

FELLOWSHIP SUMMARY REPORT

Recipient of fellowship: Guiomar Rotllant, Departament de Recursos Marins Renovables, Institut de Ciències del Mar (CSIC), Barcelona, Spain

Subject title: Investigating the humane killing of CRUStaceans through science-based INDicators (CRUSIN)

Theme number: Theme 1 Managing Natural Capital for the Future

Host institution: University of Gothenburg, Department of Biological & Environmental Sciences, Box 463, SE-405 30 Gothenburg, Sweden

Host supervisor: Lynne U. Sneddon

Dates of fellowship: 3rd June to 7th October 2022

I, Guiomar Rotllant, consent that this report shall be posted on the Co-operative Research Programme's website.

Relevance of the fellowship

Relatively little research has been conducted on crustacean welfare. Due to consumers concern on animal welfare of food products, a few countries have set up a legislation for protecting crustaceans on the “precautionary principle”, however evidence of pain in crustaceans is still in its infancy. With the increase in debates concerning environmental sustainability and management of fish stocks and aquaculture at the international and global scale, this project looks for pain indicators in crustaceans to improve their welfare and set scientific knowledge to support the European Food Safety Authority (EFSA) and governments in order to include welfare in crustacean production systems.

1. What were the objectives of the research project? Why is the research project important?

The general objective of CRUSIN project was to use a multimodal approach to explore humane stunning and killing of crustaceans and to identify biomarkers that indicate unconsciousness. Model species were the Norway lobster *Nephrops norvegicus* and the crayfish *Astacus astacus*. This innovative integrated approach was developed in the following concrete objectives:

- 1) Investigating electrical stun parameters as a noxious stimulus: intensity and stun duration, behavioural responses.
- 2) Determining analgesia conditions: dose and duration.
- 3) Identifying non-invasive animal-based Indicators proposed by EFSA:
 - a. Behavioural: locomotion and grooming.
 - b. Physiological: metabolite titters in the haemolymphs (glucose and lactate).
 - c. Neurophysiological: heart rate as a proxy of electroencephalogram (heart activity is directly controlled by brain in crustaceans).

2. Were the objectives of the fellowship achieved? Or are they on the way to being achieved?

The objectives of the fellowship were partially achieved. A review on methods to induce analgesia and anaesthesia in crustaceans has been submitted to the journal “Biology”. Regarding the research conducted at the University of Gothenburg electrical stunning conditions, analgesia procedures, behavioural parameters, glucose and lactate analyses, qPCR of genes involved in pain and heart rate analyses have been set up. The experiments planned were performed, however due to the high inter-individual variability sample size has to be largely increased and therefore some of the research is still ongoing. Dr Sneddon in collaboration with Dr Rotllant and other researchers was granted a Formas grant (Swedish government) on “Crustacean Welfare” and therefore the finalization of the experiments and publication of results are assured.

3. What were the major achievements of the fellowship? (up to three)

1. We wrote a review on methods to induce analgesia and anaesthesia in crustaceans including an on-line tool. In early 2022 UK government has recognized that crustaceans are sentient beings (<https://publications.parliament.uk/pa/bills/cbill/58-02/0255/210255.pdf>) and European Union is now revising the regulation on animal welfare for scientific purposes where crustaceans might be included. Then it will be mandatory the use of anaesthetics and we expect that this review and tool will be very useful for researchers.

2. Electroshock (1V, 10s) was proved to be a useful noxious stimulus to study pain in lobsters and crayfishes. Using this method, we showed a differential behaviour (video analyses) and heart rate between control and electroshocked crustaceans.
3. The expression of the following genes: CHH and receptors of several neurotransmitters (gamma-aminobutyric acid (GABA)_B 1 and 1, acetylcholine neuronal and nicotinic, serotonin and somatostatin) were detected in the nervous system of lobsters.

4. Will there be any follow-up work?

As indicated above, a review has been submitted to the journal “Biology” and which will be published during 2023. Regarding the research conducted in the University of Gothenburg, data which will be obtained from ongoing experiments will be merged with the data obtained from the experiments performed in the summer to be published in article to be submitted in 2023.

Moreover, we have now a four years project on Crustacea Welfare that will enhance the collaboration between the University of Gothenburg and the Institute of Marine Sciences (CSIC) in Barcelona.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

It is expected a new legislation on crustacean experimentation in the European Union and therefore knowledge on pain indicators and anaesthetics issued from this CRP grant might be very useful for researchers and policy makers. However, welfare is not only related to experimentation but it is also related to the housing and maintenance conditions of the animals. Some aquaculture companies have started to implement animal welfare measures for their farmed species because they are aware that maintaining welfare during farming, transport and slaughter improves production and the quality of the final product. There are currently several companies supplying electric stunners for crustaceans. For example, large-scale electrical stunner manufactured by Optimar (10 tons of shrimp an hour) has been used by Tesco for the shrimp *Penaeus vannamei* in Thailand. Following successful results, and a review of the out-comes by external animal welfare experts, the company was the winner of the best innovation category of Compassion in World Farming’s Good Farm Animal Welfare Awards (<https://thefishsite.com/articles/shrimp-stunning-initiative-wins-animal-welfare-award>).

6. How was this research relevant to:

We have provided evidence that electric shock was a practical way of induce noxious stimuli to lobsters and crayfishes in order to generate scientific knowledge to validate pain indicators. The duration of the locomotory activity showed a decrease after 1 and 2 hours of the electroshock while grooming was increased after analgesic treatment with lidocaine either in control or electroshocked lobsters. The heart rate used as a proxy of neurophysiological measure had increased in electroshocked crayfishes. Six genes (CHH and receptors of neurotransmitters) were differentially expressed in the brain, and subesophagic, thoracic and abdominal ganglia. Research on this topic is going on to increase sample size and get statistically significant data to confirm the best pain indicators that could be used for advisory and/or regulatory purposes (local and national administrations, EFSA and the EU commission, and among all OECD Member

Countries) to support future policy decisions related to the sustainable use of aquaculture and fishery products.

7. Satisfaction

The OECD Co-operative Research Programme fellowship fully met my expectations. My main goal was start doing research on crustacean welfare in a multidisciplinary approach (behaviour, physiology and neurophysiology) to provide a strong scientific basis to demonstrate the existence of pain in crustaceans in order to open a new research line in my home laboratory. This goal was largely achieved although we still need to increase sample size to have significant data that prove the evidence observed. We have a four years project that was approved in 2022 to go on with this relevant research that might support future legislation for crustacean welfare in Europe. My only practical problem was the low availability of the host researcher; I was expecting to have interesting scientific discussion, for instance through the review paper we were preparing, but this was very occasional and communication was complicated. Fellowship Programme works very well and this kind of issues cannot be foreseen.

8. Advertising the Co-operative Research Programme

I learned about the OECD fellowship via a colleague. I found the OECD Fellowship programme extremely well organized and appreciate the dissemination and publication of fellowship results.