This research proposal was submitted to the “Researcher Project for Young Talents” call from The Research Council of Norway in 2021.

www.forskningsradet.no/en/call-for-proposals/2021/researcher-project-for-young-research-talents

The proposal was unsuccessful but the feedback it received is included for the benefit of future applicants.

This research is ongoing, so if you find it interesting and/or consider pursuing similar projects, please reach out via our lab website (www.marineneurolab.com) and cite this proposal in your work.

# Deciphering bioluminescent communication in marine annelids

*"Bioluminescence is one of the oldest and most prevalent languages on Earth – and one that is largely alien to us"* [1]

## 1. Excellence

### 1.1 State of the art, knowledge needs and project objectives

"Life Below Water" is not only one of the UN's Sustainable Development Goals[2], it is also the origin of the most complex structure known to man: the brain. Yet, despite the strong evolutionary ties between the ocean and the nervous system, marine biology and neuroscience rarely intersect. This is due to a lack of precedence rather than a lack of pressing questions, one of which is the significance of bioluminescence. The importance of this heat-free form of light to life on our planet is immense. A large variety of species across multiple habitats are capable of producing it, but most reside in the ocean[3]. Here, bioluminescence is the primary source of light at night and at depth, and is generated by 76% of pelagic organisms[4]. As the ocean's predominant communication signal, its functions are diverse, ranging from predator defence to hunting and mating[5]. Colour is thought to play a role in mediating these functions, as a variety of hues are represented. They are, however, unevenly distributed among species. This, in turn, has led to the hypothesis that the rare yellow and red wavelengths facilitate private, intraspecies communication while the much more common blue and green also contribute to interspecies communication[5]. Whether or not this is the case remains unknown as **we still know very little about what bioluminescent signals mean and even less about the significance of colour** at both a behavioural and neuronal level**.**

This is because the majority of our interpretations regarding the use of bioluminescence is primarily based on studies of animal morphology since *in situ* observations are uncommon and experimental insights are rare[6,7]. Recent technological developments for underwater imaging are improving opportunities for *in situ* observations, highlighting the diversity of bioluminescent species, but still remain largely descriptive[8]. Behavioural experiments are rare in the study of bioluminescence, partly due to the difficulty of obtaining and maintaining specimens in good condition as pelagic organisms are often fragile[9], but also because there is minimal precedence. Such studies are, however, the only way to determine the true function of bioluminescence. Dinoflagellates, for example, are known to bioluminesce in response to mechanical disturbance. However, experimental studies were necessary to show that its function is to deter predation[10,11] by acting as a warning signal[12], eliciting high-speed swimming burst in grazing predators[13–15] and attracting secondary predators[12]. One other notable exception to the dearth of behavioural experiments is the finding that an annelid from the Tomopteridae family is able to distinguish between the colour of its own species and that of others[16]. In addition, similarities in the structure and control of the light emitting organs of the different species in this genus suggest that they may have derived from the same origin[17]. Combined, these findings indicate a **potential for complex, intra- and interspecies communication strategies in planktonic organisms**.

However, while behavioural experiments are essential to determine the relevance and significance of bioluminescence to an organism's behaviour, they **cannot distinguish whether an ambiguous response to a stimulus is due to a lack of relevance or an inability to perceive it**. For this, *in vivo* functional imaging of the underlying neural circuits is necessary. To date, this has only been achieved in two species: directly in the genetically modified polyp *hydra vulgaris*[18,19] and indirectly via the dynamics of chromatophores in cuttlefish[20]. Neither of these strategies is available to tomopterids as transgenic models do not exist and they do not have chromatophores. It is therefore **necessary to create a foundation for performing *in vivo* functional imaging in non-transgenic gelatinous organisms.**

This project will **determine how bioluminescence and its colours mediate inter- and intraspecies communication at a behavioural level and lay the foundation for future *in vivo* functional imaging studies.** This will be achieved with the use of two marine annelids from the tomopteris genus, which are ideally suited for the following reasons: first, unlike many other gelatinous organisms, they are known to remain healthy in a laboratory for weeks after collection in the wild. Second, although related, their respective

bioluminescent profiles are unique: *Tomopteris helgolandica* emits yellow, while *Tomopteris planktonis* uses the much more prevalent blue[17], allowing us to compare two potentially different communication strategies. Third, because these species are thought to derive from the same origin[17], an evolutionary perspective on the use of bioluminescent colours is attainable. Fourth, as predators they have a wide behavioural repertoire[16] that, in combination with their relatively large size (0.5-6 cm in length), enable detailed behavioural readouts. Fifth, as they live their entire lives in the open ocean, they have evolved a convenient trait to conceal themselves from predators: they are wholly transparent at all life stages (Figure 1), making them ideal models for non-invasive *in vivo* functional imaging.

This **unique combination of features, along with extensive behavioural experiments and neurobiological techniques** will allow us to fulfil four major objectives, pursued with a multidisciplinary approach:

1) **Identify naturalistic stimuli associated with bioluminescent emission**
2) **Establish whether distinct properties of bioluminescent signals elicit different behaviours**
3) **Determine if *T. helgolandica* and *T. planktonis* can communicate with each other**
4) **Lay the foundation for future *in vivo* neuronal imaging in non-transgenic gelatinous animals**

My experience with neuroscience, animal behaviour and marine biology puts me in a unique position to bridge these fields to shed light on bioluminescence, a major ecological phenomenon, and spearhead novel research lines with the overarching vision of **revealing the evolutionary, behavioural, and neuronal origins of animal communication**. Understanding how life below water communicates will also be imperative to minimise our increasing impact on this ecosystem that is the cradle of life. In addition, by going back to where it all began, this project will have implications for future research on neural function in all species, whose nervous systems all evolved from the ocean.

1.2 Research questions and hypotheses, theoretical approach and methodology

Our main objectives form the foundation for four Work Packages (WPs) that together will allow us to decipher the bioluminescent signals of two related species and lay a foundation for *in vivo* neuronal imaging. This will be achieved by identifying the stimuli associated with bioluminescent emission (WP1), whether distinct properties of the signals elicit different behaviours (WP2) and if these two species perceive each other. If so, we will determine if they understand each other or if the meaning of the signal was lost in the evolutionary mediated colour conversion (WP3). Finally, we will lay the foundation for performing *in vivo* functional imaging experiments in non-transgenic gelatinous animals for the first time (WP4).

**Methods:** For all WPs, *T. helgolandica* and *T. planktonis* specimens (Figure 1A) will be collected in the wild using plankton nets in the Trondheim fjord at 100-200 meters depth, where we frequently find them during the day. After collection and identification (in addition to their bioluminescent colour, *T. planktonis* can be distinguished from *T. helgolandica* on the basis of their smaller size, lower number of parapodia and lack of a tail[17]), they will be kept in aquariums at approximately 5°C and the water exchanged at least every two days. From our preliminary experiments, we have found them to remain healthy under these conditions for at least 10 days. With further optimisation, possible with the dedicated facilities at NTNU SeaLab, we expect to be able to extend this to several weeks[21].

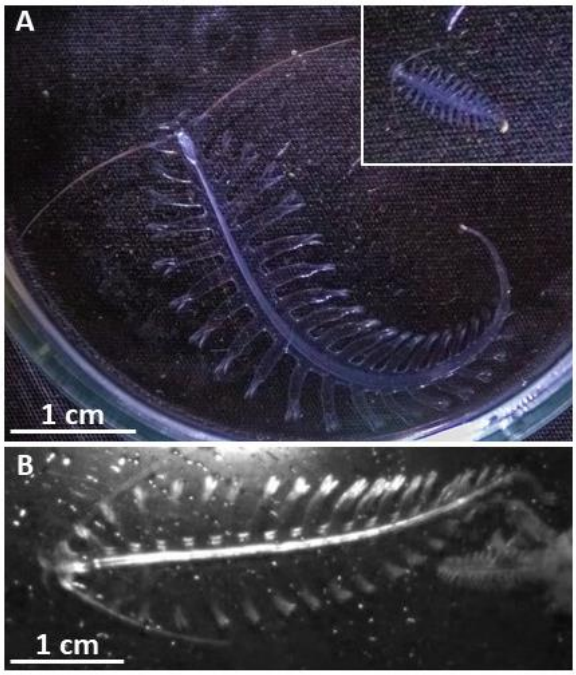


**Figure 1: *T. helgolandica* and *T. planktonis***
A) *T. helgolandica* and *T. planktonis* (insert, to scale) photographed under white light.
B) Single frame from video recording of *T. helgolandica* in darkness illuminated with infrared light. Bioluminescence was observed but not captured on camera.

All behavioural experiments (WPs1-3) will be performed by visually recording individuals in aquariums under infrared light either in 2D or 3D as appropriate with several cameras. Bioluminescent emission will be monitored simultaneously with a light sensitive camera. The 2D version of this method has already been successfully applied both in a previous study[16] and in our hands (Figure 1B). To track the behaviour of one and more individuals, we will employ recently developed algorithms at the forefront of the field, such as DeepLabCut[22].
Additional WP-specific methods are outlined in their respective descriptions below.

**WP1: Identify naturalistic stimuli associated with bioluminescent emission**
Under which circumstances do tomopterids emit bioluminescent signals? If *T. helgolandica* uses it for intraspecies communication, are they more likely to do it when in the company of others compared to when they are alone? On the other hand, is *T. planktonis* more likely to use their more common colour for interspecies communication, such as luring pray or confusing predators? Indeed, spontaneous bioluminescence is rarely observed in single-housed *T. helgolandica* specimens[16], suggesting that the presence of conspecifics may be necessary. If so, features such as sex and maturation stage likely matter, as the identification of mates is one major reason for intraspecies communication. To determine if the conditions associated with bioluminescence in these two related species are different, we will monitor individuals of both species before and after exposure to conspecifics of varying maturation stages, prey (such as smaller zooplankton found in the same net samples) as well as simulated predators (using direct mechanical stimulation and indirect water disturbances with pipettes). By comparing their movements and bioluminescent signals we will be able to observe if the evolutionary mediated change in bioluminescent colour has influenced the conditions under which bioluminescence is used.

***Hypothesis:*** *bioluminescence occurs more often in T. helgolandica when an individual is in the presence of maturation-matched conspecifics than when alone while T. planktonis' bioluminescence is associated with interactions with prey and/or predators.*

**WP2: Establish whether distinct properties of bioluminescent signals elicit different behaviours**

Do the properties of the bioluminescent signal itself (kinetics, pattern, intensity, etc.) contain information? Bioluminescent ostracods use bright, long, high frequency signals and dim, shorter, uniform pulses to differentiate between two phases of their courtship display[23], while the number of simulated dinoflagellate bioluminescent flashes modulates predator responses [15]. *T. helgolandica* is also capable of producing two different patterns, a "glow" and "flash" response[24], and they respond differently to these patterns if presented in blue but not if presented in yellow[16]. These observations suggest that the properties of bioluminescent signals are relevant for behaviour. However, it is still unknown which properties are meaningful and the extent to which they are important.

We will use small LEDs with wavelengths matching the bioluminescent signals of these species and arrange them to simulate the light organ distribution of real tomopterids. This will allow us to present individuals of both species with light stimuli that mimic real bioluminescent signals, as well as stimuli with scrambled properties, such as different inter-flash intervals and patterns. By monitoring their movements and potential bioluminescent responses, we will be able to determine whether they distinguish between signal properties in these manners and if so, which ones and what their relevance is to their behaviour.

***Hypothesis:*** *real vs scrambled pseudo-bioluminescent signals will elicit distinct behavioural responses.*

**WP3: Determine if *T. helgolandica* and *T. planktonis* can communicate with each other**

Can these two species perceive and understand each other, despite their differing bioluminescent colours? Because the vast majority of bioluminescence in the ocean is blue or green, the visual system of most species is also typically centred around these wavelengths[5]. The yellow-emitting *T. helgolandica* seems to be an exception, however, as it is able to discriminate between blue and yellow signals[16], suggesting that it may be responsive to the blue signals from *T. planktonis*. Whether the reverse is true is unknown, but one recent study suggests that yellow signals might have evolved from blue[17]. If so, we might expect that the blue-emitter *T. planktonis* is unable to discriminate colours because it represents an evolutionary stage in which yellow sensitivity has not developed.

By mimicking their bioluminescent signals with LEDs as in WP2, we can observe the behaviour of both species to determine if they respond differently to simulated intra- vs. interspecies signals. This will include signals in which all properties except colour are identical, allowing us to determine if they are able to perceive and distinguish members of their own and other species. Further, by introducing individuals and groups of both species to each other in large aquariums, we can observe how they interact in more naturalistic, spacious environments. Do their behaviours differ when they are in the vicinity of members of the same or another species? By monitoring their movements and bioluminescent signals, we will be able to determine if the colour difference between the two species means that *T. planktonis* is effectively blind to the yellow signals emitted by *T. helgolandica,* and whether the latter perceives and responds with movement or bioluminescence to the blue signals of the former, from which its yellow bioluminescence may have derived[17].

***Hypothesis:*** *signals emitted from T. planktonis results in movement and/or bioluminescent responses from T. helgolandica, but not vice versa.*

**WP4: Lay the foundation for future *in vivo* neuronal imaging in non-transgenic gelatinous animals**

While behavioural experiments are essential to determine the relevance and significance of bioluminescence to an organism's behaviour, they cannot distinguish whether an ambiguous response to a stimulus is due to a lack of relevance or an inability to perceive it. For this, *in vivo* functional imaging of the underlying neural circuits is necessary. Achieving such an ambitious goal relies on a thorough understanding of the nervous system, the identity of the cells within it, and whether the use of standard calcium and voltage sensitive dyes and methods are feasible in tomopterids. WP4 will utilise my extensive experience within neuroscience in general, and with histological approaches and *in vivo* imaging techniques in particular, to lay the foundation necessary to achieve functional imaging in non-transgenic gelatinous animals for the first time. Functional imaging of these animals' nervous systems will be a goal in future applications to the European Research Council (ERC). In addition, by performing these experiments in both *T. helgolandica* and *T. planktonis,* we will take the first step to uncover whether the neurobiology underlying bioluminescent communication is conserved.

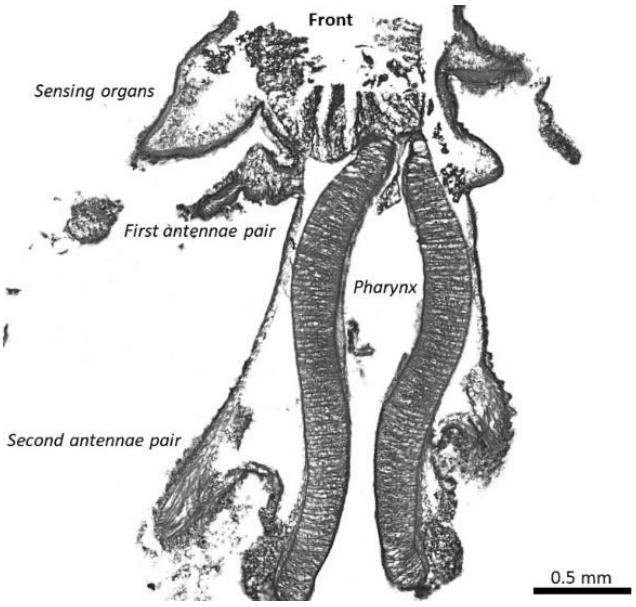


**Figure 2: Horizontal section of *T. helgolandica***
20 µm section stained with cresyl violet to visualise general anatomy.

First, the general neuroanatomy of both species will be determined by preserving individual specimens and making thin (10-40 µm) sections of their entire bodies. These will be stained with cellular staining methods such as cresyl violet (Figure 2) and DAPI[25] to characterise the overall structure of their nervous systems. Second, molecular markers (such as serotonin, glutamate and GABA) will be used to determine neuronal identities and how these are distributed. Figure 2 demonstrates the feasibility of using cresyl violet. Additionally, previous studies show that common molecular markers used in other animals can also be applied to tomopterids[21,25]. Third, calcium and voltage sensitive dyes (such as Oregon Green BAPTA and RH155) will be introduced to the identified neurons via injection in menthol anaesthetised animals to establish the feasibility of performing functional imaging of them. Brightfield, darkfield, epifluorescence and confocal microscopy will be used to visualise anatomical features and to determine the best approach for future *in vivo* functional imaging.

***Significance:*** *laying a foundation for in vivo imaging will significantly improve the feasibility of neural circuit investigations in marine invertebrates. This will not only lead to an unprecedented understanding of how bioluminescence is perceived – resultant findings will also have extensive implications for nervous function in all species, whose nervous systems evolved from the same habitat in which these marine annelids still live.*

The results obtained under these WPs will provide one of the first experimental insights into how bioluminescent signals are used, how members of the same and other species respond to them, as well as the significance of colour. In addition, a foundation for future *in vivo* functional imaging in non-transgenic gelatinous organisms will be formed for the first time. As behavioural experiments with marine organisms are few and far between, this project will also set an important and timely precedent for experiments in other species. Such experiments will be crucial to determine if our findings on bioluminescent communication generalise to other species, and to increase the much-needed research effort into an ecosystem on which we are having an unprecedented impact.

**Risks**

Our preliminary data (Figure 1), and one previous study[16] using one of the species we will employ here, demonstrate the feasibility of performing behavioural experiments with tomopterids (WPs1-3). Rather than being methodological, the risks associated with them are therefore conceptual: behavioural responses observed in the lab may not be identical to those in the wild, leading to inconclusive results. We will minimise this risk by replicating the conditions observed in the wild as closely as possible, including fresh seawater collected from similar depths at which these annelids are found and using spacious aquariums to support their pelagic nature.

Furthermore, the feasibility of performing neuroanatomical investigations (WP4) is demonstrated with our preliminary data (Figure 2) and previous studies[21,25]. My extensive experience with histological and *in vivo* imaging methods will also limit the risks associated with these experiments and the development of a foundation for *in vivo* imaging.

Although related, the individual WPs can be performed independently and do not depend on each other for success, further minimising the overall risk of the project (Table 1).

**Table 1: Risk assessment**

Overview of the novelty, gain and risks associated with each WP. High values indicate a more significant return than low values.

| **Work Package** | **State of the Art** | **How this project goes beyond the State of the Art** | **Novelty** (1-5) | **Gain** (1-5) | **Risk** (1-5) |
|---|---|---|---|---|---|
| 1. Conditions for bioluminescence | Inferred from morphology and physiology | First experimental study | 5 | 3 | 3 |
| 2. Signal properties and behaviour | Theories that "glow" and "flash" signals have different functions | First experimental study | 5 | 5 | 3 |
| 3. Inter- and intraspecies communication | Limited behavioural evidence for yellow serving as intraspecies signal | First study to directly compare inter- and intraspecies communication | 4 | 5 | 3 |
| 4. Foundation for *in vivo* imaging | Possible in one transgenic species and indirectly with chromatophore species | First attempt (that I am aware of) in non-transgenic gelatinous organisms | 5 | 5 | 4 |

**Ethics**

As this proposal aims to decipher bioluminescent communication, the use of animals is necessary. However, the marine annelids in this proposal are considered zooplankton and most of the experiments involve non-invasive procedures. The ethical concerns relating to animal experiments are therefore minimal. Nonetheless, we will firmly comply with the Three Rs of humane experimental technique by using a simple animal model (replacement), the lowest number of animals possible by using the same individuals for multiple experiments (reduction) and optimising our housing and handling conditions to minimise distress and enhance animal welfare (refinement).

## 1.3 Novelty and ambition

Behavioural studies on bioluminescence are extremely rare because these organisms are often fragile and there is minimal precedence. This project will overcome these challenges by using relatively robust species whose suitability has been demonstrated[16], including by our preliminary data (Figure 1B). As a result, novel insights into how bioluminescence is used will be gained that **go beyond morphological and physiological inferences**. Further, this project will **bridge two rarely overlapping fields**, marine biology and neuroscience, which will shed light on a major ecological phenomenon and determine the roles that bioluminescence and its colours play in mediating inter- and intraspecies communication. In combination with a foundation *for in vivo* neuronal imaging, we will **spearhead novel research lines that will reveal the evolutionary, behavioural and neuronal origins of animal communication**.

Finally, it will **provide the essential foundation for my independent research career**. My ambition is to build on these behavioural and neurobiological experiments to **unravel the underlying neurophysiology of bioluminescent communication:** by leveraging my extensive imaging experience and the unique features of these species, my future aim is to perform non-invasive imaging of their entire brain. This will allow me to reach my overarching ambition of taking my principal field, neuroscience, into the future by going back to where it all began: the ocean.

# 2. Impact

## 2.1 Potential for academic impact of the research project

This project will determine how bioluminescence and its colours mediate inter- and intraspecies communication at a behavioural level. Additionally, it will lay the foundation for future *in vivo* functional imaging at a neuronal level. While previous morphological and physiological studies of bioluminescence are valuable, they remain largely descriptive and cannot tell us how or why bioluminescence is actually used. Knowing that humans produce sound does not tell us what those sounds are for, let alone if they are important. **The ambitious behavioural experiments in this project (WPs1-3) will be among the first in the field and help us finally decipher what bioluminescent signals mean and their importance for the organisms themselves**. In addition, by comparing two related annelid species, *T. helgolandica* and *T. planktonis*, this project will significantly contribute to our understanding of the evolution of both behaviour and that of the nervous system (WP4) in general. **Laying the foundation for *in vivo* imaging studies, this project will pave the way for a future ERC application with a goal to achieve direct readouts of neural function in non-transgenic gelatinous animals, which has not yet been achieved**. Using state-of-the-art techniques, our cross-disciplinary approach will advance both the fields of marine biology and neuroscience, providing novel insights into the functions and neuroanatomy of bioluminescence on the one hand, and the neural circuits underlying animal communication on the other. Further, this novel, interdisciplinary research line will have extensive implications for our understanding of nervous function in all species, whose nervous systems evolved from the same habitat in which these marine annelids still live.

## 2.2 Potential for societal impact of the research project

The importance of the ocean is more evident than ever, as highlighted by its inclusion in the UN's Sustainable Development Goal (SDG) number 14, "Life Below Water"[2]. Only with increased scientific knowledge (SDG target[26]) and a deeper understanding of how life in the oceans communicate can we minimise our increasing impact on this ecosystem. In turn, this will lead to the realisation of another SDG target: to prevent and reduce marine pollution[26], specifically light pollution, which is known to dramatically affect the behaviour of organisms in naturally dark waters[27]. A deeper understanding of the importance of bioluminescence for the oceans' inhabitants will help us design less harmful, and limit the use of, artificial light as our enterprises expand into this vast territory.

## 2.3 Measures for communication and exploitation

To make our results public (dissemination), promote (communication) and make concrete use of them (exploitation), we will target scientific and public audiences through several avenues, including conference presentations and online platforms, as presented in Table 2 below.

**Table 2: measures for communication and exploitation**

Acronyms: NRSN (Norwegian Research School in Neuroscience), FENS (Federation of European Neuroscience Societies), SfN (Society for Neuroscience), EMBS (European Marine Biology Symposium), ISBC (International Society for Bioluminescence and Chemiluminescence)

| | Target audience | Scientific | Public |
|---|---|---|---|
| **Activities and scope** | **Dissemination**<br>*Scope: throughout project period* | - Important results presented at national and international conferences, such as those organised by NRSN and ISBC<br>- Publication of at least 6 open access articles in journals such as eLife and Current Biology<br>- Data, code and analysis made available in open repositories, such as EBRAINS, GitHub and Figshare | - Activities, results, and information on scientific methods presented in accessible manner on online platforms, such as the lab website and Twitter account<br>- Engagement through discussions on Twitter and public outreach activities (e.g. writing for gemini.no and forskersonen.no, and presentations at events such as Forskningsdagene) |
| | **Communication and engagement**<br>*Scope: throughout project period* | - Availability of results, data, code, and analysis announced on lab website and Twitter account<br>- Discussions at conferences (organised by e.g. FENS, SfN, EMBS, ISBC), on Twitter and in seminars held at both departments affiliated with the project | |

| | | | |
|---|---|---|---|
| | **Exploitation**<br>*Scope: towards end of project and after* | - Generation of new experimental protocols for bridging marine biology and neuroscience research<br>- Sharing knowledge, skills, and data | - Sharing knowledge, skills, and data in an accessible, jargon free manner to increase societal awareness of light in the oceans |

# 3. Implementation

## 3.1 Project manager and project group

**Project manager**

My educational background and most of my research have been within the field of neuroscience. Inspired by notable neuroscientists such as Kandel, Hodgkin and Huxley, I firmly believe that marine life holds the key to many unanswered questions about the brain. Ever since my undergraduate degree, my dedication to research has led me to seek out and participate in a large variety of research activities. This included volunteering at a shark conservancy in order to familiarise myself with research methods in marine biology. My aim has been to gain the knowledge, skills and experiences necessary to establish my own research group and to take my principal field, neuroscience, into the future by going back to where it all began: the ocean.

After training in international world-leading research institutes with top scientists within the field of neuroscience and immersing myself in cutting-edge techniques, I finally made my dream a reality. In 2019, I started contacting experts within marine biology, researching candidate organisms and unresolved questions in the field, and establishing national and international collaborations. With this foundation in place and the generosity of my collaborators, I started the first experiments with the organisms of my choice in August 2020 and simultaneously launched the Marine Neuroscience Laboratory. At the end of January 2021, I secured the first funding grant for the lab, which allows me to rent and purchase essential equipment.

Funding from the Researcher Project for Young Talents will allow me to take my dream one step further by allowing me to 1) continue to build the behavioural and neuroanatomical foundation on which my research plan and future funding opportunities (e.g. from the ERC) rely upon, 2) elevate my lab from spare-time ambition to full-time endeavour, and 3) gain extensive experience with marine organisms. Combined, these elements will help me to continue to establish myself as an independent researcher that bridges and leads both marine biology and neuroscience into novel directions.

**Project group**

Apart from my full-time commitment to the project, it will also be supported by a PhD student (in-kind donation from NTNU) and a part-time technician. As I have extensive experience with all the techniques employed, I will train the PhD student to perform all the proposed experiments with me. I will also train the technician to provide support, mainly centred on maintenance and optimisation of housing conditions for our animals for WPs1-3 and preparation of stock solutions for WP4. Both new team members will be recruited based on the interests and skills they bring to the lab. Their positions will be advertised widely through my and my collaborators' networks to ensure they reach a wide and diverse audience. My support of both will be tailored according to their independent personal and career-related needs, ensuring they receive the training and experience necessary to perform the project tasks at hand, as well as prepare them for the career step they would like to pursue next.

## 3.2 Project organisation and management

### Work plan

This project will last four years and consist of four complementary WPs (Figure 3). Communication and exploitation measures outside the writing and publication of research manuscripts will take place throughout. The first months will be spent acquiring the necessary equipment, building setups, and recruiting team members. Once recruited and trained by me, all team members will contribute to all tasks. The building and optimisation of setups for WPs2-3 is expected to be faster as they share commonalities with WP1 and all team members will be sufficiently trained by then.

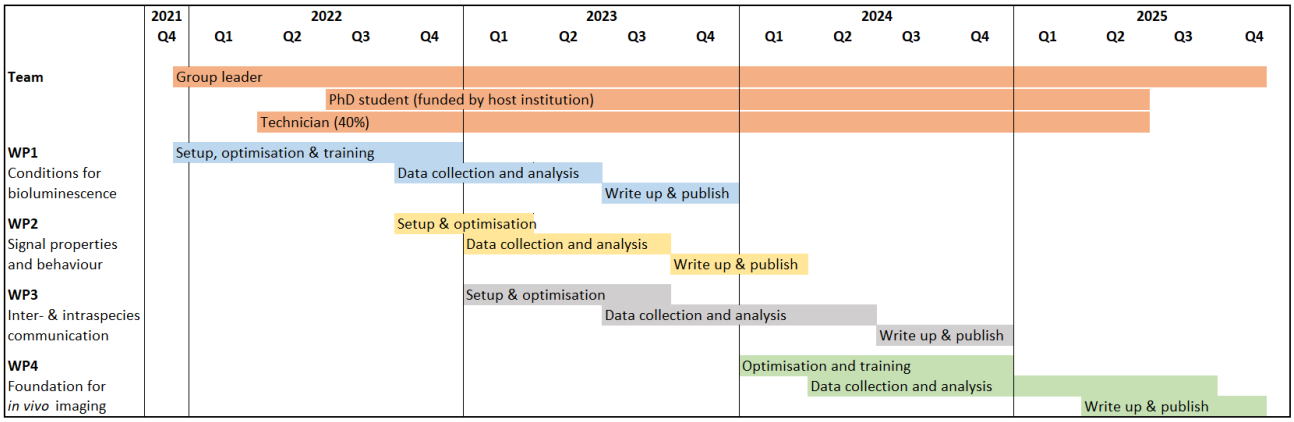


**Figure 3: Gantt chart of project work plan**

### Research infrastructure and organisation

The multidisciplinary nature of this project means that it will take place in two locations individually optimised for their respective fields. Behavioural experiments (WPs1-3) will be performed at SeaLab in the Faculty for Natural Sciences (NTNU). SeaLab has the necessary infrastructure and expertise for marine biology research. This includes fresh seawater pumped up from the sea floor and climate-controlled rooms for housing a variety of organisms and performing experiments. Neuroanatomical characterisations and the foundation for future *in vivo* imaging (WP4) will be performed at the Kavli Institute for Systems Neuroscience in the Faculty of Medicine and Health Sciences (NTNU), where advanced facilities and expertise for the processing and imaging of neural tissue are available, including equipment for sectioning and a wide variety of microscopes through NORBRAIN III. An affiliation with both places has already been established through my postdoctoral work at Kavli and independent collaboration at SeaLab. The team will be managed by the project manager and group leader, Ragnhild Irene Jacobsen.

# References


1. Jabr, F. The Secret History of Bioluminescence. *Hakai Magazine* (2016).
2. Oceans – United Nations Sustainable Development. Available at: https://www.un.org/sustainabledevelopment/oceans/. (Accessed: 31st January 2021)
3. Shimomura, O. *Bioluminescence: Chemical Principles and Methods*. (Singapore: World Scientific Publishing Co. Pte. Ltd, 2006).
4. Martini, S. & Haddock, S. H. D. Quantification of bioluminescence from the surface to the deep sea demonstrates its predominance as an ecological trait. *Sci. Rep.* **7**, 1–11 (2017).
5. Haddock, S. H. D., Moline, M. A. & Case, J. F. Bioluminescence in the Sea. *Ann. Rev. Mar. Sci.* **2**, 443–493 (2010).
6. Widder, E. A. Bioluminescence in the ocean: Origins of biological, chemical, and ecological diversity. *Science* **328**, 704–708 (2010).
7. Herring, P. J. Review. 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**, 829–842 (2007).
8. Haddock, S. *et al.* Insights into the Biodiversity, Behavior, and Bioluminescence of Deep-Sea Organisms Using Molecular and Maritime Technology. *Oceanography* **30**, 38–47 (2017).
9. Raskoff, K. A., Sommer, F. A., Hamner, W. M. & Cross, K. M. Collection and culture techniques for gelatinous zooplankton. *Biol. Bull.* **204**, 68–80 (2003).
10. White, H. H. Effects of dinoflagellate bioluminescence on the ingestion rates of herbivorous zooplankton. *J. Exp. Mar. Bio. Ecol.* **36**, 217–224 (1979).
11. Esaias, W. E. & Curl, H. C. Effect of dinoflagellate bioluminecence on copepod ingestion rates1. *Limnol. Oceanogr.* **17**, 901–906 (1972).
12. Hanley, K. A. & Widder, E. A. Bioluminescence in Dinoflagellates: Evidence that the Adaptive Value of Bioluminescence in Dinoflagellates is Concentration Dependent. *Photochem. Photobiol.* **93**, 519–530 (2017).
13. Buskey, E., Mills, L. & Swift, E. The effects of dinoflagellate bioluminescence on the swimming behavior of a marine copepod1. *Limnol. Oceanogr.* **28**, 575–579 (1983).
14. Buskey, E. J. & Swift, E. Behavioral responses of oceanic zooplankton to simulated bioluminescence. *Biol. Bull* **168**, 263–275 (1985).
15. Buskey, E. J. & Swift, E. Behavioral responses of the coastal copepod Acartia hudsonica (Pinhey) to simulated dinoflagellate bioluminescence. *J. Exp. Mar. Bio. Ecol.* **72**, 43–58 (1983).
16. Gouveneaux, A., Gielen, M. C. & Mallefet, J. Behavioural responses of the yellow emitting annelid Tomopteris helgolandica to photic stimuli. *Luminescence* **33**, 511–520 (2018).
17. Gouveneaux, A., Flood, P. R. & Mallefet, J. Unexpected diversity of bioluminescence in planktonic worms. *Luminescence* **32**, 394–400 (2017).
18. Dupre, C. & Yuste, R. Non-overlapping Neural Networks in Hydra vulgaris. *Curr. Biol.* **27**, 1085–1097 (2017).
19. Szymanski, J. R. & Yuste, R. Mapping the Whole-Body Muscle Activity of Hydra vulgaris. *Curr. Biol.* **29**, 1807-1817.e3 (2019).
20. Reiter, S. *et al.* Elucidating the control and development of skin patterning in cuttlefish. *Nature* **562**, 361–366 (2018).
21. Gouveneaux, A. *et al.* Morphology and fluorescence of the parapodial light glands in Tomopteris helgolandica and allies (Phyllodocida: Tomopteridae). *Zool. Anz.* **268**, 112–125 (2017).
22. Mathis, A. *et al.* DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281–1289 (2018).
23. Rivers, T. J. & Morin, J. G. Complex sexual courtship displays by luminescent male marine ostracods. *J. Exp. Biol.* **211**, 2252–2262 (2008).
24. Gouveneaux, A. & Mallefet, J. Physiological control of bioluminescence in a deep-sea planktonic worm, Tomopteris helgolandica. *J. Exp. Biol.* **216**, 4285–4289 (2013).
25. Heuer, C. M., Müller, C. H., Todt, C. & Loesel, R. Comparative neuroanatomy suggests repeated reduction of neuroarchitectural complexity in Annelida. *Front. Zool.* **7**, 13 (2010).
26. #Envision2030 Goal 14: Life Below Water. Available at: https://www.un.org/development/desa/disabilities/envision2030-goal14.html. (Accessed: 2nd February 2021)
27. Berge, J. *et al.* Artificial light during the polar night disrupts Arctic fish and zooplankton behaviour down to 200 m depth. *Commun. Biol.* **3**, 1–8 (2020).

# Summary of marks

| Criterion | Mark |
|---|---|
| Excellence | 6 |
| Impact | 5 |
| Implementation | 4 |
| Overall assessment of the referee/panel | 5 |

# Criteria

## Excellence

.

The extent to which the proposed work is ambitious, novel, and goes beyond the state-of-the-art
• Scientific creativity and originality.
• Novelty and boldness of hypotheses or research questions.
• Potential for development of new knowledge beyond the current state-of-the-art, including significant theoretical, methodological, experimental or empirical advancement.

The quality of the proposed R&D activities
• Quality of the research questions, hypotheses and project objectives, and the extent to which they are clearly and adequately specified.
• Credibility and appropriateness of the theoretical approach, research design and use of scientific methods. Appropriate consideration of interdisciplinary approaches.
• The extent to which appropriate consideration has been given to ethical issues, safety issues, gender dimension in research content, and use of stakeholder/user knowledge if appropriate.

A highly creative and original project that will investigate a widespread but largely neglected area, bioluminescence and colour mediated inter- and intraspecies communication along with the neuronal circuits underlying this form of communication in two deep-sea tomopterid annelids.

The project is well written, and the research questions are clear, logical and well-articulated with the state of the art. The rationale for the project is well-presented and justified by current knowledge about bioluminescent organisms.

The study holds significant likelihood of producing unique new knowledge and the claim that the project may open several new research lines and experimental procedures of cross-cutting relevance is credible.

The state of the art provides a solid and logical basis for the workplan proposed. The choice of the model is well justified since the unique transparent characteristics of tomopterids will be exploited and are advantageous for the aim of the project.

The four main hypotheses are clearly presented and are appropriate to address the biology of bioluminescent signalling.

The WPs are described in a very superficial manner. Only a general description of the approach undertaken in each of the WPs is presented. There is minimal details of methods, number of replicates, endpoints to measure. As such, the credibility and appropriateness of the approach, design and methods cannot be adequately evaluated.

A relevant risk analysis is presented, although risks linked to aquaria-based maintenance and behavioural changes caused by captivity or feeding issues and survival linked to maintaining wild animals in aquaria and mitigation measures would have been beneficial.

Ethics have been considered and even though not covered by regulations research will follow 3R principals, which is commendable.

Selected mark : 6 - Excellent
The proposal successfully addresses all relevant aspects of the criterion. Only minor shortcomings are present.

# Impact

.

Potential impact of the proposed research
• Potential for academic impact:
The extent to which the planned outputs of the project address important present and/or future scientific challenges.
• Potential for societal impact (if addressed by the applicant):
The extent to which the planned outputs of the project address UN Sustainable Development Goals or other important present and/or future societal challenges.
• The extent to which the potential impacts are clearly formulated and plausible.

Communication and exploitation
• Quality and scope of communication and engagement activities with different target audiences, including relevant stakeholders/users.

The potential academic impact of the planned outputs of the proposal is high, as knowledge about marine annelid bioluminescence and related behaviors is very limited. In addition, the proposal aims to lay the foundations to develop methodologies that will greatly help address important scientific questions related to the neurological basis of animal communication.

The proposal will contribute to the UN's Sustainable Development Goal basically by increasing scientific knowledge on basic aspects of life below water. Other argued benefits, a) the prevention and reduction of light pollution and b) understanding how life in the oceans communicate to minimize anthropogenic impacts are unconvincing.

Actions for communication are presented but are over general and include scientific publications, presence at conferences, etc. Communication with the general public is foreseen and involves mainly social media and on-line platforms. Measures for monitoring successful project impact would have been beneficial.

The compliance with FAIR principles in relation to data release is clearly stated, which is commendable.

Selected mark : 5 - Very good
The proposal addresses the criterion very well. A small number of shortcomings are present.

# Implementation

.

The quality of the project manager and project group
• The extent to which the project manager has relevant expertise and experience, and demonstrated ability to perform high-quality research (as appropriate to the career stage).
• The degree of complementarity of the participants and the extent to which the project group has the necessary expertise needed to undertake the research effectively.

The quality of the project organisation and management
• Effectiveness of the project organisation, including the extent to which resources assigned to work packages are aligned with project objectives and deliverables.
• Appropriateness of the allocation of tasks, ensuring that all participants have a valid role and adequate resources in the project to fulfil that role.
• Appropriateness of the proposed management structures and governance.

The project manager (PM) has a moderate scientific output. Some more evidence of recent outputs and leadership positions would have strengthened the demonstration of leadership potential.

One shortcoming of the workplan management is the potential risks as the PM may underestimate the difficulty of establishing functional imaging in the species chosen. The proposed methodology includes injections of voltage or calcium dyes into identified neurons in the worms. However, it is not clear how neurons will be identified in the live worms (immunostaining will not be of too much help) and it is unclear how the relevance of these neurons in bioluminescent signalling will be determined. It might have been better to first focus on the eyes or the bioluminescent organs and their neuronal control.

The project depends on the ability of the experimental organisms to survive for enough time under lab conditions. Although this has been reported by others, evidence from preliminary tests under the lab setting would have reinforced the feasibility of the project workplan.

The PM has the necessary expertise to implement all aspects of the project workplan and will train the proposed PhD and technician. However, experience in project management is less clear and although a senior scientist will assist if necessary their CV is absent and their exact role in the project is not defined, which is a shortcoming.

Despite the small size of the consortium (PM, PhD and technician) a better outline of the management structure and strategy would have been beneficial. For example, clearer indication of specific milestones and deliverables would have been beneficial.

The necessary infrastructure for implementation of the project is well demonstrated and the requested funding is fully justified.

Selected mark : 4 - Good
The proposal addresses the criterion well. A number of shortcomings are present.

# Overall assessment of the referee/panel

.

Overall assessment of the referee/panel based, on the criteria Excellence, Impact and Implementation.

A creative and original project that is well written with clear and logical research questions that are well-articulated with the state of the art. The rational for the project is well-presented and justified by current knowledge about bioluminescent organisms. The hypothesis to be tested related to bioluminescence and colour mediated inter- and intraspecies communication along with the neuronal circuits underlying this form of communication in tomopterids (gelatinous organisms) can be expected to deliver high impact and significantly advance both science and technology. While the potential for development of new knowledge is high, the quality of the proposed R&D activities is lower since the project had inadequate details about the data collection and data analysis methodology and therefore the credibility and appropriateness of the approach, design and methods could not be appropriately evaluated. The workplan implementation and management failed to clearly outline the management strategy and structure.

Selected mark : 5 - Very good
The proposal is very good. The criteria are very well addressed. A small number of shortcomings are present.

# Special points to consider

Comments to special points to consider