Fisheries Challenge Fund Grant – Importation of live lobsters into the U.K. – An assessment of disease transfer to European lobsters

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Executive summary

Aim: The aim of this project was to evaluate the risk posed by importation of live American lobsters (*Homarus americanus*) into the U.K. with particular reference to a newly emerging disease of these animals, termed epizootic shell disease. In particular, it sought to determine if European lobsters (*Homarus gammarus*) showed any susceptibility to this disease under aquarium conditions.

Approach: The initial approach was to examine imported American lobsters on arrival in the U.K for characteristic symptoms of epizootic shell disease. Subsequently, aquarium-based experiments were carried out to determine whether epizootic shell disease can be transferred to our native European lobsters.

Main findings: Limited site visits to exporters of live American lobsters to the U.K. showed that no animals displaying gross symptoms of epizootic shell disease were exported. However, animals displaying sub-clinical infections could be present at the time of shipping hence not ruling out the risk of disease spread. Juvenile European lobsters held together with American lobsters in the same aquarium system did not develop any clear symptoms of epizootic shell disease. However, the susceptible American lobsters were also not affected with this disease during the experimental period. Experimental abrasion and wounding of both American and European lobsters produced small cuticular lesions. Initial studies showed that these contained different populations of microbes than intact cuticle – some of which may have been involved in the development of the lesions.

Potential implications: This initial study did not rule out the possibility that European lobsters may be susceptible to epizootic shell disease. Further long-term experiments are recommended to provide a robust assessment of the risk posed to native stocks of European lobsters.

1. Background to project

There have been over 26 confirmed reports of the non-native, American lobsters (*Homarus americanus*) found in British waters since 1988 (Moore et al., 2011). Presumably, their presence has resulted from the accidental loss of imported live American lobsters into the wild. Most of the imported American lobsters into the U.K. come from Canada and America through a number of exporters mainly based on the eastern seaboard of North America. The importation of live American lobsters in 2009 was in the order of 1,900 tonnes, while the landings of native, European lobsters (*Homarus gammarus*) in the same year was *ca*. 2,800 tonnes with an estimated value of £26.5 m to the U.K. lobster fishing industry.

Although American lobsters are susceptible to a range of microbial and macrobial diseases (see Cawthorn, 2011 for review), epizootic shell disease is arguably of the greatest potential threat to our native European lobster (*H. gammarus*). Epizootic shell disease (ESD) has caused significant economic losses in lobster catches of the American coast in the last decade (Castro et al., 2006). The disease manifests itself by deep, unsightly, spreading lesions from the dorsal carapace (see **Figure 1A/B**). The nature of the causative agent of the disease is still unclear but is probably bacterial in nature (e.g. Chistoserdov et al., 2005, 2012). One of the candidate bacterial species involved in the early stages of the disease is *Aquimarina homari* (Chistoserdov et al., 2012) although whether this is the main causative agent (i.e. the primary pathogen) is unclear. Other environmental factors appear to play a largely undefined role in the disease and these include temperature (Glenn & Pugh, 2006), poor diet (Tlusty et al., 2008), and pollutants including alkylphenols (Laufer et al., 2005). In a recent review, Castro et al. (2006) postulated the potential role of such environmental stressors in leaving lobsters more susceptible to diseases including shell disease, amoebiasis and calcinosis.

There are no reports of European lobsters (*Homarus gammarus*) that inhabit British waters showing symptoms similar to those of ESD. However, these lobsters do show mild versions of what is commonly referred to as enzootic shell disease or ‘black spot’ which affects other crustaceans in our waters including edible crabs (*Cancer pagurus*) (Vogan et al., 2008). In recent field studies made by the PI as part of another project funded by the EU Interreg B Ireland-Wales programme, superficial cuticular shell disease lesions were seen in lobsters collected from Lundy Island (**Figure 2**). These lesions are unlike those found in ESD especially in size and depth of penetration. Furthermore, they are unlikely to lead to death of afflicted lobsters while ESD is seen as a more acute disease causing economic loss to fishers.

2. Main objective of this project

This project examined whether the current importation of live American lobsters (*Homarus americanus*) into the U.K. could cause disease outbreaks in native European lobsters (*Homarus gammarus*).

The specific aims of the project were:

AIM 1. To assess the health status of imported American lobsters – in particular whether they harbor significant levels of ESD

AIM 2. To assess whether European lobsters are susceptible to ESD.

3. Results

Aim 1

With the assistance of staff from the Shellfish Association of Great Britain (the Stakeholders) several U.K. – based companies involved in importation of live lobsters into the U.K. were approached. Two reported that they no longer import lobsters with the others failing to respond to the request to examine their imported live lobsters. Because of this difficulty, Dr Paul Stebbing (Fish Health Inspectorate at Cefas in Weymouth) and Janet Farmer (Commercial Officer, Agriculture & Fisheries, Canadian High Commission, London) gave additional assistance. Despite this, we failed to gain access to either lobsters prior to export or upon arrival in the U.K. Dr Michael Tlusty in the New England Aquarium, did however, facilitate a site visit of Dr Miranda Whitten (the PDRA on the grant) to one main exporter in the USA during November 2011. Below is a summary of the main findings from this visit:

- Medium sized lobsters destined for export and the home market (sold as live) were very clean and in good condition with no obvious signs of ESD.
- Lobsters were maintained for 2-3 days prior to shipping. They were held in good quality water at 3-4ºC (see **Figure 3**) with effective sanitation.
- Personnel in the plant were aware of ESD and its characteristic lesions and they did not ship any lobsters that clearly manifested the disease.
- Lobster fishers had not reported much ESD during 2011. Most cases appeared to have been found in Maine.
- Lobsters with ESD usually were more apparent when processing large catches where overcrowding and damage could have occurred during transit

to the plant. (No such large shipments were present at the time of the site visit)

Aim 2

In early August 2011 a total of 36 European lobsters were shipped to the collaborators at the New England Aquarium in Boston for these experiments. To check if lobsters at different stages showed differential susceptibility to ESD, some small individuals (*ca.* 1.8 cm total body length) together with larger juveniles as summarised in **Table 1**, were also sent.

Table 1: Characteristics of lobsters (*Homarus gammarus*) shipped to the New England Aquarium, Boston, USA

Stage & (number of individuals)	Size (cm)	Source
Stage 5 (23)	1.5 - 2	The National Lobster Hatchery, Padstow, Cornwall UK
Immature (2)	3.5, 3.7	The National Lobster Hatchery, Padstow, Cornwall UK
Juvenile (11)	12-20	Centre for Sustainable Aquatic Research, Swansea University

The small (Stage 5) lobsters were all apparently disease free and showed no signs of shell disease on their surfaces. The juvenile lobsters reared in Swansea were similarly from disease free stock but had some small shell disease lesions mainly on their claws (see **Figure 4a,b**).

Simultaneous to these lobsters held in Boston, we maintained similar cohorts of animals in the main aquarium system in Swansea University as summarised in **Table 2.**

Table 2: Characteristics of lobsters (*Homarus gammarus*) held in the main aquarium system of Swansea University between July 2011-January 2012

Stage & (number of individuals)	Size (cm)	Source
Stage 5 (20)	1.5 - 2	The National Lobster Hatchery, Padstow, Cornwall UK
Juvenile (12)	17-21	Centre for Sustainable Aquatic Research, Swansea University

The European lobsters shipped to Boston were housed in an aquarium system in which the bacteria thought to be associated with ESD are naturally present. Hence, the imported European lobsters were in a system where they were exposed to the putative causative agent of this disease state.

During the initial phase (*Phase 1*) of the experiments in both Boston and Swansea, lobsters were reviewed at a weekly basis for lesion progression and any affected areas photographed. Detailed records were also made of moulting timings, any mortalities and water quality. Although not originally planned at the time of the grant application, swabs were taken of lesions, normal cuticle, tank biofilms from both Boston and Swansea facilities for later molecular analysis of microbial communities (these are not currently fully completed).

During the period August – October the European lobsters held in Boston showed no obvious signs of ESD. There were also no mortalities. Although lesion progression was observed in the European lobsters on the claws and tail fan (see **Figure 5**) these were no different to observations made on the other cohort of European lobsters held in Swansea. Overall, it is concluded that European lobsters held in aquarium conditions where the potential causative agents associated with ESD are thought to be present, showed no clear evidence of susceptibility to the disease. However, during the period of the experiments, none of the American lobsters held in the same aquarium system in Boston showed ESD.

Phase 2 of the experiments commenced in late October 2011. As some cases of ESD may be faciltated by handling damage and abrasion, this process was mimicked under laboratory conditions. Both American and European juvenile lobsters were abraded on one side of the thorax (see **Figure 6**) using sterile 400 grit wet-dry paper (3M). On one claw, a deep puncture wound was made using a sterile Philips-type screwdriver while the other claw was left untreated. A total of 11 juvenile European and 10 juvenile American lobsters housed in Boston were treated in this

manner. On the return of Dr Whitten from Boston in November 2011, the Swansea-based European lobsters (12 individuals) were treated in the same manner. A systematic sampling regime was used where damaged areas were photographed before and on fortnightly intervals after treatment, and samples of damaged cuticular material was taken on swabs and FDA® cards (Whatman; these bind and selectively preserve DNA) for molecular analysis of microbial communities. Finally, at the end of the experimental period, damaged areas were photographed and fixed in 70% ethanol or sea-water formalin for scanning electron microscopy. Underlying tissues were fixed in Davidson's seawater fixative for later histology.

The main findings of *Phase 2* experiments are:

- Initial observations revealed that the carapace of the juvenile American lobsters was smoother and with fewer pores than that of their European counterparts. Furthermore, the cuticle of American lobsters was more easily cracked while that of the European lobsters was more flexible. These differences in cuticle morphology may be of significance in determining susceptibility to ESD.

- During the abrasion experiments, it was noted that to gain the same amount of damage between the two species of lobsters, the abrasion time had to be extended for European lobsters. This implies that European lobsters are less susceptible to abrasion injury (at least at the site examined) - which may be of key importance in the susceptibility to shell disease including ESD.

- The claws and carapace of American lobsters had much less biofilm than that of the European lobsters.

- With the European and American lobsters held in the same container and aquarium system in Boston, it was noted that the European lobsters spent a greater time abrading themselves on the edge of their containers which resulted in greater numbers of lesions on the claws and tail fans.

- Abraded carapace from both European and American lobsters became progressively melanised after damage (**Figure 7A,B**). In some cases (ca. 25% of animals) small lesions developed (**Figure 7C**) during the experimental period.

- Similarly, claw damage showed progression of melanisation at later times (**Figure 8**). Some of these also showed additional lesion formation or necrosis that penetrated through the cuticle.

- Overall, however, **no major differences** were seen in lesion development between American and European lobsters held in Boston. Similarly, the

European lobsters held in both Swansea and Boston showed similar patterns of lesion development.

- Molecular analyses (temperature gradient gel electrophoresis) were used to compare the bacterial flora of intact and damaged shell (abraded carapace or cracked/punctured claw), comparing healthy and necrotic / lesioned samples. As shown in **Figure 9**, to date, an average of sixteen bands corresponding to different bacterial species have been routinely visualized from each sample, and the majority are present irrespective of the health of the carapace. However, three bands were associated exclusively with damaged carapace (and are possible pathogens), and two further bands were absent from necrotic shell but present in healthy samples (and are possibly beneficial species). The genotypic identification of these bacteria is pending. Whether any of these are related to the potential causative agent of ESD (*A. homari*) will also be determined.

- Initial scanning electron microscopical studies of lesions on lobsters showed a rich bacterial community at the margins of the lesion (**Figure 10 B**). Within the melanised lesion itself, there were few visible microbial agents. These studies are currently incomplete awaiting the arrival of samples from Boston for analysis of experimentally induced lesions.

Results from related studies

As well as the planned aims of this project, a survey of whether U.K. fishers have caught American lobsters was initiated. This survey has principally been carried out as part of another grant held by the PI (Susfish, funded by the EU Interreg Ireland Wales programme) which looks at shell disease in crabs. The survey was sent to all fishers in the U.K. and Eire in collaboration with regional fishing organisations and SAGB (see *Fishing News* report appended in Interim Report). One of the questions in the survey was designed to determine if any persons had found such escaped lobsters in the wild. To date, three respondents (two in the U.K. and one in Eire) have reported catching American lobsters in the wild. The locations were the Cutler Bank, Felixstowe, Suffolk (2005/6), Dun Laoghaire harbour, Nr Dublin (2009) and the Isle of Wight (1996 and 1997). There has also been a further potential capture of an American lobster from the Milford Haven area in Wales. Whether all of these sightings in our survey are also incorporated in the report of Moore et al. (2011) is unclear. The Susfish survey is still on-going.

4. Discussion

Shell disease syndrome (both enzootic and epizootic) is a poorly understood condition where the causes are still unclear. Like many aquatic diseases, there is a strong link between environmental 'stressors' and the presence of the disease. It is possible therefore that ESD will only manifest itself following such exposure to unidentified stressors.

The studies for Aim 1 were only partially successful as imported lobsters arriving in the U.K. were not surveyed. However, the site visit in the USA and suppliers' practices seems to indicate that animals displaying clear ESD are not shipped. This reduces the risk to accidental spread of disease in the U.K., although animals displaying sub-clinical lesions are likely to enter by importation hence presenting some risk. To our knowledge, none of the sightings of Americal lobsters in British waters have revealed the presence of ESD although this is a small sample size. One unconfirmed report has shown that an American lobster found in Norwegian waters developed ESD under aquarium conditions (P. Stebbing, personal communication). If correct, then this is a worrying finding but it does not imply that European lobsters are at direct risk. There is also a report of hybrid progeny from European and American lobsters produced under aquarium conditions in the same laboratory in Norway. To our knowledge, these reports have not been subject to peer review via the usual scientific route of publication.

Our preliminary studies showed no evidence that European lobsters housed in the same aquarium system as American lobsters developed lesions similar to those in terms of morphology and distribution to the characteristic symptoms of ESD. However, the short duration of the experiment (*ca*. 3 months) and the lack of ESD in American lobsters held in the system during the time of the experiments, may imply that additional studies are required to fully address this key question.

One of the most important observations made in the current work comes from the simple gross observations on the morphology of the cuticle from European and American lobsters. Clear differences existed including the ability to abrade the carapace where the European lobster was more resistant to this process. It is widely accepted that some forms of shell disease may be linked to abrasion injury (e.g. Sindermann & Rosenfield, 1967; Vogan et al., 1999). For example, in edible crabs, Vogan et al. (1999) reported that the back-burrowing behaviour of these animals caused abrasion/damage of the cuticle that resulted in the formation of shell disease lesions. Other species of crabs, such as shore crabs (*Carcinus maenas*) living in the same location, showed no symptoms of shell disease either due to differences in the cuticle and/or their behaviour. It is noteworthy that the prevalence and severity of shell disease in general appears to depend on the crustacean species involved (with some showing no susceptibility to the disease) and their geographical location. It is

possible that the European lobster may prove to be totally resistant to ESD simply due to differences in the morphology and chemical make-up of the cuticle although our experiments to date neither prove nor disprove this.

5. Main implications of study

This study aimed to give initial indication of the potential danger of importation of live American lobsters into the U.K. Reports from both the U.K. and Norway have shown that such animals can survive accidental transfer to our coastal waters. While the numbers of lobsters sighted are small they may not fully reflect the extent of the problem in numerical terms. Observations by Norwegian scientists that hybrids of *H. gammarus* and *H. americanus* are possible (at least under laboratory conditions) and the unsubstantiated finding of ESD in escaped American lobsters in captivity are both worrying and should necessitate a review of the situation by appropriate statutory bodies in the U.K.

While it is clear that exporters are not shipping American lobsters into the U.K. with gross symptoms of ESD, we cannot rule out the possibility that sub-clinical specimens routinely appear in the U.K. Hence, this trade is not without risk to native stocks of European lobsters.

European lobsters in an aquarium system with previous ESD outbreaks did not shown any clear symptoms of ESD but the timescale of the experiment (limited by the timescale of the grant) did not allow us to fully answer the question posed as we are unsure as to how long it may take for lesion progression to occur.

6. Possible future work

A major problem with all studies on shell disease, including ESD, is a full appreciation of the nature of the disease in terms of epidemiology and causative agents. Hence, although *A. homari* has been linked with lesion development in American lobsters with ESD, its presence alone is not proof of the disease. This lack of fundamental information hampers studies on evaluating the risk of disease spread from American to European lobsters.

The current study has shown that abrasion or puncture of the cuticle in both American and European lobsters results in changes in the microbial populations in the affected areas. While the nature of these changes is still not fully appreciated, it is clearly of importance to determine these. Also, whether *A. homari* is one of the bacteria that make an appearance in lesions in both European and American lobsters needs to be addressed.

In terms of imported lobsters, it might be prudent to sample animals at the time of import by an appropriate statutory body. Some of the molecular approaches developed in this grant might aid these experiments.

7. References

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8. Acknowledgements

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Figure 1. Characteristic lesions of a heavily infected American lobster (*H. americanus*) with ESD. Image from the 100 lobster project of Professor J. Shields and co-workers, Virginia Institute of Marine Sciences, USA. (http://www.vims.edu/research/departments/eaah/programs/crustacean/research/lobster_shell_disease/Lobster-Project-Resources/Photos/index.php)

Figure 2. Examples of the shell disease lesions (left and right) found on the claws of European lobsters (*Homarus gammarus*). The claw in the middle is from a newly moulted individual that is free of shell disease. Photograph courtesy of Dr. Emma Wootton and colleagues.

Figure 3. Example of lobster holding facility in New England, USA.

Figure 4 a,b. Photographs of a European lobster shipped to Boston showing superficial shell disease lesions (unlabelled arrows) on the claw of one of the lobsters at moult. Such lesions are unlike those of epizootic shell disease in terms of location and severity.

Figure 5. Lesion progression in a juvenile European lobster held in the New England Aquarium in Boston. Upper photograph was taken on 17th October while the lower photograph shows the development of a lesion formed on 2nd abdominal segment photographed on 22nd January 2012.

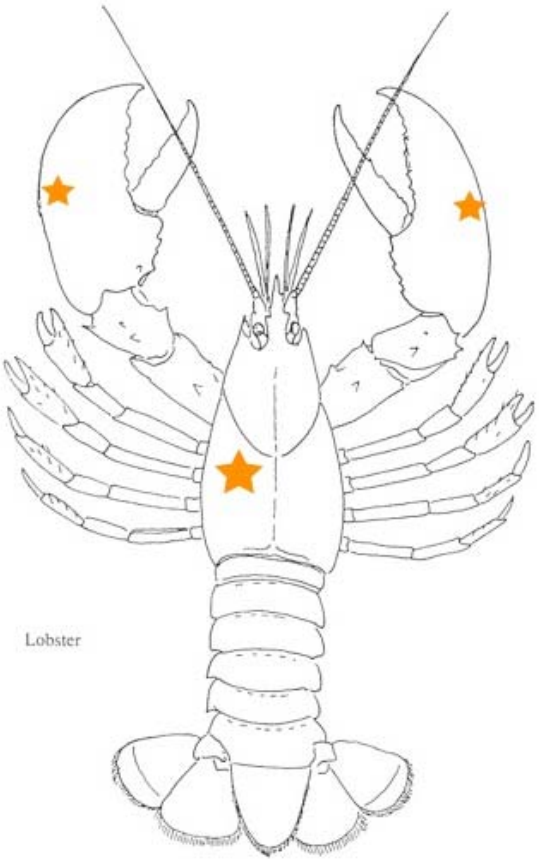

Figure 6. Location of experimental abrasions (large star) and claw damage (small stars) to juvenile American and European lobsters.

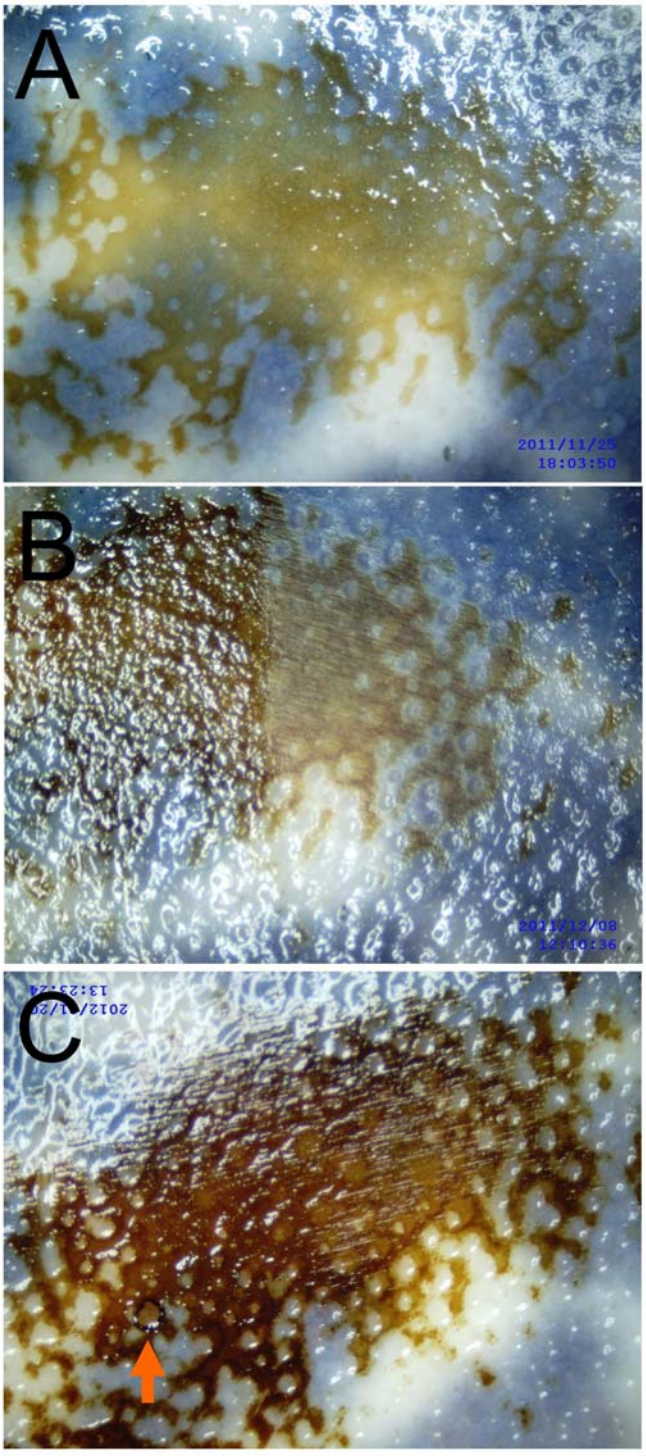

Figure 7. Progression of cuticle damage following abrasion in European lobsters. A = 2 days post abrasion, note initial melanisation of abraded area. B = 4 weeks post abrasion, note darkened (melanised) area. C = 8 weeks post abrasion. Note presence of a developing lesion with melanised outer area (arrow).

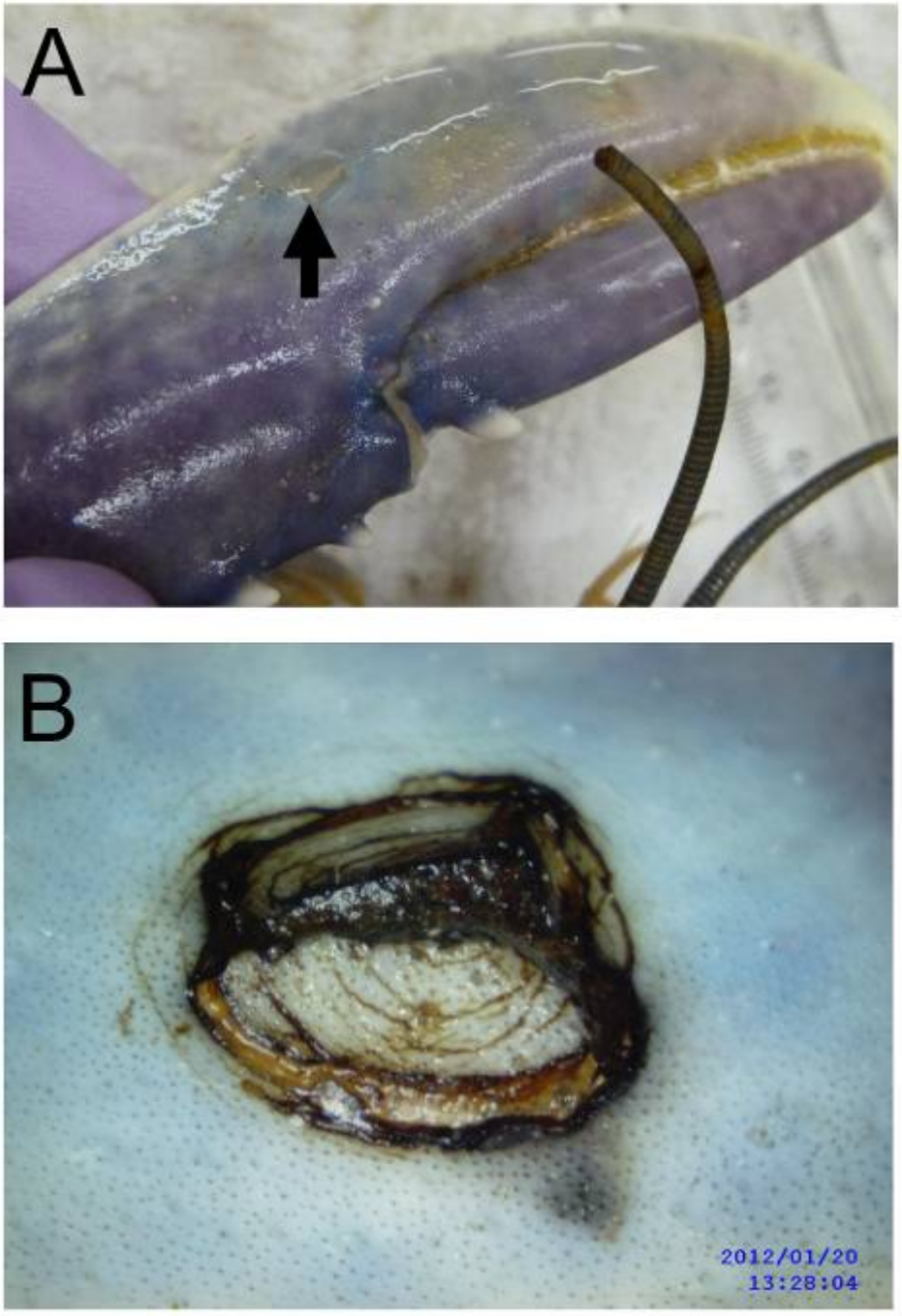

Figure 8. Example of puncture damage to lobsters. (A). Puncture damage immediately post-puncture (arrow). (B). Close up view of damaged area on the claw 8 weeks later. In this animal there are no additional lesions formed similar to those in Figure 7C.

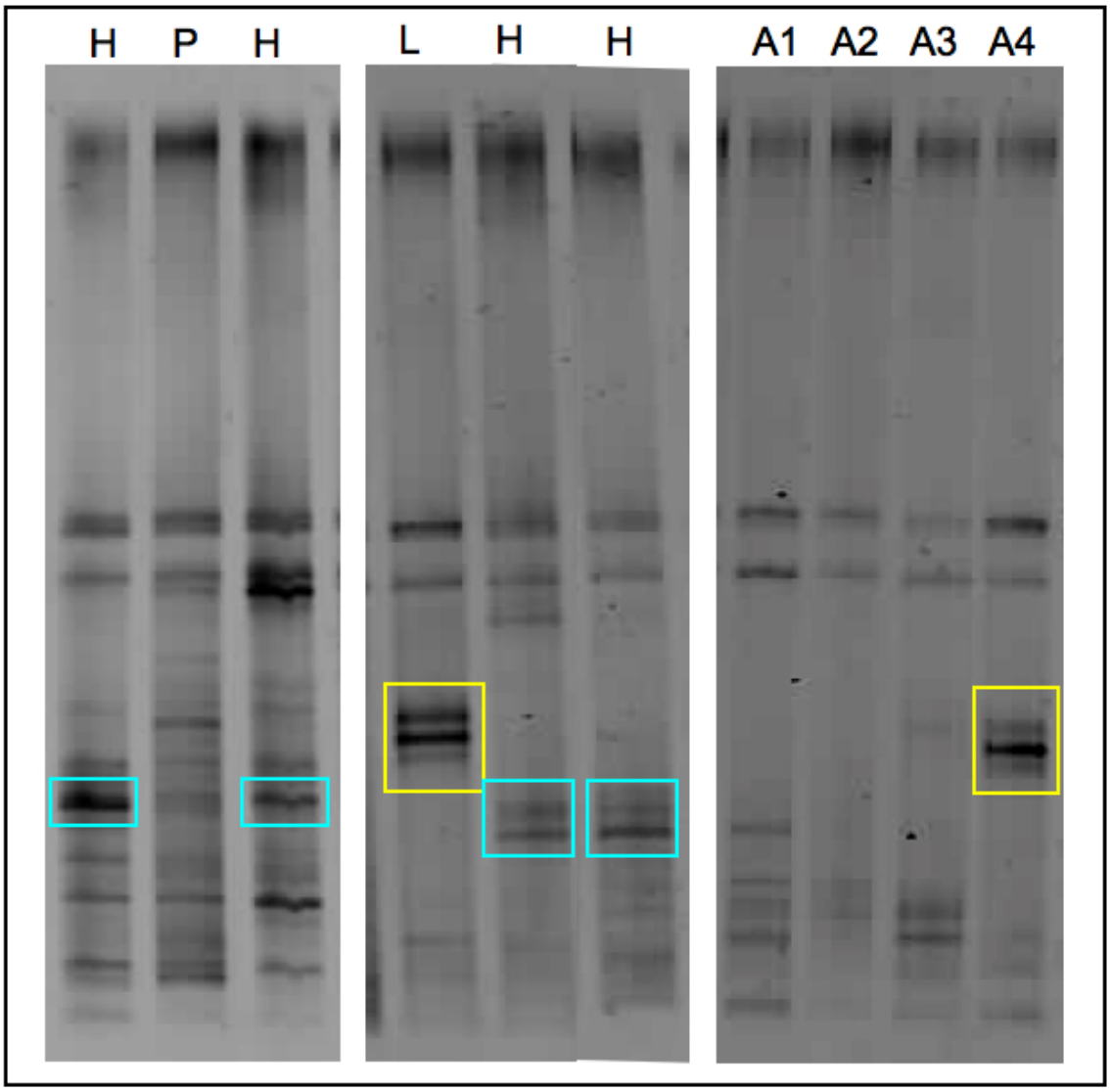

Figure 9. TGGE gel indicating bacterial species in swabbed shell samples. H = healthy control shell samples; P = punctured claw; L = lesioned abrasion; A1 to A4 = abraded shell samples at 2 week intervals post abrasion. Lesions first observed 6 weeks post-abrasion (lane A3). Boxed bands (yellow - appearing in lesions; blue - disappearing) will be cloned & sequenced for genotypic identification.

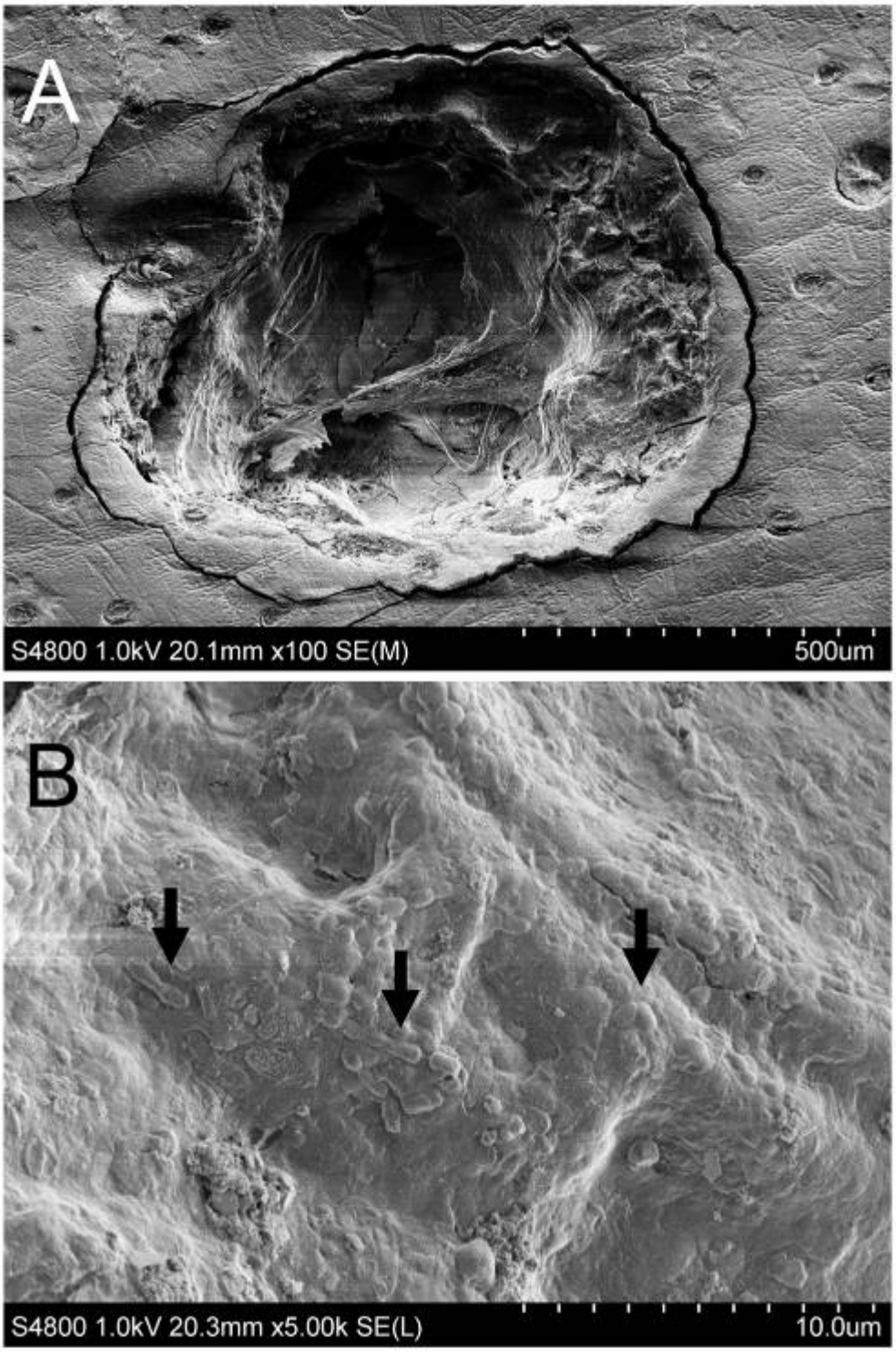

Figure 10. Scanning electron micrographs of a natural wound on the claw of a juvenile European lobster held in the Swansea aquarium. (A). Low power view of whole lesion with cratered appearance. (B). High power view showing bacterial community (unlabelled arrows) at the margin of the lesion shown in 'A'.