoe*	No	Broch, 1913
	Paraphyllina ransoni	Animalia	Cnidaria	Scyphocoa	Coronatae	Paraphyllinidae	*Paraphyllina*	No	Russel, 1956
	Clytia gracilis	Animalia	Cnidaria	Hydrozoa	Lepototthecata	Campanulariidae	*Clytia*	Yes	Sars, 1851
	Aglantha Digitale	Animalia	Cnidaria	Hydrozoa	Trachymedusae	Rhopalonematidae	*Aglantha*	Yes	O.F. Müller, 1776
	Eutonina Indicans	Animalia	Cnidaria	Hydrozoa	Leptothecata	Eirenidae	*Eutonina*	Yes	Romanes, 1876
	Nanomia cara	ANimalia	Cnidaria	Hydrozoa	Siphonophorae	Agalmatidae	*Nanomia*	Yes	Agassiz, 1856



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