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*Publication date:*  
2011

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*

Wulff, T., & Nielsen, M. E. (2011). The effect of wounding on protein expression in rainbow trout muscle.. Poster session presented at Tissue Repair & Regeneration, New London, NH, USA, .

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# **The effect of wounding on protein expression in rainbow trout muscle**

**Tune Wulff and Michael Engelbrecht Nielsen**

The present investigation was initiated in order to look for protein changes as a result of wounding in rainbow trout muscle. Rainbow trout is the most important fish in aquaculture in Denmark and of significant commercial interest. Tissue damage is a naturally occurring phenomenon in fish grown in aquaculture, and can occur as a consequence of mechanical damage like handling, interaction with other fish or contact with its surroundings. The fish is only slaughtered when the wound is completely healed; however the wound and healing process will leave lasting changes in the tissue and can thereby impact quality. Increased knowledge of the healing process in rainbow trout will therefore be of great importance for the aquaculture industry.

## **Experimental set-up**

The investigation uses a very simple concept in which the fish is used as its own control. This effectively eliminates variability originating from differences between individuals. The division of the fish is possible because the wound can be inflicted on one side of the fish (wound) without harming the control side of the fish (internal control). Even though the wound healing process primarily is a local process, the possibility of systemic effects cannot be ruled out, and the experimental setup will also include an independent group of fish which are not challenged (external control). The experimental design included 7 fish that were injured and 7 fish that were uninjured. Injure were inflicted with a 2 mm needle, while the fish were completely sedated. Samples were taken 7 days after time of injury. Before sampling fish were killed with an overdose of benzocaine. Proteins were separated by 2-dimensional gel electrophoresis, which allows for investigation of more than 900 protein spots in a single study. Changes in protein expression between the wound, internal- and external control were found using a t-test.

## **Discussion and Results**

The number of proteins changing were: wound vs. Internal control (210), wound vs. External control (200) and internal vs. external control (23). The result shows that a number of proteins are indeed changing in response to wounding. Interestingly only a limited number of proteins changed in abundance in response to more than one of the different comparisons, and no single protein changed in response to all three comparisons. This shows the importance of using both and internal and external control. The comparison of the external- and internal-control allows for an investigating of the systemic effect of the wound, and showed that there clearly is an systemic effect of the wound. Identification of proteins is an ongoing process and currently proteins involved in cytoskeletal regulation, the immune system and iron binding have been identified.

All procedures were conducted in accordance with the regulation set forward by the Danish Ministry of Justice and animal protection committees by Danish Animal Experiments Inspectorate permit 2007/561-1302 and in compliance with European Community Directive 86/609.