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Final Report: Comparative trials on the effectiveness of anti-melanotic products from Xyrex Ltd. on cooked langoustines

A Scientific Report by

**Mr Andrew McBride
Dr Amaya Albalat
Professor Douglas Neil**

May 2011



**Frozen-thawed
Undipped
5 days**



**Frozen-thawed
Dipped in Prawn-Fresh™
5 days**



**University
of Glasgow**

Comparative trials on the effectiveness of anti-melanotic products from Xyrex Ltd.

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Introduction

Xyrex Ltd is a Scottish company that is a market leader in the production of antimelanotic treatments for shellfish, including Prawn-Fresh™, which has been approved by the EU (Directive 2006/52/EC article 19) and provides sulphite-free melanosis control in crabs and lobsters. The company now wishes to adapt its Prawn-Fresh™ product for use on commercially-important aquacultured prawn species, which are lucrative exports from South American and Asian countries. Of particular concern to both processors and particularly to food manufacturers is the effectiveness of antimelanotic treatments on cooked products, since there appears to be an increase in the aggressiveness of the melanosis following cooking, especially when the product has been previously frozen.

Original objectives of the project

The project will use indwelling temperature probes to determine the rate of change of temperature in the internal tissues of fresh langoustines as they are heated to different controlled water temperatures up to boiling point. Objective indices will then be used to score the rate of subsequent melanosis development, and the thermal thresholds for activating and de-activating the melanosis process will this be determined. In a further series of trials, the same regime will be applied to product that has previously been freeze/thawed, a process known to further exaggerate melanosis. Finally, the dosage and treatment time of Prawn-Fresh necessary to suppress exaggerated melanosis due to cooking will be determined.

Additional project objectives

By agreement between the company and the researchers at the university, a number of additional objectives were incorporated into the project. These included a study of the effects of pre-heating Prawn-Fresh+™ on its subsequent performance as an anti-melanotic treatment, an evaluation of the performance of Prawn-Fresh+™ when reconstituted from concentrates and a comparison of the performance of Prawn-Fresh+™ with other commercially available anti-melanotic treatments,

Expected Deliverables

- An evaluation of the effects of different cooking temperatures and times on the temperature profile of the prawns
- The effects of different cooking temperatures and times on the development of aggressive melanosis within them
- An understanding of how these factors interact with prior freezing of the product
- A determination of the dosage of Prawn-Fresh™ and the treatment procedures to be recommended to food manufacturers for effective suppression of melanosis in prawns that have been heated to different temperatures during cooking protocols

and additionally

- An evaluation of the effects of pre-heating Prawn-Fresh+™ on its subsequent performance as an anti-melanotic treatment
- An evaluation of the performance of Prawn-Fresh+™ reconstituted from concentrates
- A comparison of the performance of Prawn-Fresh+™ with other commercially available anti-melanotic treatments when applied under strictly comparable conditions

Experimental Trials

Trial 1: Efficacy of Prawn-Fresh +™ in delaying melanosis development when treated langoustines are heated in boiling water.

Trial 2: Efficacy of Prawn-Fresh +™ in delaying melanosis development when treated langoustines are heated in water to various temperatures below boiling point.

Trial 3: Efficacy of Prawn-Fresh +™ in delaying melanosis development in langoustines after the solution has been pre-heated to 75°C or 90°C

Trial 4: Efficacy of reconstituted Prawn-Fresh +™ from concentrate in delaying melanosis development in fresh langoustines.

Trial 5: Comparison of the effectiveness of Prawn-Fresh +™, Hasenosol and sodium metabisulphite in delaying melanosis development in fresh langoustines (*Nephrops norvegicus*)

Presentation of Results

For all experimental trials, blackening was assessed using digital images and quantified using the Melanosis Index Score. Melanosis development is displayed in the Results sections of each trial as plots of the Melanosis Scores, and the digital images from which these scores were derived are contained within an accompanying Appendix.

Experimental Trial 1: Comparison of effectiveness of Prawn-Fresh + TM in delaying melanosis development in langoustines (*Nephrops norvegicus*) heated in boiling water

Methodology

Langoustines (*Nephrops norvegicus*) were collected by otter trawl in the Clyde Sea by the research vessel 'Actinia' from the University Marine Biological Station Millport (UMBSM). Once on board animals were carefully washed and separated into groups of 60 animals. These animals were then further subdivided into groups of 12 and placed in plastic bags before being transported on ice to the facilities at the University of Glasgow. Once in the laboratory, animals were heated to 100°C in boiling water for three minutes. Different groups were subjected to the following protocols:

- Un-dipped and heated in boiling water
- Dipped in Prawn-Fresh + TM (diluted 1/1000 for 12 minutes - as manufacturer's instructions) then heated in boiling water
- Heated in boiling water then dipped in Prawn-Fresh + TM

[A further series of protocols was applied involving dipping/heating/freezing/thawing in various combinations]

Animals were then stored at 4-5°C for the rest of the trial. Melanosis development was scored on days 3, 5, 7, 12 and 16. Blackening was assessed using digital images and quantified using the Melanosis Index Score.

Results and Conclusions

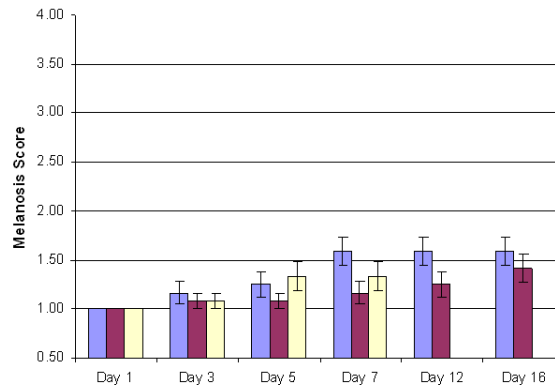
The procedure of heating animals in boiling water for three minutes prevented any melanosis development, in both undipped and dipped animals (Figure 1) and with the other protocols (data not shown). This suggests that the enzyme involved, phenoloxidase, was denatured by heating to 100°C.

Because no melanosis was apparent in undipped animals, as expected Prawn-Fresh + TM could exert no further antimelanotic action (although CF dorsal un-dipped animals in some cases looked 'duller' than dipped animals). It is thus concluded that dipping in Prawn-Fresh + TM would serve no purpose if applied to product that had been brought to boiling point for three minutes. However, the possibility exists that melanosis might develop at lower temperatures, and therefore the effectiveness of Prawn-Fresh + TM under those conditions was investigated in a separate trial (Experimental Trial 2).

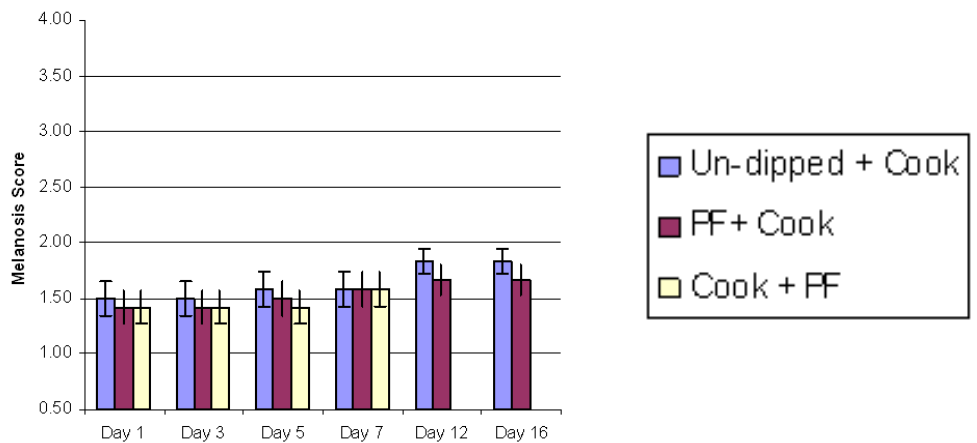
For the same reason, comparison of the cook/dip and dip/cook protocols in this trial at 100°C in fact allows no conclusion to be drawn about whether the heating process reduces the

antimelanotic action of Prawn-Fresh + TM itself. This possibility was therefore tested in a separate trial (Experimental Trial 3).

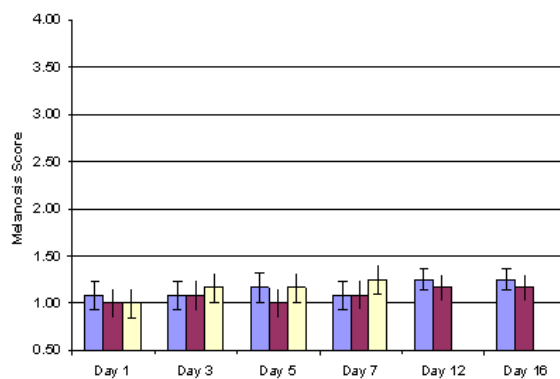
A) Cephalothorax dorsal



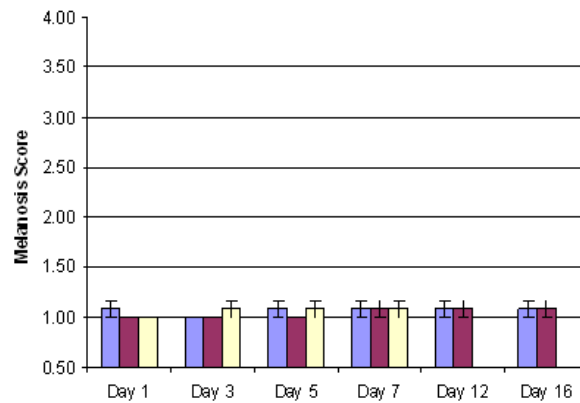
B) Cephalothorax ventral



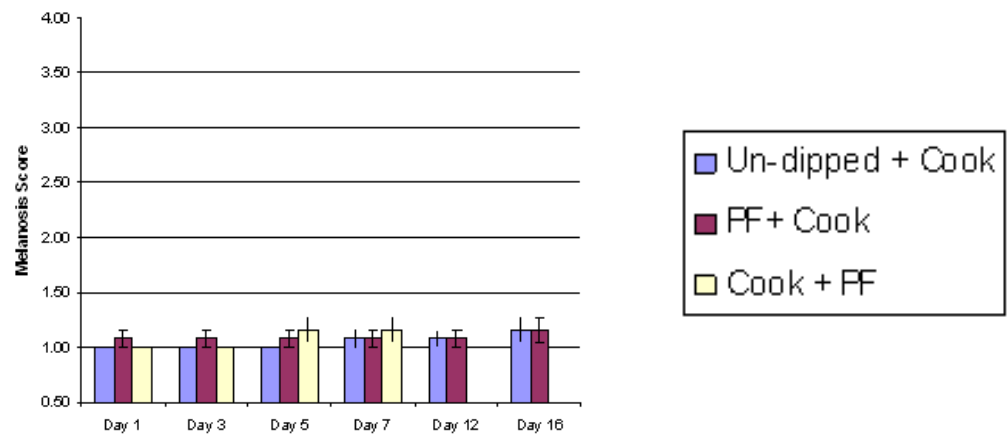
C) First Clawed Legs



D) Tail



E) Tail fan



F) Pleopods

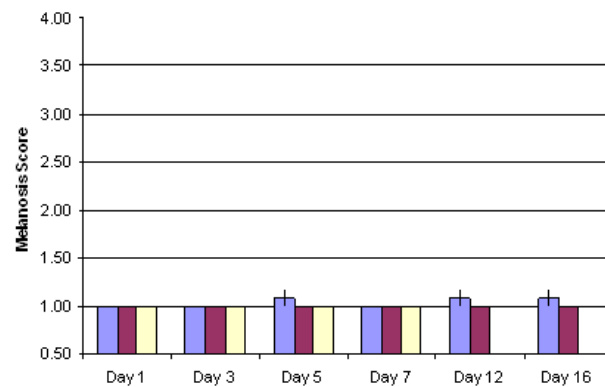


Figure 1 – Melanosis scores for Prawn-Fresh +TM treated or untreated langoustines heated to a temperatures of 100°C in boiling water before undergoing a freeze/thaw cycle and scored

over 16 days. Values are the mean melanosis score \pm SEM for each group of animals.

Experimental Trial 2: Comparison of effectiveness of Prawn-Fresh + TM in delaying melanosis development in langoustines (*Nephrops norvegicus*) heated in water to various temperatures

Methodology

Langoustines (*Nephrops norvegicus*) were collected by otter trawl in the Clyde Sea by the research vessel 'Actinia' from the University Marine Biological Station Millport (UMBSM). Once on board animals were carefully washed and separated into groups of 60 animals which were treated as follows:

- Undipped (control group)
- Dipped in Prawn-Fresh + TM diluted 1/1000 for 12 minutes (as manufacturer's instructions)

These animals were then further subdivided into groups of 12 and placed in plastic bags before being transported on ice to the facilities at the University of Glasgow. Once in the laboratory, animals were warmed slowly to the ambient temperature (~20°C) then heated in a water bath until their internal tissues reached the desired temperature. The water temperature was monitored using a thermistor temperature probe in the water, and the internal temperature of the animal was monitored by thermistor temperature probes inserted into the abdominal musculature of two animals per batch.

Animals were then stored at -18°C for 4 week. Defrosting was done overnight at 4-5°C, and the animals were stored at this temperature for the rest of the trial. Melanosis development was scored on days 1, 5, 7, 12 and 14 following defrosting. Blackening was assessed using digital images and quantified using the Melanosis Index Score.

Results and Conclusions

At each of the tested temperatures between 60°C to 90°C, the batch of 12 lobsters was placed in a water bath with the water at the desired temperature (Figure 2A). This caused an initial slight lowering of the water temperature (by <5°C), but it returned to its controlled value within a few minutes. The temperature within the animals' tissues increased from its initial value around ambient to reach the temperature of the water in a period of 10 – 12 minutes (Figure 2A). This equilibration time was characteristic for the size of the animals, the tails of which had diameters of 12 -15 mm.

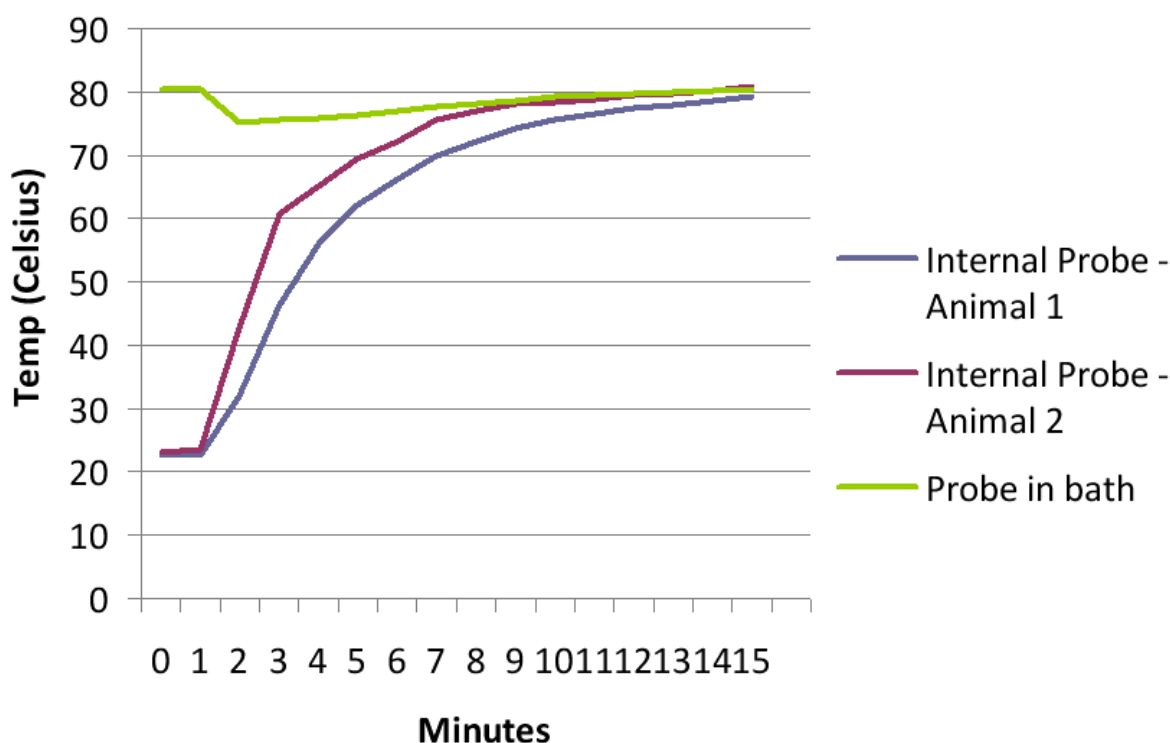
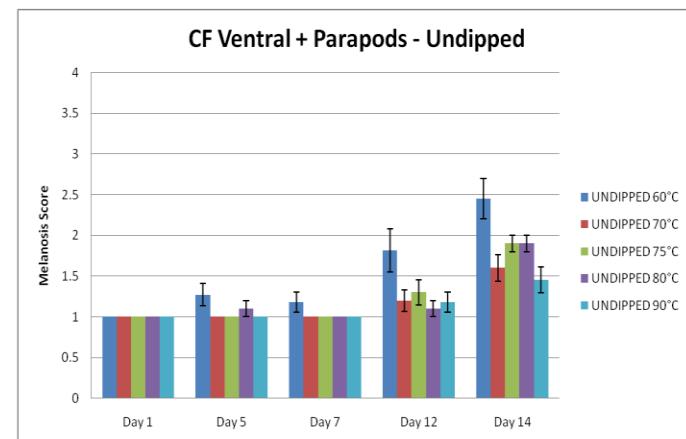
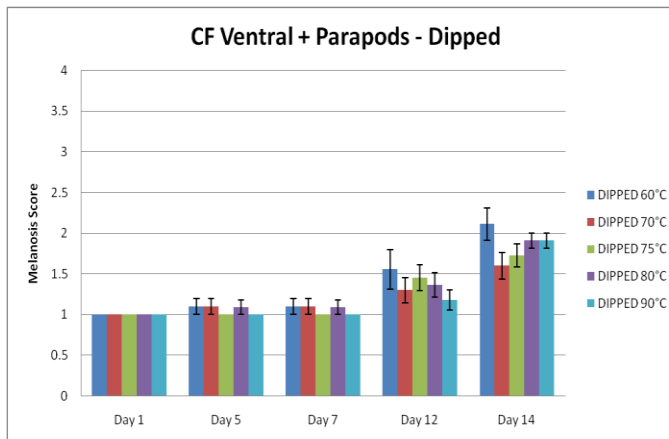
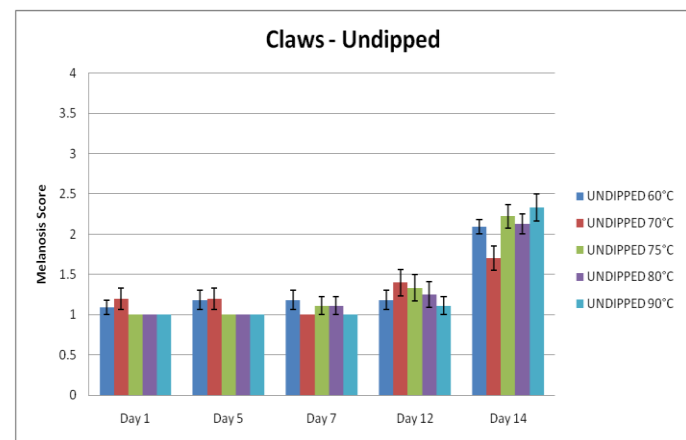
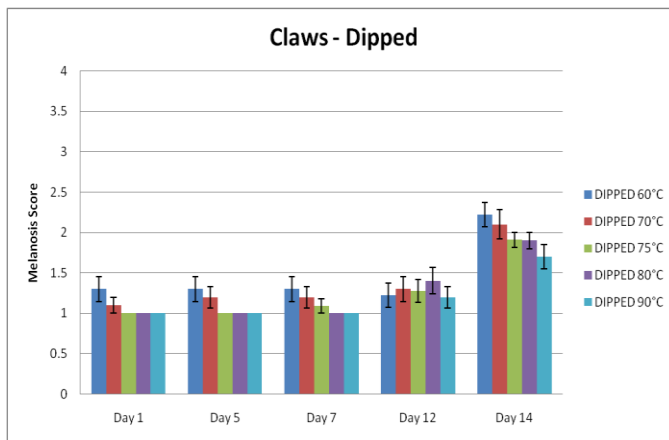
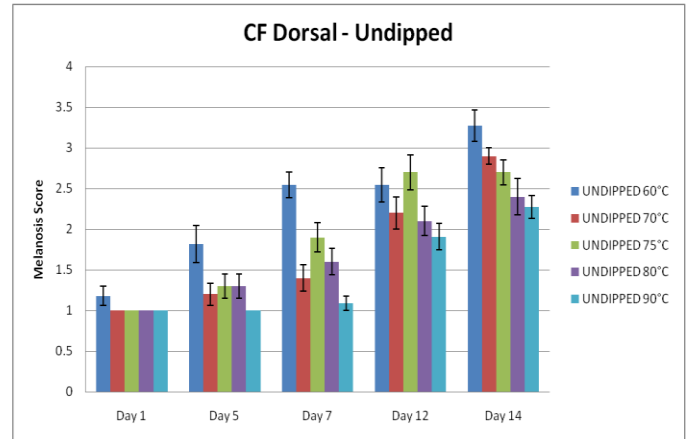
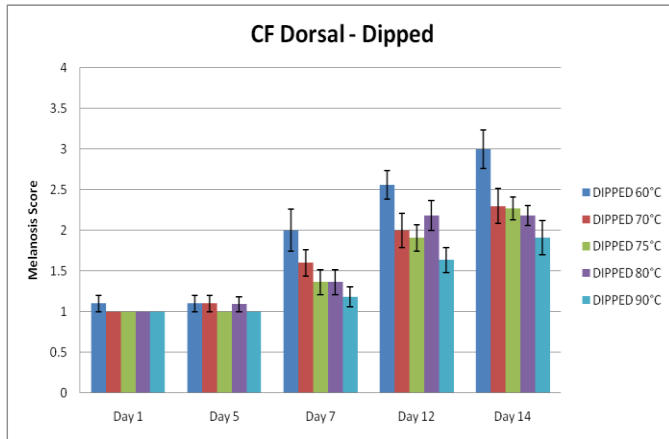


Figure 2A – Temperature profiles for the water in the water bath and for the internal tissues (abdominal musculature) of two animals in a typical heating trial (to 80°C).

Heating in water to temperatures in the range 60°C to 90°C had an almost complete inhibitory effect on melanosis in the tails, pleopods and tail fan of the lobsters, but in the ‘head’ region (cephalothorax) there was only a partial inhibition, which was temperature-dependent (Figure 2B). A clear inverse correlation exists between the temperature to which the animals were heated and the degree of blackening, as the melanosis scores for the ‘head’ region were highest when the animals were heated to 60°C and lowest at 90°C. This suggests that in this region of the body there is a progressive inactivation of the phenoloxidase enzyme with temperature in the range tested, leading ultimately to complete denaturation of the enzyme at 100°C as shown in Experimental Trial 1.

In terms of the effect of Prawn-Fresh + TM on the ‘head’ region (CF dorsal, Claws and CF ventral in Figure 2B), it was found that at all the temperatures tested there was reduced melanosis development in animals treated with Prawn-Fresh + TM compared with the untreated animals.



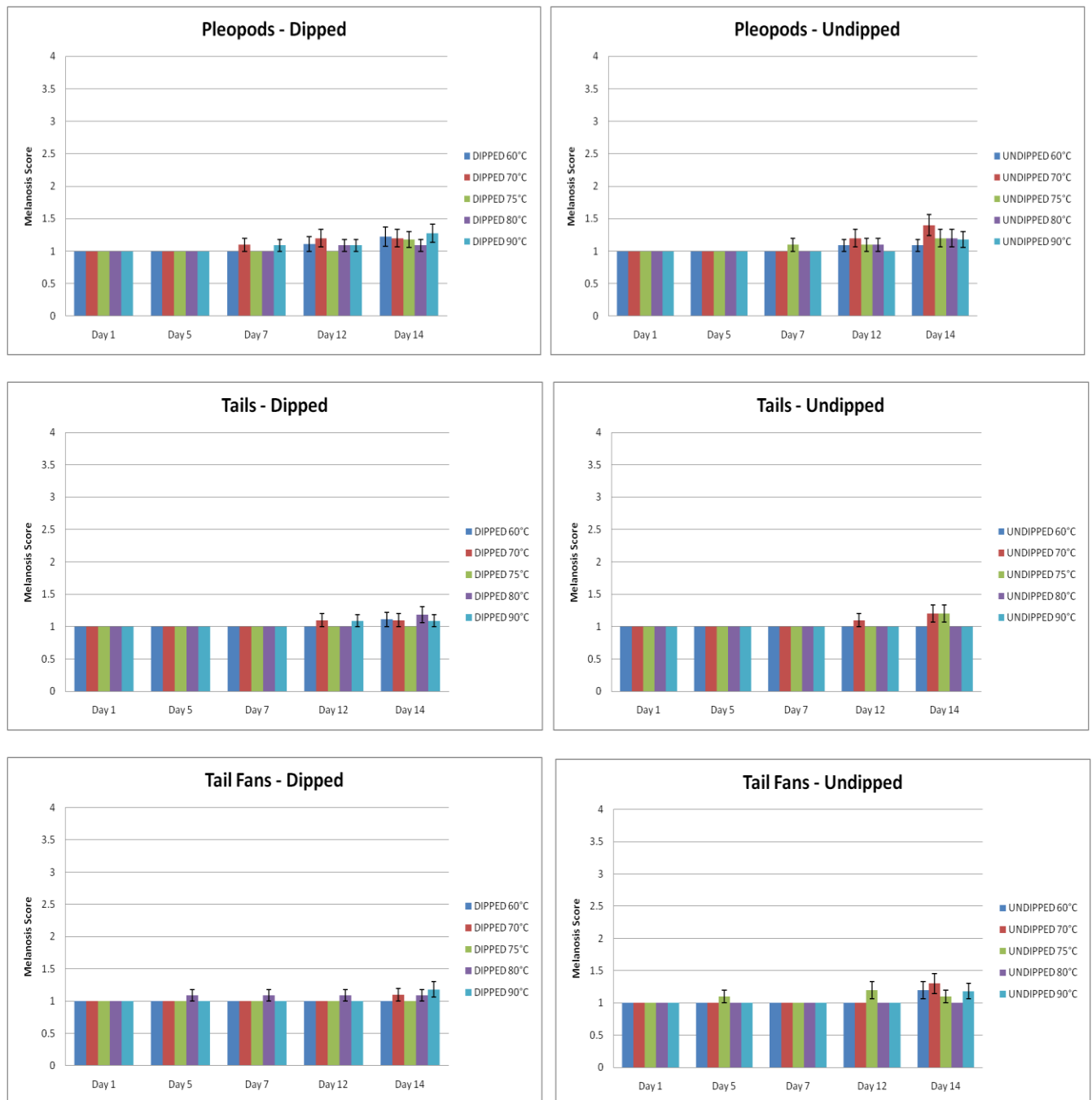


Figure 2B – Melanosis scores for Prawn-Fresh + TM treated or untreated langoustines heated to temperatures between 60°C and 90°C before undergoing a freeze/thaw cycle and scored over 14 days. Values are the mean melanosis score ± SEM for each group of animals.

Experimental Trial 3: Effectiveness of Prawn-Fresh + TM in delaying melanosis development in langoustines when the solution was preheated to 75°C or 90°C

Methodology

Langoustines (*Nephrops norvegicus*) were collected by otter trawl in the Clyde Sea by the research vessel 'Actinia' from the University Marine Biological Station Millport (UMBSM). Once on board animals were carefully washed and separated into groups of 12 animals which were treated as follows:

- Dipped in Prawn-Fresh + TM diluted 1/1000 for 12 minutes (as manufacturer's instructions)
- Dipped in Prawn-Fresh + TM, preheated the previous day to 75°C, diluted 1/1000 for 12 minutes (as manufacturer's instructions)
- Dipped in Prawn-Fresh + TM, preheated the previous day to 90°C, diluted 1/1000 for 12 minutes (as manufacturer's instructions)

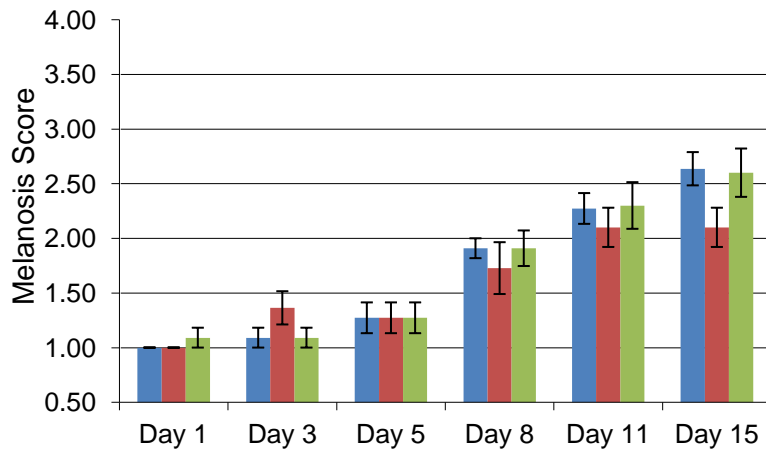
Animals were placed in plastic bags and transported on ice to the facilities at the University of Glasgow. The animals were stored at -16°C for 2 weeks, before being thawed and subsequently stored at 4-5°C. Melanosis development was scored on days 1, 3, 5, 8, 11 and 15 following defrosting. Blackening was assessed using digital images and quantified using the Melanosis Index Score.

Results and conclusions

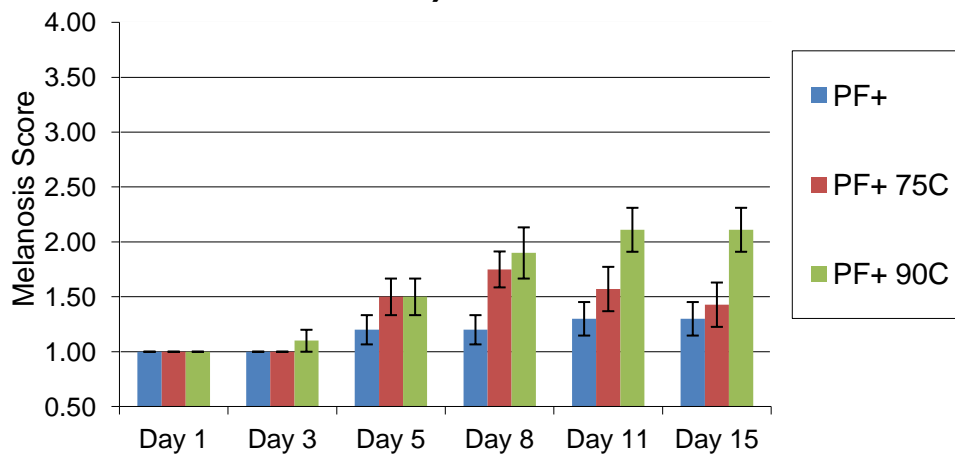
Prawn-Fresh + TM and Prawn-Fresh + TM pre-heated to 90°C performed comparably with regard to reducing melanosis (Figure 3). This indicates that heating Prawn-Fresh + TM to this high temperature does not affect its action.

Prawn-Fresh + TM preheated to 75°C was the most effective of the treatments, with reduced blackening seen on almost every area of the animal scored. The fact that heating it to 75°C produced better results than the unheated Prawn-Fresh + TM is not easily explained, but could be due to experimental error.

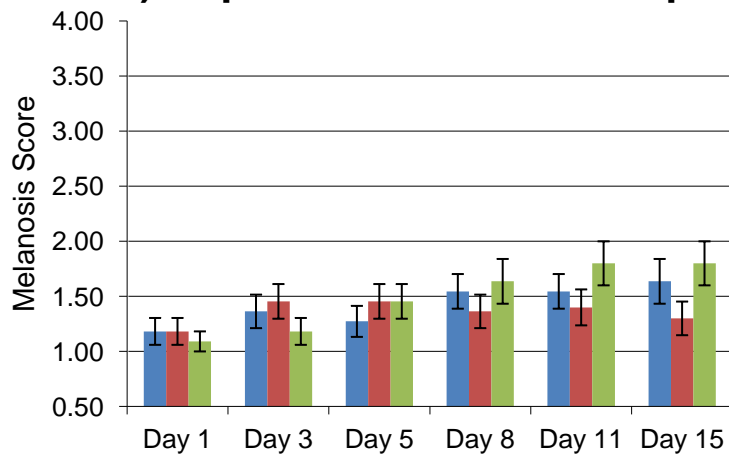
A) Cephalothorax dorsal



B) Claws



C) Cephalothorax ventral + parapods



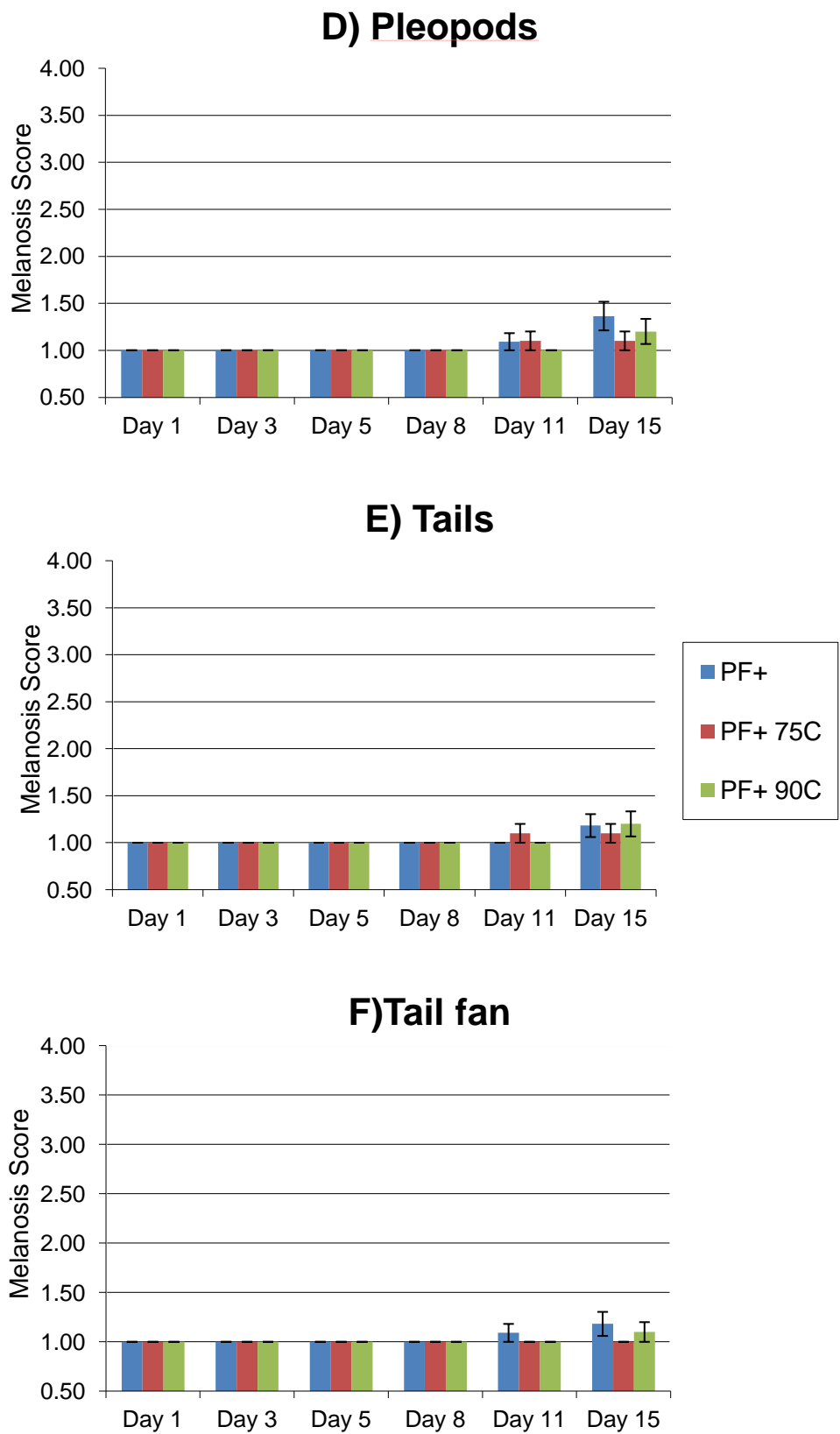


Figure 3 -Melanosis scores for langoustines treated with Prawn-Fresh + TM, Prawn-Fresh + TM preheated to 75°C, or Prawn-Fresh + TM preheated to 90°C before undergoing a freeze/thaw

cycle and then being scored over the course of 15 days. Values are the mean melanosis score \pm SEM for each group of animals

Experimental Trial 4: Comparison of effectiveness of Prawn-Fresh + TM, Prawn-Fresh + TM from 50% concentrate and Prawn-Fresh + TM from 100% concentrate in delaying melanosis development in fresh langoustines (*Nephrops norvegicus*)

Methodology

Langoustines (*Nephrops norvegicus*) were collected by otter trawl in the Clyde Sea by the research vessel 'Actinia' from the University Marine Biological Station Millport (UMBSM). Once on board animals were carefully washed and separated into groups of 12 animals which were treated as follows:

- Undipped (control group)
- Dipped in Prawn-Fresh + TM diluted 1/1000 for 12 minutes (as manufacturer's instructions)
- Dipped in Prawn-Fresh + TM50% diluted 1.5/1000 for 12 minutes (as manufacturer's instructions)
- Dipped in Prawn-Fresh + TM100% diluted 2/1000 for 12 minutes (as manufacturer's instructions)

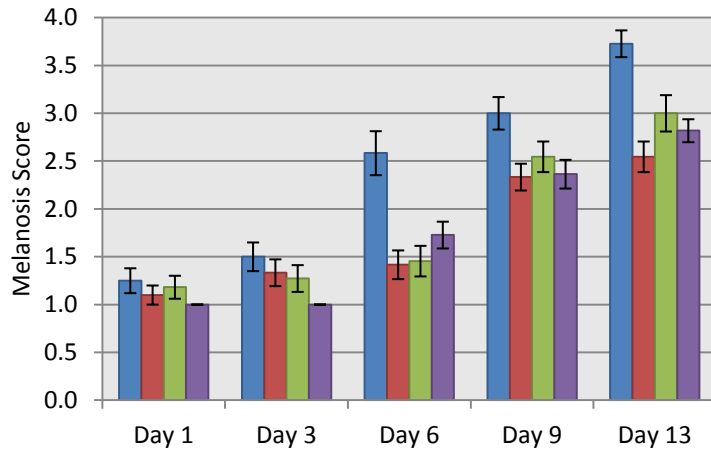
Animals were placed in plastic bags and transported on ice to the facilities at the University of Glasgow. Once in the laboratory, animals were stored at 4-5°C for 13 days. The animals were assessed for melanosis development on days 1,3,6,9 and 13 of storage. Blackening was assessed using digital images and quantified using the Melanosis Index Score.

Results and Conclusions

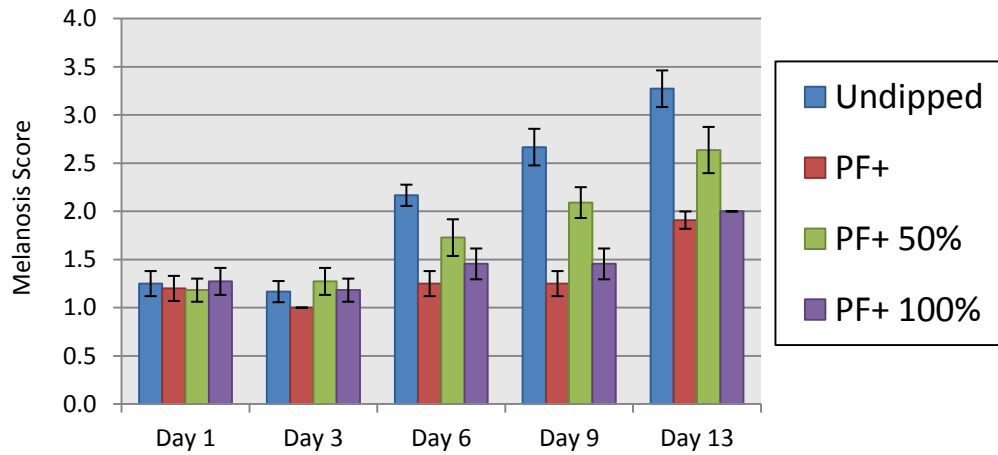
All Prawn-Fresh + TM formulations showed a significant reduction in melanosis development in comparison to untreated animals (Figure 4). Prawn-Fresh + TM and Prawn-Fresh + TM from 100% concentrate showed comparable effectiveness in reducing melanosis development, although Prawn-Fresh + TM from 50% concentrate appeared less effective. It can be concluded that Prawn-Fresh + TM reconstituted from concentrate can be as effective as the standard formulation.

The reason for the anomalous results with Prawn-Fresh + TM from 50% concentrate cannot be explained, but may have been due to some feature of the batch or the experimental procedure as the more dehydrated 'Prawn-Fresh + TM 100%' worked as effectively as Prawn-Fresh + TM. This trial could be repeated if required.

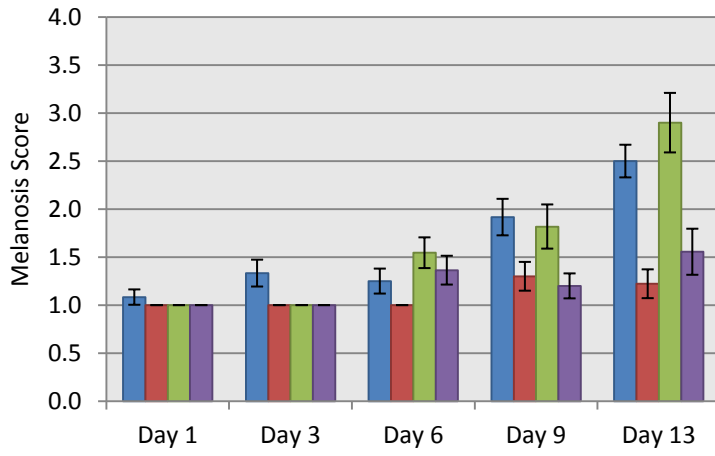
A) Cephalothorax dorsal



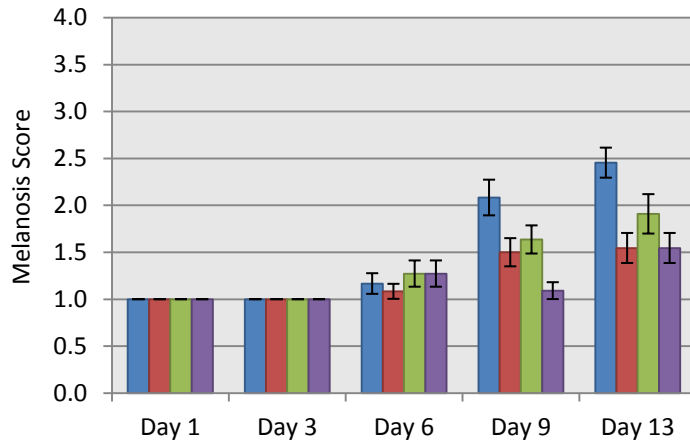
B) Cephalothorax ventral & parapods



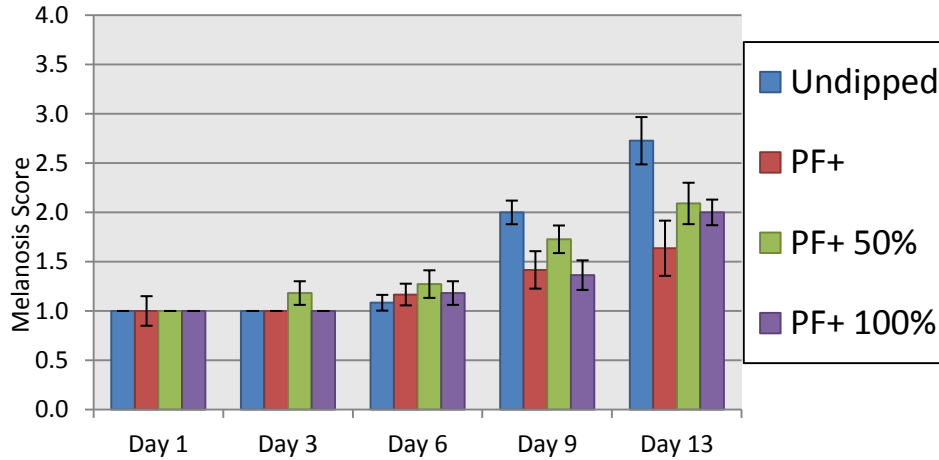
C) First clawed legs



D) Tails



E) Tail fan



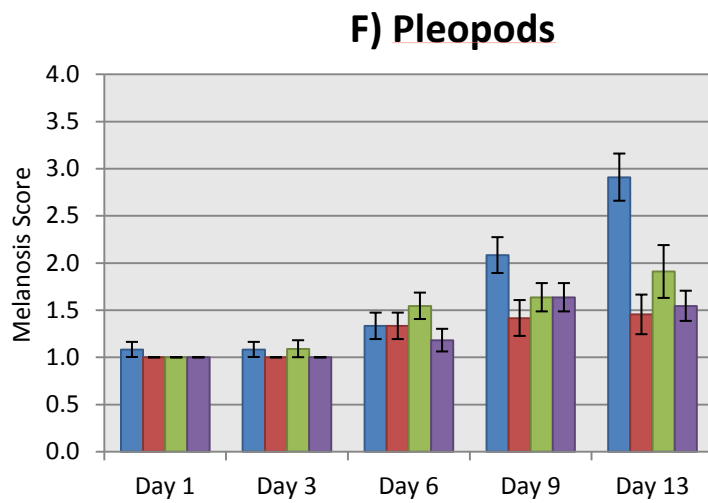


Figure 4 - Melanosis scores in fresh langoustines undipped or dipped using Prawn-Fresh + TM, Prawn-Fresh + TM 50%, or Prawn-Fresh + TM 100% and stored at 4-5°C for up to 13 days. Values are the mean melanosis score \pm SEM for each group of animals.

Experimental Trial 5: Comparison of effectiveness of Prawn-Fresh + TM, Hasenosa and sodium metabisulphite in delaying melanosis development in fresh langoustines (*Nephrops norvegicus*)

Methodology

Langoustines (*Nephrops norvegicus*) were collected by otter trawl in the Clyde Sea by the research vessel 'Actinia' from the University Marine Biological Station Millport (UMBSM). Once on board animals were carefully washed and separated into groups of 12 animals which were treated as follows:

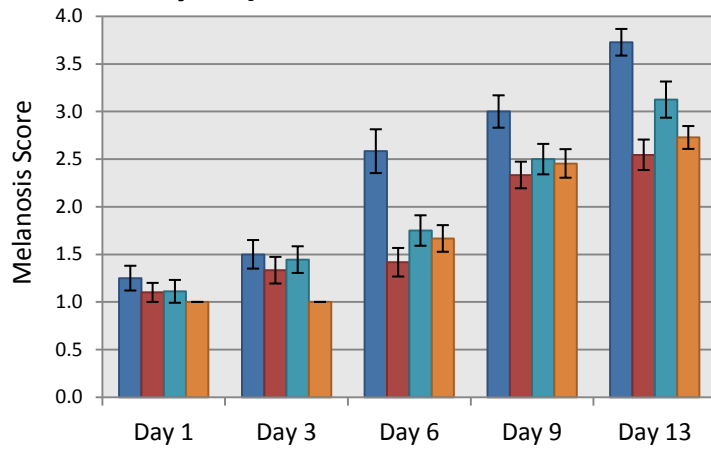
- Undipped (control group)
- Dipped in Prawn-Fresh + TM diluted 1/1000 for 12 minutes (as manufacturer's instructions)
- Dipped in Hasenosa at a concentration of 2% for 5 minutes (as manufacturer's instructions)
- Dipped in sodium metabisulphite (NaMet) at a concentration of 2.5% for 3 minutes (as manufacturer's instructions)

Animals were placed in plastic bags and transported on ice to the facilities at the University of Glasgow. Once in the laboratory, animals were stored at 4-5°C for 13 days. The animals were assessed for melanosis development on days 1,3,6,9 and 13 of storage. Blackening was assessed using digital images and quantified using the Melanosis Index Score.

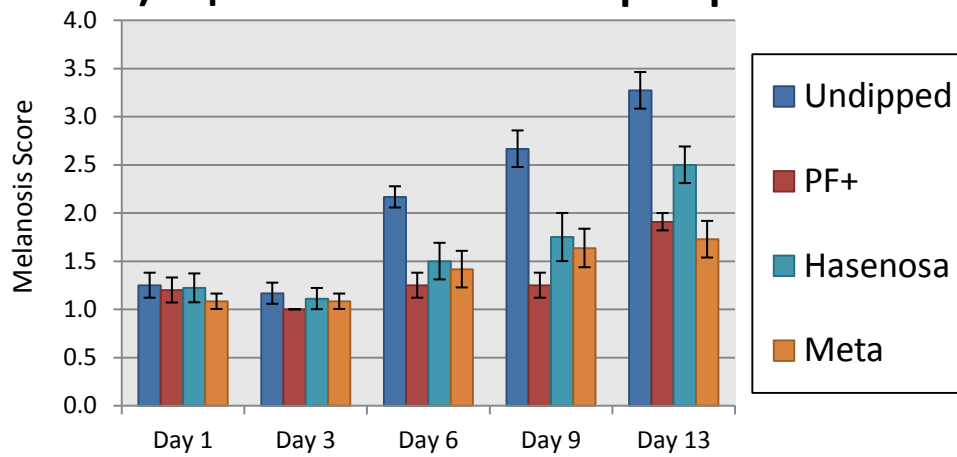
Results and Conclusions

Prawn-Fresh + TM was the most effective treatment for delaying melanosis development in langoustines (Figure 5). Sodium metabisulphite and Hasenosa were not as effective, with metabisulphite possibly performing the better of the two.

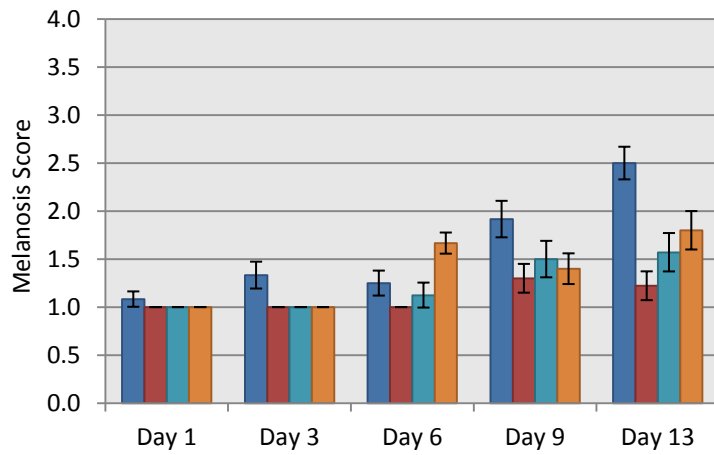
A) Cephalothorax dorsal



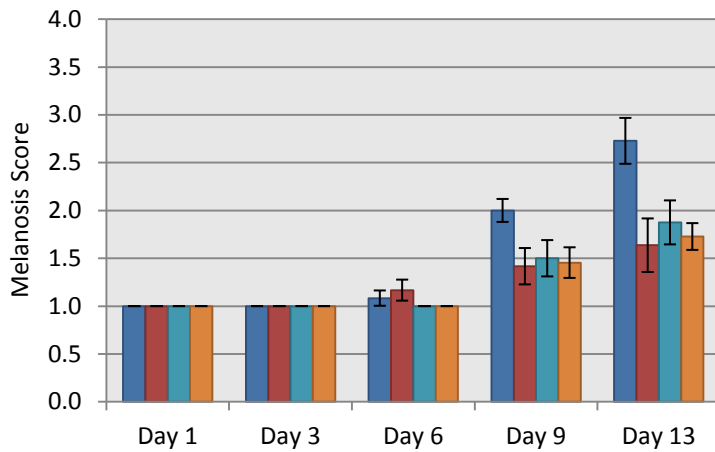
B) Cephalothorax ventral + parapods



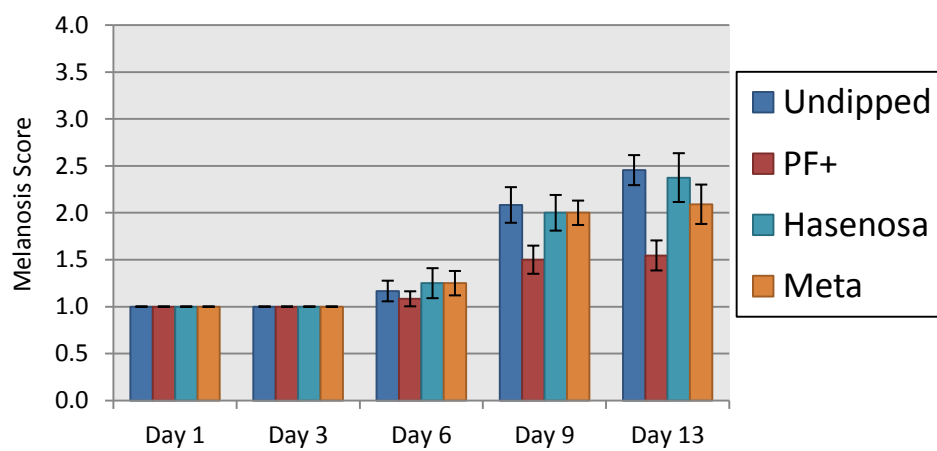
C) First clawed legs



D) Tail fan



E) Tails



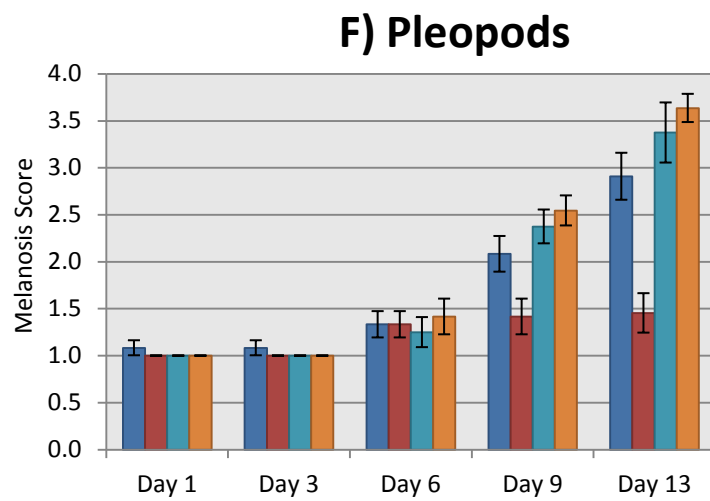


Figure 5 - Melanosis scores in fresh langoustines undipped or dipped using Prawn-Fresh + TM, Hasenos or metabisulphite and stored at 4-5°C for up to 13 days. Values are the mean melanosis score \pm SEM for each group of animals.

Conclusions

- Heating to 100°C in boiling water completely prevents the development of melanosis in untreated langoustines, and dipping in Prawn-Fresh + TM can therefore provide no further mitigation.
- The rate of change of temperature in the internal tissues of fresh langoustines as they were heated to different controlled water temperatures between 60°C and 90°C indicates that the equilibration time is 10-12 minutes for a body diameter of ~15mm. This equilibration time will be shorter in smaller shrimp and prawn species.
- Heating in water to between 60°C and 90°C demonstrated that in this temperature range there is a good correlation in langoustines between reduced melanosis and increased heating temperature.
- Moreover, in this temperature range Prawn-Fresh + TM was observed to reduce melanosis in the 'head' of the animals, compared to undipped animals, even at temperatures as high as 90°C.
- Pre-heating Prawn-Fresh + TM to high temperatures prior to use does not reduce its effectiveness. This suggests that Prawn-Fresh + TM applied to product in conjunction with a cooking protocol will continue to be effective in suppressing melanosis.
- Prawn-Fresh + TM appeared to work as effectively as the standard product when reconstituted from concentrate, since in these trials this was the case for the more concentrated 'Prawn-Fresh + TM 100%'.
- This suggests that it would be feasible to ship Prawn-Fresh + TM in a concentrated form in order to save transport costs, and following re-constitution expect it to act as effectively as the standard product.
- Although the result for 'Prawn-Fresh + TM 50%' appeared to show the product performed slightly less well than the standard product, it is hypothesised that this was most likely due to experimental error.
- Prawn-Fresh + TM is the most effective of the tested products for delaying melanosis in fresh langoustines.

Outcome

The project has provided a detailed understanding of the thermal relationships that underlie the observed increase of melanosis within the prawns during cooking, and a determination of the appropriate dosage and treatment time or Prawn-Fresh™ needed to suppress it. With this knowledge the company will be able to provide an appropriate anti-melanotic product to food manufacturers in S. America and Asia who supply pre-cooked prawns to important export markets. This will thereby lead to new commercial opportunities for Xyrex Ltd, which will also bring benefits to the Scottish economy.

Follow-on work

Follow-on work could use the results of this study as a general guide to the best practice for using antimelanotic treatments in combination with the cooking of products from shrimp and prawn aquaculture. Also, with precise information on particular cooking protocols it will be possible to tailor antimelanotic treatments to best match the temperature profiles used.

Further targeted studies could assess the degree to which exaggerated aggressiveness of melanosis is dependant upon the particular prawn species used in aquaculture systems, with a view to identifying species in which the effect is least prevalent.

Funding for this follow-on work will be sought through a Knowledge Transfer Partnership or from international sponsorship by industrial partners involved in prawn aquaculture.

Acknowledgements

The authors of the report are grateful to the crew members of the research vessel ‘Actinia’ from the University Marine Biological Station Millport for the supply of the animals. This work was funded in part by First Steps Award FSA-045 from the University of Glasgow.