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Fluorescence for non-contact detection of living salmon lice on salmon skin

Kari Anne Hestnes Bakke*, Trine Kirkhus, and Jon Tschudi

SINTEF Digital, Smart Sensors and Microsystems, Forskningsveien 1, 0373 Oslo, Norway

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Abstract. This work presents a promising method for automatic, non-contact, detection and counting of salmon lice infested on salmon in an aquacultural farm setting. The method uses fluorescence from chitin in the visual part of spectrum to enhance the contrast between fish skin and salmon lice, and show that the fluorescence is even strong enough to give a real-time view of the digestive and reproduction system in live lice without use of staining dyes. The wavelengths used are compatible with an underwater measurement system.

Keywords: Fluorescence, Imaging, Underwater, Salmon lice.

1 Introduction

One of the main threats to salmon farming in Norway is a copepod, the salmon lice *Lepeophtheirus salmonis* (*L. salmonis*) [1]. It occurs naturally in seawater all over the northern hemisphere. It lives and reproduces on salmon and trout in seawater, and lice are found both on salmon in aquaculture sites and on trout and salmon in the fjords and along the coast, throughout the year. Lice infestation in salmon can create wounds in the fish skin and the welfare of the salmon will decrease if there are larger attacks. Over the years, the lice have developed resistance to several medical treatments [1] and in some cases, the only solution is down slaughter in a specific farming site. To keep the welfare at a high level and to hinder spread of salmon lice between sites, the Salmon farmers are required to do weekly lice counts on a representative set of salmons to ensure that the number of lice attached to salmon within the cage is on average less than 0.5 fertile female lice per salmon. Numbers must be reported to the authorities. Systems for automatic counting of lice are therefore much sought. Performing live lice counting using an underwater camera in the cage, is, however, demanding. The counting system should count or image the lice (2–6 mm) on the live salmon (60 cm) from 1 to 2 m distance through seawater, while it swims at a speed of approximately 1 fishlength/sec.

Other studies, that focus on identifying and enumerating planktonic *L. salmonis* stages in plankton samples in the lab. Thompson et al. [2] have investigated if a specific combination of excitation and emission filters would result in *L. salmonis* fluorescing, while suppressing fluorescence from non-target planktons. The results are described by an excitation and emission matrix. The resulting matrix presented in [3] is however limited to excitation below 580 nm and emission below 600 nm. Based on this they propose an excitation wavelength of 380 nm with emission detection at emission wavelength 474 nm or excitation at 450 nm with detection at 516 nm for *L. salmonis* larvae identification in laboratory imaging systems.

2 Methods and experiments

In our study fluorescence properties of salmon and lice were assessed, to find a method to enhance the image contrast between lice and skin, in a spectral area that can be applied under water. This will ease the image analysis and computational needs, reduce the need for optical resolution given that the method is specific enough. We had access to newly slaughtered salmons with living lice which was delivered in seawater. Properties of lice, fish skin and seawater were investigated. The fish skin has large colour variations and has spots coloured white, grey, and black. The lice itself is semi-specular and partly transparent, and the colour of the non-transparent part will vary between lice. The contrast between lice and fish skin in an ordinary camera image is therefore very limited. High resolution camera images of lice on fish skin in air are given in Figure 1. Due to the low contrast, high quality images would be needed for identifying the lice. It is very difficult to get images of sufficient quality (low noise and high resolution) in a real situation, and it would also be hard to automate the process of identifying the lice. To enhance the contrast, addressing fluorescence properties are especially attractive since the

* Corresponding author: kari.a.bakke@sintef.no

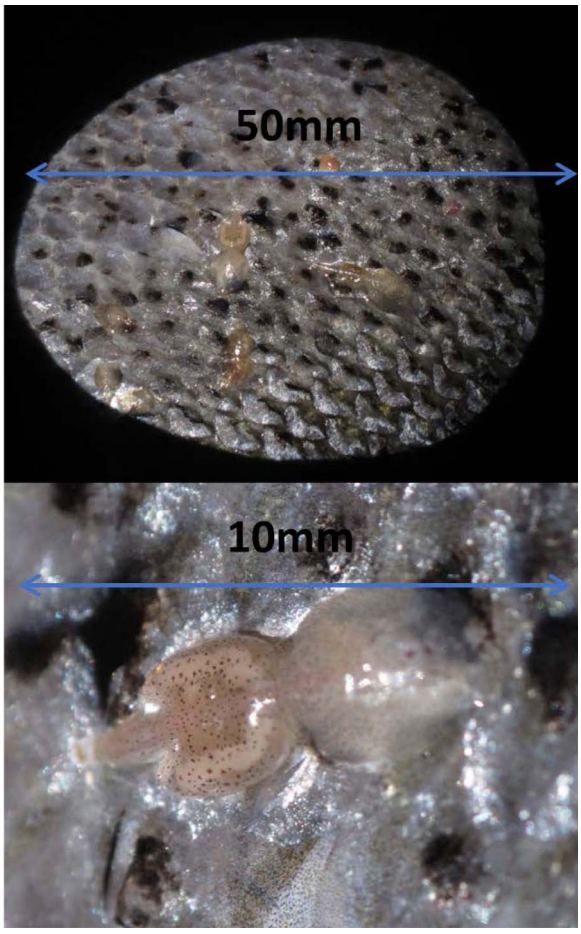

Figure 1. Top: Seven live lice at salmon skin, Bottom: One 8 mm lice at salmon skin.

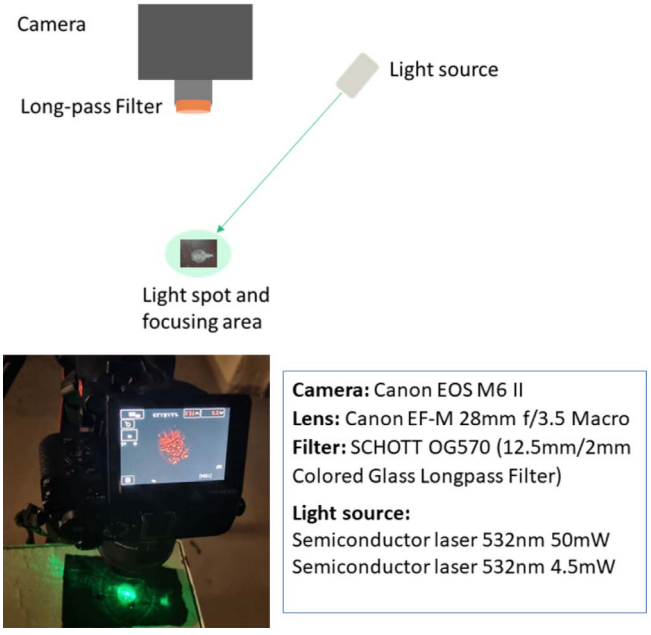

Figure 2. Scheme, imaging setup with live view of salmon lice.

fluorescence will depend on the difference in chemical properties of lice and salmon. The chemical substance of the lice copepod is different from the fat salmon and its fish skin.

Through our investigations we discovered that red fluorescence emission (> 600 nm) could be obtained from lice using a 532 nm, green, excitation wavelength. This set of excitation/emission wavelengths also enhances contrast from salmon skin, since salmon skin fluorescence is not excited by use of this wavelength [4]. A main concern in an underwater imaging system is also the absorption of light in the water column itself.

Excitation at 380 nm centre wavelength with emission above 410 nm was tested in our lab. Our measurements confirmed that salmon lice have a fluorescence signal when excited in this wavelength range. The wavelengths are however not compatible with the other restrictions in an image enhancing system separating lice from salmon skin in an underwater imaging setup working at a distance. The contrast between lice and fish skin is not enhanced since the salmon skin also give a fluorescence signal, and the absorption of UV-light in seawater is unpredictable due to varying organic matter in the water column. The best transmission window in seawater is within the 500–700 nm range [4] and the wavelengths that we propose are well suited for underwater use due to low absorption. Within the 500 nm–700 nm range, 80–95% of the light will be transmitted to the detector. This is in contrast to the wavelength range below 425 nm, where we have measured less than 20% transmitted light though a 1 m water column.

Based on these findings we set up a fluorescence imaging system (see Fig. 2): Semiconductor lasers at 532 nm were used for excitation. A long-pass, glass filter with cut-on wavelength of 570 nm was used in front of the Canon EOS M6 II camera to block the light from the laser, resulting in the visible wavelengths above 570 nm reaching the camera chip. A 28 mm macro lens was included for high image resolution. Two lasers with different power were used for excitation. The diameter of the illuminated spot areas were about 4 mm and 12–14 mm, giving similar intensity for both lasers.

3 Results

In Figure 3; three lice on salmon are imaged, using the setup described in Figure 2. They are 3–4 mm of size, and the field of view is about 10 mm times 15 mm. In the upper image a white light source and RGB camera have been used. In the lower image (Fig. 3) the proposed fluorescence imaging system have been used. In the fluorescence image we can observe complex details of the salmon lice's anatomy. Our expectations were that we should observe the exoskeleton made by chitin, and were surprised by the detailed image of the lice anatomy. After we had observed these details, we learned from literature [5] that copepods have chitin not only in their exoskeleton but also as part of their digestive and reproductive systems. These systems are clearly visible in the fluorescence images. The contrast between

Figure 3. Three salmon lice on salmon skin. Setup described in Figure 2 were used. Top: White light source and RGB camera Bottom: 532 nm laser source, long-pass filter on RGB camera.

the lice and salmon skin is high, like we could expect from our initial fluorescence measurements.

In our setup, we used a rather long exposure time of 0.4–0.8 s and a high-resolution RGB camera. The quality of these images is far better than needed for detection of lice, both in the aspect of resolution and low image noise. A commercial system will need to compromise on the image quality to get images of sufficient quality at a much shorter exposure time.

In addition to the images obtained with the settings above, we also did full resolution, low framerate video imaging at 0.1 frame pr sec. Given a circular illuminated area of radius 0.9 cm, the total 50mW laser power is distributed over 2.5 cm^2, which gives a mean optical power of ~20 mW/cm^2 and 2mJ/cm^2 per image frame. The resulting images have a contrast of about 10. In a commercial real-time lice counter the optical resolution should be a compromise between needed area to cover per frame and the size of the details to be studied. A resolution of 0.2 mm per pixel will give 15 × 15 pixels for typical lice of 3 mm length, and this should be suitable for detecting the lice through image analysis. Effort must also be put in selecting a proper laser pulse frequency and peak power effect, as the salmon can swim at a speed of 1 m/s. There will be a need to take images in a stop-motion like manner to avoid blurring. To minimize the movement of the fish during the acquisition of the image we will need a pulsed laser with a pulse width of around 0.5 ms and repetition rate of 50 Hz. We can then image the fish in slices of 2 cm by 30 cm, as the fish swims by. Each pulse must then have a peak effect of 240 W to obtain the optical energy of 2 mJ/cm^2 per slice. To image the requested area of 2 cm times 30 cm at 0.2 mm resolution, a camera chip with at least 100 × 1500 pixels will be needed.

Further investigation is still needed to determine the minimum laser power level that is required and the actual configuration of laser or set of lasers and camera for best performance in a real-time lice counter.

4 Conclusion

L. salmonis' produce a fluorescence signal when excited with green light (532 nm). The detailed images underpin that the fluorescence origin from chitin, which is part of both the exoskeleton and the digestive and reproduction system [5]. We have demonstrated that a simple camera system, like the one we have set up in the lab, can be used to study the anatomy in living lice without using staining dyes like Congo red as fluorescence marker [6].

The way forward is to perform tests and experimental work in a larger scale, including measurements in seawater tank with living lice and living salmon to confirm that the imaging principle is scalable and can be realised as a live viewing and real-time counting of salmon lice in a salmon farm production facility.

Further work will include investigations on fluorescence from other particles in the water column that could disturb the image analysis, including callanus and fluorescing feed particles from salmon farms.

Conflict of interest

The authors declare no conflict of interest.

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