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Executive Summary

This report describes the activities and results in Project MINOUW related to Technological Innovation Task 2.7: the use of lights as novel stimuli. This task was lead by Amit Lerner (IOLR) and specifically addresses case studies:

1.2 – Algarve deep-water trawl fishery (Lead: CCMAR) and

3.7 – Norwegian gadoid pots (Lead: IMR).

The work was organised, and is reported in, three distinct parts:

- Determining the visual capacities of target and by-catch species using the opto-motor response (OMR)(Lead: IOLR);
- Case Study 1.2: Behavioural responses of target and non-target species in the Algarve deep-water trawl fishery to artificial light (Lead: CCMAR); and
- Case Study 3.7: Behavioural Responses of Krill and Cod to Artificial Light (Lead: IMR)

The first aim of Task 2.7 was to determine the visual capacities of polarization sensitivity, sensitivity threshold to light intensity, and temporal and spatial resolution, in the most important target, bait and principal bycatch species in two commercial fisheries. Then for each case study controlled experiments would determine the behavioural responses of target and bycatch species to selected light stimuli. In CS 1.2 (Portugal) the target species are rose shrimp (Parapenaeus longirostris) and Norway lobster (Nephrops norvegicus), while the bycatch species are the Blue jack mackerel (Trachurus picturatus) and the Atlantic chub mackerel (Scomber colias). In CS 3.7 (Norway), the target species Atlantic cod (Gadus morhua) and Northern krill (Meganyctiphanes norvegica), a principal prey species for cod. The knowledge gained in this phase of the research will be used in the following stages to develop light sources to be implemented on the fishing gear in Task 2.8 to both increase catch efficiency of the target species and reduce by-catch.

<u>Determining the visual capacities of target and by-catch species using the opto-motor response (OMR)</u>

The goal of this part of Task 2.7 was to define the visual capacities of commercial and by-catch species, namely polarization sensitivity, threshold of intensity sensitivity, and temporal and spatial resolutions, to possibly use this information in the future to differentiate the target and by-catch species using artificial light sources on the fishing gear based on their vision.

These experiments have, using the opto-motor response (OMR), attempted to determine the visual capacities for cod (Gadus morhua), chub mackerel (Scomber colias) and blue jack mackerel (T. picturatus), with respect to polarization sensitivity, threshold of intensity sensitivity, and temporal and spatial resolutions. However, rose shrimp (Parapenaeus longirostris) and the krill (Meganyctiphanes norvegica) did not

respond in the OMR apparatus and alternate tests such as escape response or optokinetic (eye movement) response may be more appropriate techniques for crustacea.

For Case Study 1.2 (Algarve deep-water trawl), chub mackerel had a critical flicker fusion frequency (CFF) which ranged between 20-70 Hz. Therefore, light with a flickering rate of ~10-20 Hz (i.e. <20-70 Hz) could be used to repel chub mackerel from deepwater trawls without affecting the target crustacean species, Nephrops. Polarized light may also be useful to repel non-target blue jack mackerel from shrimp fishing gear, although only one individual responded to polarized light in this study.

For case study 3.7 (Norwegian gadoid pots), we recommend that the light intensity to stimulate cod could range from water surface intensities of 1 µE cm-2 s-1 nm-1] and down to ~10-12 µE cm-2 s-1 nm-1and less. Light polarization may be useful for attracting cod to pots, as we have provided evidence to suggest that cod may possess the ability to detect polarized light and could use this capacity to target their prey. Furthermore, cod have a CFF ranging between 20-100 Hz depending on light intensity, therefore if a flickering light is used to attract potential prey (i.e. krill) to a pot, the light should have a flickering rate higher than 20-100 Hz to avoid repelling the target cod.

These results will, as part of task 2.7, be used to further investigate the behavioural response of these species to artificial light sources, as well as design and test light sources mounted on fishing gears in Task 2.8: Alternative Fishing Gears.

Case Study 1.2: Behavioural responses of target and non-target species in the Algarve deep-water trawl fishery to artificial light.

The main target species of the deep-water trawl fishery are the rose shrimp, *Parapenaeus longirostris* and the Norway lobster, *Nephrops norvegicus*. Blue whiting and small pelagic species constitute most of the unwanted by-catch. The strategic goal of this case study is to achieve by-catch minimization solutions with minimum losses of crustacea. One approach is the use of lights to promote avoidance behaviour in unwanted fish species. An alternative approach is to improve the catchability of N. norvegicus traps (already a viable commercial fishery) and to introduce efficient traps to catch P. *longirostris*. The planned work involves laboratory tests and field work. At present, only the laboratory tests to determine the visual capacity of selected species have been completed (see OMR results).

Plans for ongoing work to complete this case study include:

- Identify attraction/repulsion behaviour for *N. norvegicus* and *P. longirostris* using behavioural experiments – September to December 2016.
- Undertake field tests using lights to decrease by-catch in trawlers.– March to October 2017.
- Test the use of attractant lights to improve catchability of already operating N. norvegicus traps – February to April 2017.
- Evaluate the possibility of using lights as attractant in traps for *P. longirostris* – January 2017.

The results from this continuing work will be reported in D2.13 - Reports on the use of novel stimuli to minimise unwanted catch.

Case Study 3.7: Behavioural Responses of Krill and Cod to Artificial Light

The goal of this case study is to increase the number of cod that enter pots using artificial illumination tuned to the species' vision and visually-guided behaviour. This can be achieved by attracting either the cod and/or their prey krill. Thus, to find which light characteristics are most attractive for krill, we first tested the effect of light intensity, wavelength composition and flickering fusion frequency on krill's attraction to a light source. The most promising light stimuli for krill were then tested to determine whether they would have repulsive or attractive effects on cod.

Results from our investigation support the hypothesis that artificial light is most attractive to krill across a range of wavelengths (448 nm to 530 nm) that include peak visual sensitivity (490 nm, Frank and Widder 1999), as well as the wavelength of its bioluminescence (470 nm, Kay 1962; 1965). The most attractive individual wavelength was 530 nm, while broadband "White" light was an equally attractive light source. The intensity of the emitted light did not appear to have a direct effect on attraction to the light source; however it did significantly increase swimming activity among the observed krill.

Cod demonstrated no strong behavioural response to the artificial light source. Since krill were most attracted to steady light at 530 nm wavelength, and the difference between steady and 2 Hz strobe was insignificant for cod, we recommend that a steady 530 nm light, with an irradiance (at 1m from the source) of approximately 0.25 µE m-2 s-1, should be tested in commercial fishing pots in our field fishing study to be undertaken as part of Task 2.8: *Alternative Fishing Gears*. Furthermore, this experiment should use cameras to demonstrate that krill are aggregating around the light source, and take stomach samples from the cod to determine whether the cod have been feeding on krill.

1. Introduction

This report describes the activities and results in Project MINOUW related to *Technological Innovation Task 2.7: the use of lights as novel stimuli*. This task was lead by Amit Lerner (IOLR) and specifically addresses case studies:

1.2 – Algarve deep-water trawl fishery (Lead: CCMAR) and

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in the most important target, bait and principal bycatch species in two commercial fisheries. Then for each case study controlled experiments would determine the behavioural responses of target and bycatch species to selected light stimuli. In CS 1.2 (Portugal) the target species are rose shrimp (*Parapenaeus longirostris*) and Norway lobster (*Nephrops norvegicus*), while the bycatch species are the Blue jack mackerel (*Trachurus picturatus*) and the Atlantic chub mackerel (*Scomber colias*). In CS 3.7 (Norway), the target species Atlantic cod (*Gadus morhua*) and Northern krill (*Meganyctiphanes norvegica*), a principal prey species for cod. The knowledge gained in this phase of the research will be used in the following stages to develop light sources to be implemented on the fishing gear in Task 2.8 to both increase catch efficiency of the target species and reduce by-catch.

Project Objectives

This work has contributed to the following project objectives:

O2.7 Use novel stimuli to avoid unwanted catches; and

O2.8 Development alternative fishing techniques to avoid unwanted catches.

and to the technological innovation tasks:

T.2.7. – Using artificial light as a novel stimuli;

T.2.8. - Alternative fishing methods.

Deliverables & Milestones

This work has contributed directly to the following project deliverables and milestones:

D 2.12 Behavioural Responses to Novel Stimuli

MS18: Innovative solutions to reduce post-catch losses

MS19: Innovative solutions to reduce pre-catch losses

MS26: Technological solutions to unwanted catches: developed as planned

MS27: Technological solutions to unwanted catch: defined/benchmarked

2.1. Background to Case Studies

Case Study 1.2 - Algarve deep-water trawl fishery.

Main target species of the deep-water trawl fishery are the rose shrimp, *Parapenaeus longirostris* and the Norway lobster, *Nephrops norvegicus*. Blue whiting and small pelagic species constitute most of the unwanted by-catch.

Bottom trawling for deep-water crustaceans, the rose shrimp, *Parapenaeus longirostris* and the Norway lobster, *Nephrops norvegicus* involves a total of 26 Portuguese trawlers (end of 2015), constituting a well-defined fleet component licensed for cod-end mesh sizes of 55mm (targeting rose shrimp) and 70mm (targeting Norway lobster), which operates off the south and southwest coasts of Portugal (ICES Functional Units 28 and 29) at depths from 150 to around 750m. Additionally, 5 fishing licenses are distributed among 9 Spanish trawlers, also operating within this area within the framework of a fishing agreement.

According to their relative abundance and market value, the fleet may re-direct the fishing effort preferentially to one of these species, as shown by the existence of two distinct species-oriented landing profiles in the fishery (Campos *et al.*, 2007), one at lower depths, where rose shrimp is the main target species and a second one where the proportions of Norway lobster and rose shrimp are similar.

There is a substantial capture of by-catch in addition to the target species, including high commercial value species such as the European hake, *Merluccius merluccius*, and the monkfishes, *Lophius* spp. However a large subset, comprising a miscellaneous collection of fish, crustaceans, cephalopods and other invertebrates is discarded at sea, together with undersized individuals of commercial species. Discards data from different studies estimated discard rates (*per* fishing trip in weight) from 38% (Monteiro *et al.*, 2001) to 70% (Borges *et al.*, 2001). The main species discarded were hake, due either to the catch of an important fraction under the MLS or quota reasons; and small-spotted dogfish, conger and boarfish due to their low commercial value. The blue whiting was found to be the most important discard in the first study, representing 30% of the total weight discarded. Prista and Santos (2012) identify horse mackerel and hake as the main discard species in hauls targeting rose shrimp, while blue whiting was found to be the main discard when the Norway lobster was targeted.

Deepwater crustaceans are almost exclusively caught with bottom trawls. Only the Norway lobster has a small percentage of catches made with creels by vessels in the multi-gear fleet. Although the amounts are lower than those obtained by trawling (about 14% by weight in the last 5 years), its relative importance in value is much higher (larger individuals and sold alive) accounting for 27% over the same period. If the income is compared, it can be concluded that fishing with creels has higher economic returns than those of trawling, which is highly vulnerable to fuel price fluctuations and declines in the value of the product (Leocádio *et al.*, 2012). If the environmental impacts of both gears are considered, creels are incomparably less harmful from an environmental point of view, being selective for the target species (Adey, 2007; Kinnear *et al.*, 1996).

Within the current CFP reform, a new topic was introduced in European fisheries, the discard ban or “Landing Obligation”. This entails a transformation to a policy where all

fish caught count on the TAC/quotas as opposed from present management where only the fish landed count. When enforced, a discard ban will strongly affect crustacean trawling, for which discard rates are typically high. The extent to which this fishery will be affected will depend on the ability to deal with the by-catch problem. Although a number of fishing strategies and tactics can contribute to avoid by-catch, the most effective way to promote by-catch reduction in a fishery-by-fishery basis is the improvement in gear selectivity.

Case Study 3.7 - Norwegian gadoid pots.

Behavioural observations have shown that despite baited pots attracting sufficient numbers of targets, too few fish enter and remain in the pots to make them an economically viable alternative to other fishing methods (Furevik 1994, Rose et al 2005, Thomsen et al 2010). Thus the main challenge is to increase the efficiency of this environmentally responsible gear, by developing suitable stimuli to encourage the target species like cod (*Gadus morhua*) into the pot.

Cod are predators, not scavengers. So, while the odour from inanimate bait may attract them to a pot, it will not present the visual appearance of a potential prey and therefore may not provide a sufficiently strong stimulus for them to enter the pot. Recent experiments conducted in the Baltic Sea have shown that pots fitted with green lights increase catch weight by 80% (Bryhn et al, 2014). Furthermore, a study in Iceland yielded comparatively high catch rates (9-10 kg/day/m^3 of trap volume) for pots equipped with light only (Einar Hreinsson, Icelandic Institute of Marine Research, pers. comm.). The artificial light attracted large quantities of krill ***Meganyctiphanes norvegica)*** into the pots, and the cod captured in these pots were observed to feed on the krill.

The goal of this case study is to increase the number of cod that enter pots using artificial illumination tuned to the species' vision and visually-guided behaviour. This can be achieved by attracting either the cod and/or their prey krill.

Thus, to find which light characteristics are most attractive for krill, we first tested the effect of light intensity, wavelength composition and flickering fusion frequency on krill's attraction to a light source. The most promising light stimuli for krill were then tested to determine whether they would have repulsive or attractive effects on cod.

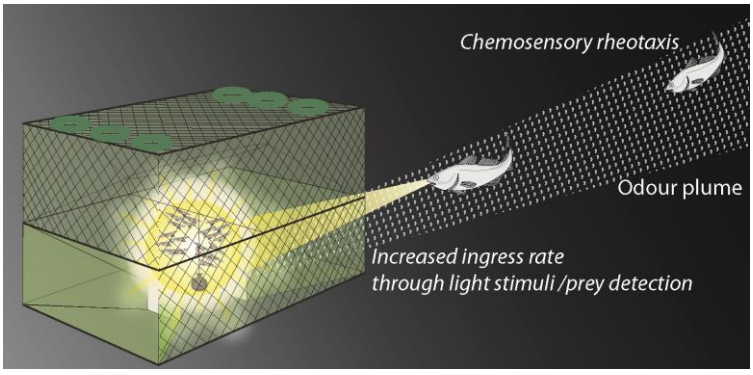

Figure 1.1: Increasing the catch efficiency of baited pots by using artificial light to control behaviour of cod: increased ingress motivation by switching from chemosensory to visual foraging towards light attracted krill at night. Two chamber pot dimensions: 1,5 x 1.0 x 1.2 m (length x width x height; Furevik et al 2008).

2. Determining the visual capacities of target and by-catch species using the opto-motor response (OMR)

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2.2. Introduction

Different taxa of marine species possess different visual capacities often determined by their habitat and way of life. For example, crustaceans that live close to the ocean bottom commonly possess low visual resolution (spatial and temporal) and high sensitivity to light, while open water predator species (fish, cephalopods) possess high visual resolution. Another possible differentiating visual characteristic between taxa is the perception of polarized light. Polarization vision is a common visual capacity in many invertebrate groups such as Arthropods (Crustaceans) and Mollusks (Cephalopods) but rarely documented with unknown mechanism of perception in vertebrates such as fish (Horváth and Varjú 2004, Horváth 2014). Therefore, artificial light sources projecting a differentiating level of one or more of these characteristics of light can potentially be used to attract/repel target and by-catch species from different taxa from/to fishing gear in commercial fishery and, consequently, increase target species catch efficiency or reduce by-catch.

The first aim of Task 2.7 is to test the visual capacities of polarization sensitivity, sensitivity threshold to light intensity, and temporal and spatial resolution, in the most important target, bait and principal bycatch species in two commercial fisheries. In CS 3.7 (Norway), sensitivity was tested for the target species Atlantic cod (*Gadus morhua*; Gadidae) and Northern krill (*Meganyctiphanes norvegica; Euphausiidae*), a principal prey species for cod. In CS 1.2 (Portugal) the target species is rose shrimp (*Parapenaeus longirostris*; Penaeidae) and the bycatch species are the Blue jack mackerel *Trachurus picturatus* (Carangidae) and the Atlantic chub mackerel *Scomber colias* (Scombridae). The knowledge gained in this phase of the research will be used in the following stages to develop light sources to be implemented on the fishing gear in Task 2.8 to increase catch efficiency of the target species and reduce the by-catch ones.

2.3. Methodos

Test species

The studies in CS 3.7 were conducted at the Austevoll field station of the Institute of Marine Research (IMR), Norway. Krill were captured in the nearby fjord and kept in a tank with running sea water. Juvenile cod were bred and kept at the station facilities in

dark conditions in a 50-l tank and fed twice a day (morning and evening). Larger wild cod were also caught, however were too big to use in the OMR apparatus.

Studies conducted under CS 1.2 were performed at the laboratories of the University do Algarve, Faro, Portugal. Rose shrimp, Atlantic chub mackerel, and blue jack mackerel fish were collected by commercial fishermen and kept in a dark room under 12:12 hours light:dark conditions.

The OMR apparatus

An optomotor response (OMR) apparatus was used to assess the visual capacities of all species tested. The apparatus consists of a drum (50 cm in diameter used on the cod and the chub mackerel, and 40 cm in diameter used on the blue jack mackerel) which is rotated by a controllable motor (Fig. 1A; Li 2001). A vertical stripe pattern (e.g. unpolarised white and black stripes) was placed in the interior wall of the drum. A glass tank (40 cm in diameter used for the polarization and all chub mackerel experiments, and 23 cm in diameter used for the cod sensitivity, resolution and all blue jack mackerel experiments) containing seawater and a single test animal was placed on a stationary platform at the center of the drum (Fig. 1B). A video camera was suspended over the tank to record the animal's movement. The optomotor apparatus was illuminated from behind the pattern using a controlled strip of white LED lamps (LED STRIP DC24V 5050, Bright lighting Ltd, Zhuhai, China) surrounding the drum to avoid reflections from the polarized stripes that will reveal them to polarization insensitive visual system (Fig. 1C; S1). A magnetic speedometer at the top of the platform measured the drum's speed.

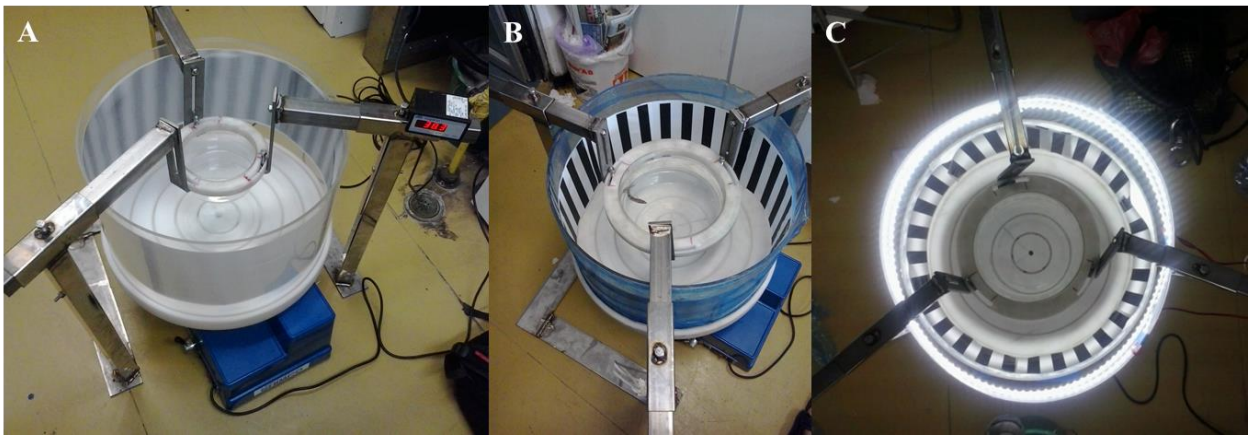

Fig. 1. The OMR apparatus. (A) The drum and the controllable motor. (B) The removable striped pattern and the glass tank suspended by the platform. (C) The stripes being illuminated by a LED strip light positioned outside of the drum.

Experiment 1 – sensitivity to light polarization

OMR apparatus with polarized grating has been demonstrated as a useful method to determine species polarization sensitivity in past studies both for cephalopods and fish (Darmaillacq and Shashar 2008, Talbot and Marshall 2010, Berenshtein et al. 2014). To test for response to polarized cues, the test animals were exposed to a polarized vertical stripe pattern of 25.4 mm width (POL, M318, American Polarized Inc., Reading. PA, USA; Fig. 2). The pattern cannot be seen by polarization insensitive visual systems

like the human eye or a common camera. The partial polarization projected from the stripes was > 80% throughout the 400-700 nm wavelength range. The stripes included polarization e-vector orientations of 0°, 45°, 90°, and 135°.

Controls

Each individual was tested against some controls to determine its response to the rotating grating. The controls included the following: (a) response to unpolarised (black and white) stripes that are visible to the human eye, to ensure there was a response from the animal to the apparatus, (b) no response to blank ("white") sheet with no stripes, to ensure that the animal did not follow other cues than the rotating grating. In the 'rest' periods, during which the drum was not rotated, the fish movement was recorded to assure that its movement is different than its movement when the stimulus is provided (the drum rotation). In general, the fish performed a lower, non-constant swimming speed during this period. Only animals that successfully responded to all the controls were tested against polarized grating to check for polarization sensitivity.

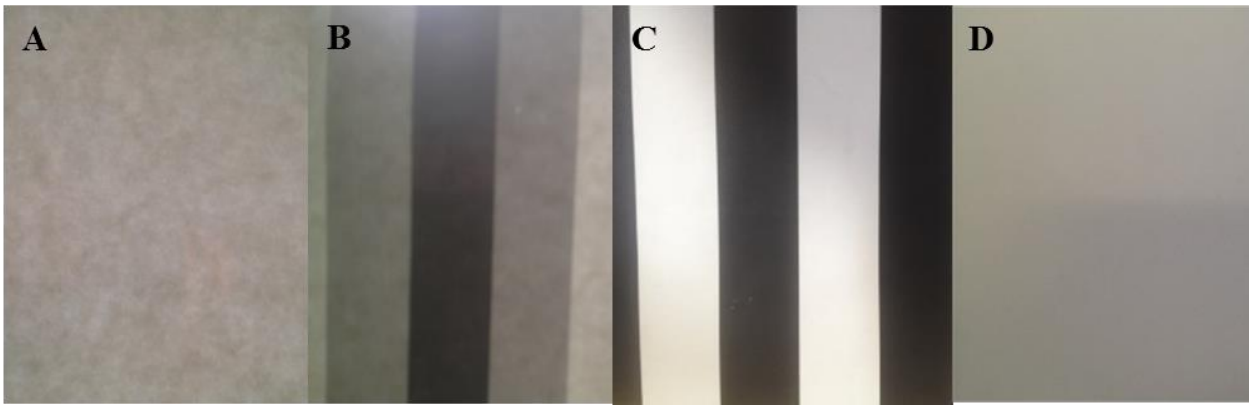

Fig. 2. The three removable stripe patterns used in the polarization sensitivity experiment. (A) Linear polarization grating ("POL") photographed without a polarization filter, (B) The linear polarization grating photographed through an analyzer (polarization filter) on the camera, (C) Black and white pattern ("unpolarised "), (D) plain ("W") sheet with no grating.

Experimental protocol

The experimental protocol conducted on each individual for all the experiments and tests included the following: (1) placing the animal in the experimental tank and leaving it there for 10 minutes for acclimatization, (2) 2-minutes rotation in randomly chosen direction, (3) 2-minutes rest (rotation stopped), (4) 2-minutes rotation in the opposite direction of stage (2), (5) 2-minutes rest, (6) 2-minutes rotation in the same direction as stage (2) (the first direction). The movement of the animal during the full protocol was video recorded for further analyses. Animal response to each experiment was scored as positive, if the animal swam in at least one continuous circle around the tank with the drum's rotational direction in each of the test stages (2, 4 and 6) and passed all controls.

Experiment 2 – sensitivity threshold to light intensity

Individuals were exposed to three levels of light intensities of 1×10^{-6}, 1×10^{-7}, 1×10^{-12} [µE cm^{-2} sec^{-1} nm^{-1}], to check for the sensitivity threshold of the species (Figure. 3A). The 25.4 mm black and white vertical stripes pattern was used and the drum was rotated at low speed of 6 rpm. The contrast was calculated for each intensity level and was above the value of 0.3 throughout the 400-700 nm wavelength range (Fig. 3B).

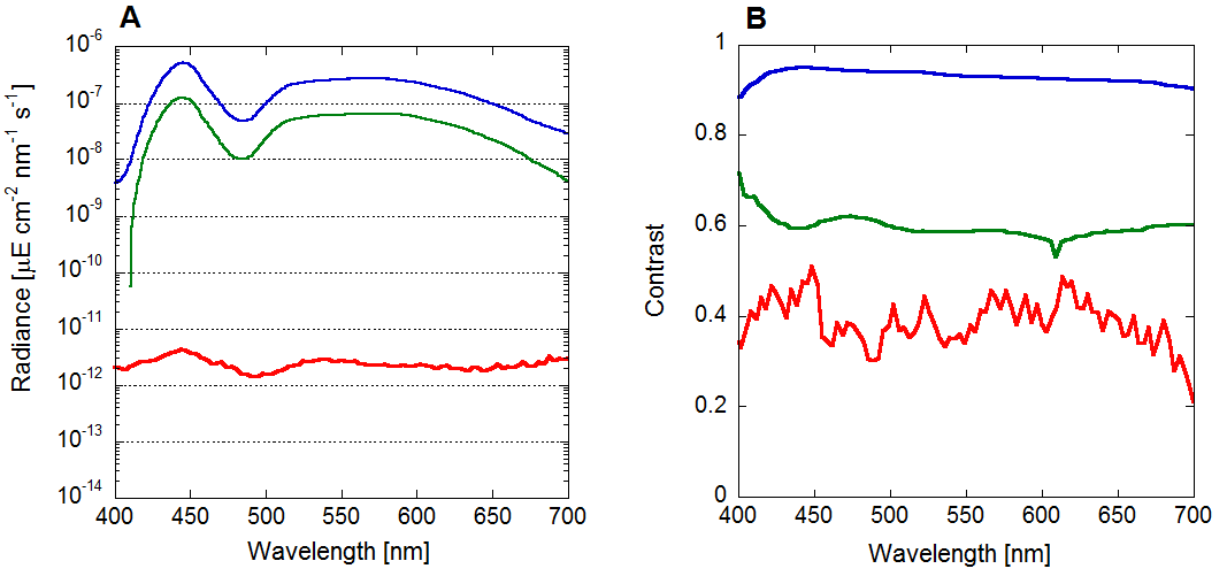

Figure 2.3. The radiance (A) and contrast (B) between the black and white stripes projected under the three levels of light intensity 1×10^{-6} (Blue line), 1×10^{-7} (green line), and 1×10^{-12} (red line) [µE cm^{-2} s^{-1} nm^{-1}] in the intensity sensitivity experiment. Light intensity applied to the chub mackerel was 1×10^{-9} [µE cm^{-2} s^{-1} nm^{-1}].

Experiment 3 – temporal and spatial resolution threshold

To check for the spatial and temporal resolution of the species, the response to vertical stripes of widths 25.4, 3.2 and 0.8 mm for cod, and 25.4, 12.7, 6.4 and 0.8 mm for chub mackerel were checked, at all light intensities. Individuals were tested for each width and the highest drum speed for positive response was determined. The spatial resolution, represented by the viewing angle α in degrees, was calculated using:

$$\alpha[^\circ] = 2\arctan\left|\frac{sw/2}{d}\right| \times \frac{180^\circ}{\pi[rad]} \quad (1)$$

where *sw* is the stripe width [cm] and *d* = 13.5 and 5 cm is the distance [cm] between the fish (cod and mackerel respectively) and the stripes taken from the fish tank edge (as the fish mostly swam close to the tank edge). The temporal resolution, *v* [Hz], was calculated as:

$$v[Hz] = \Omega_{max}\pi D \quad (2)$$

where Ω_{max} [rps] is the highest drum angular velocity to which the animal responded, and *D* = 50 cm is the drum diameter.

Light measurements

The spectrum of the light emitted from each stripe in the pattern was measured at 1-nm nominal wavelength resolution in the range of 350-750 nm through the three different pattern sheets, unpolarised , W and polarized using a spectrophotometer and optical fiber (600 µm, Ocean Optics, USB2000+, Dunedin, FL, USA) covered by a 5° custom-made restrictor (Lerner et al. 2008).

OMR analysis

The video recordings of individuals that passed the controls were analyzed using Microsoft Expression Encoder 4 (Microsoft Corporation, Redmond, WA, USA). The time that each fish positively responded (i.e. swam with the drum speed and direction) was calculated and the number of positive responses was counted. A sequence of 20 seconds during which the animal swam with the drum direction was selected for each animal and the angular position of the animal (in degrees from a start point at time zero) and the drum were sampled. The fish position was determined by placing an image of a protractor on the movie in the program. The drum and animal position during each second was plotted and fitted with a linear regression model to calculate the slope (angular velocity). Then, a gain was calculated as the ratio between the animal and the drum angular velocities (Talbot and Marshall 2010). A gain value of one reflects an exact match between the animal movement and the drum, while a case where the animal did not move with the pattern would yield a gain of zero.

2.4. Results

Krill OMR

Nine individuals of Krill were tested in the OMR apparatus against unpolarised (black and white) vertical stripes (body length mean ± s.e. = 2.0±0.1 cm). None of the individuals responded to the apparatus. The krill did not respond to the first setting that included a 50 cm diameter drum with 25.4 mm stripes and drum angular velocity of 8 rpm and maximum light intensity of 8×10^{-11} [µE cm^{-2} sec^{-1} nm^{-1}]. In this trial, the krill was placed inside a 15 cm diameter tank. In an attempt to elicit a response from the krill several adjustments were made: the tank size was changed to larger tanks (23 cm and 40 cm diameter), the drum speed was varied from 5 to 120 rpm, thinner stripes of 12.7 mm and 6.4 mm were applied, and the light intensity was lowered to 1×10^{-12} [µE cm^{-2} sec^{-1} nm^{-1}]. None of the individuals tested in any combination of the setting responded to the rotating drum.

Rose shrimp OMR

Sixteen individuals of rose shrimp (body length mean ± s.e. = 15.0±0.1 cm) were tested in the OMR apparatus against unpolarised (black and white, Control 1) vertical stripes. None of the individuals responded to the apparatus. The shrimp did not respond to the first setting that included a 50 cm diameter drum with 25.4 mm stripes and drum

angular velocity of 8 rpm and minimum light intensity of 1×10^{-15} [µE cm^{-2} sec^{-1} nm^{-1}]. In this trial, each shrimp was placed inside a 23 cm diameter tank. Again, several adjustments were made in an attempt to elicit a response from the shrimp: the tank size was changed to a larger tank (40 cm diameter), the drum speed was varied from 5 to 120 rpm, thinner stripes of 6.4 mm and 0.8 mm were applied, and the light intensity was increased to 1×10^{-12} and 1×10^{-6} [µE cm^{-2} sec^{-1} nm^{-1}]. Nonetheless, none of the individuals tested in any combination of the setting responded to the rotating drum.

Experiment 1 - Polarization sensitivity of Atlantic cod, blue jack mackerel and Atlantic chub mackerel

Seventeen cod fish (total length average ± s.e. = 16.8 ± 0.5 cm), over seventy blue jack mackerel (total length average ± s.e. = 20.4 ± 0.7 cm) and thirteen Atlantic chub mackerel (total length average ± s.e. = 25.9 ± 0.8 cm) were tested for their response to unpolarised (black and white, positive control) and plain pattern (W, negative control). Twelve cod passed all controls. Only five of these individuals responded positively to the polarized grating (S1 – movies 1 and 2 in supplementary data). Several setups of the OMR apparatus were tested to evoke response to polarized grating from the blue jack mackerel. The set that gave the best results was the 23 cm diameter tank with a 40 cm diameter drum, and with drum speed of 48 °/s and 25.4 mm unpolarised stripe width. Out of the eight individuals that passed all controls, only one responded to the polarized grating. Ten Atlantic chub mackerels passed all the controls, but none of them responded to the polarized grating.

Table 2.1 summarizes of the response of the five cod individuals and one blue jack mackerel that passed the controls and responded to the polarized grating. Out of a total of 360 seconds of drum rotation (i.e. protocol stages 2, 4, and 6), cod responded (swimming with the drum rotation) for an average ± s.e. of 174 ± 36 seconds to the unpolarised grating and 76 ± 29 seconds to the polarized grating. Time responding out of 360 seconds with rotating grating of the blue jack mackerel was 206 and 183 seconds, the gain was 0.71 and 0.99, and the fish angular velocity was 34.2 and 48.8 °/s for the unpolarised and polarized grating respectively (Tables 1 and 2).

Table 2.1. Summary of the cod (*Gadus morhua*) and blue jack mackerel (*Trachurus picturatus*) response time to the unpolarised and polarized grating.

	B&W pattern (unpolarised)		**Polarized grating (POL)**	
Cod #	**response**	**time of positive response out of 360 seconds**	**response**	**time of positive response out of 360 seconds**
1	Y	307	Y	35
2	Y	154	Y	45
3	Y	89	Y	47
4	Y	161	Y	61
5	Y	160	Y	190
Average ± s.e.		**174±36**		**76±29**

Blue jack mackerel	Y	206	Y	183

The five cod fish showed a strong response to both unpolarised and polarized patterns as presented by the gain calculated and presented in Table 2.2. The average ± s.e. of the gains of the cod to unpolarised and polarized grating were 1.0 ± 0.2 and 1.2 ± 0.6 respectively, and they were not significantly different from each other (paired t-test, $T_{4,0.05}$=-1.108, p=0.33). The average ± s.e. angular velocity (degrees/sec) of the fish against the unpolarised and polarized grating was 49 ± 4.1 and 61.8 ± 11.6 °/s respectively, while the drum angular velocity was 36 °/s (6 rpm).

Table 2.2. Angular velocity, Gain and linear fit coefficient R^2 of each individual fish responded to unpolarised and polarized grating for each fish position with time shown in Fig. 4. The average of the slope was calculated using circular statistics. The drum angular velocity was 36 and 42 °/s for the cod and the mackerel respectively.

	B&W pattern (unpolarised)				**Polarized grating (POL)**		
Cod #	**Slope [°/s]**	**Gain**	**R^2**	**White (W)**	**Slope [°/s]**	**Gain**	**R^2**
1	66.49	1.39	1.00	0	76.69	1.60	0.99
2	39.62	0.83	0.99	0	32.13	0.67	0.98
3	43.67	0.91	0.99	0	30.45	0.64	0.99
4	50.62	1.05	0.99	0	97.53	2.03	0.96
5	48.18	1.00	0.99	0	71.95	1.50	0.99
Average±s.e.	**49.7±4.1**	**1.0±0.2**			**61.8±11.6**	**1.2±0.6**	
Blue jack mackerel	34.2	0.71	0.99	0	48.8	0.99	1

The positions (angular distance from a start reference point) of the 5 cod and the blue jack mackerel during 20 seconds against each pattern, related to the position of the drum, are shown in Figure 2.4. All cod swam with the drum speed and direction when presented with either unpolarised or polarized stripes. They swam opposite the drum direction or did not swim at all when no grating was presented ("White").

Experiment 2 - sensitivity threshold to light intensity

All 10 cod individuals responded under all assessed light intensities, down to our instrument's measurement limit of 1×10^{-12} [µE cm^{-2} s^{-1} nm^{-1}], at stripe width of 25.4 mm. The contrast at this light intensity between the black and the white strips was 0.4 (Fig. 3B). Due to time constraints, neither blue jack mackerel nor the chub mackerel could be tested for light sensitivity threshold.

Experiment 3 – temporal and spatial resolution threshold

All cod individuals tested (10/10) responded to all the stripe widths provided (25.4, 3.2, 0.8 mm) at the high light intensity level (1×10^{-6} [µE cm^{-2} s^{-1} nm^{-1}]). Only seven out of ten cod individuals responded at the lowest light intensity of 1×10^{-12} [µE cm^{-2} s^{-1} nm^{-1}] to the 3.2 mm stripes and only 3/10 cod individuals responded to the 0.8 mm wide

stripes. The average±s.e. of lowest and highest temporal resolution of the cod was 20.9±22.0 and 109.4±17.3 [Hz] respectively (Fig. 5A). All *S. colias* individuals tested (9/9) responded to all the stripe widths provided (0.8, 6.3, 12.7, and 25.4 mm) at the light intensity level (1×10^{-9} [µE cm^{-2} s^{-1} nm^{-1}]). The average ± s.e. of lowest and highest temporal resolution of the Atlantic chub mackerel was 22.4 ± 5.8 and 62.7 ± 6.7 [Hz] respectively (Fig. 5B). Again, due to time constraints, the blue jack mackerel could not be tested for temporal or spatial resolution threshold.

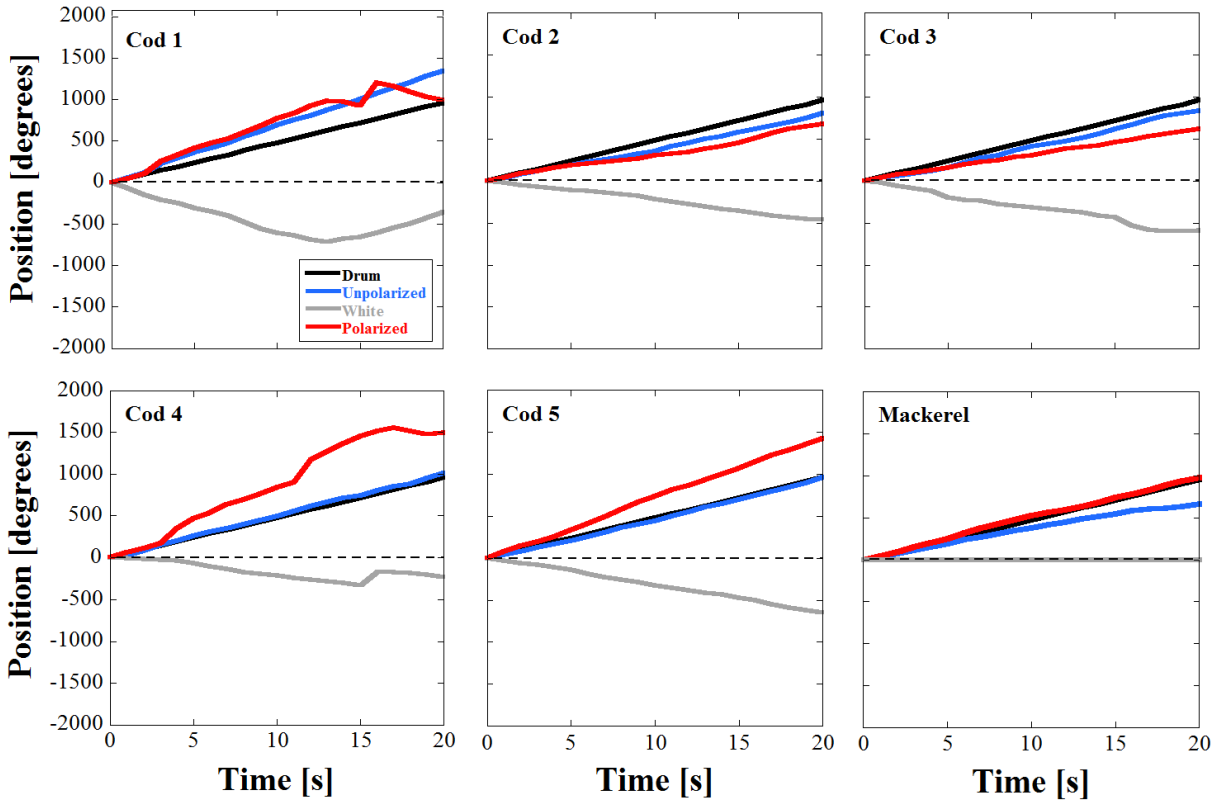

Figure 2.4. Fish and drum position during 20 seconds of drum rotation of each of the 5 Atlantic cod (*G. morhua*) and the blue jack mackerel (*T. picturatus*) that responded to all control and polarization patterns (Experiment 1). Positive position represents swimming with the drum rotation direction, while negative position represents swimming against the direction of the drum rotation. Zero position (dash line) represents no movement. Lines represent movement of the drum (black), and of the fish against unpolarised (blue), control (i.e. no stripes)(gray) and polarized (red) patterns.

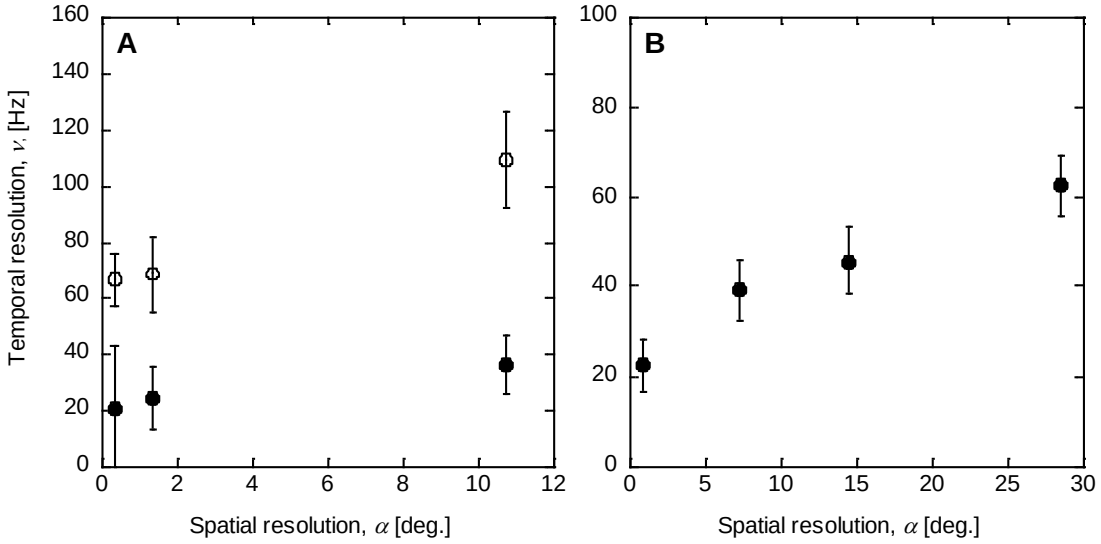

Figure 2.5. Temporal resolution (average ± s.e.) vs. spatial resolution measured for the cod (A) and the chub mackerel (B). Light intensities applied in (A) are 1×10^{-6} and 1×10^{-12} µE cm^{-2} sec^{-1} nm^{-1} (open and filled circles respectively), and in (B) is 1×10^{-9} µE cm^{-2} sec^{-1} nm^{-1}. The stripe widths used in (A) are 0.8, 3.2 and 25.4 mm, and in (B) are 0.8, 6.3, 12.7, and 25.4 mm.

2.5. Discussion

The goal of this part of Task 2.7 was to define the visual capacities of commercial and by-catch species, namely polarization sensitivity, threshold of intensity sensitivity, and temporal and spatial resolutions, to possibly use this information in the future to differentiate the target and by-catch species using artificial light sources on the fishing gear based on their vision.

Out of the two taxa (crustacean and fish) tested in the OMR apparatus in this study, only the fish responded. The rose shrimp and krill tested in this study did not respond and did not pass the crucial control of moving against black and white (unpolarised) stripes. To the best of our knowledge this study is the first one to test shrimps in an OMR apparatus. The non-response of the shrimp to this apparatus may be explained either by the poor condition of the individuals during the tests, as small marine crustaceans are highly sensitive to physical injury and hard to maintain in captivity, or due to their particular slow-moving lifestyle with a sedentary component to their normal behavior, which may make them poorly suited to this type of OMR test. Alternate tests such as escape response, successfully tested in crayfish (Tuthill and Johnsen 2006), or optokinetic (eye movement) response (Li 2001) may therefore be more appropriate techniques for shrimps. Due to time and funding limitations, we could not test these alternative methods on our crustaceans. A future study should concentrate on crustaceans to successfully evoke an optomotor response, or use optokinetic or escape responses.

Nonetheless, the Atlantic cod, the Atlantic chub mackerel and the blue-jack mackerel did respond to the OMR apparatus. Our study is the first we are aware of that shows a response in cod and mackerel to a polarized grating, although only one mackerel individual responded. This is an important observation, as only a small number of

vertebrate species have thus far been shown to possess polarization sensitivity (Horváth 2014). Although, there is currently no consensus on the mechanism for polarization vision in vertebrates, except for anchovies in which the mechanism resembles that of invertebrates (Horváth 2014). The cod individuals in our experiment responded with a high gain value to the polarized stripes, which did not differ from the gain value recorded in response to the unpolarised (black and white) stripes. The optomotor response to the polarized grating did, however, appear to be weaker than for the unpolarised grating (cod time spent less time swimming with the polarized grating than the unpolarised grating). This suggests that polarization perception is more complex than intensity vision (unpolarised).

The fact that only a small number of individuals responded to the polarized light, should not dismiss a conclusion about polarization sensitivity in these species. Most studies of polarization sensitivity demonstrate this capacity or the lack of it in a small number of individuals (Shashar et al. 1998, Darmaillacq and Shashar 2008, Talbot and Marshall 2010).

Species such as cod that prey on zooplankton and small crustaceans in their early life stages (Keats and Steele 1992, Demain et al. 2011) may use polarization vision to better detect prey, as polarization vision has the potential to improve visibility in water and increase the range at which small prey can be detected (Shashar et al. 1998, Novales-Flamarique and Browman 2001, Schechner and Karpel 2004, Sabbah and Shashar 2006, Johnsen et al. 2011). Polarized light reaches depths greater than 100 m in significant quantities (Ivanoff and Waterman 1958, Sabbah et al. 2005, Johnsen et al. 2011), while unpolarised light is more rapidly attenuated. Polarized light may, therefore be a useful visual cue for cod and mackerel, visual predators, which are often found at these depths. Therefore, it may be possible to exploit the polarization vision of cod to attract the fish to pots either by using live bait (small crustaceans) or by artificial lures which are polarization active against an unpolarised background. The latter strategy has been tested successfully on juvenile squid in laboratory conditions (Shashar et al. 1998).

Our attempt to determine the visual sensitivity threshold to light intensity was unsuccessful due to limitations in the sensitivity of the instrument used to measure the radiance during the tests. Available ocean optics light radiometers were developed to measure light intensities typical of day time measurement and photosynthetic activity, which is in several order of magnitudes higher than the sensitivities of animal visual systems. To the best of our knowledge, no “shelf” radiometer exists today that can measure radiance below 10^{-12} µE cm^{-2} s^{-1} nm^{-1} or irradiance (180° acceptance angle) below 10^{-5} µW cm^{-2}]. The cod visual sensitivity is expected to be 4-6 orders of magnitude lower than these values. Therefore, the light intensity to stimulate cod could range from water surface intensities of 1 µE cm^{-2} s^{-1} nm^{-1} and down to ~10^{-12} µE cm^{-2} s^{-1} nm^{-11} and less; Although observations during our trials suggest that cod avoid high intensity levels of artificial light during feeding, probably due to a decrease in contrast, and photoreceptor bleaching.

Not surprisingly, cod and chub mackerel temporal resolution were found to be dependent on light intensity, and to a lesser extent on stripe width. As demonstrated in this and past studies (Land and Nilsson 2012), the contrast of the stripes decreases with light intensity. Our measurements indicate that the temporal resolution for cod

ranges between 20-110 Hz depending on stripe width. Atlantic chub mackerel temporal resolution was measured only under light intensity of 1×10^{-9} µE cm^{-2} s^{-1} $nm^{-1}$and ranged between 22-63 Hz depending on stripe width (Fig. 5 B). These ranges are in agreement with the temporal resolution found in other open water predator fish species such as swordfish and tuna (Fritsches et al. 2005).

Flickering light is known to repulse fish and may also cause stress (e.g. Richards et al 2007). For example, strobe lights were used to prevent fish entering a navigation lock, with a success rate of over 85% reduction in fish entrance (Johnson and Bouchard 2005). Therefore, if species possess different temporal resolution, the flickering rate of a light could be used to induce differential responses and possible influence the selectivity of a fishing gear.

Due to time constraints, the temporal resolution (i.e. critical flickering fusion frequency, CFF) was measured here on cod and chub mackerel only. Cod and chub mackerel CFF ranged between 20-100 Hz and 20-70 Hz respectively, depending on light intensity. Therefore, in case study 1.2, light with flickering rate of ~10-20 Hz (i.e. <20-70 Hz) could be used to repel chub mackerel, a by-catch species, while Nephrops (the target species) should remain unaffected, as many crustaceans have a temporal resolution lower than 20 Hz (Johnson et al, 2000). Conversely, in case study 3.7, if a flickering light is used to attract potential prey to a pot, to avoid repelling the target cod, the light should have a flickering rate higher than 20-70 Hz.

2.6. Conclusions & Recommendations

These experiments have, using the opto-motor response (OMR), attempted to determine the visual capacities for cod (*Gadus morhua*), chub mackerel (*Scomber colias*) and blue jack mackerel (*T. picturatus*), with respect to polarization sensitivity, threshold of intensity sensitivity, and temporal and spatial resolutions (table 2.3). However, rose shrimp (*Parapenaeus longirostris*) and the krill (*Meganyctiphanes norvegica*) did not respond in the OMR apparatus and alternate tests such as escape response or optokinetic (eye movement) response may be more appropriate techniques for crustacea.

Table 2.3: Result summary of the species tested.

species	polarized vision	temporal resolution (Hz)	light intensity resolution (µE vm^{-2} sec^{-1} nm^{-1})
Cod	V	20-100	< $1X10^{2}$
chub mackerel	/	20-70	N/A
blue jack mackerel	/	N/A	N/A

For Case Study 1.2 (Algarve deep-water trawl), chub mackerel had a critical flicker fusion frequency (CFF) which ranged between 20-70 Hz. Therefore, light with a flickering rate of ~10-20 Hz (i.e. <20-70 Hz) could be used to repel chub mackerel from deepwater trawls without affecting the target crustacean species, Nephrops. Polarized light may also be useful to repel non-target blue jack mackerel from shrimp fishing gear, although only one individual responded to polarized light in this study.

For case study 3.7 (Norwegian gadoid pots), we recommend that the light intensity to stimulate cod could range from water surface intensities 1 µE cm^{-2} s^{-1} nm^{-1} and down to ~10^{-12} µE cm^{-2} s^{-1} $nm^{-1}$and less. Light polarization may be useful for attracting cod to pots, as we have provided evidence to suggest that cod may possess the ability to detect polarized light and could use this capacity to target their prey. Furthermore, Cod have a CFF ranging between 20-100 Hz, therefore if a flickering light is used to attract potential prey (i.e. krill) to a pot, the light should have a flickering rate higher than 20-70 Hz to avoid repelling the target cod.

These results will, as part of task 2.7, be used to further investigate the behavioural response of these species to artificial light sources, as well as design and test light sources mounted on fishing gears in Task 2.8: A*lternative Fishing Gears*.

2.7. Acknowledgement

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3. Case Study 1.2: Behavioural responses of target and non-target species in the Algarve deep-water trawl fishery to artificial light.

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3.1. Introduction

The main target species of the deep-water trawl fishery are the rose shrimp, *Parapenaeus longirostris* and the Norway lobster, *Nephrops norvegicus*. Blue whiting and small pelagic species constitute most of the unwanted by-catch.

The strategic goal is to achieve by-catch minimization solutions with minimum losses of crustacea. One approach is the use of lights to promote avoidance behaviour in unwanted fish species. An alternative approach is to improve the catchability of *N. norvegicus* traps (already a viable commercial fishery) and to introduce efficient traps to catch *P. longirostris*.

The planned work involves laboratory tests and field work. At present, only the laboratory tests to determine the visual capacity of selected species have been completed.

3.2. Methods and preliminary results

The methodological approaches were defined to address the **specific objectives** for using lights in the present case study. These included:

Using artificial light as a novel stimulus:

1) Identify visual capacities of target species (*N. norvegicus* and *P. longirostris*) and by-catch species (*Trachurus trachurus*, *T. picturatus* and *Scomber colias*) using a OMR apparatus (see section 2.0);
2) Identify attraction/repulsion behaviour for *N. norvegicus* and *P. longirostris* using behavioural experiments;
3) Undertake field tests using lights to decrease by-catch in trawlers.

Alternative fishing methods:

4) Test the use of attractant lights to improve catchability of already operating *N norvegicus* traps;
5) Evaluate the possibility of using lights as attractant in traps for *P. longirostris*.

Definition of species to be studied and capture of individuals for the experiments

The experiments required animals in good condition and acclimated to the lab. The option of bringing up specimens with the trawl net was only possible for crustaceans

(not affected by sudden depth changes). Two different sampling strategies were developed.

For crustaceans, a crustacean trawler was contracted to capture rose shrimp or Norway lobster using ½ hour tows (to minimize damage in the net), at the end of the day (to minimize exposure to light when arriving on deck and guarantee speedy transport to the lab). Rose shrimps, at arrival on deck, were immediately put into dark containers with cold (11˚C) aerated water. Norway lobsters were put in insulated refrigerated trays, designed to avoid air exposure and guarantee a cold and highly humid environment. The individuals were immediately transported to the lab; an operation that lasted between 3.5 (rose shrimp) and 5 hours (Norway lobster).

For fish, individuals were provided by a company using the tuna trap off Olhão. When the pelagic species entered the tap, individuals that were of interest for this case-study were selected. Initially 3 species were considered, the silver horse mackerel (*Trachurus trachurus*), the Atlantic chub mackerel (*Scomber colias*) and the blue jack mackerel (*Trachurus pictoratus*). Since there was no time to study all three, it was decided to use only two, depending on the availability. In the end, the chub and blue jack mackerels were used.

The fish were transported from the tuna trap to nearby port facilities belonging to the same company. These facilities are used for acclimation and quarantine of fish sold alive to aquariums and other businesses, and thus had the correct facilities to maintain the fish for a period of one to two weeks. They were then transported to the lab (20 minute drive) in containers with temperature control and water aeration.

Since only one species could be tested at a time, the sequence of species tested was the following: 1- rose shrimp, 2 – chub mackerel, 3 – blue jack mackerel. Time constraints did not allow the testing of Norway lobster.

Laboratory setup

Three independent closed circuits were prepared for rose shrimp, Norway lobster and the fish, each including: holding tanks, mechanical filter, skimmer, biological filters, refrigeration system and pump. The water quality was monitored daily (ammonia, nitrites, nitrates, pH, salinity and temperature) and approximately ¼ of the water volume was changed twice a week.

The rose shrimp holding tank was 750 litres and the shrimp were maintained in net cylinders inside the tank (to facilitate capture of the individuals and cleaning of the tank). The circuit for Norway had 100 individual tanks each with an approximate volume of 7.5 litres (total volume of the system including supply and filter tanks were 1700 litres). In both systems prepared for crustaceans the water temperature was maintained 13˚C and the holding tanks were kept in total darkness. When necessary, red light was used to inspect the tanks and perform maintenance tasks.

The fish had two separate 200 litre round holding tanks with water temperature at 17˚C and 12/12 hours dark/light cycle.

In all cases the experimental animals exhibited the expected behaviour for the species. The rose shrimp stayed close to the bottom of the mesh cylinders and would swim when disturbed. The mackerel maintained a school behaviour swimming around the tanks. Norway lobsters had not been used at the point of preparing this report.

Mortality was only observed for the rose shrimp. On a steady basis, 2-6 individuals would die every day, either without completing the moult or right after the moult. The last individuals to die had lived for 28 days in the tank (Figure 3.1). The individual shrimp did not eat the provided food, consisting of pellets for aquaculture shrimp. Further experiments will require some adjustments in the tanks, namely placement of the specimens in individual tanks to better study their response to captivity, feeding responses and adjustment to captive conditions.

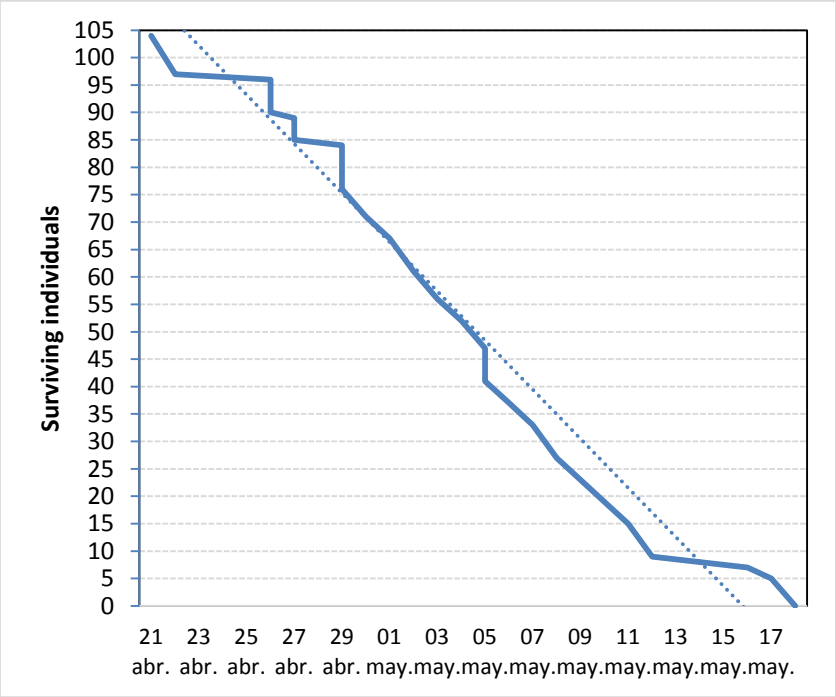

Figure 3.1 – Survival curve for the rose shrimp, Parapenaeus longirostris, for 104 individuals initially brought to the lab (dashed trend line).

The fish were fed with pellets, live zooplankton and fish eggs when staying it the lab more than 2 days. The individuals ate the provided food well, which was considered, in combination to the school swimming behaviour, as indicative of moderate to good adaptation to the captive conditions and relatively low levels of stress.

Experiments with the OMR apparatus

An abridged version of the methodologies and results for the OMR apparatus experiments is presented here.

Rose shrimp (*Parapenaeus longirostris*):

Sixteen individuals of rose shrimp were tested in the OMR apparatus against unpolarised black and white vertical stripes. Several settings involving changes in size of the tank (23 and 40 cm), drum angular velocity (5 to 120 rpm), stripe width (25.4 and 6.4 and 0.8 mm) and light intensity (1×10^{-15}, 1×10^{-12} and 1×10^{-6} µE cm^{-2} sec^{-1} nm^{-1}). None of the individuals responded to the apparatus.

Chub mackerel (*Scomber colias*):

A total of 25 individuals were tested. Of these, 10 passed all the controls, but none of them responded to the polarized grating. With respect to the temporal and spatial

resolution threshold, the average ± s.e. of lowest and highest temporal resolution of the Atlantic chub mackerel were 22.4 ± 5.8 and 62.7 ± 6.7 [Hz] respectively. Due to time constraints, the chub mackerel could not be tested for light sensitivity threshold.

Blue jack mackerel (*Trachurus pictoratus*):

A total of 75 individuals were tested. Several setups of the OMR apparatus were tested to evoke response to polarized grating from the blue jack mackerel. The set that gave the best results was the 23 cm diameter tank with a 40 cm diameter drum, and with drum speed of 48 °/s and 25.4 mm unpolarised stripe width. Eight individuals passed all controls but only one of these responded to the polarized grating. Due to time constraints, the blue jack mackerel could not be tested for light sensitivity threshold or for temporal or spatial resolution threshold.

In summary, most fish specimens tested did not pass the controls, that is, they either failed to response to unpolarised black and white stripes that are visible to the human eye or responded to the blank sheet with no stripes (indicating the animal followed cues other than the rotating grating). Most of the blue jack mackerel tested, failed the second control what implies they could see the light. Adding to this, one blue jack mackerel individual responded to polarized light. This result indicates that light cues should not be discarded as tools to reduce by-catch.

Despite these interesting preliminary results, the information obtained does not allow the definition of light properties to use in field experiments in the near future. Also there is no indication of which light characteristic will induce attraction or repulsion.

With respect to crustaceans, the OMR apparatus does not seem to be the right approach to study responses to light stimuli.

3.3. Future work

This section was organized by specific objectives, listed at the beginning of the *Methods and preliminary results* section.

Using artificial light as a novel stimuli

1) Identify visual capacities of target species (*N. norvegicus and P. longirostris*) and by-catch species (*Trachurus trachurus, T. picturatus and Scomber colias*) using an OMR apparatus – April to June 2016.

   This objective was only partially achieved for the species *T. picturatus* but the results are not enough to specify light characteristics to be used in future field work. We suggest the use of lights in trawling nets, to repel fish species, is based on an extensive literature review.

   With respect to crustaceans, the laboratory is ready to receive Norway lobsters and rose shrimp. No problem with the adaptation and survival of *N. norvegicus* is expected, and for this species, the identification of light properties that may trigger a response that can be combined with the identification of attraction/repulsion behaviour.

With respect to *P. longirostris,* some practical aspects related with survival in captivity need to be further studied, namely the best holding conditions and food, before further experiments can proceed.

2) Identify attraction/repulsion behaviour for *N. norvegicus* and *P. longirostris* using behavioural experiments – September to December 2016.

   The work will proceed with behavioural experiments on *N. Norvegicus*.

   A new attempt at maintaining and feeding *P. longirostris* will be made while the *N. norvegicus* behaviour experiment goes on, and if success is achieved, behavioural experiments will also be conducted with *P. longirostris*.

3) Undertake field tests using lights to decrease by-catch in trawlers.– March to October 2017.

   This specific objective is related with by-catch reduction in trawling nets

   Standard duration tows (at most three hours) will be carried out, alternating hauls with and without lights installed on the headline, to evaluate the effect of strobe light in eliciting fish avoidance behaviour. Fishing grounds with high abundance of small pelagic by-catch species will be favoured.

Alternative fishing methods

4) Test the use of attractant lights to improve catchability of already operating *N. norvegicus* traps – February to April 2017.

   Depending on results from specific objective 2), field trials for *N. norvegicus*, using lights in traps, will be conducted during the spring of 2017.

5) Evaluate the possibility of using lights as attractant in traps for *P. longirostris* – January 2017.

   Lights will be used during the experiments to test trap performance. Since positive preliminary results were previously obtained with fluorescent light sticks (Eichert, 2015), lights will be used even if no relevant information is obtained with the behavioural experiments. The lights used in Mediterranean trawl fisheries to increase deep-water shrimp catches will also be tested.

The results from this continuing work will be reported in D2.13 - Reports on the use of novel stimuli to minimise unwanted catch.

3.4. Critical Resources

Personnel

OMR experiments, finished during June 2016, were conducted by IOLR researchers (Amit Lerner, Ben Brinberg) in cooperation with CCMAR researches (Margarida Castro, Moritz Eichert and Lino Marques).

Future laboratory (behavioural experiments) and field work will be carried out by CCMAR researchers (Aida Campos, Margarida Castro, Moritz Eichert, Paulo Fonseca and Lino Marques).

Planning of the experiments will have the cooperation of SafetyNet Technologies (Dan Watson) and IMR (Michael Breen).

Equipment

Holding facilities for the rose shrimp and Norway lobsters are set up and experimental animals will be brought to the lab during the month of September 2016.

Experimental structures to test behavioural response of *N. norvegicus* and *P. longirostris* to light will be planned and built during September 2016.

A flexible light source will be designed by SafetyNet Technologies for the lab experiments and be ready for operation in early October 2016.

A deep-water camera system is being developed by SafetyNet Technologies and expected to be operational until October 2016.

A lander to fit the camera and allow time lapse photos of the different traps is being developed and will be built during October-November 2016.

Other small equipments are in the process of acquisition such as temperature sensors and a current meter (INFINITY-EM Electromagnetic current meter).

Semi-floating traps (partially acquired in previous projects, new traps will be built locally) will be available until December 2016.

Lights to be used in trawling nets will be designed in cooperation with SafetyNet Technologies and will be available February 2017.

Other resources

Vessels for Nephrops trap experiments – commercial vessels already practicing this métier will be used, no major costs anticipated.

Vessels for experiments with semi-floating traps – contacts will be made to charter a vessel for this purpose. A vessel with experience in deep-water trap fishing will be selected, in order to provide the materials necessary to deploy trap fleets.

Charted crustacean trawler – a consultation will be initiated during 2016 to select a trawler for the field experiments.

3.5. Time line & Milestones

April, May and June 2016	OMR apparatus experiments
September 2016	Development of lights and structures for lab behavioural experiments for *N. norvegicus* and *P. longirostris*.
October and November 2016	Behavioural experiments for crustacean species (lab)
November 2016 to and January 2017	Building/acquisition of lights for the field experiments, setting up of lander with fitted equipment (camera, current meter, temperature sensors), finishing all consultation processes for chartered vessels.
January 2017	Field tests for rose shrimp (traps)
March to April2017	Field tests for Norway lobster (traps)
March to October 2017	Field tests for by-catch reduction on a commercial trawler(s)

3.6. References

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4. Case Study 3.7: Behavioural Responses of Krill and Cod to Artificial Light

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The goal of this case study is to increase the number of cod that enter pots using artificial illumination tuned to the species' vision and visually-guided behaviour. This can be achieved by attracting either the cod and/or their prey krill.

Thus, to find which light characteristics are most attractive for krill, we first tested the effect of light intensity, wavelength composition and flickering fusion frequency on krill's attraction to a light source. The most promising light stimuli for krill were then tested to determine whether they would have repulsive or attractive effects on cod.

4.1. Krill attraction study - effect of wavelength and flickering

4.1.1. Introduction

Studies on krill (***M. norvegica)*** wavelength sensitivity has demonstrated that they have a single receptor class with sensitivity peaks around 488 - 490 nm (Denys and Brown 1982; Frank & Widder 1999, Fig. 1). This means they have light sensitivity comparable with most other deep sea creatures (Denton and Warren 1957). Earlier studies showed wavelength sensitivity that peaked at 470 nm and 515 nm, suggesting two visual pigments (Boden et al. 1961, Fisher and Goldie 1960). However, these findings are thought to be incorrect due to some contaminating factors present in euphausiid eyes that were not known at the time of the original work (Denys and Brown 1982). The λ_{max} 488 nm sensitivity is close to their bioluminescence emission λ_{max} 468 nm (Kay 1962; 1965). We hypothesise that they might be more attracted to light composed of comparable wavelengths to their bioluminescence emission at 468nm or alternatively to their peak sensitivity at 490nm. To test this we looked at *M. norvegica's* attraction to light at selected wavelengths between 410 nm and 625 nm in addition to white light (see Fig. 2). Clark et al. (1962) found that *M. norvegicus* emit bioluminescence in short to prolonged glows lasting 4-22 sec. Thus we choose to test only lower levels of light flickering (2 and 8 Hz, plus steady light - 0Hz).

Objective: determine the behavioural responses of krill to different properties of light, including: wavelength, intensity and flicker.

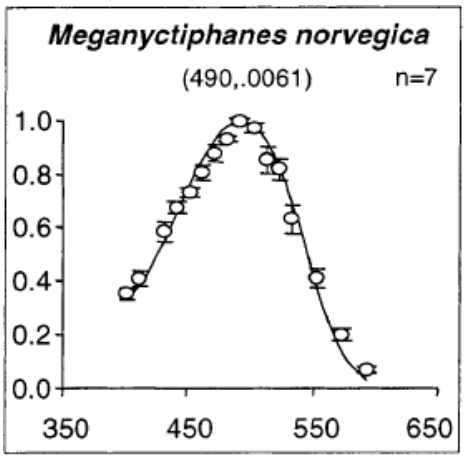

Figure 4.1. Spectral selectivity curve for *Meganyctiphanes norvegica*, from Frank and Widder 1999. Y-axis shows Relative sensitivity or Absorbance, X-axis wavelength.

λ_{max}	Reference
490 nm	Frank and Widder 1999
460 nm, 465 nm	Fisher and Goldie 1959
462 nm	Fisher 1967
460 nm, 490 nm, 515 nm	Boden et al. 1961
488 nm	Denys and Brown 1982 Kay 1962; 1965
**468 nm	

Table 4.1. Wavelength sensitivity found in previous studies on Comparison of previous and current studies on M*eganyctiphanes norvegica.* ** is the bioluminescence signature wavelength.

4.1.2. Methods

Experimental animals: Krill (Meganyctiphanes norvegica) were caught weekly by a large plankton net (MIC-net, Figure 4.3) at depths of 150 to 180 m in Langenuen, near Austevoll in the outer Hardanger fjord, Western Norway, in January – February 2016. After capture, they were transported to the lab in black 30 litre buckets covered by black plastic. In the lab they were held in black 80 litre buckets in a cold room with dim lighting. Stocking density was < 20 individuals per 80 litres. At least 80% of the water was exchanged daily together with a fresh supply of *Artemia*. Krill do not cope well with handling. Therefore, between each replicate treatment, we checked the experimental individuals to see if they were still in good condition. This was done by squirting a water wave towards them using a plastic pipette. If a krill did not swim and/or re-orientate in a normal manner after this treatment, it was replaced with a new healthy krill.

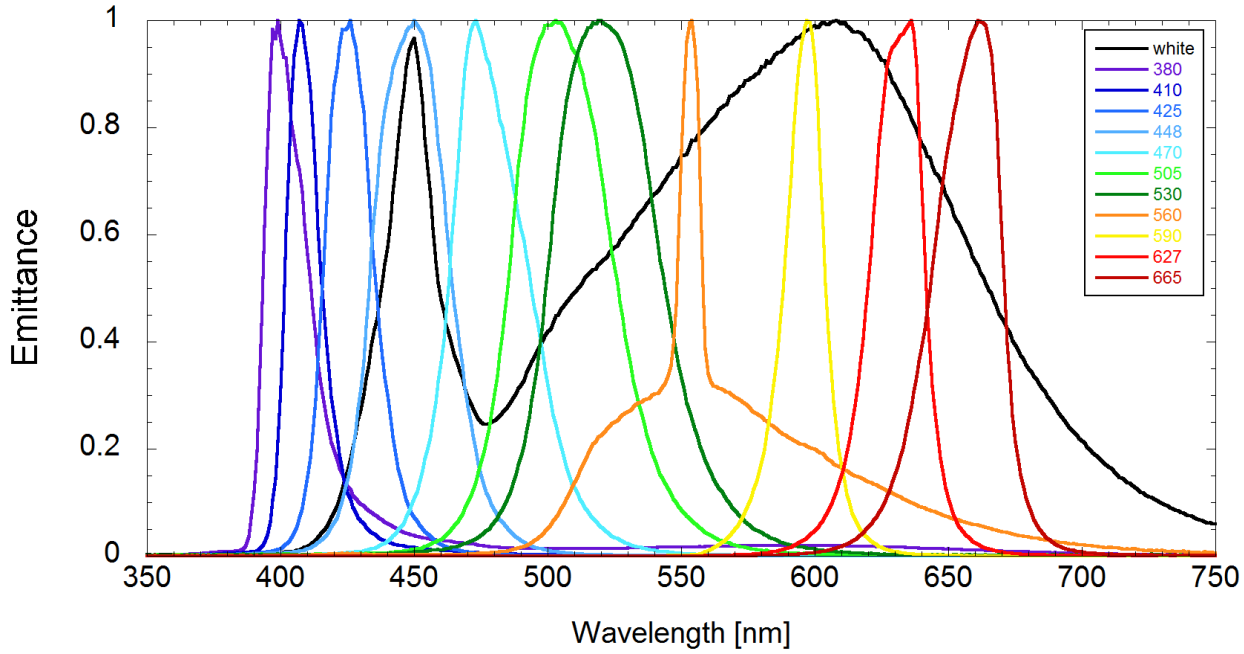

Figure 4.2. Wavelength and emittance spectre of the different lights tested in the krill study.

Figure 4.3. MIC net used to catch live krill (two left pictures). At the end of the net there is a 30 litre cup in which the catch is collected and preserved (far right picture).

Experimental procedures: This experiment used the distribution of krill in a small aquarium tank, with a light source at one end, to assess the degree of attraction to light of different properties, including: wavelength (410, 425, 448, 470, 505, 530, 560, 590, 625nm and White light), intensity (0.25, 0.5 and 1.0 $\mu E\ cm^{-2}\ s^{-1}\ nm^{-1}$) and flicker frequency (2 and 8 Hz, as well as steady light, 0 Hz).

All experiments were conducted in a totally dark room. The attraction experiments were conducted in a black 80 litre tank, the bottom of which was covered with white masking tape (Fig. 4), to provide a contrasting surface enabling the experimenter to view the krill. The experimental light source (Fig. A1 in Appendix) was placed at one end and lines drawn on the bottom marked the distance from the lamp, at 18 cm intervals.

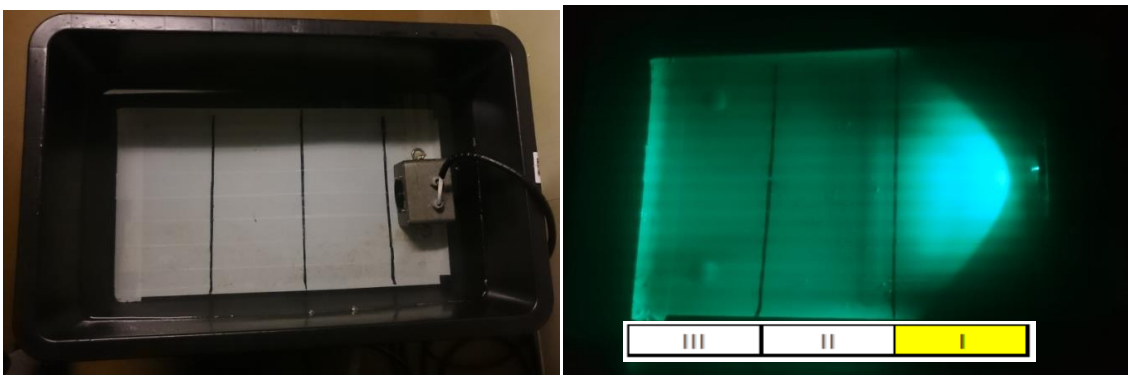

Figure 4.4. Experimental tank in light conditions (left picture), with the experimental light positioned by the right wall, and the division of the tank into three equal sections. Experimental tank during an experiment (right picture). The number of krill positioned in the two rectangular sections (areas II & III) or the section closest to the light source (D-formed light area in front of the light source)(area I) was noted every 30 sec.

A total of eight replicate groups consisting of three krill each, were tested for their behavioural response to the experimental treatments. Three krill of similar size (30-35 mm total length) were placed in the dark experimental tank for acclimatization at least 20 min prior to the start of the experiment. Only active krill were selected for use in the experiment. If a krill became consistently inactive during the experiments, it was replaced (see comment under "experimental animals" above).

The experiment started by turning on the experimental light at a pre-set wavelength, flickering and intensity treatment. As a measure of krill's attraction to the light source, we noted the number of krill positioned in the three different sections of the tank every 30 seconds for 5 minutes (Fig. 5). . Only krill positioned in the "D-shaped" illuminated area in front of the lamp were considered to be attracted to the light.

The treatments (wavelength, intensity and flicker) were presented in random order to twelve different groups of three krill. The original aim was to obtain a balanced data-set with eight replicates per treatment combination. However, resource limitations (i.e. the available number of viable krill and time) meant that it was necessary to prioritise the treatments in the following order: wavelength (at 0.25 µE & no flicker); intensity (with no flicker) and flicker. This resulted in an unbalanced data-set (see Table 4.2), were:

- All wavelengths have 8 replicates in a balanced data set at 0.25 $\mu E.m^{-2}.s^{-1}$ and no flicker (broadband "white" has 7 replicates);
- All light intensity treatments (0.25, 0.5 and 1.0 $\mu E\ cm^{-2}\ s^{-1}\ nm^{-1}$) have 8 replicates for most wavelengths (410, 425, 448, 470, 505, 530, and 625nm) and no flicker; but
- The three flicker frequency treatments (no flicker, 2 & 8Hz) have only single replicates for a small number of wavelengths and intensities (see Table 4.2).

Table 4.2 – the number of replicates per light treatment combination: Wavelength (nm), Intensity ($\mu E\ m^{-2}\ s^{-1}$) and Flicker Frequency (Hz).

Intensity (µE.m-2.s-1)	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1
Flicker Frequency (Hz)	0	0	0	2	2	2	8	8	8
Wavelength (nm)									
410	8	8	8						
425	8	8	8	1				1	
448	8	8	8			1			1
470	8	8	8		1			1	
505	8	8	8			1			
530	8	8	8			1			1
560	8	8		1			1		
590	8			1			1		
625	8	8	8						
White	7	7	8					1	

Statistical analysis: A Pearson's Chi-Squared Test was used to assess whether the observed distribution of krill in each of the visible areas in the tank (areas I, II and III) deviated significantly from an "expected" distribution (i.e. assumed to be random). The "expected" distribution was estimated by dividing the total numbers of observed active krill per treatment group proportionally between each area, based on the relative area of visible tank that each area represented (i.e. Area I: 0.2941; Area II: 0.3529; and Area III: 0.3529). The reduced area in Area I was estimated by subtracting two right-angled triangles (9 x 14cm), representing a dark area outside the light beam, from the total area of Area I (18 x 42cm). P values from this analysis were adjusted using the Bonferroni correction to account for the increased risk of type I inference errors due to multiple comparisons.

The effects of the different treatments (Wavelength, Intensity and Flicker) upon both activity (i.e. the proportion of krill that were recorded as active during each observation) and attraction (i.e. the proportion of active krill that were observed in area I during each observation) were modelled using a General Linear Model (GLM). As the response variables were proportions, each model was fitted using a logit linking function and assuming the residual error distribution were binomial. There was significant over-dispersion in both final models (activity & attraction) and the dispersion parameters (Φ) were estimated to be 3.311 and 4.900, respectively. In both cases, the final models excluded any interaction terms due to non-significance. During the analysis it was noted that there was substantial variation in the attraction responses between krill groups, which would warrant the use of a Generalised Linear Mixed Model (GLMM) to account for this random error, however attempts to fit such a model failed due to singularities; presumably due to the unbalanced dataset. The GLM modelling was conducted using R version 3.2.2 (R Core Team, 2015).

Within each krill group, each replicate wavelength treatment was ranked from highest to lowest count of krill attracted to the light (i.e. into area I). The mean scores per treatment were them compared to give an indication of the preferred light source properties.

4.1.3. Results

The results of this work indicate that at least some krill are attracted to artificial light sources, and that both their swimming activity and degree of attraction to the light source may be related to the wavelength of the emitted light. With the limited data

available, there is also evidence to suggest that light intensity (in the range 0.25-1.0 µE $m^{-2}.s^{-1}$) influenced the behaviour of the krill by modifying swimming activity, but did not increase the degree of attraction.

The krill were not randomly distributed in the tanks during the treatments (Fig. 5). Their distribution appeared to be determined by two effects: firstly, some individuals moved towards the light; secondly, others - while still active - appeared unable to manoeuvre away from the corners of the tank. As a result, for most combinations of wavelength and intensity treatments there was a bimodal distribution, which is dominated by area I (closest to the tank) and area III (furthest from the tank). A Pearson's Chi Squared Test confirmed that in many treatment combinations these observed distributions differed significantly from the expected distributions, if it was assumed that the animals were simply randomly distributed (Fig. 5; Table 4.3). In particular, 530 nm and 448 nm, as well as broadband "white" light, appeared to consistently have the greatest numbers of krill in area I (i.e. closest to the light) in comparison to both areas II and III.

The proportion of active krill varied significantly between different wavelength treatments, and increased with increasing light intensity (Fig. 7; Table 4.4). Activity peaked at 530 nm and broadband "white", but was conspicuously low at lower wavelengths.

The proportion of active krill in Area I also appears to vary between wavelengths (Fig. 8), as suggested by the Chi-squared analysis (Table 4.3 and Fig. 6); again with a peak at 530 nm, which shifts to 448 nm at 1.0 µE $m^{-2}.s^{-1}$. However, these differences were only marginally significant when tested using a GLM, and there was no significant effect or interaction due to light intensity (or flicker frequency) (Table 4.5). Although mean proportions of krill in area I of more than 0.6 were observed, supporting the observations that krill were attracted to the light source, individual replicate results were highly variable, suggesting that there is considerable variation between individual krill, hence the effect being only marginally significant in the GLM.

Flicker had no apparent effect on attraction but there was evidence of a marginally significant effect upon krill activity (figures 6b; tables 3b & 4). However we have little confidence in these results, given that they based on a very small number of observations.

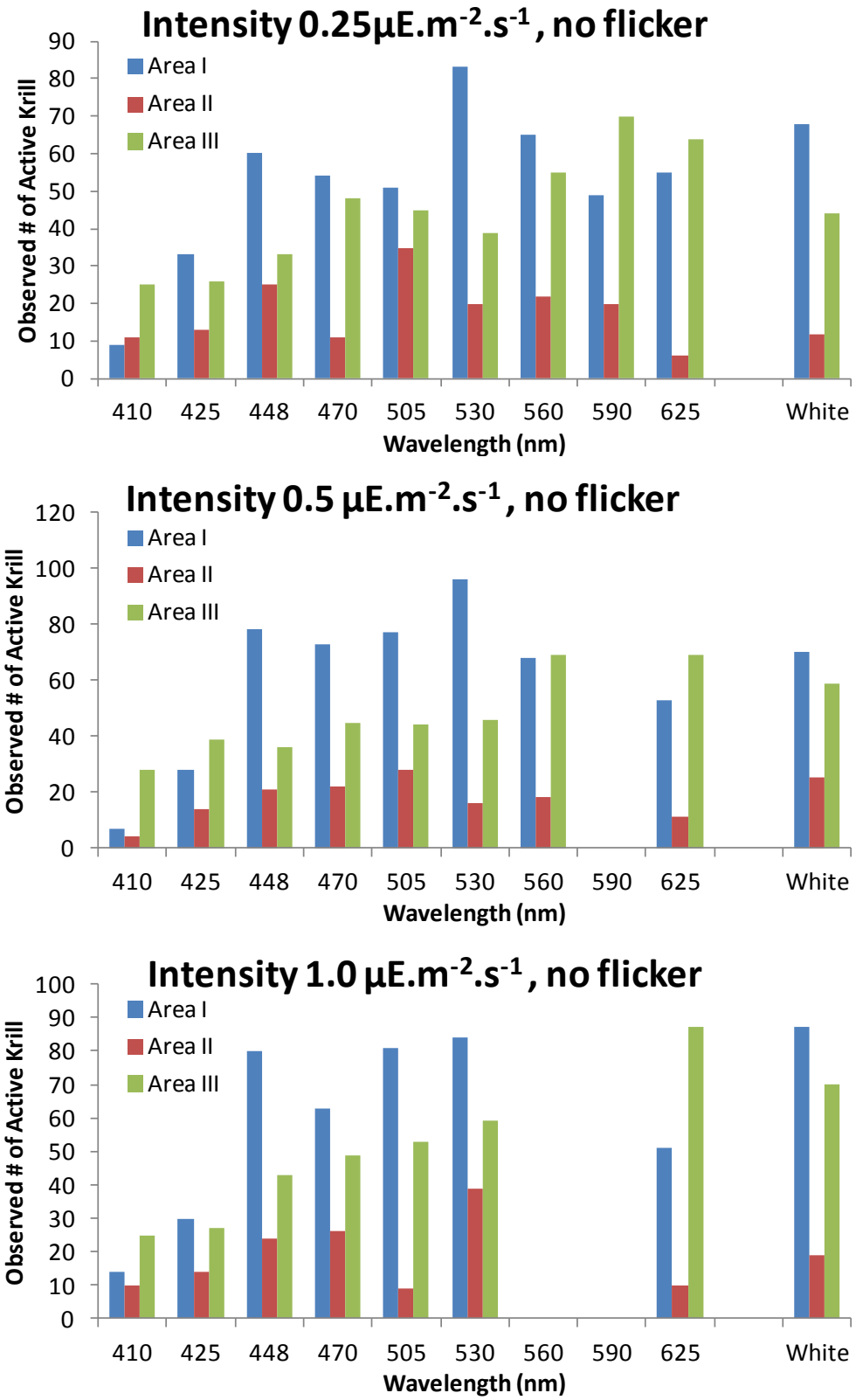

Figure 4.5: The number of active krill counted in each area of the observation tank when exposed to different wavelengths and intensities of light, including "white" (i.e. broad-spectrum – see Figure 4.2). Area I is closest to the light source, while area III is furthest away.

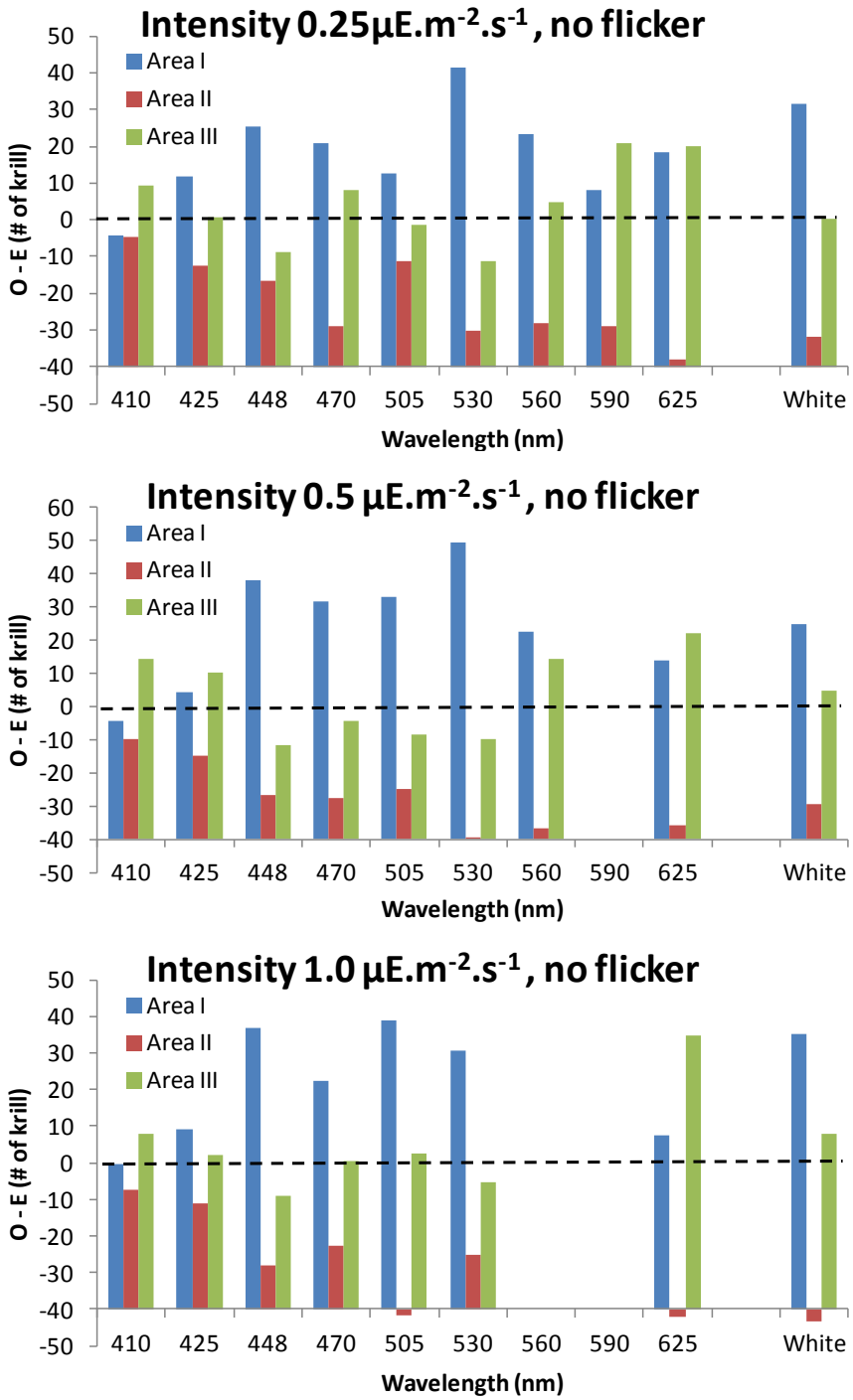

Figure 4.6: The difference between the observed and expected (i.e. when assuming a random distribution) numbers of krill in each area of the observation tank when exposed to different wavelengths and intensities of light, including "white" (i.e. broad-spectrum – see Figure 4.2), Area I is closest to the light source, while area III is furthest away. Chi-squared P values reproduced from Table 4.3.

Chi-Squared Test P values (Wavelength & Intensity)

Intensity	410	425	448	470	505	530	560	590	625		White
0.25	0.9993	0.0108	0.0000	0.0000	0.0005	0.0000	0.0000	0.4909	0.0000		0.0000
0.50	0.6985	0.9890	0.0000	0.0000	0.0000	0.0000	0.0002		0.0082		0.0000
1.00	1.0000	0.0918	0.0000	0.0000	0.0000	0.0000			0.0100		0.0000

Table 4.3: Bonferroni corrected P values from a Pearson's Chi-Squared Test to whether the observed distribution of krill differed significantly for an expected distribution, which assumed that the krill were randomly distributed in the tank. The magnitude of these differences can be seen in Figure 4.6.

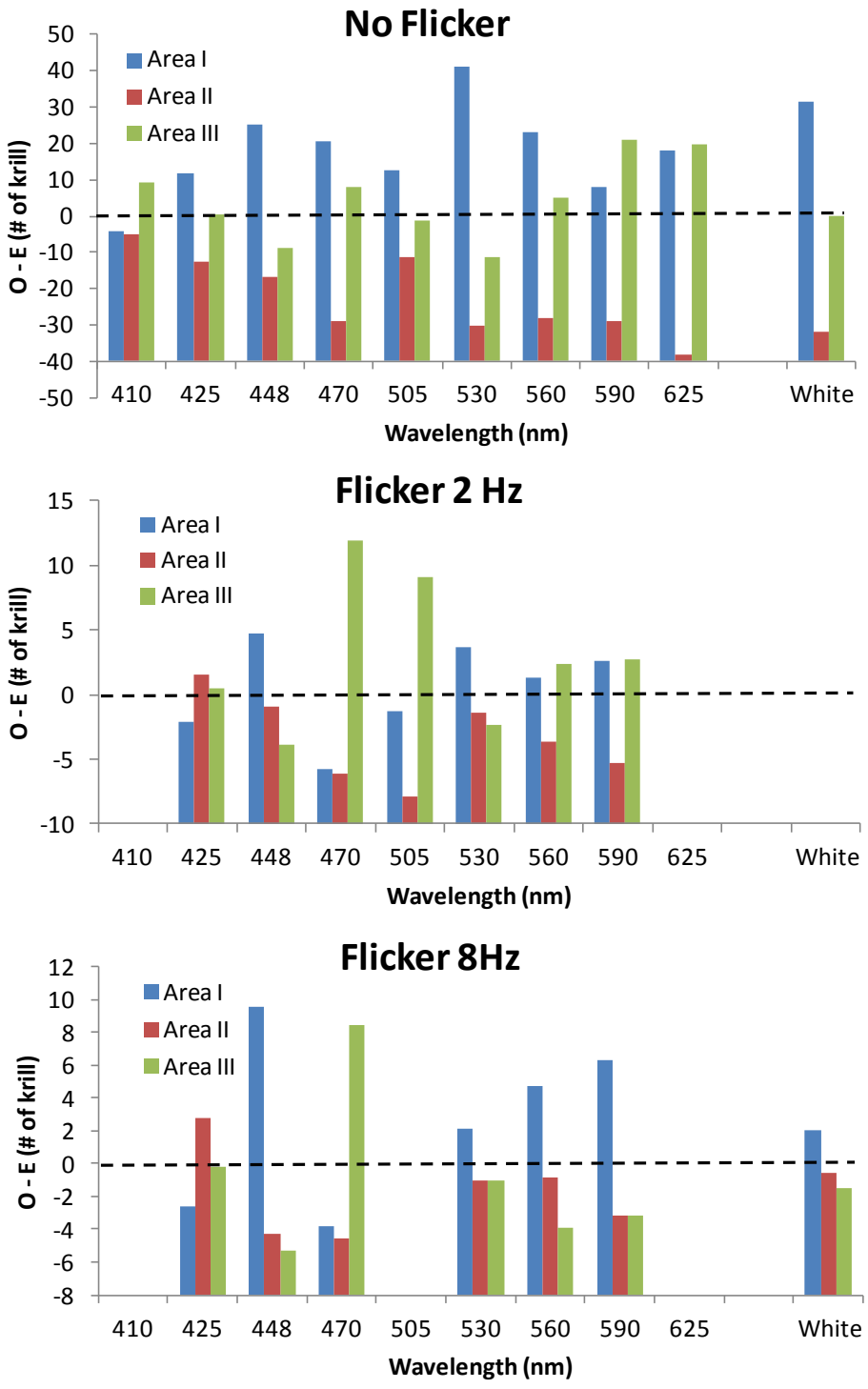

Figure 4.6b: The difference between the observed and expected (i.e. when assuming a random distribution) numbers of krill in each area of the observation tank when exposed to different

wavelengths and intensities of light, including "white" (i.e. broad-spectrum – see Figure 4.2), Area I is closest to the light source, while area III is furthest away. Chi-squared P values reproduced from Table 4.3.

Chi-Squared Test P values (Wavelength & Flicker)

Flicker	410	425	448	470	505	530	560	590	625		White
Steady	0.4113	0.0517	0.0000	0.0000	0.6389	0.0000	0.0000	0.0000	0.0000		0.0000
2Hz		0.8805	0.0344	0.0000	0.0053	0.7758	0.7870	0.1399			
8Hz		0.5067	0.0000	0.0001		0.9996	0.0344	0.0002			0.9748

Table 4.3b: Bonferroni corrected P values from a Pearson's Chi-Squared Test to whether the observed distribution of krill differed significantly for an expected distribution, which assumed that the krill were randomly distributed in the tank. The magnitude of these differences can be seen in Figure 4.6b.

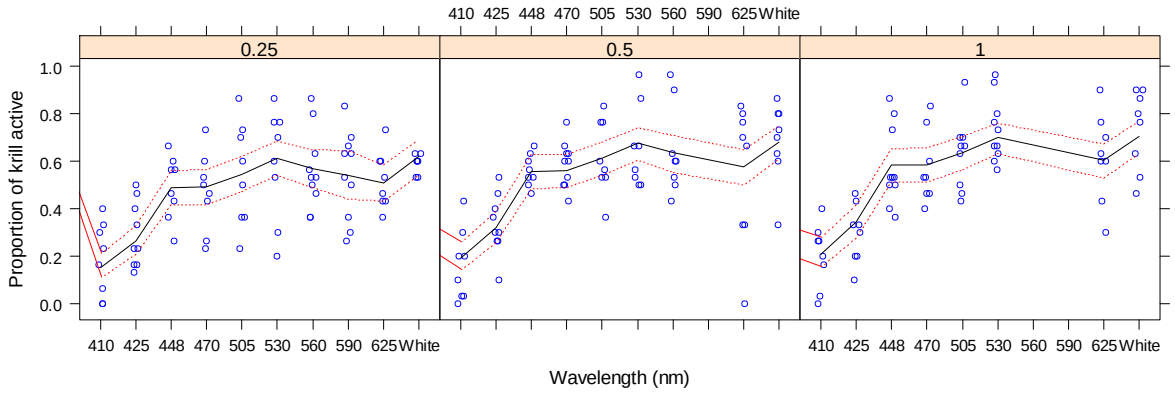

Figure 4.7: the proportion of krill observed to be active during replicate treatments (blue circles, jittered) and GLM fitted values (Black line) with 95% confidence intervals (Red dashed line), with respect to wavelength and light intensity (0.25, 0.5 & 1.0 µE $m^{-2}.s^{-1}$). X-axis is not to scale, and for comparison broadband "white" light (400-800nm) is display on the right side.

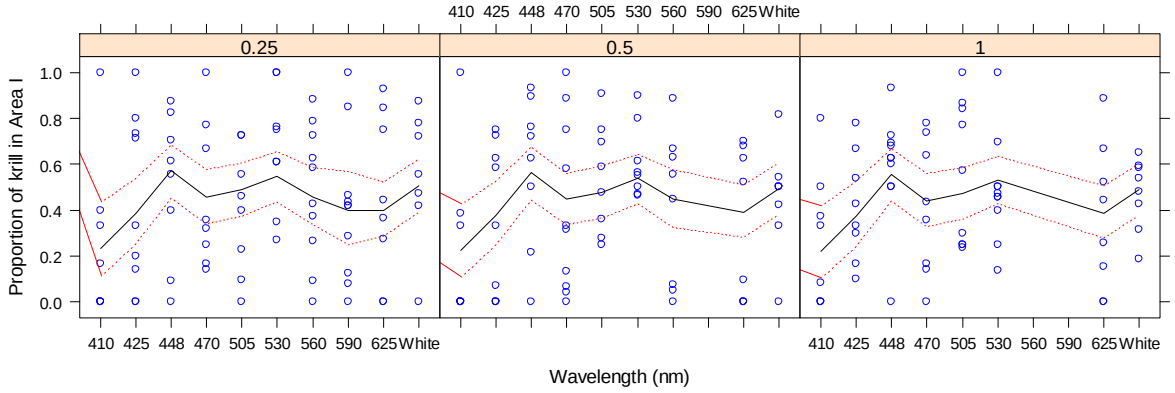

Figure 4.8: the proportion of active krill observed in Area I during replicate treatments (blue circles) and GLM fitted values (Black line) with 95% confidence intervals (Red dashed line), with respect to wavelength and light intensity (0.25, 0.5 & 1.0 µE $m^{-2}.s^{-1}$). X-axis is not to scale, and for comparison broadband "white" light (400-800nm) is display on the right side.

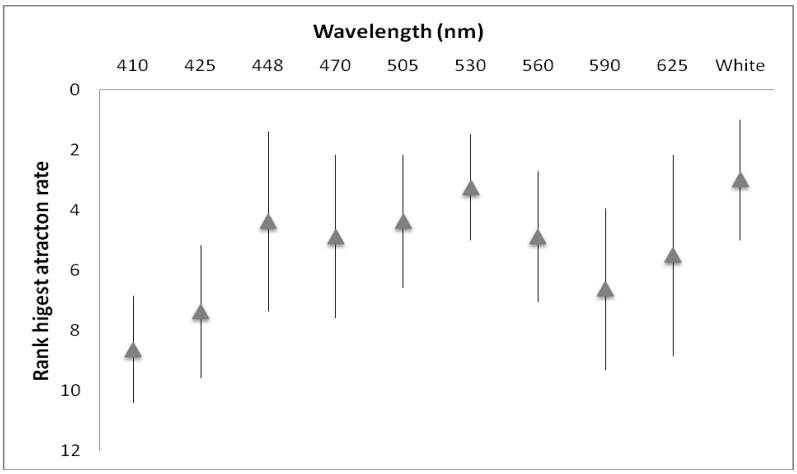

Figure 4.9. Ranked attraction scores to the light source with respect to wavelength. The mean (triangles) and standard deviation (bar) of the ranking scores (1 – highest to 10 – lowest) are presented for each wavelength and "white" light. The highest scoring wavelengths are "white" (3.00), 530 nm (3.25), 505 nm and 448 nm (4.38). X-axis should be read as a category axis and not to scale.

Table 4.4: Analysis of Deviance from a GLM analysis of Krill Activity (i.e. the proportion of krill that were recorded as active during each observation) in response to three light treatments: wavelength (nm), intensity (µE $m^{-2}.s^{-1}$) and flicker (Hz).

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL	227	1505.8				
Wavelength	9	669.68	218	836.12	22.4735	<0.0001
Intensity	2	36.69	216	799.44	5.5405	0.0045
Flicker	2	21.73	214	777.71	3.2808	0.0395

Table 4.5: Analysis of Deviance from a GLM analysis of Krill Attraction (i.e. proportion of active krill in area I) in response to three light treatments: wavelength (nm), intensity (µE $m^{-2}.s^{-1}$) and flicker (Hz).

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL	222	1324.7				
Wavelength	9	82.336	213	1242.4	1.8670	0.0585
Intensity	2	0.485	211	1241.9	0.0495	0.9517
Flicker	2	11.31	209	1230.6	1.1541	0.3174

4.1.4. Discussion

Light between 448 and 560 nm, as well as white light, appear to be the most suitable wavelengths to attract krill (Fig. 9). In particular, 530 nm and 448 nm, as well as broadband "white" light, appeared to consistently have the greatest numbers of krill in the area closes to the light in comparison to the rest of the tank. This matches well with the relative sensitivity curve of Frank and Widder 1999 (Fig. 1), which shows a relative sensitivity of more than 60% at 450 nm and up to 530 nm. Krill having monochromatic vision (one pigment with λ_{max} 488 nm, Frank and Widder 1999), the attractive nature of the white light is probably a result of the fact that a large amount

of the emitted light (Fig. 2, white light is the black curve) consists of wavelengths which falls within the sensitivity curve of the krill's visual pigment (Fig. 1).

The proportion of active krill varied significantly between different wavelength treatments, as well as it increased with increasing light intensity (Fig. 7; Table 4.4). Activity was clearly lowest at lower wavelengths and peaked around 450 to 560 nm, though being highest at 530 nm (green light) and broadband "white". Increased activity could be a response to a perceived increased risk of predation due to increased visibility. Bioluminescence of marine animals falls within the range 440-560 nm (Haddock et al., 2010), which matches the range of where we found an increased activity in krill. Like krill the copepode *C. finmarchicus,* a common prey of krill, increase their swimming activity at wavelengths between 460 and 560 nm (Busky and Swift 1985). Similar to krill, copepods emit bioluminescence at 472 – 492 nm (Latz et al. 1987).

The peak activity shown at 530 nm (green light), matches exactly the wavelength of the light used in a successful cod pot study in Sweden (Bryhn et al 2014). They found an 80 % increase in catch of cod in pots with light. However, attraction and presence of krill or plankton in these pots was not controlled for in this study. In an Icelandic study they managed to keep cod through the winter in a net pan without actively feeding them, by using white light to attract prey. Camera observations confirmed that cod were feeding on krill attracted to the light (ICES, 2012). These previous findings support our finding of an increase in krill activity and attraction to green (530 nm) and white light.

Flickering light should be less detectable for the compound eye of a crustacean, thus our finding of a tendency of lower activity in flickering light was as expected, though this tendency was not seen for attraction. A larger sample size tested over a greater range of flickering frequencies and particularly a greater range of intensities (orders of magnitude difference) would be needed to adequately test for both flickering and intensity effects. Concerning flickering, we know from the literature that flickering light is commonly used to repel fish. There are to our knowledge no studies that have showed flickering light to attract fish, though fishing hooks equipped with flashing light are on sale for recreational fishing. However, these lure lights are of very low light intensity compared to the flickering strobe lights used to repel fish (e.g. 600 W, Johnson et al. 2005). Where krill and plankton is scarce, it might be necessary to use a relatively strong light source to attract sufficient numbers, using a flickering light might repel the target fish.

Using a different experimental set-up could also have improved the outcome of our study. For example, a tube (cylinder) with a light at one end, introducing the krill half way along the tube would be a simpler design for assessing whether the krill are attracted or repelled by the light. The cylinder form would remove the bias of the corner effect that this study suffered from.

4.1.5. Conclusion

Results from our investigation support the hypothesis that artificial light is most attractive across a range of wavelengths (448 nm to 530 nm) that include peak visual

sensitivity (490 nm, Frank and Widder 1999), as well as the wavelength of its bioluminescence (470 nm, Kay 1962; 1965). The most attractive individual wavelength was 530 nm, while broadband "White" light was an equally attractive light source. The intensity of the emitted light did not appear to have a direct effect on attraction to the light source; however it did significantly increase swimming activity among the observed krill.

4.2. Cod attraction study - effect of wavelength and flickering

4.2.1. Introduction

The aim of this case study is to improve the capture rate of cod in fish pots by attracting krill into the pots, and thus providing a source of live bait. From the krill study we have seen that krill are attracted to artificial light of various properties, but more so to *White light* and blue and green lights of 448 nm and 530 nm wavelengths. Thus, the next step is to assess whether this type of light stimuli will also attract cod, or least not repel them. Given that the most important effect of adding light to a pot is to attract krill, we only tested the light which krill displayed most affinity for. Our OMR studies showed that cod are sensitive to flickering in the range of 20-110 Hz, see OMR results on cod). However, it is well known that high frequency flickering light repels fish (e.g. Patrick et al, 1985). Light in the range of 300-600 flashes per min (equivalent to 5-10 Hz and above) is known to repel fish (e.g. Johnson et al. 2005; Sager et al. 2000). Thus, we tested lower flickering frequencies (2 Hz) similar to krill's own light signalling frequency, and one higher within the range that is known to repel some fish (8 Hz).

Wavelength sensitivity of cod is very similar to that of krill though cod has a dichromatic vision (Valen et al 2014), one peak at 450 nm and one at 517 nm (Fig. 10 and Table 4.6).

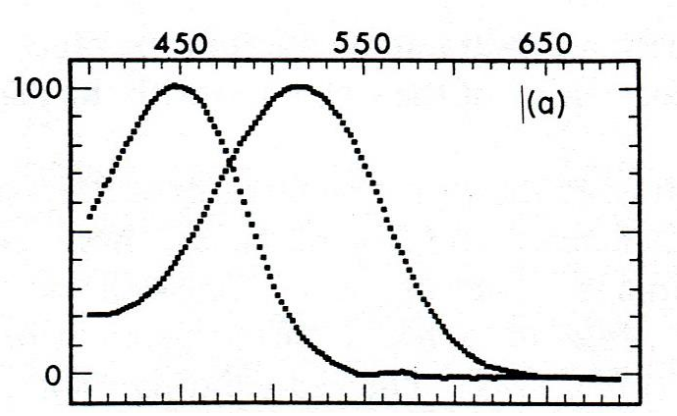

Figure 4.10. Spectral selectivity curve for *Gadus morhua*, from Bowmaker 1990. Y-axis shows Absorbance(%), X-axis wavelength.

Table 4.6. Wavelength and light intensity sensitivity found in previous studies on cod (*G. morhua*). *Change from scotopic to photopic vision, **Light threshold.

λ_{max}	Method	Reference
446 nm, 517 nm	Microspectrophotometer	Bowmaker 1990

490 nm, 450 nm	Conditioning exp	Anthony and Hawkins 1983
*8 x 10^{-6} W sr^{-1} m^{-2}	Conditioning exp	Anthony 1981
**0.01 - 0.001 µ E m^{-2} s^{-1}	Behavioural, herding effect of trawl gear	Blaxter 1970

Objective: determine the behavioural responses of cod to different properties of light, including: wavelength, intensity and flicker; in particular with reference to the properties that are attractive to krill.

3.2.2. Methods

Experimental animals: Cod (*Gadus morhua*) was caught using fyke-nets in the outer Hardangefjord (Os and Austevoll), western Norway. Upon capture they were placed in a 5000 litre transportation tank in the fishing boat before being released into the holding tank (10,000 litres) at IMR's Research Station in Austevoll. The holding tanks had a constant supply of seawater, and the fish were fed daily on frozen shrimp and feed pellets.

Experimental procedures: Two fish were taken from the holding tank the evening before the experiment and one placed in each of the two experimental tanks. This gave them an acclimation time of 16-18 hours. The fish were not fed during the experiment. The only source of lighting in the experimental room was the experimental stimulus and infra-red lights placed above the two experimental tanks. The experimental light source (Fig. A1 in Appendix) was placed at one end of the tank. The infra-red light was used to illuminate the tanks to enable video recording of cod behaviour, as cod are not able to see infra-red light (Valen et al. 2014). The two experimental tanks were lined with black fabric to minimize reflection from the infra red light (Figure 4.10). A wide-angle camera was mounted centrally above the two tanks. Both the camera and light source were controlled from a nearby laboratory.

Each fish experienced the same twelve treatments, which were a combination of four different wavelengths (448 nm, 505 nm, 530 mm and White light) and three different flickering rates (steady, 2 Hz, 8 Hz) all at an intensity of 0.25 µE $m^{-2}.s^{-1}$. The order of the treatments was randomized between the replicate fish. A total of nine fish were tested, for 10 min with twelve different light settings (treatments) and twelve controls. Both tanks were observed simultaneously (see Fig. 10), this enabled us to also observe the fish that was not experiencing the light – which acted as a control for its next treatment with light stimuli.

Analysis: The video was analysed by noting which side the two cod (i.e. treatment and control) were at every 30 sec for 10 minutes. A standardised measure of the proportion of time ("Residency Time") that a fish spent in the side of the tank containing the light (Side I) was defined by subtracting the proportion of time spent in Side I under control conditions (i.e. without the light) from the proportion of time spent in Side I during the treatment (i.e. with light on). Due to the apparent lack of response in the experimental subjects towards the light source, no formal statistical analysis was conducted on this data. However, the mean standardised residency times for each treatment are presented in Figure 4.11, along with their 95% confidence intervals.

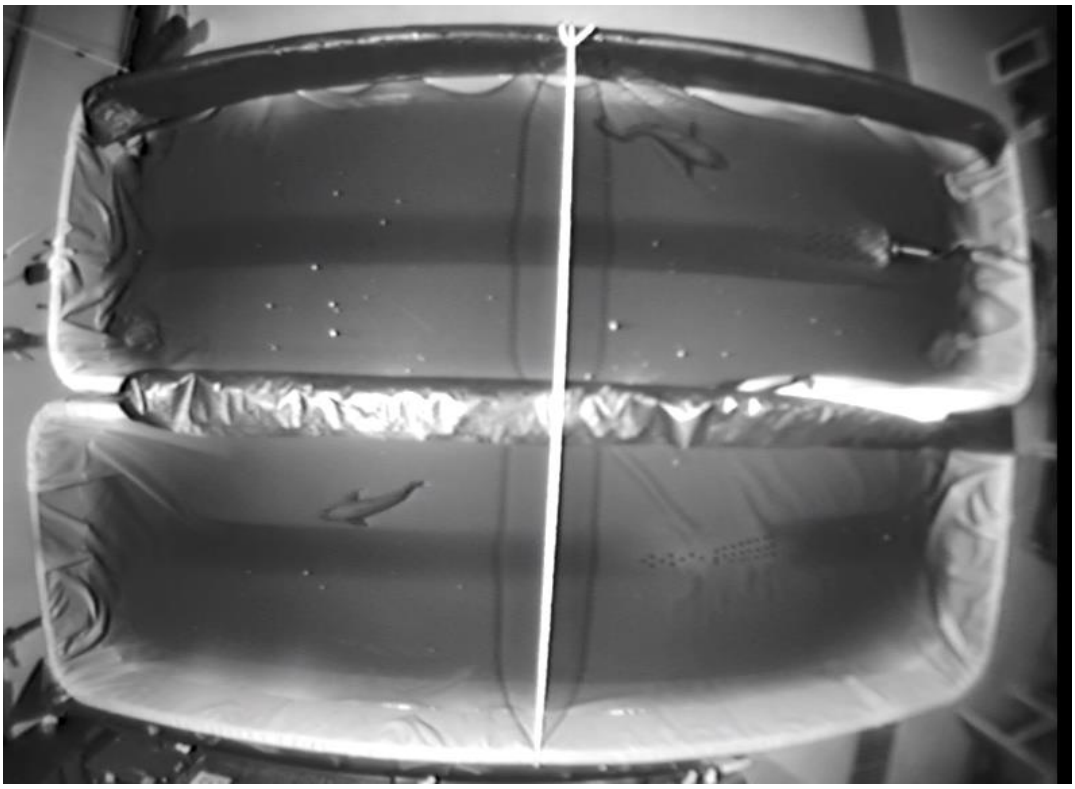

Figure 4.11. Experimental set-up. Tanks are 400 cm long, 150 cm wide and 100 cm deep. The light source was placed in one tank at a time (here seen in top tank in the right end). A rope was tied across splitting the two tanks in two equal parts. Infra-red lighting and overview camera were mounted to the ceiling above the two tanks.

3.2.3. Results

In general, the light stimuli appeared to have a slightly negative effect on the distribution of cod, in that for most trials fish spent on average 10 – 30 % less time in the lamp side of the tank (side I) when the lamp was present compared to the control (Figure 4.12). However, due to a small effect size and substantial individual variation most of these differences were not statistically significant (Figure 4.12). In the only significant deviation from the control (wavelength 448 nm and no flicker), cod were 28 % less likely to be in the lamp side of the tank compared to the control. Conversely, for 530 nm there appears to be a slightly positive effect on the distribution, when it was flickering at 2 Hz, although this is non-significant.

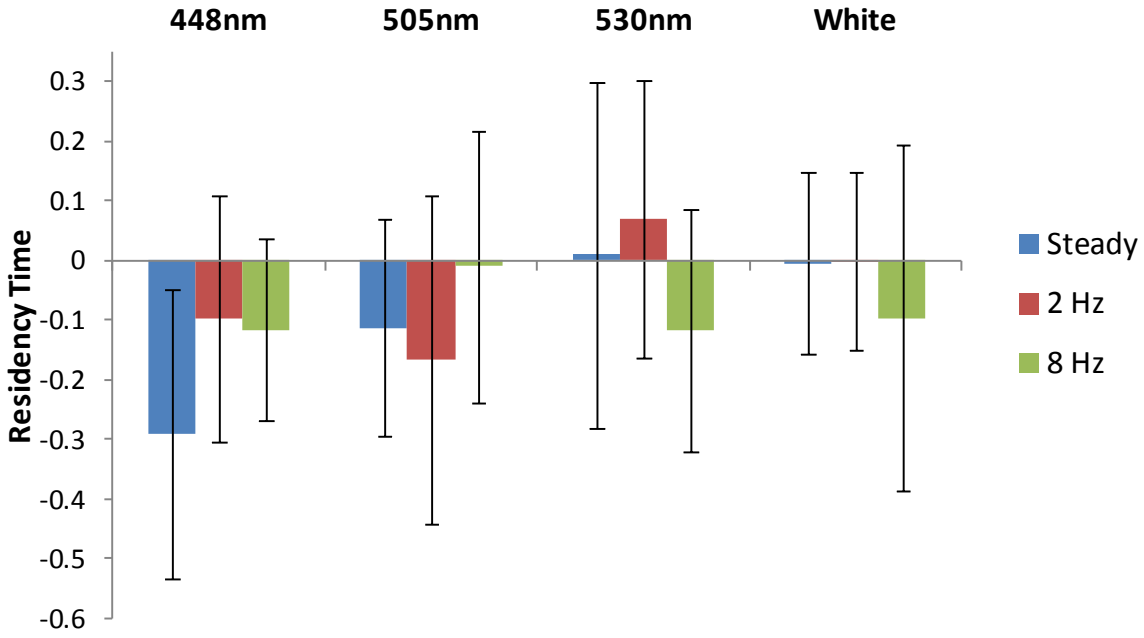

Figure 4.12. Effect of wavelength and flickering on cod distribution. "Residency time" is the relative time cod spent on the lamp side of the tank when light was present compared to when not present. 448 =448 nm, 505 = 505 nm, 530= 530 nm, White = white light, see Figure 4.1 for colours.

3.2.4. Discussion

The cod in this experiment did not react significantly to the presence of a light at the intensities and flicker frequencies tested. At times cod were observed on top or just in front of the lamp. However, pooling all the recorded data from the videos indicates some avoidance with the exception of 530 nm, where there could be some attraction effect when flickering at 2 Hz and indifference to 0 Hz flickering. From the literature (Table 4.6) we know that cod (*Gadus morhua*) has a two-peaked sensitivity curve with a λ_{max} at 446 nm and at 517 nm (Fig. 10), both in the range of the peak sensitivity of krill (*M. norvegica*) (Fig. 1). Thus, all tested wavelengths 448 nm, 505 nm, 530 nm, as well as the white light, should be within the range of the peak sensitivities of cod.

In reviewing our observations, it is may be unreasonable to expect that cod should be attracted to s simple light source just because it is a visual predator. Firstly, a predator's ability to use vision to hunt does not necessitate phototaxis. Secondly, cod use visual and olfactory senses, as well as touch (in their lips/mouths) when selecting bait (Løkkeborg, 1998). Also, they are very sensitive to auditory stimuli (Chapman & Hawkins, 1973). They may have been indifferent to light in this case simply because the light alone was just not interesting enough. However, having a swarm of krill around the light might have made it more attractive.

3.2.5. Conclusion

In this experiment cod demonstrated no strong behavioural response to the artificial light source. However, there is some marginal evidence that they may have avoided

close encounter with a steady (i.e. non-flickering) light at a wavelength of 448 nm and intensity 0.25 $\mu E\ cm^{-2}\ s^{-1}\ nm^{-1}$.

Since krill were most attracted to steady light at 530 nm wavelength, and the difference between steady and 2 Hz strobe was insignificant for cod, we recommend that a steady 530 nm light, with an irradiance (at 1m from the source) of approximately 0.25 $\mu E\ m^{-2}.s^{-1}$, should be tested in commercial fishing pots in our field fishing study to be undertaken as part of Task 2.8: A*lternative Fishing Gears*. Further, this experiment should use cameras to demonstrate that krill are aggregating around the light source, and take stomach samples from the cod to determine whether the cod have been feeding on krill.

3.2.6. Acknowledgements

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Appendix

Figure A1. Experimental light source ("The Magic Lantern"). Left: Control panel, for setting required wavelength (380, 410, 425, 448, 470, 505, 530, 560, 590, 625, 665nm and "White" (400-800nm) light), flash rate (steady light or 0.1-100Hz, with 50% duty cycle square wave) and intensity (controlled via input current in mA, where the resultant irradiance, $\mu E.m^{-2}.s^{-1}$, from each LED was calibrated against input current, see figure A2); and right: the underwater housing with LED light sources visible behind the glass (max depth 5m).

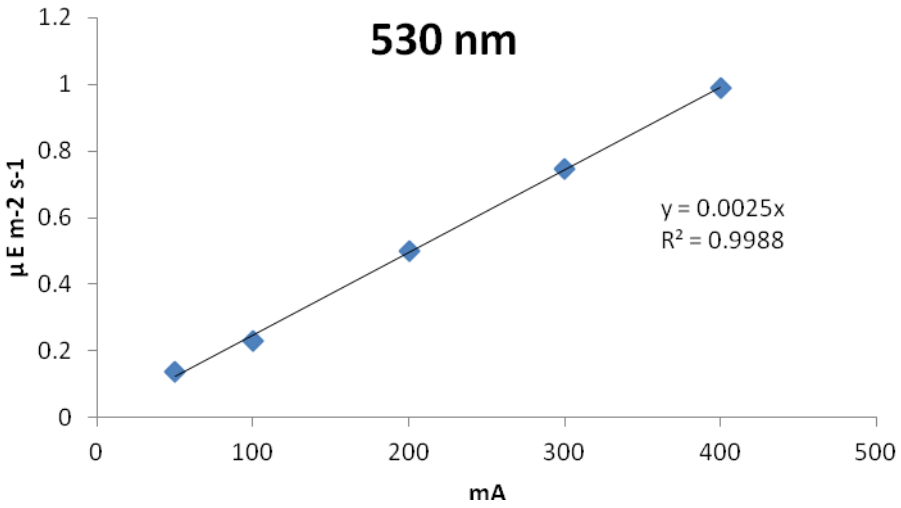

Figure A2. Example of the calibration for an LED (530 nm) in the experimental light source, showing the linear relationship between input current (mA) and resultant irradiance ($\mu E.m^{-2}.s^{-1}$).

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