The Lobster Pregnancy Test

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Abstract

The illegal practice of 'scrubbing' the eggs of berried (egg bearing) lobsters has come about as a result of new laws which has prohibited the landing of them into English ports. This evasion of the law is causing serious depletion of stocks ('R130: Detecting berried lobsters', 2022).

This project aims to detect traces of exposed yolk antigen left behind from this process by swabbing European Lobsters (*Hommarus gamarus*) and testing the sample using the ELISA protocol. Ultimately leading to the development of a field kit which can non-invasively and rapidly confirm this illegal practice, aiding enforcement and prosecution.

Methods

Using the indirect Enzyme-linked immunosorbent assay

- A 96 well plate was coated in either crushed lobster egg sample, swabs taken from scrubbed berried females, swabs taken from males, swabs taken from unknown landed females
- Externally manufactured primary antibody raised in rabbits (taken at 72 days) introduced to the well and incubated
- Secondary conjugated anti-rabbit antibody introduced and incubated
- Alkaline phosphatase substrate introduced to react with conjugated enzyme, which will have bound if lobster antigen resent in the bottom of the well
- Intensity of colour change is measured by obtaining the optical density on a plate reader

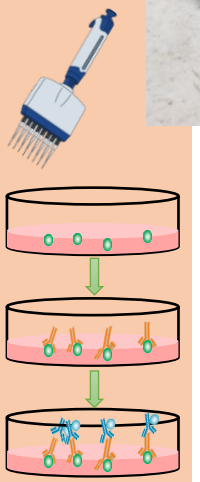
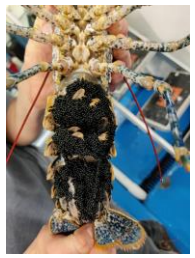


Figure 1: Diagram showing wells in ELISA protocol process. (Lucy Irwin)

Photograph taken by Lucy Irwin at Newlyn landing port of a female lobster

Photographs taken by Lucy Irwin of a berried and non-berried lobster at the National Lobster Hatchery in Padstow



Discussion

- It is important that swabs on naturally spent lobsters are taken to eliminate the possibility of protein detection on these specimens.
- The risk of contamination between individuals in the pot must also be assessed by swabbing positive females and negative male individuals who have shared a tank over time.
- It must be determined whether proteins on the surface of the lobster degrade over time. This will be done in a series of protein degradation experiments. Here, egg proteins will be left in both dry and wet conditions (to simulate storage in or outside seawater) and swabbed from glass slides (to simulate lobster abdomen) and an ELISA will be run on the samples. Protein degradation will reflect in the optical densities obtained from this.

Results

Overall results show high detection of proteins in both crushed eggs and in scrubbed females immediately after scrubbing, while male only background detection was shown in male swab samples.

Crushed eggs

- Optical densities at various stages of pregnancy, an average of 0.25nm
- Pooled egg antigen gives an overall positive control value of 0.36

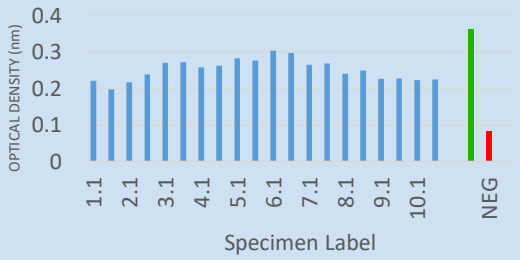


Figure 1: Crushed Lobster Egg Protein Assay Optical Density for 12 Specimens (2 samples from each)

Male lobsters

- Negative cut-off value of 0.099 established
- All male optical density results indicate no proteins detected. Low optical density due to background 'noise'

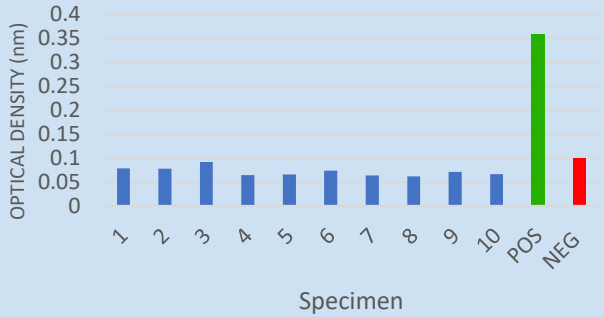


Figure 2: Optical Densities from Male Lobster swabs as a negative control

Scrubbed females at 4 hr time intervals

- Optical densities match that of crushed eggs when lobsters were swabbed immediately after scrubbing
- In most cases, optical density drops to around or below the negative cut-off value after 4 and 8 hours

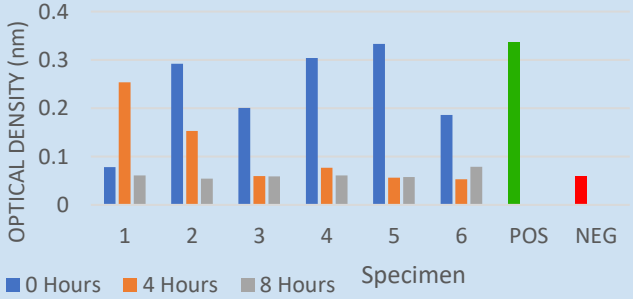


Figure 3: Optical Densities for each specimen over time from a protein assay

Reference

GOV.UK. 2022. *R130: Detecting berried lobsters*. [online] Available at: <https://www.gov.uk/government/publications/r130-detecting-berried-lobsters/r130-detecting-berried-lobsters> [Accessed 31 May 2022].

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