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A comparison of the structure of American (*Homarus americanus*) and European (*Homarus gammarus*) lobster cuticle with particular reference to shell disease susceptibility

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ABSTRACT

The integument of arthropods is an important first-line defence against the invasion of parasites and pathogens. Once damaged, this can be subject to colonisation by microbial agents from the surrounding environment, which in crustaceans can lead to a condition termed shell disease syndrome. This condition has been reported in several crustacean species, including crabs and lobsters. The syndrome is a progressive condition where the outer cuticle becomes pitted and eroded, and in extreme cases is compromised, leaving animals susceptible to septicaemia. This study examined the susceptibility of juvenile American (*Homarus americanus*) and European (*Homarus gammarus*) lobsters to shell disease, as a result of mechanical damage. Scanning electron microscopy was used as a method to identify differences in the cuticle structure and consequences of mechanical damage. Claw regions were aseptically punctured, whilst carapaces were abraded using sterile sandpaper, to mimic natural damage. After a period of between 10 and 12 weeks, lobsters were sacrificed, fixed and stored for later examination. The carapace and claws of juvenile American lobsters were shown to be thinner and more vulnerable to abrasion damage than their European counterparts. In addition, the number and distribution of setal pits and pore canal openings also differed between the two species of lobster. Mechanical damage resulted in the formation of shell disease lesions on the claw and carapace of both lobster species. However, American lobsters, unlike their European counterparts, had extensive bacterial colonisation on the margins of these lesions. Overall, it is concluded that the cuticle of the American lobster is more susceptible to damage and resulting microbial colonisation. This may have implications for susceptibility of both species of lobster to shell disease syndrome.

1. Introduction

The chitin-containing exoskeleton, or integument, is a key feature to all arthropods, with its development of great importance to their evolutionary success (Neville, 1975). It typically consists of a mineralized, fibrous, chitin-based tissue composed of several different layers. The outermost layer, the epicuticle, is a tri-layer consisting of a protective lipoprotein layer, a waxy layer that provides waterproofing, and a cuticulin layer that is primarily composed of protein (Brusca and Brusca, 2003). Underneath the epicuticle is the thicker procuticle, composing the exocuticle and the endocuticle, and containing a lattice of protein and chitin (Smolowitz et al., 2005). The endocuticle is a bilayer; the outer part is calcified, while the inner is non-calcified and in close association with an underlying monolayer of epidermal cells that secrete the entire cuticle (Raabe et al., 2005).

The arthropod integument has a number of functions, ranging from support and structure, the maintenance of water balance, and as a barrier against disease causing agents (Brusca and Brusca, 2003). Few microbes, with the obvious exception of fungi and oomycetes, have the ability to actively penetrate this cuticle (Söderhäll and Unestam, 1978), however, if it is breached or damaged, a wide range of opportunistic pathogens can colonise the cuticle and surrounding tissue (Stewart, 1984). One such example of microbial degradation of the cuticle is shell disease syndrome, thought to be brought about by the activities of chitinolytic and

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non-chitinolytic bacteria (Vogan et al., 2008). This condition has been reported in a wide range of crustaceans worldwide (Sindermann, 1989; Vogan et al., 2008). The identity of the bacteria associated with the progression of shell disease is poorly known, but is thought to include vibrios (Vogan et al., 2002) and members of the Flavobacteriaceae (Chistoserdov et al., 2005, 2012). Knowledge of what triggers bacteria normally found in the surrounding environment to colonise and degrade the crustacean cuticle is also lacking. However, fighting injuries (Sindermann, 1989) and abrasion against hard substrates (Vogan et al., 1999; Quinn et al., 2012), both of which are natural behaviours for wild and captive lobsters, are thought to play a part in the initiation of the disease, primarily as a result of damage to the epicuticle. A range of environmental stressors including pollutants (Young and Pearce, 1975; Laufer et al., 2012) and temperature (Malloy, 1978; Castro et al., 2006; Glenn and Pugh, 2006; Tlusty and Metzler, 2012) may also play a role in crustacean susceptibility to the syndrome.

The severity and outcome of shell disease syndrome are highly variable across the Crustacea. For example, in European lobsters, *Homarus gammarus*, the condition has been reported to result in small pinpoint lesions that do not appear to penetrate through the cuticle and cause any further disease within its host (Wootton et al., 2012). In contrast, in edible crabs, *Cancer pagurus*, Vogan et al. (1999) showed that lesion development may result in breaching of the cuticle and septicaemia. Similarly, a new form of shell disease found in the American lobster, *Homarus americanus*, termed epizootic shell disease (ESD), results in the formation of large lesions that may cover a significant percentage of the cuticle (Shields et al., 2012). Although this new condition may not directly result in death of the host, it dramatically affects its marketability (Landers, 2005). ESD was first reported in American lobsters caught off New England as early as 1998 (Castro and Angell, 2000; Glenn and Pugh, 2006). Since then, the condition has spread through the Eastern seaboard of the United States, causing significant economic loss to the lobster fisheries (Castro et al., 2006). While the causative agents of ESD are considered complex and multifactorial, there is some evidence that several species of bacteria, including *Aquimarina 'homaria'* and *Thalassobius* sp., may play a role in the disease (Chistoserdov et al., 2012; Whitten et al., submitted). It has also been hypothesized that ESD has a polymicrobial basis during a "dysbiotic shift" of the normal flora (Bell et al. 2012; Meres et al., 2012; Shields, 2013). Recent research has suggested that there are four distinct forms of shell disease lesions in lobsters including those formed in ESD, namely, impoundment, enzootic, diet-induced and black spot (Quinn et al., 2013).

Concerns have been raised recently over the importation of live American lobsters into Europe due to the potential for disease transfer to their European counterparts (*H. gammarus*). The two species are closely related, with only minor genetic differences (Hedgecock et al., 1977). Since 2000, over ninety-one *H. americanus* individuals have been found in European waters (Jørstad et al., 2011; Stebbing et al., 2012), presumably as a result of importation. Of some potential concern is a report of an American lobster found with ESD-like symptoms in Norwegian waters (van der Meeren, 2008), implying that diseased animals have been inadvertently imported into Europe.

The aim of the current study was to assess whether European lobsters are susceptible to ESD-like shell disease. To achieve this, a model experimental system was employed in which the cuticle of both European and American lobsters was abraded or punctured, and the lobsters then held together in an aquarium system harboring bacteria thought to be associated with ESD development (Quinn et al., 2012), and in which 'outbreaks' of ESD have been observed. This approach simulates both abrasion and fighting injury, which are considered shell disease initiators. The ensuing lesion development was assessed both morphologically (present study) and in terms of its microbial community (Whitten et al., submitted). In the present paper, we report on the cuticle structure of European and American lobsters following co-location. We also examine the outcome of abrasion and puncture damage to the structural integrity and microbial communities of the surrounding cuticle in these two species.

2. Materials and methods

2.1. Animals

Juvenile European lobsters (*H. gammarus*) were raised in the Centre for Sustainable Aquatic Research, Swansea University, UK. A total

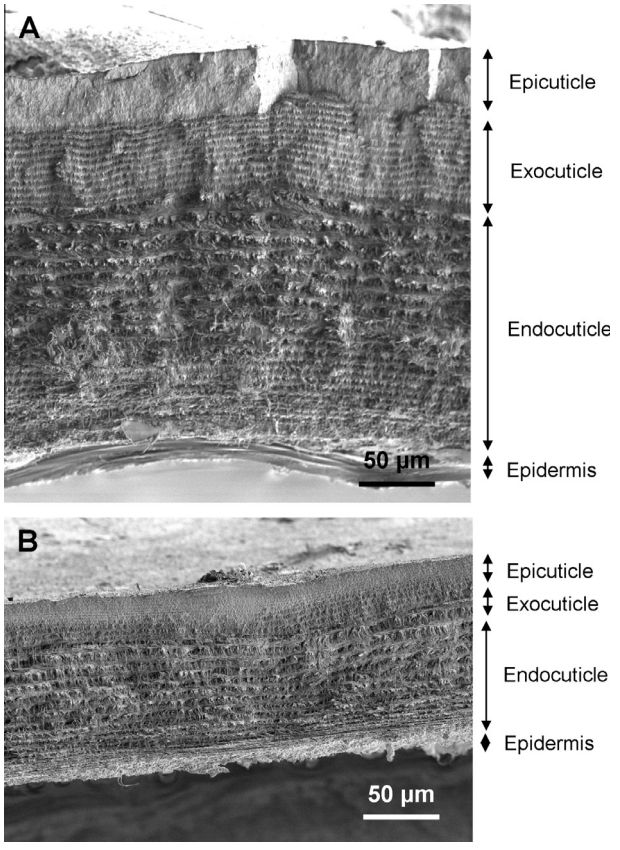

Fig. 1. (A and B). Low power scanning electron micrographs of cross sectional views of the control carapace from (A) a European lobster, *Homarus gammarus* and (B) an American lobster, *Homarus americanus*. The main areas of the cuticle are shown.

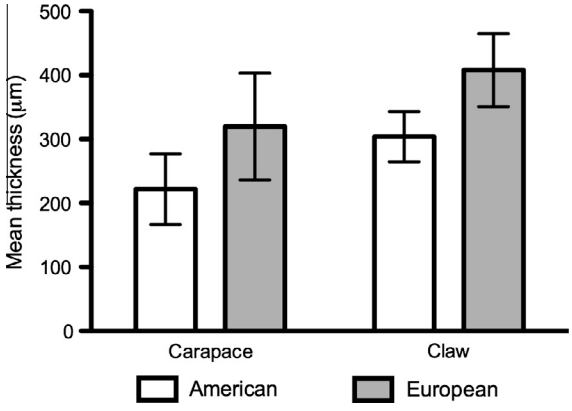

Fig. 2. A comparison of the mean thickness of the carapace and propodus region of claws of American and European lobsters. Values shown are means with 95% CI, $n = 10$ for each species.

of 11 animals with a mean carapace length of 4.4 ± 0.9 cm (mean value ± SD) were shipped to the New England Aquarium (Boston, USA), where they were allowed to acclimate for *ca.* 2 months prior to experimentation. They were maintained within the same aquarium system as juvenile American lobsters (*H. americanus*; mean carapace length of 4.1 ± 0.4 cm (mean value ± SD), which were hatchery-raised at the New England Aquarium. All lobsters were held in individual containers in one aquarium tank within a 1132 L recirculating seawater system. Water quality parameters were maintained at pH 7.8–8, with temperatures varying from 14.5 to 10.5 °C (according to seasonality). All lobsters were fed daily a gel-based diet containing brine shrimp and krill (Tetra marine mix gel and Mazuri® gel diet), as well as mussels.

2.2. Experimental procedures

The cuticle of European and American lobsters were damaged to simulate natural puncture injuries or abrasions as detailed in Whitten et al. (submitted). For details of the two damage locations see Fig. S1 in Supplementary File. Briefly, in the case of punctures, the dorsal propodus of the claw (cheliped) was perforated by a sterile flathead or Phillips screwdriver. Abrasions on the dorsal surface of the carapace were formed by abrading one 2 cm^2 area of one side of the carapace with sterile (UV-irradiated) 400-grit wet/dry sandpaper (3 M) for 30 s (Europeans) and 20 s (Americans). The difference in abrasion time was due to the differences in cuticle texture and the time it took to reach the inner

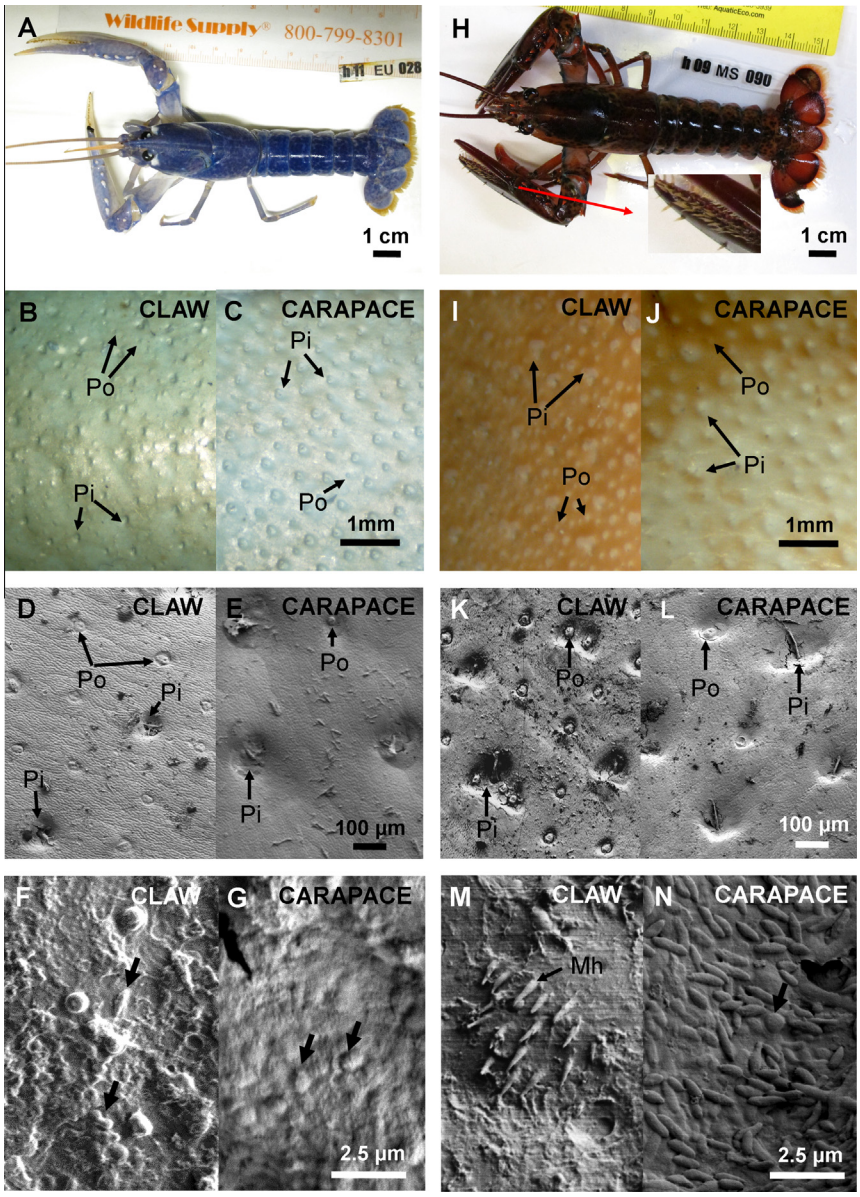

Fig. 3. (A-M). Structure of the surface and bacterial colonization of the healthy carapace and claw regions of European (A-G) and American (H-N) lobsters. (A, H): Light micrographs of the uninjured specimens of *H. gammarus* and *H. americanus* respectively. Note colour difference and the presence of large numbers of hairs on the claws of American lobster (insert). Macrophotographic record of typical appearance of the undamaged claw (B, I) and carapace (C, J) regions of European and American lobsters respectively. Note presence and distribution of the larger and more prominent setal pits (Pi) and the smaller, less obvious pores (Po). The uniform distribution of setal pits is more apparent in the European (C) compared with its American counterpart. (J). Low power scanning electron micrographs of the surface of the claws and carapace from European (D, E) and American lobsters (K, L) showing the setal pit openings (Pi) and the smaller pores (Po). (F, G, M and N). High power scanning electron micrographs showing the paucity of bacteria (unlabelled arrows) on the uninjured surface of European lobsters (F, G) and more extensive colonization by rod-shaped bacteria (unlabelled arrows) of some regions of the American lobster particularly around the setal pits (N). Note the species specific 'micro hairs' (Mh) on the surface of the claw of an American lobster (M).

exocuticle. Subsequently, damage was examined every 14 days for up to 12 weeks and any lesions recorded photographically. At the end of this time, lobsters were euthanized by the injection of 1–2 ml of potassium chloride solution (149 mg ml^{-1}) into the cardiac sinus as detailed by Battison et al. (2000). All lobsters were fixed by an injection of *ca.* 10 ml of Davidson's seawater fixative. Small areas (*ca.* 1 cm^2) of carapace from both damaged and control areas of claw and carapace were excised and post-fixed in 100% analytical grade ethanol for scanning electron microscopy. If a lobster moulted during the experiment, the number of days since last moult was recorded, and the moult was used to examine the structure of the lesions. In the case where moulting occurred, there was no significant difference between the time since last moult between the two species of lobsters (*t* test; $P = 0.35$).

Scanning electron microscopy (SEM) was used to assess the following attributes in control (i.e. uninjured) *vs.* damaged (or diseased) cuticle: density and morphology of bacteria, thickness of cuticle, distance of bacteria from lesion edge, morphological characteristics of lesion margins and necrotic tissue, and pore and setal pit density. Samples of excised carapace and claw were first photographed using a low power light microscope in order to assess damaged tissue prior to their preparation and examination by SEM. For the latter, the majority of samples were dried under vacuum and coated with *ca.* 3 nm of gold using an Edwards Sputter Coater S150B. Scanning electron microscopy was carried out using a Hitachi S4800 scanning electron microscope. Standard imaging conditions using an accelerating voltage of 1.0 kV and a working distance of 8 mm were employed. Photographed samples consisted of abraded carapace, control carapace, punctured claw, control claw and natural lesions (i.e. those lesions which developed without assisted interference). Control (uninjured) regions of carapace and claw were initially examined and photographed. The thickness of the cuticle in the claw and carapace of European and American lobsters ($n = 10$ for each species) was measured at 50× magnification with 15 independent measurements made of each randomly chosen area, avoiding pits, pores scratches and other obvious imperfections which could alter typical thickness. It was difficult to quantify bacterial numbers on the surface of the cuticle due to most being cosseted in an amorphous matrix, so it was assessed qualitatively.

Counts of setal pits and pore canals were made at low magnification by SEM (*ca.* 50×), and were used to determine the quantity of pits and canals in a known carapace surface area. Uninjured carapace and claw regions of both lobster species were examined ($n = 5$ in both species). Image J software (v. 1.47d Mac) was used to measure 15 × 1 mm^2 squares for each animal. For SEM examination of cuticle cross sections, samples of carapace or claw ($n = 10$ for each species) were fractured rather than cut to reduce the appearance of artefactual stress cracks in the sample. The thickness measurements were compared statistically (2-way ANOVA, matched by animal, with Bonferroni post-test) using Prism 6.0 (GraphPad Software, San Diego, CA, USA). Cuticle samples were also examined at low magnification to determine the extent of damage, followed by high magnification to record the presence of any bacteria around the margins of lesions.

3. Results

3.1. Comparison of the normal structure of the carapace and claws of American and European lobsters (Figs. 1–4)

To determine the thickness and structure of the different regions of cuticle, cross sections of control cuticle from the carapace and claws were examined and measured by SEM (Fig. 1A and B). The carapace of European lobsters was on average 1.4× thicker while the claw was 1.2× thicker than those of American lobsters (Fig. 2). Two-way ANOVA, matched by individual, showed a significant effect for both species (European *vs.* American; $F_{1,18} = 9.154$, $P = 0.0073$; Fig. 2; Table S1 in Supplementary File) and location (carapace *vs.* claw; $F_{1,18} = 22.18$, $P = 0.0002$; Fig. 2; Table S1 in Supplementary File) in cuticle thickness, with no interaction between the two factors. Therefore, the juvenile European lobsters examined possessed thicker cuticles than those in equivalent size American lobsters, in both the claw and carapace areas. Furthermore, the cuticle in the claws was thicker than that found in the carapace region for both species (Fig. 1A and B and Fig. 2).

Initial low magnification (macrophotographic) examination of the cuticle of American and European lobsters showed obvious differences in pigmentation (Fig. 3A and H) and the appearance of 'hairs' on the claws of American lobsters (Fig. 3H), which formed a dense fringe on both the dactyl and propodus. Macrophotographic and low power SEM examination of the surface of the carapace and claws showed apparent differences in the proportion and distribution of tegumental gland openings (pores) to setal hair pits (pits) (compare Fig. 3B–E with 3I-L). On the carapace of European lobsters, the pits and the pores appeared to be in a regular arrangement (Fig. 3C), while in American lobsters they were more randomly distributed (Fig. 3J).

Between species, there was a significant difference between the numbers of pores on the claw of American lobsters compared with that on European lobsters, with American lobsters having significantly more. Furthermore, within American lobsters, claws possessed significantly more pores per unit area than the carapace.

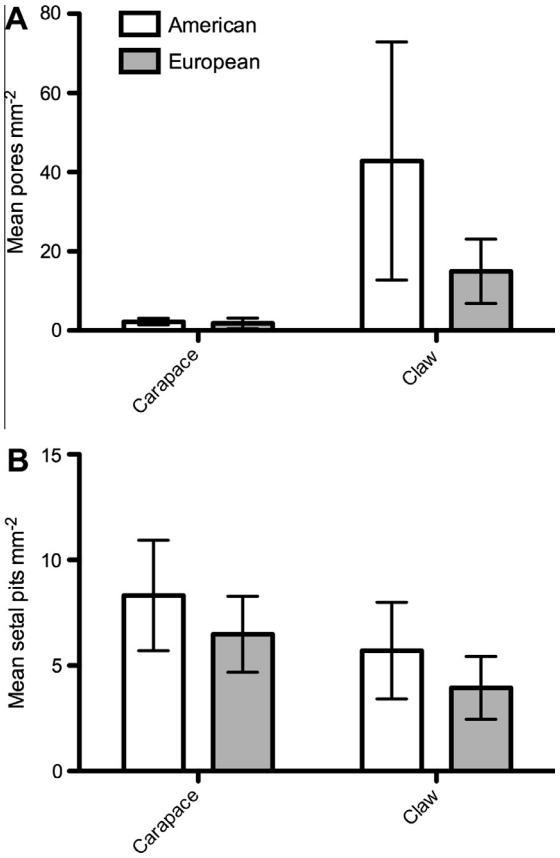

Fig. 4. (A and B). Comparison of the density of pores (A; $F_{1,8} = 22.65$, $P = 0.0014$) and setal pits (B; $F_{1,18} = 22.18$, $P = 0.0002$) on the carapace and claw regions (mean per mm^2) of American and European lobsters using SEM. Values are means ± 95% CI, $n = 5$ for each species (i.e. carapace and claw examined from five American and five European lobsters).

Two-way ANOVA, matched by individual, showed a significant effect for both species (European *vs.* American; $F_{1,8} = 6.423$, $P = 0.0350$; Fig. 4A; Table S2) and location (carapace *vs.* claw; $F_{1,8} = 22.65$, $P = 0.0014$; Fig. 4A; Table S2) and a significant interaction ($F_{1,8} = 5.920$, $P = 0.041$) in the number of pores mm^{-2}. Bonferroni multiple comparisons test revealed that the claws of American lobsters possessed more pores mm^{-2} than the claws of European lobsters ($P < 0.05$; Table S2). It was also observed that there were more setal pits on the carapace than on the claws, irrespective of species; two-way ANOVA, matched by individual, showed a significant effect for location (carapace *vs.* claw; $F_{1,8} = 4.463$, $P = 0.0039$; Fig. 4B; Table S3 in Supplementary File) but not species in the number of setal pits mm^{-2} and no interaction between these two factors.

Observation of the control (i.e. uninjured) carapace and claw of both species by SEM showed minimal microbial presence (Fig. 3F, G, M and N). Most areas examined possessed no (or very few) bacteria. Most of the bacteria observed were found around the base of the hairs in the setal pits and in the depressions of the pores, and appeared to be embedded in an amorphous matrix (e.g. Fig. 3G). Although not quantified due to layering and clumping, there appeared to be more bacteria on the cuticle of American lobsters compared to their European counterparts (compare Fig. 3F and G with Fig. 3M and N). American lobsters also possessed 'micro-hairs'

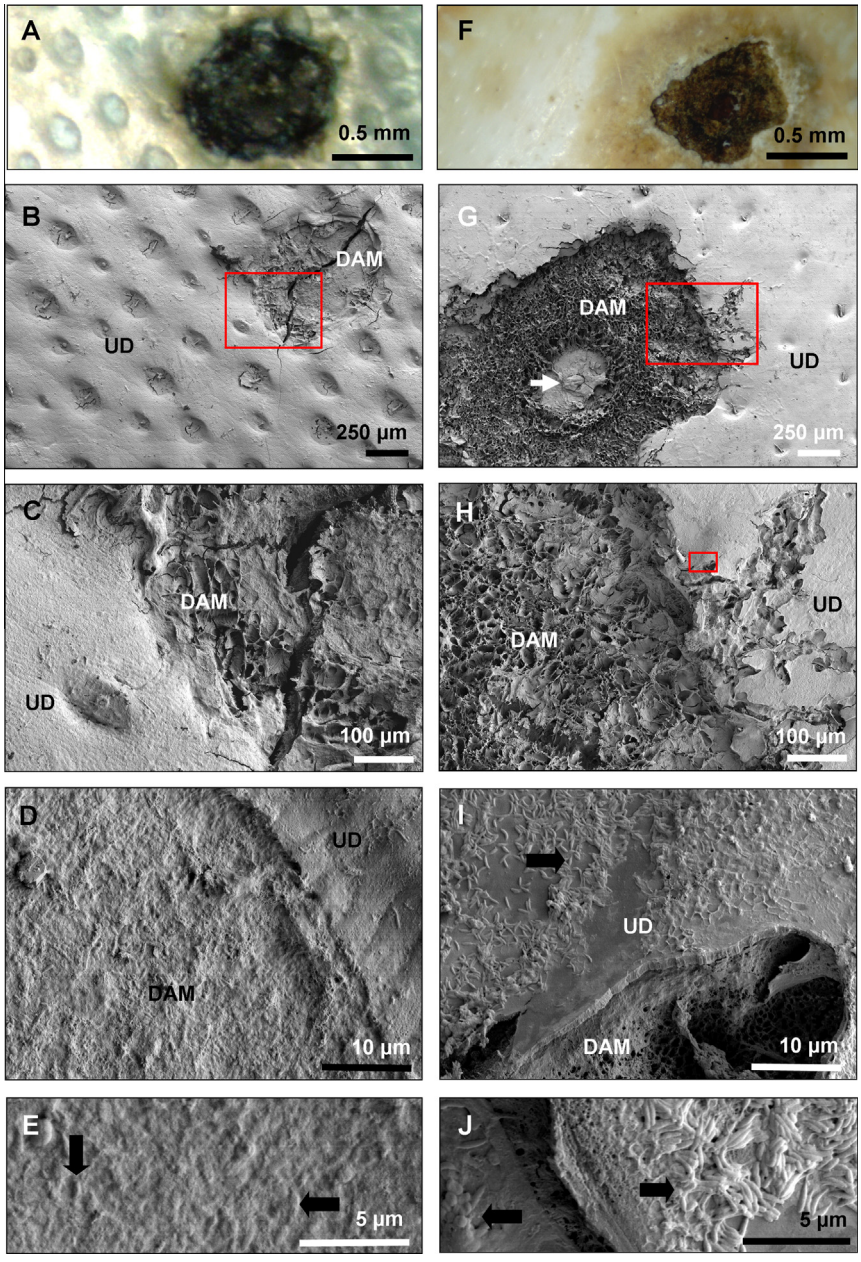

Fig. 5. (A-J). Natural lesions found on the carapace region of European (A-E) and American (F-J) lobsters. (A, F). Light micrographs showing the appearance of typical natural lesions. Note their melanised nature. (B, G). Low power scanning electron micrographs showing the appearance of the lesions shown in A and F. These lesions probably originate from setal pits (see white unlabelled arrow on 'G'). Note the damaged (DAM) and undamaged (UD) regions of the cuticle surface. The crack in (B) is probably an artifact of preparation. Boxed areas show enlargements in C and H. (C, H). Higher power scanning electron micrographs showing the cavernous nature of the cuticle in the lesion in the damaged (DAM) area. Boxed area in H enlarged in I. (D, E, I and J). High power scanning electron micrographs showing the paucity of epibionts (unlabelled arrows) on the surface and surrounded undamaged (UD) region in European lobsters (D, E) compared with the extensive bacterial colonization (unlabelled arrows) in the damaged (DAM) and undamaged (UD) margins of lesions from American lobsters (I, J).

on their claws, but not their carapace (Fig. 3M). These 'micro-hairs' accommodated a number of coccus-shaped bacteria (not shown), however due to the presence of an amorphous matrix surrounding these bacteria, they were difficult to quantify and identify. These 'micro-hairs' were absent from all European lobsters examined.

3.2. Spontaneous (natural) lesions

During the study, both lobster species showed the development of natural (spontaneous) lesions, which were independent of those caused by abrasion or puncture procedures. These were prominent due to their melanised appearance (Fig. 5A and F) and found on multiple regions but especially on the claws. It should be remembered that the melanisation seen by light microscopy is not visible under SEM as it is a pigment located below the outer surface. Scanning electron microscopy showed that these lesions tended to develop around setal pits (Fig. 5B and G). The damaged area of the lesions in both species showed extensive degradation of the epicuticle and even the exocuticle in some places, leaving a cavernous appearance (Fig. 5C and H and Fig. 6), presumably due to the breakdown of exo- and endo-cuticle components. Lesions in American lobsters appeared to result in greater erosion of the exocuticle (compare Fig. 5D and I). There were large numbers of bacteria at the margin of the lesions in the American lobsters (Fig. 5I and J) however these were less apparent in the same region of natural lesions in European lobsters (Fig. 5D and E). The necrotic, melanised cores of the lesions were rarely seen to contain bacteria in either lobster species.

3.3. Lesions associated with abrasion injury to the carapace

Examination of abraded areas by light microscopy showed melanisation initiation in the underlying exposed cuticle after approximately 3 days. In American lobsters, setal pits and pores became heavily melanised within the abraded area (Fig. 7E), whilst in the Europeans this was less apparent (Fig. 7A). SEM examination revealed that abrasions in European lobsters were superficial, with the abrasion only affecting the epicuticle. There was limited damage to the setal pits, however, in some instances pore openings were completely erased (Fig. 7B). In comparison, and despite shorter abrasion times, more extensive damage was observed to the setal pits and pores in American lobsters (Fig. 7F). Examination of the abraded areas at higher magnifications showed extensive colonisation of the abraded cuticle of American lobsters by a variety of rod-shaped bacteria (Fig. 7G and H). The equivalent area in European lobsters had only small numbers of rod-shaped bacteria, which were also encased in an extracellular matrix (Fig. 7C and D).

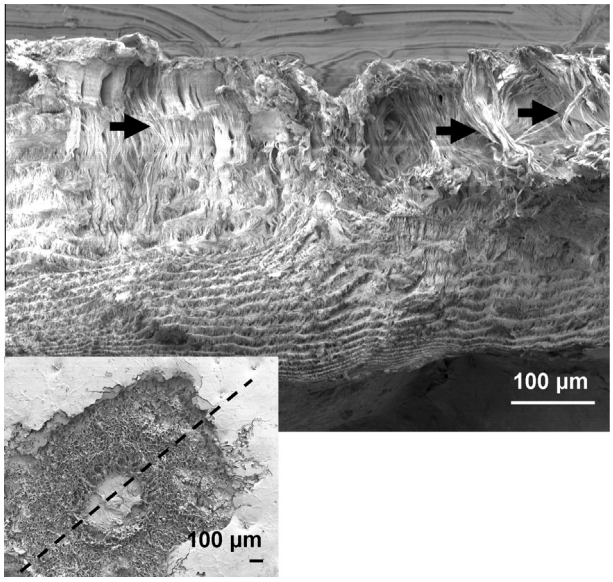

Fig. 6. Scanning electron micrograph of a snapped cross section through a natural lesion and a surface view (shown in insert) in an American lobster showing the cavernous nature of the damaged cuticle, with exposed chitin fibrils (unlabelled arrows).

3.4. Lesions associated with puncture injury to the claw

Light microscopical examination revealed that punctures in the claws caused extensive damage to the integrity of the cuticle with melanisation and associated hairline cracking (Fig. 8A and D). In the American lobsters, the puncture margins developed as the experiment progressed and possessed a surrounding colourless, non-pigmented zone (Fig. 8D). This zone was commonly absent from European lobsters (Fig. 8A). Scanning electron microscopy revealed no clear differences in the structure of the puncture sites between American and European lobsters (compare Fig. 8B with E). Similarly, both species had only limited microbial populations in the margins of puncture sites (compare Fig. 8C with F). Due to the puncture depth and associated problems with charging during SEM observation, it proved difficult to examine the puncture core for microbial populations.

4. Discussion

The current study has shown clear differences in the ability of the carapace of juvenile American and European lobsters to withstand mechanical abrasion used to initiate lesions on the claws and carapace. In essence, the carapace of juvenile European lobsters was more resistant to abrasion injuries than the equivalent region on American lobsters. For example, when abrading the European lobsters with sandpaper for 30 s, the equivalent abrading time in American lobsters resulted in deeper penetration of the carapace. Therefore to keep the injuries between the two species equivalent, the latter animals were only abraded for 20 s. Even after this procedural adjustment, SEM images of the abraded regions revealed increased damage to the American lobster cuticle. As a general observation, the cuticle of the juvenile American lobsters studied was more friable than that of the Europeans. These observed differences in ability to withstand abrasion injuries may have a profound influence on the development of cuticular lesions, which may be the forerunner of more extensive shell disease. It must be remembered, however, that the differences seen in the cuticle of the juvenile European and American lobsters in this study may not be representative of those animals across the entire life history.

A further key finding of the study was the difference in cuticle thickness and the number of pores per unit area between the two lobster species. The cuticle of European lobsters (at least at the juvenile stage examined) was significantly thicker than that of juvenile American lobsters apparently at the same stage of development. Furthermore, there were more pores per unit area in American than European lobsters. The pores, sometimes termed dermal or tegumental gland openings, run perpendicularly through the cuticle, and serve as a pathway for the transport of ions during cuticle mineralisation after moulting (Cameron, 1989). Smolowitz et al. (2005) noted that shell disease infections in American lobsters often extended from the cuticular pores/wax canals rather than the setal pores (pits). The setal pits, containing hairs, also known as sensory canals, neural glands (Kunkel et al., 2012) or pore canal fibres (Cheng et al., 2008), showed no significant difference in frequency between the two species of lobster. Both types of

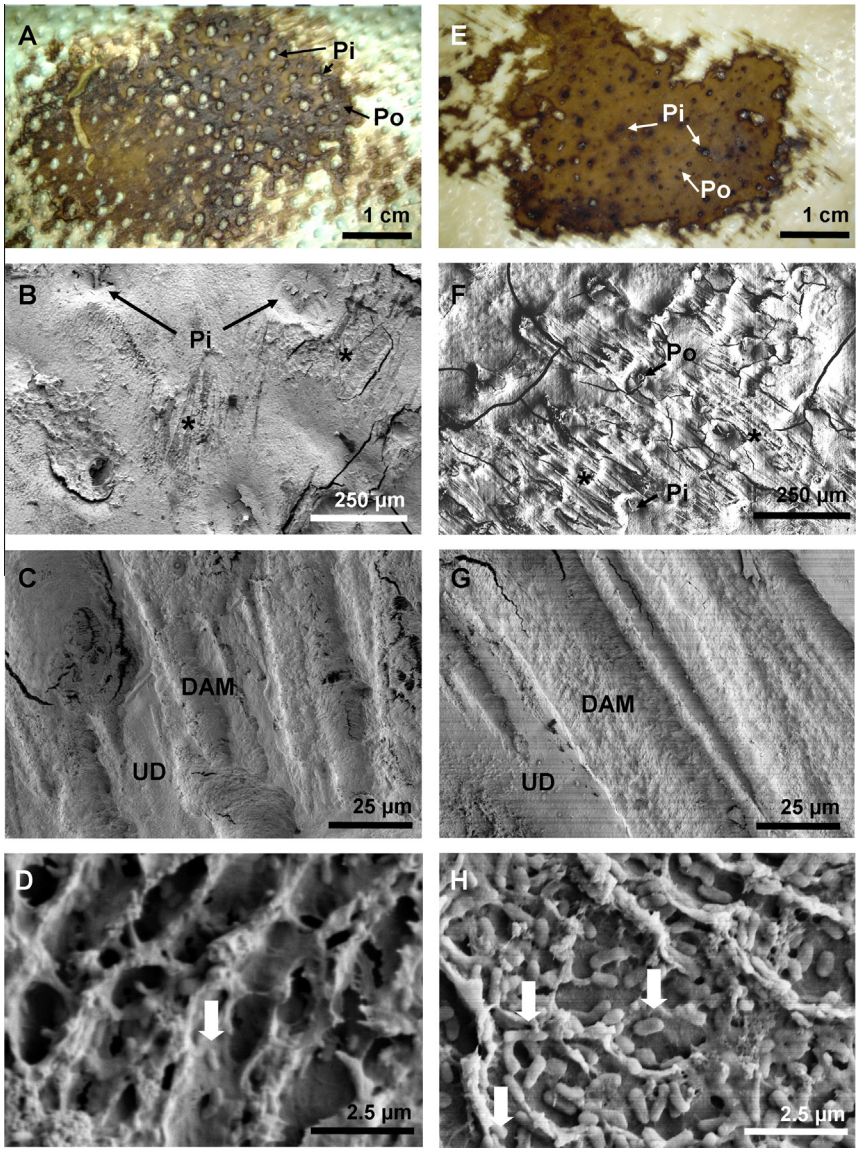

Fig. 7. (A-H). Abraded carapace (*) of European (A-D) and American (E-H) lobsters. (A, E). Light micrographs of abraded region of carapace showing scratched nature of the carapace. Note in the European lobster that the setal pits (Pi) and some pores (Po) are apparently undamaged and not melanised while those in the equivalent area of an American lobster are heavily melanised. (B, C, F and G). Scanning electron micrographs of the abraded area showing abrasions consisting of damaged (DAM) and undamaged (UD) regions. (D, H). High power scanning electron micrographs showing the cavernous nature of the damaged carapace in both European and American lobsters and bacteria-like particles (unlabelled arrows) in this region.

openings (pores and pits) represent potentially vulnerable areas of the carapace, where bacteria may find refuge or gain entry to underlying tissues. Hence, it would be expected that these areas might have additional chemico-physical barriers against such invasion. Indeed, Kunkel and Jercinovic (2013) hypothesised that the walls surrounding various "canals" penetrating the lobster cuticle had an insulated layer in comparison to the more acid soluble calcium carbonate found in the main endocuticle, which is considered more susceptible to bacterial degradation. Our knowledge of the antimicrobial activity of the crustacean cuticle is limited to a few studies (e.g. Mars, 2010; Kunkel et al., 2012), but both the prophenoloxidase activating system and its products (Nappi and Vass, 1993), together with low molecular weight antimicrobial peptides (Mars, 2010), may be important in protecting against bacteria associated with shell disease syndrome. Indeed, Noga et al. (1994), Mars (2010) and Homerding et al. (2012) suggested that deficiencies in the antimicrobial armory may leave crustaceans vulnerable to various forms of shell disease. Thus, future studies should compare the cuticle-based antimicrobial armory in American and European lobsters to ascertain if differences exist between the two species, and subsequently their vulnerability to that microbial attack.

There have been several ultrastructural studies of the microbial populations associated with various forms of shell disease in crustacean species, including the American lobster, *H. americanus* (Hsu and Smolowitz, 2003; Porter, 2004; Smolowitz et al., 2005) but to our knowledge, no such studies have been carried out in the European lobster, *H. gammarus*. In the American lobster, Porter (2004) noted that both rod-shaped and coccoid bacteria were found on the normal (uninjured) cuticle, whilst in the lesions the bacteria were mainly rod-shaped. Porter also reported that most bacteria were found at the margins of cuticular lesions. Similarly, bacteria were observed on the leading edge of natural lesions in American lobsters by Hsu and Smolowitz (2003) and Smolowitz et al. (2005) in their studies of shell disease. Our current study also found a similar marginal distribution of bacteria in healthy and injured cuticle in both American and European lobsters.

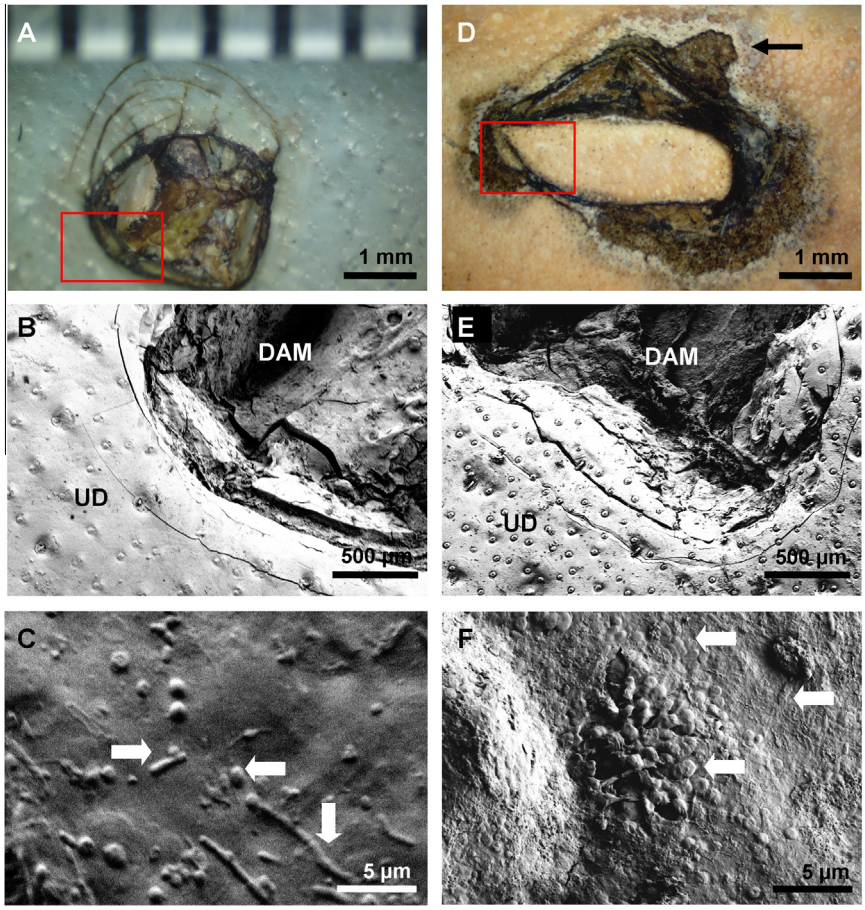

Fig. 8. (A-F). Punctured claw of European (A-C) and American (D-F) lobsters. (A, E). Light micrographs of typical punctures post-damage showing extensive damage and melanisation. Note the white, necrotic area at the margin of the damaged area in the American lobster (unlabelled arrow) with melanised pore openings. Boxed regions in A and D show areas enlarged in B and E, respectively. (B, E). Low power scanning electron micrographs of punctured (DAM) and undamaged (UD) areas of the claw from European (B) and American (E) lobsters. The concentric cracks apparently result from the puncture procedure. (C, F). High power scanning electron micrographs showing bacteria (unlabelled arrows) in the margins of the puncture area. Note diversity of bacterial morphology in C and the apparent presence of an amorphous matrix around the bacteria in F.

Although larger numbers of bacteria were found in association with cuticular lesions in American than European lobsters, this should not be taken as evidence of greater degradative activity as a result of bacterial secretion of proteases, lipases and chitinases. Not all bacteria observed by SEM are necessarily actively involved in cuticular breakdown, because some species may be commensal or mutualistic with their metabolic activities protecting the cuticle from putative invasion pathogens. Although it may be unwise to make a comparison between the functional significance of the lobster cuticle microbiome and that of the human skin, the latter serves as a timely reminder of the different roles that bacteria play on the integument of animals (Grice et al., 2008).

The main objective of our study was to assess the potential risk of the spread of ESD to native European lobsters resulting from the importation of live American lobsters into Europe. This is imperative following the finding of an American lobster in Norwegian waters showing ESD symptoms (van der Meeren, 2008). Our experimental protocol placed European lobsters in an environment where bacterial species potentially involved in ESD are present, together with their American lobster counterparts. Both the lesion morphology reported in the present study, and the nature of bacterial communities on lobster carapaces exhibiting lesions reported in Whitten et al. (submitted), differ between the two lobster species. Our results suggest that the European lobster, at least at this stage of development, may be better equipped to deal with exposure to bacteria associated with ESD, and other forms of shell disease, due to its thicker carapace and smaller number of openings, which are thought to be points of refuge or lesion initiation for these organisms. However, due to the high commercial value of European lobsters in the UK (£32.6 million in 2010; MMO, 2011), it is important to monitor the levels of American and hybrid lobsters found in European waters, and to be vigilant in assessing these individuals for the symptoms of ESD. Unfortunately, as the nature of the causative agents of ESD is still unclear, it is difficult to predict the risk of ESD to European lobsters.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jip.2014.01.001.

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