Journal of Fish Diseases 2000, **23,** 251–263

A rapid onset, post-capture muscle necrosis in the Norway lobster, *Nephrops norvegicus* (L.), from the West coast of Scotland

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Abstract

A post-capture, abdominal muscle necrosis of rapid onset has been identified in Norway lobsters, *Nephrops norvegicus* (L.), captured off the West coast of Scotland. Economic losses, as a result of the mortality of these animals in transport, were encountered by Scottish wholesalers during the summer and autumn of 1999. Affected animals show a characteristic whitening of individual muscle fibres and fibre bundles of the abdomen within hours of capture, with a progression towards complete opacity of the abdominal musculature within a number of days. The pathology causes a loss of the normal function of the abdomen; thus, preventing the normal 'tail flip' swimming. Electron microscopy failed to reveal any obvious causative agent but showed that affected tissue displayed a progressive disruption of sarcomere organization, loss of Z-line material, condensation of myofibrils and infiltration of necrotic regions by granulocytes. SDS–PAGE of affected muscle tissue showed that there was a great reduction of most of the major contractile proteins. The condition most closely resembles idiopathic or spontaneous muscle necrosis, a pathology previously reported from both wild and cultured crustaceans. Damage to the integument in conjunction with exposure to various stressors during and immediately after capture is the most likely cause of the pathology. The rapid onset of the pathology has implications for the post-capture handling procedure for *N. norvegicus* and their subsequent vivier transport to market. It may also be partially responsible for the high mortality rate of undersized *N. norvegicus* returned to the sea after capture and aerial emersion.

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Introduction

Pathological damage to crustacean muscle has been widely reported and is known to have various causative agents, including microsporidians (Findley, Blakeney & Weidner 1981; Langdon 1991; Kabre 1992; Dennis & Munday 1994; Childers, Reno & Olson 1996), ciliates (Cawthorn 1997), bacteria (Stewart 1980), dinoflagellates (Shields 1994; Stentiford, Neil & Coombs 2000) and viruses (Arcier, Herman, Lightner, Redman, Mari & Bonami 1999). A number of other studies have described muscle wastage with unknown aetiology (Lindqvist & Mikkola 1978). While host-induced (idiopathic) muscle necrosis has been reported to occur in conditions of natural or artificial stress, both in the wild and under aquaculture situations (Akiyama, Brock & Haley 1982; Momoyama & Matsuzato 1987; Nash, Chinabut & Limsuwan 1987; Anderson, Nash & Shariff 1990; Evans, Fotedar, Fan & Jones 1999). Idiopathic muscle necrosis (also known as 'tail rot', muscle opacity, muscle necrosis, and spontaneous muscle necrosis) is characterized by focal to gross opaque lesions in the striated musculature of the abdomen. The lesions are composed of necrotic muscle cells with disorganized myofibrils, loss of sarcomeres, loss and atrophy of mitochondria and damage to the sarcoplasmic reticulum (Akiyama *et al.* 1982). Late-stage necrosis is characterized by fragmentation and condensation of myofibrillar bundles (Nash *et al.*

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1987). Haemocytic infiltration of the damaged musculature is also commonly observed, these participating in aggregation, encapsulation and, in the late stages of necrosis, myophagia (Momoyama & Matsuzato 1987; Nash *et al.* 1987; Anderson *et al.* 1990).

Nephrops norvegicus (L.) supports a major fishery in the UK, with the Scottish fishery contributing over 76% of this (£48 million in 1998—FRS 1999). The majority of the landings are from trawler capture, with the animals being 'tailed' for sale as 'scampi'. Larger animals are also captured by baited creels and are often exported to continental Europe by specially designed 'vivier' vehicles. In order to ensure sale, exported live lobsters must be in good condition on arrival at market.

During the summer and autumn of 1999, creel-caught lobsters from the Sound of Jura (West Scotland) and other west coast sites were reported to be dying or moribund during vivier transport, with some catches being refused at market because of the opaque appearance of their tail meat (Mr G. Goldsworthy, personal communication). Signs of this condition were also noted in animals taken directly from creels, and in trawl-caught animals from the Clyde sea area (West Scotland) which were subsequently maintained in aquaria at the University of Glasgow.

This study was carried out to establish the epidemiology of this condition in the Scottish West coast *N. norvegicus* fishery and to describe its pathology and progression by using histological and biochemical techniques.

Materials and methods

Norway lobsters showing clinical signs of the described condition (opaque abdominal musculature and inability to flex the abdomen) when caught in creels in the Sound of Jura (see Fig. 1) were collected from Loch Fyne Fisheries Co., Tarbert, West Scotland. Trawl-caught lobsters from the Clyde sea area (Fig. 1), were maintained post-capture in the aquarium at the Division of Environmental and Evolutionary Biology, University of Glasgow. Clyde lobsters were fed *ad libitum* on mussels, *Mytilus edulis* (L.), until preparation of abdominal muscle for histology and SDS–PAGE, while muscle tissue from the Sound of Jura lobsters was immediately prepared for histology.

In order to establish the rate of progression of this condition, 200 lobsters captured in the Clyde sea area in January 2000 by standard otter trawling (Fig. 1) were selected randomly from a sub-sample and assessed for signs of muscle opacity immediately after capture, and again 4 h post-capture, after holding them on the deck in a standard fish box covered with a damp sack. In those showing signs of the condition, individual segments of the abdomen were visually assessed for the location of muscle opacity. In order to investigate possible reasons for the onset of the condition within the abdomen, any damage to the integument in the vicinity of the necrosis was recorded by probing the area with a blunt dissection needle. All lobsters

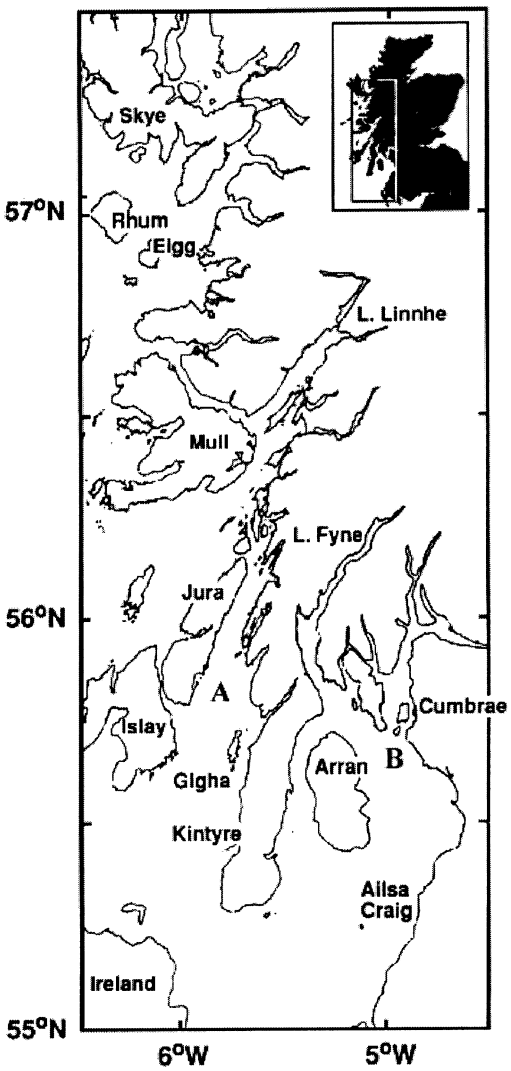

Figure 1 Map of western Scotland showing the capture sites in the Sound of Jura (A) and the Clyde sea area (B). The University Marine Biological Station Millport is situated on the Isle of Cumbrae in the Clyde estuary.

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were also staged for infection by the dinoflagellate parasite *Hematodinium* sp., which is known to affect *N. norvegicus* in these regions (Field & Appleton 1995). All lobsters were diagnosed as stage 0 (uninfected). Histological samples from these animals were compared with previously collected muscle samples from *Hematodinium*-infected lobsters.

Histology

The deep flexor and medial superficial flexor muscles from the abdomen of *N. norvegicus* were exposed by removal of the abdominal cuticle and underlying extensor muscles. Small blocks of opaque muscle and muscle fibre bundles showing opacity along part of their length were removed under a dissecting microscope. Muscle tissue samples were also taken from animals diagnosed with *Hematodinium* infection. Muscles were fixed for 2 h in a solution containing 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, with 2% sucrose and 1.5% sodium chloride for 2 h at room temperature. Fixed samples were then rinsed in 0.1 M phosphate buffer with 4% sucrose and post-fixed in 1% osmium tetroxide in phosphate buffer for 1 h. Specimens were washed in several changes of distilled water and block stained in 0.5% uranyl acetate for 1 h. After dehydration through an ethanol series, specimens were embedded in Spurr resin (Spurr 1969). Thick sections (1 μm) were stained with toluidine blue for viewing with a light microscope and suitable areas were cut and mounted on uncoated copper/palladium grids and stained with uranyl acetate and lead citrate. Thin sections (60–70 nm) were examined in a Zeiss 902 transmission electron microscope (Leo Electron Microscopy Ltd., Cambridge, UK).

SDS–PAGE

For SDS–PAGE analysis of deep abdominal flexor muscle from lobsters showing signs of muscle opacity, individual muscle fibres were dissected out under calcium-free *Nephrops* physiological saline and placed into 200 μL of SDS-sample buffer, denatured at 95 °C for 4 min and stored at −20 °C until electrophoretic protein separation (see Neil *et al.* 1993). Discontinuous SDS–PAGE was performed according to the method of Laemmli (1970) with gels containing 12.5% acrylamide separating gel and 4% acrylamide stacking gel. Electrophoresis running conditions were as for those described by Neil *et al.* (1993), followed by overnight incubation in 10% TCA and staining for 30 min in Coomassie-blue solution. Stained gels were later imaged with an Appligene™ gel imager (Appligene, Durham, UK). Opaque muscle fibres were compared with fibres from lobsters showing no signs of muscle opacity.

Results

In the early stages of the condition in *N. norvegicus*, individual muscle fibres of the abdomen appeared opaque when viewed through the ventral membrane, with the opacity progressing to whole muscle fibre bundles and eventually the whole abdomen. In advanced cases, the abdomen did not respond to normal physical stimuli and, thus, affected lobsters could not initiate a normal 'tail-flip' response, while the thoracic limb system appeared to function normally. Progression of the condition was rapid, leading to death of affected lobsters within days.

Light microscopy revealed that in the abdominal superficial flexor muscles of animals showing early stages of the pathology (individual opaque muscle fibres), the peripheral areas of the muscle appeared damaged, with disruption to the sarcolemma and infiltration of the damaged regions by granulocytes (Fig. 2A). However, other areas contained intact myofibrillar structure, with regular sarcomere arrangements. The deep abdominal flexor muscles, which form the major muscle mass within the abdomen, were also progressively affected by the necrosis, and in severe cases there was a complete loss of sarcomeric structure and a condensation of myofibrillar bundles (Fig. 2B). In some instances, the pathology was clearly progressing along individual myofibrillar bundles, with areas of semi-intact muscle being found immediately adjacent to areas of complete muscle degeneration, the latter being heavily infiltrated by granulocytes (Fig. 2C).

Examination of the necrotic tissue with electron microscopy revealed that the normal sarcomeric structure observed in unaffected animals (Fig. 3A) was initially altered by expansion of the space occupied by the tubular system and the appearance of granular material in the cytoplasm (Fig. 3B). Regions of the muscle with a fairly intact myofibrillar structure also displayed some erosion of the sarcomeres, with degeneration originating in

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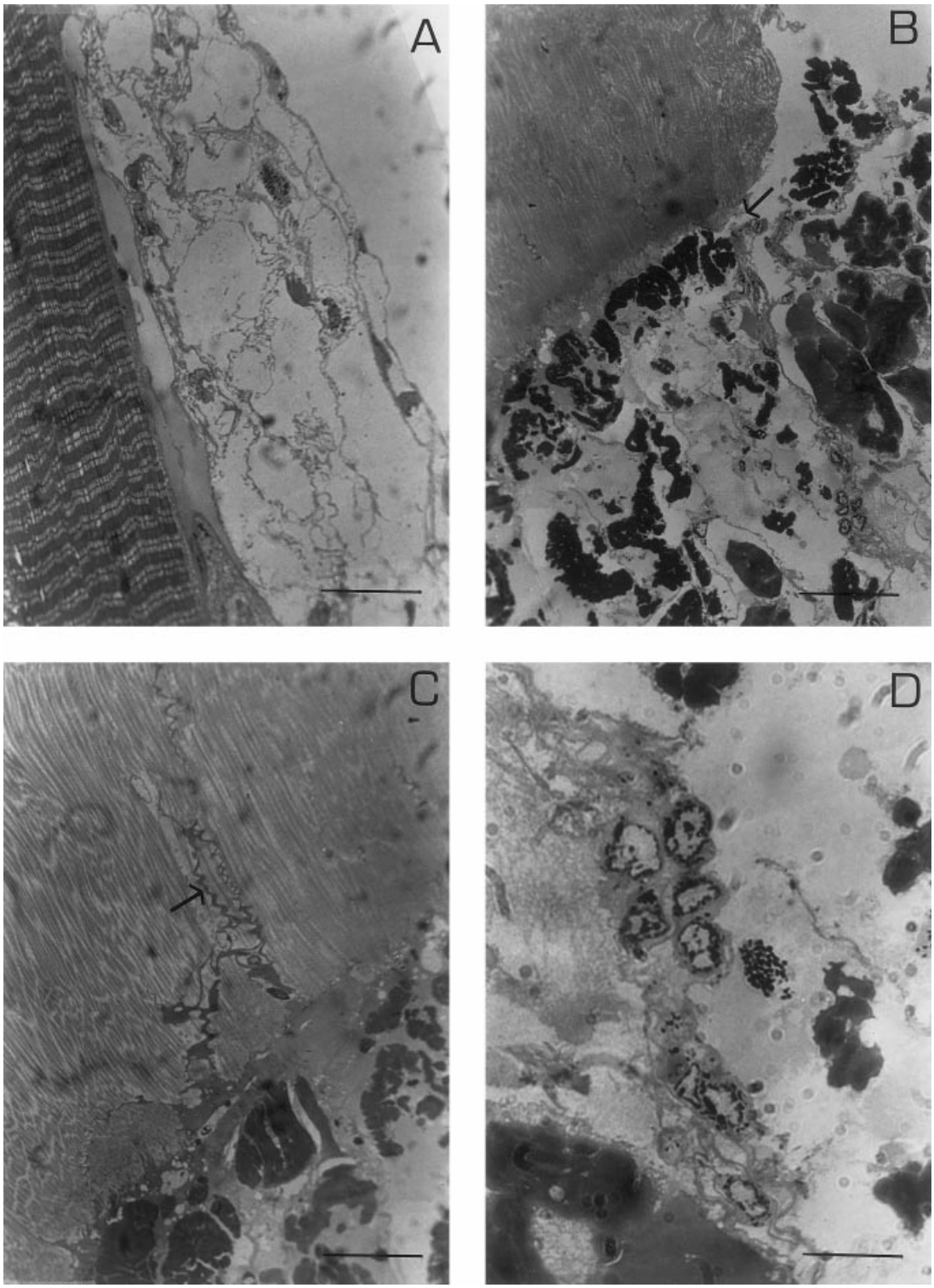

Figure 2 (A) Superficial flexor muscle from lobster showing early signs of pathology. Note the intact sarcomeres and adjacent region of necrotic muscle showing infiltration by granulocytes (bar = 40 μm). (B) Deep flexor muscle from lobster showing late stage necrosis. Note the junction between relatively intact and disorganized muscle (arrow), condensed myofibre bundles and granulocyte infiltration (bar = 80 μm) (see also Fig. 2D). (C) Fibrous processes at the junction between intact and disorganized muscle (arrow) (bar = 30 μm). (D) Infiltration of granulocytes into necrotic regions of DAF muscle (bar = 20 μm).

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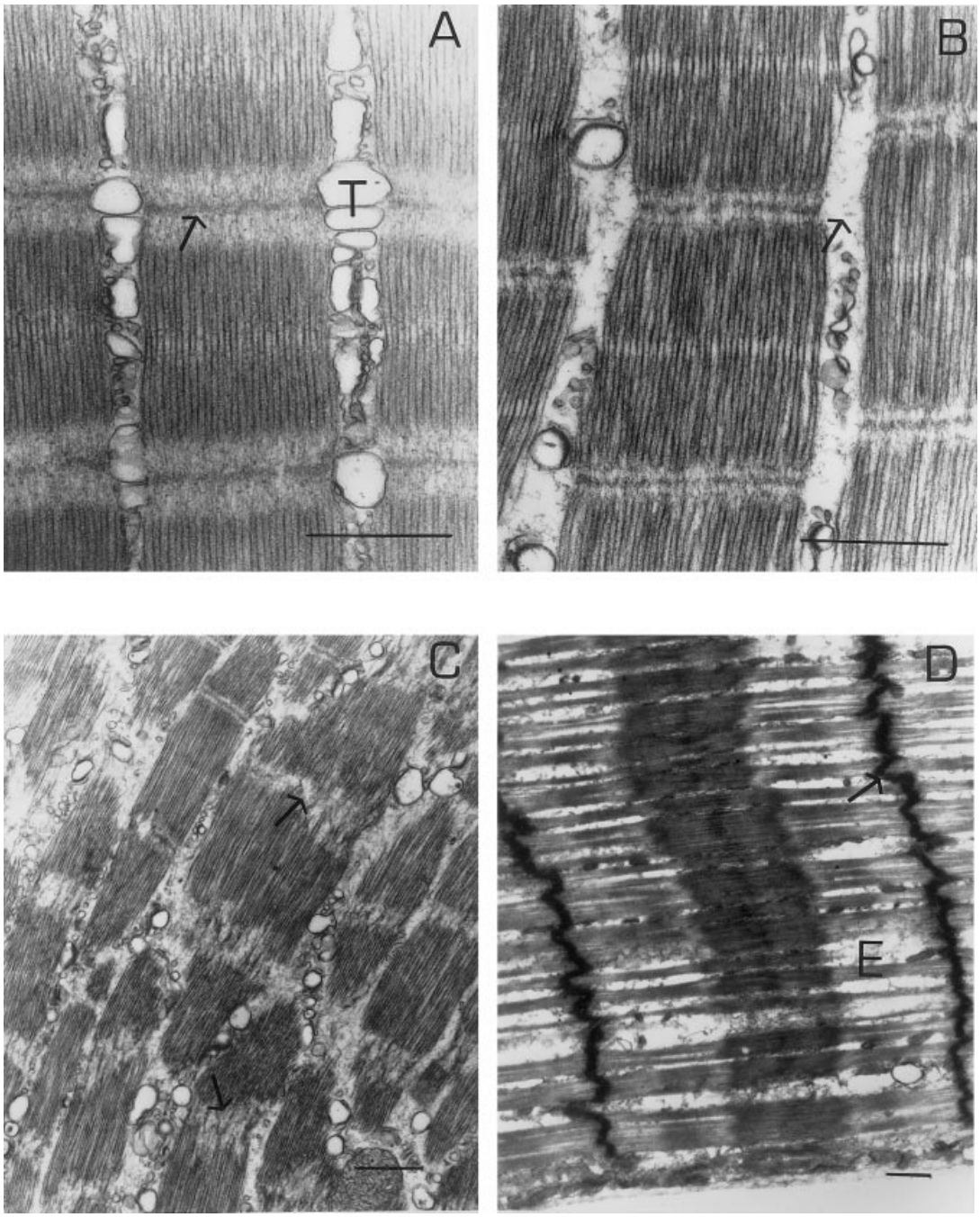

Figure 3 (A) Electron micrograph of normal DAF muscle showing regular sarcomeric structure, clear Z-line (arrow) and well-developed tubular system (T) (bar = 1 μm). (B) Electron micrograph of DAF muscle from lobster showing early signs of muscle necrosis. Note the expansion of the spaces between adjacent fibre bundles, disruption of the tubular system and appearance of granular material in the cytoplasm (arrow) (bar = 1 μm). (C) Electron micrograph from lobster showing progressing signs of necrosis. Note the disorganized appearance of myofibre bundles, expansion of the inter-bundle spaces and degeneration originating at the Z-line (arrows) (bar = 1 μm). (D). Electron micrograph of superficial flexor muscle from *Hematodinium*-infected lobster. Note the clear erosion of material from within the sarcomere (E) and the intact Z-lines (arrows) (bar = 1 μm).

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the vicinity of the Z-line (Fig. 3C). Penetrating between the myofibre interstices of relatively unaffected bundles were sinuous structures, containing filaments (Figs 2D & 4A). In regions of pathological progression, the junctions between intact and degenerated muscle (see also Fig. 2B) were characterized by a dissolution of myofibrillar bundles into granular material (Fig. 4B).

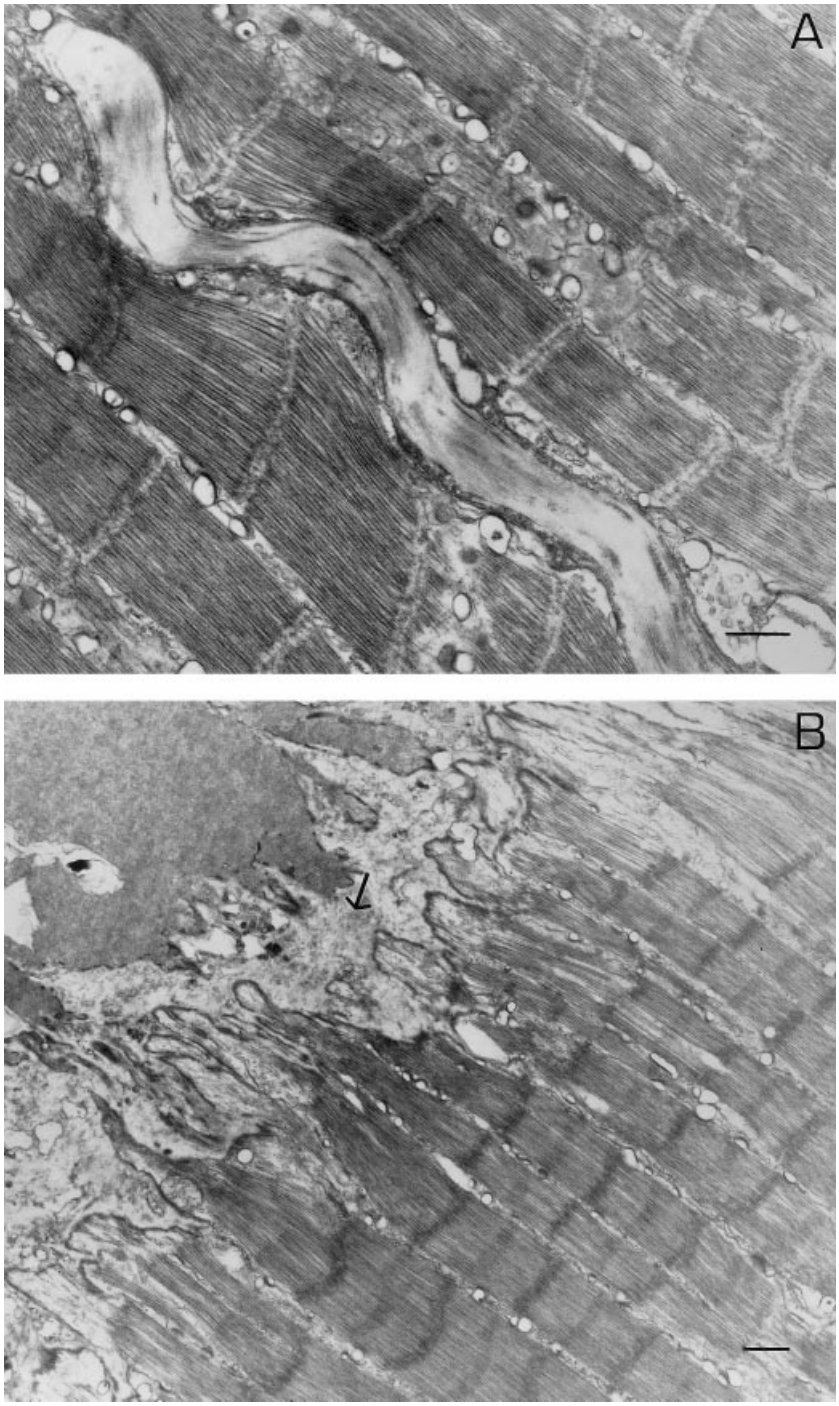

Figure 4 (A) Electron micrograph showing fibrous protrusion in DAF muscle of lobster showing early signs of necrosis (bar = 1 μm). (B) Electron micrograph of junction between relatively intact and severely necrotic DAF muscle. Note the loss of normal sarcomeric organization and appearance of granular material in the region of the junction (arrows) (bar = 1 μm).

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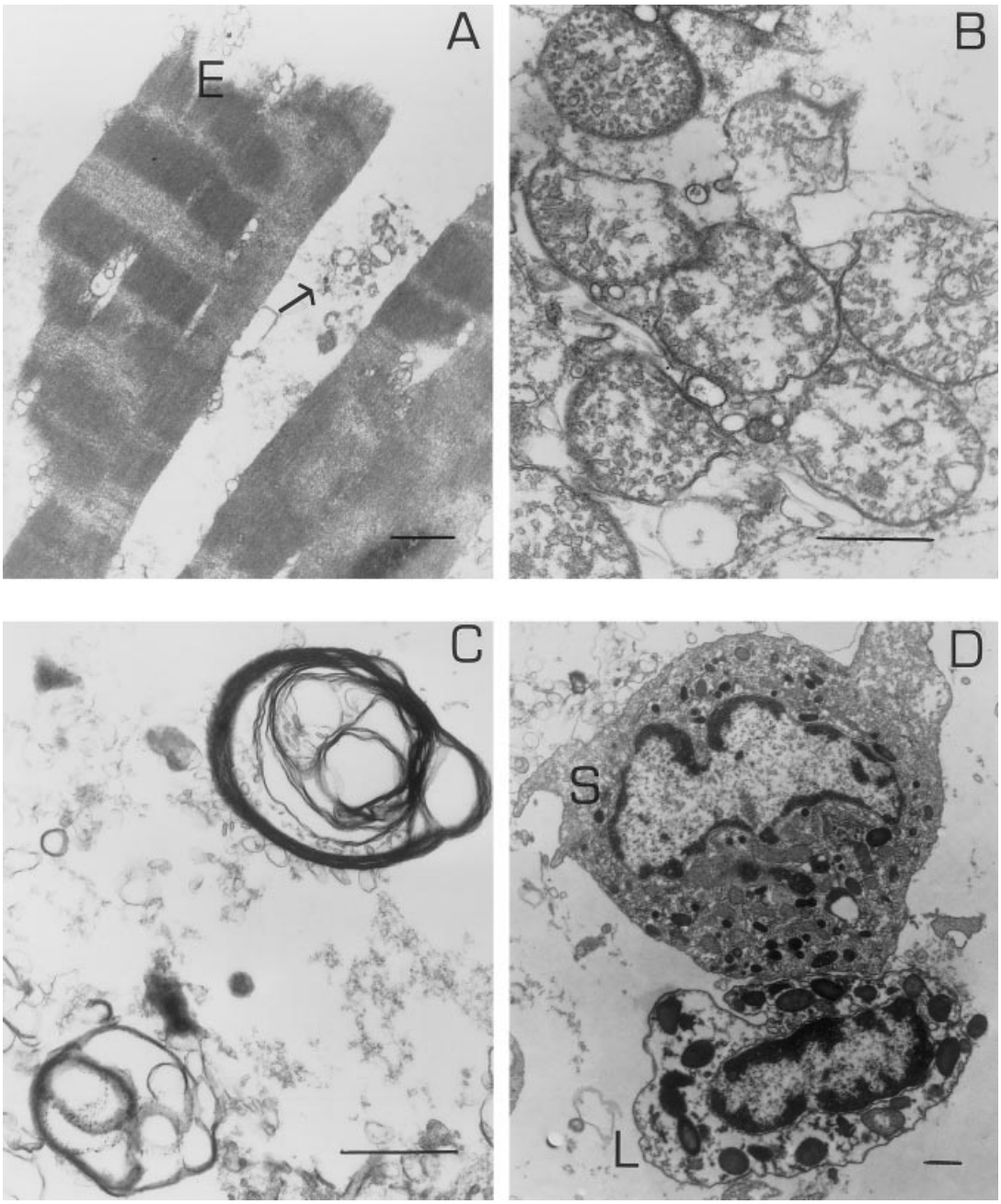

Figure 5 (A) Electron micrograph of densely staining DAF muscle from lobsters showing severe signs of necrosis. Note the erosion of bundles (E) and the presence of granular material (arrow) (bar = 1 μm). (B) Electron micrograph of atrophied mitochondria within the granular cytoplasm of heavily necrotic DAF muscle (bar = 1 μm). (C) Electron micrograph of myelin figures within the granular cytoplasm of heavily necrotic DAF muscle (bar = 1 μm). (D) Electron micrograph of a large (L) and a small (S) granulocyte in the cytoplasm of heavily necrotic DAF muscle (bar = 1 μm).

In areas of severe muscle necrosis in *N. norvegicus*, there was a partial to complete loss of myofibrillar and sarcolemmal structure, with components of the myofibrillar membrane system often the only recognizable feature in the granular cytoplasm separating islands of densely-staining, condensed myofibrillar bundles (Fig. 5A). Necrotic regions of muscle were also characterized by aggregations of atrophied mitochondria within the granular cytoplasm (Fig. 5B), the appearance of myelin

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figures (Fig. 5C) and the infiltration of the damaged tissue by granulocytes (Figs 2C & 5D). Such areas occasionally contained unidentified inclusions measuring 100–150 nm in diameter (Fig. 6).

A certain degree of muscle breakdown was also found to occur in the abdominal muscles of *N. norvegicus* heavily infected by the dinoflagellate parasite *Hematodinium* (Fig. 3D). However, in contrast to the effects of necrosis, this mainly involved erosion of groups of myofilaments within the sarcomeres, with the Z-line region remaining relatively unaffected.

SDS–PAGE analysis of the necrotic condition revealed a major loss of contractile proteins from opaque deep abdominal flexor muscle fibres compared with their amounts in unaffected fibres (Fig. 7). The myosin heavy chain was completely absent, while paramyosin, troponin-T, actin, tropomyosin, troponin-I isoforms and the myosin α-light chain were significantly reduced in quantity.

In a study of trawled *N. norvegicus*, immediately after capture the onset of the necrotic pathology was seen in at least one abdominal muscle fibre bundle in 8% of the 200 lobsters. When these

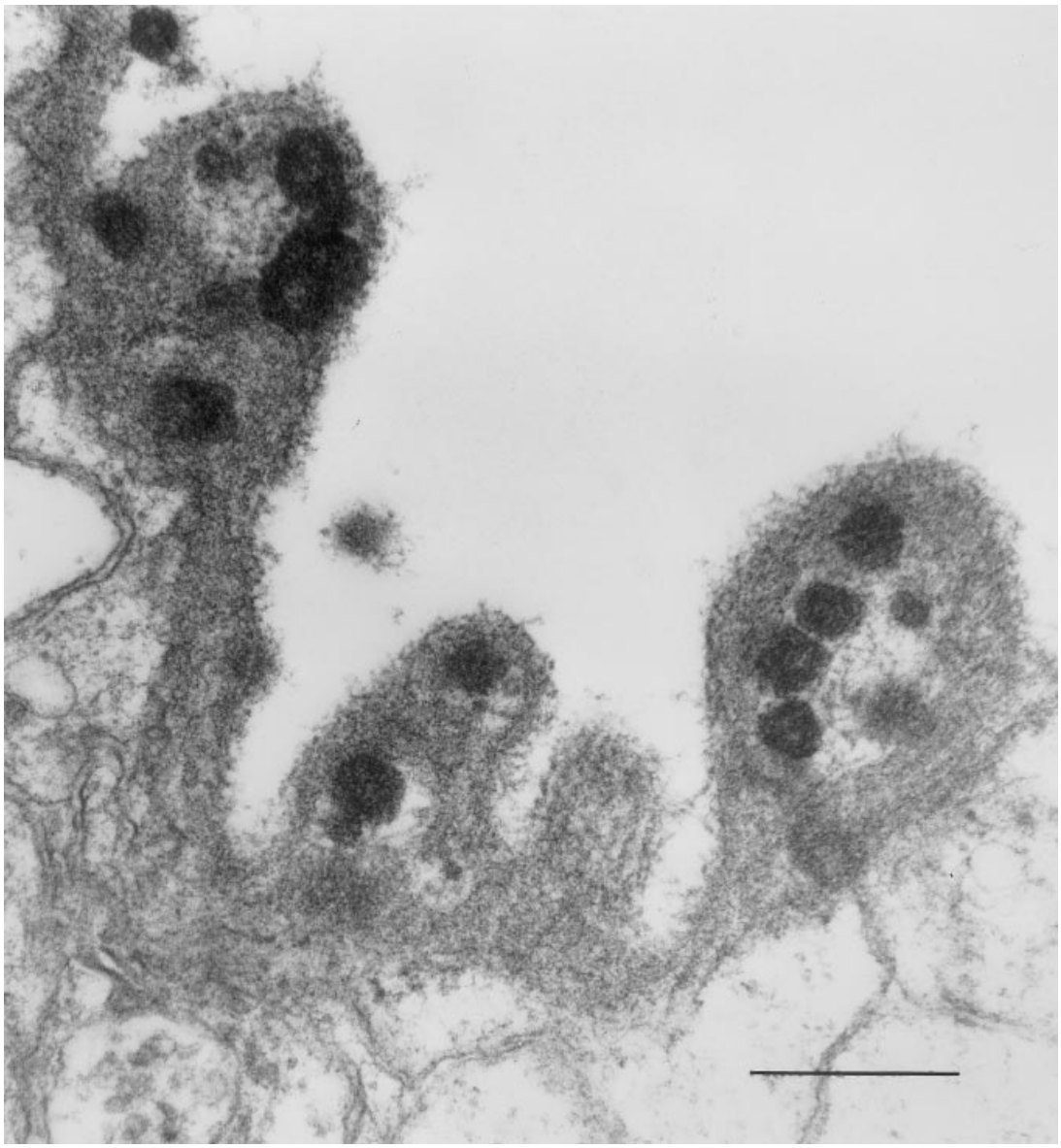

Figure 6 Electron micrograph of unidentified bodies in the cytoplasm of heavily necrotic DAF muscle (bar = 1 μm).

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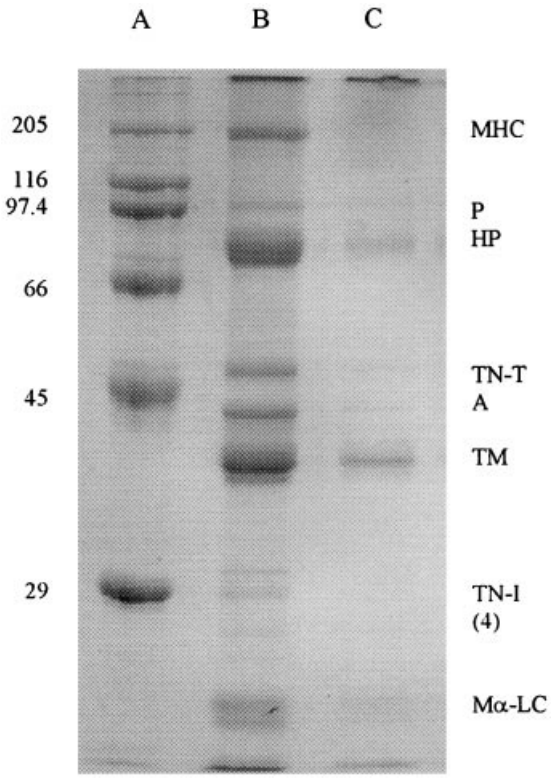

Figure 7 SDS–PAGE (12.5%) gel of contractile proteins from an unaffected muscle fibre (lane B) and a necrotic muscle fibre (lane C) from *N. norvegicus*. Lane C contains a molecular weight marker (weights in kDa given on left of gel). Key: MHC, myosin heavy chain; P, paramyosin 1 and 2; HP, haemolymph proteins; TN-T, troponin-T; A, actin; TM, tropomyosin; TN-I, troponin-I; and Mα-LC, myosin α-light chain.

animals were re-examined 4 h later, after holding them in a covered fish box on deck, this prevalence had increased to 29% (Fig. 8A). In the affected lobsters, opacity was found in various abdominal segments, with those adjacent to the cephalothorax being the most commonly affected (Fig. 8B). It is perhaps significant that visible damage to the abdominal integument (tearing or puncturing) was observed in the vicinity of opaque lesions in 46.5% of the lobsters with signs of muscle necrosis. Small, undetectable lesions or minor damage to the main carapace may have been present in other animals, although this was not investigated.

Discussion

Lack of causative agent

The current study has identified a post-capture, abdominal muscle necrosis of rapid onset in the Norway lobster, *N. norvegicus* captured from the West coast of Scotland. Externally, the condition resembles that of a microsporidian infection. These are common pathogens of crustacean tissue, often causing severe softening, opacity and general degeneration of the musculature (Breed & Olson 1977; Findley *et al.* 1981; Olson & Laman 1984; Langdon 1991; Dennis & Munday 1994; Childers *et al.* 1996). However, a number of features of the observed necrosis are counter-indicative of microsporidian involvement. First, progression of microsporidiosis tends to be much slower than that observed for the condition in *N. norvegicus*. Breed & Olson (1977) report that progression from a light to a heavy microsporidian infection takes approximately 120 days in crangonid shrimps. The condition observed in *N. norvegicus* in this study had a much shorter time course, progressing significantly over a period of 4 h after trawl capture, and leading to death within a further 3–4 days (a feature also noted for spontaneous muscle necrosis in *Penaeus aztecus*—Rigdon & Baxter 1970). Secondly, no microsporidian life stages were visible in

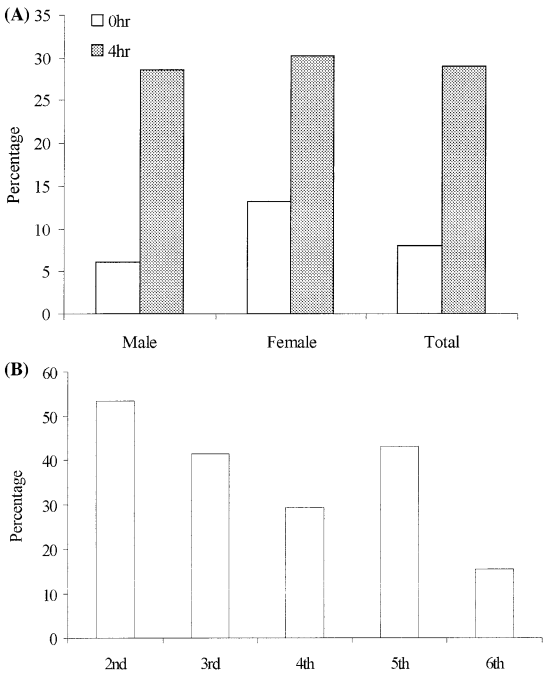

Figure 8 (A) Chart showing the prevalence of muscle opacity symptoms in the abdomens of *N. norvegicus* immediately after (0 h) and 4 h after trawl capture. (B) Chart showing the percentage of *N. norvegicus* exhibiting muscle opacity in different abdominal segments 4 h after trawl capture. Addition of percentages to above 100% indicates that lobsters regularly showed muscle opacity in more than one abdominal segment.

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any of the histological sections of abdominal muscle tissue, examined by either light- or electron-microscopy.

Indeed, no other pathogenic agents were detected within the abdominal musculature, although in certain specimens some unidentified bodies of approximately 150 nm in diameter were detected within severely damaged regions of muscle (see Fig. 6). The possibility that these bodies are viruses cannot be ruled out at present, especially as viruses are known to cause a similar necrosis condition in post-larval stages of the freshwater prawn, *Macrobrachium rosenbergii* (Arcier *et al.* 1999). The identity of these bodies and their possible involvement in the muscle necrosis in *N. norvegicus* requires further investigation. There is also no evidence for any bacterial involvement in the pathology seen in *N. norvegicus*. Although bacteria have also been implicated in a similar muscle necrosis and rapid death syndrome in *M. rosenbergii* (Cheng & Chen 1998), and may also be a secondary consequence of necrosis in the same species (Nash *et al.* 1987).

The pathology of necrosis

Due to the absence of any obvious causative agent, the seasonal nature of the condition in creel-caught animals (appearing in the summer and autumn) and the propensity for trawled animals to become affected even during the winter months, the opaque muscle condition noted in *N. norvegicus* resembles the spontaneous, idiopathic muscle necrosis noted in a number of crustacean species; in *Cherax terminatus* (Evans *et al.* 1999), *M. rosenbergii* (Delves-Broughton & Poupard 1976; Akiyama *et al.* 1982; Nash *et al.* 1987; Anderson *et al.* 1990), *P. aztecus* (Rigdon & Baxter 1970; Lakshmi *et al.* 1978), *Penaeus japonicus* (Momoyama & Matsuzato 1987), *Procambarus clarkii* (Lindqvist & Mikkola 1978) and also in the fish *Stizostedion vitreum* (Holloway & Smith 1982).

Indeed, histological features of the condition in *N. norvegicus*, including a general loss of myofibrillar and sarcomeric structure, coupled with differential reaction of necrotic muscle tissue to histological staining, are similar to those reported in muscle necrosis of *P. japonicus* (Momoyama & Matsuzato 1987) and *P. aztecus* (Rigdon & Baxter 1970). The appearance of sinuous filaments with a fibrous structure is similar to that noted during muscle necrosis in *P. japonicus*, where the production of collagenous fibres was extensive in mid to late progression of the condition (Momoyama & Matsuzato 1987). The presence of myelin figures and atrophied mitochondria in conjunction with infiltration of degenerated areas of tissue by granulocytes is consistent with the features ascribed to idiopathic muscle necrosis in *M. rosenbergii* (Nash *et al.* 1987).

Loss of Z-line material, which is a prominent feature of necrosis in *N. norvegicus*, is reported to occur in a number of pathological and physiological conditions (Kumudavalli Reddy, Etlinger, Rabinowitz & Fischman 1975) and also represents an early step in pre-moult muscle atrophy in crustaceans (Mykles & Skinner 1990a). The calcium-dependent proteases involved in pre-moult atrophy are localized in the sarcoplasm (Mykles & Skinner 1990b) and it is conceivable that the necrotic condition observed in *N. norvegicus* involves activation of these proteases, leading to the initial breakdown of Z-line material. Interestingly, the breakdown of abdominal muscle fibres induced by *Hematodinium* infection in *N. norvegicus* does not disrupt the Z-line, but rather involves the erosion of groups of myofilaments within the sarcomeres. This suggests that the proteolytic processes that occur in this parasitic infection are different to those occurring in necrosis.

Factors which induce the onset of necrosis

This study has shown that muscle opacity can be observed in all segments of the abdomen, but with a tendency for the condition to occur in the segments adjacent to the cephalothorax. A similar feature was noted in idiopathic muscle necrosis in *P. japonicus* (Momoyama & Matsuzato 1987) and *C. tenuimanus* (Evans *et al.* 1999), while others have reported the necrotic condition to be associated with the more distal abdominal segments in *P. aztecus* (Rigdon & Baxter 1970; Lakshmi *et al.* 1978) and *M. rosenbergii* (Akiyama *et al.* 1982; Nash *et al.* 1987). The mechanism underlying the location of lesions is not known, but it is possible that breaches in the integument (seen in almost half of those lobsters exhibiting signs of the pathology) may provide foci for initiation of necrosis, the progression of which is exacerbated by subsequently imposed stressors.

It is well known that crustaceans are exposed to a considerable array of stressors during and after capture (including crowding, air exposure, light exposure, heat exposure and mechanical damage)

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and a number of studies have considered the expression of biochemical and molecular stress indicators during commercial handling of crustaceans (see Jussila, Jago, Tsuetnenko, Dunstan & Evans 1997; Paterson & Spanoghe 1997; Chang, Chang, Keller, Sreenivasula Reddy, Snyder & Spees 1999). *Nephrops norvegicus* will certainly be exposed to such stresses during trawl capture. Most studies of spontaneous muscle necrosis in crustaceans also report an induction of hyperactivity in animals prior to the onset of necrosis. Such hyperactivity in animals which are habitually sedentary is a common response to a number of stressors, and is followed by exhaustion and elevated levels in haemolymph L-lactate, which can remain elevated for 24–48 h after the stressor has been removed (see Nash *et al.* 1987). Extreme exertion in strongly glycolytic muscles such as the deep abdominal flexor muscle also causes rapid utilization of glycogen, generating local heat and lactic acid, both of which are known to have degenerative effects on the affected and the surrounding muscle fibres (Hulland 1985). It is, therefore, conceivable that during capture and handling of *N. norvegicus*, the repetitive 'tail flipping' that is commonly induced could be causing these types of effect. Initiation of the condition, possibly caused by damage to the abdominal integument, may then be exacerbated by further stressful post-capture holding conditions.

Once the condition in *N. norvegicus* muscle is established and begins to progress, it does so extremely rapidly, with complete necrosis of the tail muscle and death within days. It has been suggested that the rapid progression of necrosis may be linked to the infiltration of the muscle by activated granulocytes and their subsequent production of superoxide radicals which induce lipid peroxidation, damage membranes and kill cells (Fridovich 1978; Nash *et al.* 1987; Di Giulio, Washburn, Wenning, Winston & Jewell 1989). However, the role of O_2^- and other reactive oxygen intermediates in the necrosis of *N. norvegicus* muscle remains to be established.

It has been noted in *P. aztecus* that the appearance of muscle necrosis can be reversed if the environmental stressors are removed soon after onset of the condition (Rigdon & Baxter 1970; Lakshmi *et al.* 1978). The data presented in this study for the prevalence of muscle opacity immediately after trawl capture, and 4 h later (Fig. 8a) reinforce the hypothesis that the holding conditions of the animals in the period immediately after capture are crucial in determining whether the necrotic condition develops or regresses. This has important consequences both for the vivier transport of live *N. norvegicus* and the quality of the meat in 'tailed' lobsters. It may also contribute to the high mortality of discarded *N. norvegicus* which are returned to the sea after trawl capture and several hours of emersion (Ulmestrand, Valentinsson, Sangster, Bova, Kynoch, Breen, Graham, Soldal, Cruikshank, Moth-Poulson & Lowry 1998). The apparent effect of damage to the integument and the reported reversal of the similar necrotic condition in *P. aztecus* requires further investigation in *N. norvegicus* and may lead to advisory measures for the post-capture handling of this commercially important species.

Acknowledgements

This work was supported by a contract from the Ministry of Agriculture, Fisheries & Food (MAFF) of the United Kingdom (Contract No. CSA 4172, Project code MFO221). The authors wish to thank Mr Mick Parker and the crew of the RV '*Aora*' (University Marine Biological Station Millport) for assistance in the collection of trawled animals, Mr Gordon Goldsworthy of Loch Fyne Fisheries, for notification of the condition from the West coast fishery and the supply of creel-caught animals from the Sound of Jura, Ms Margaret Mullin for assistance with light and electron microscopy and Professor Keith Vickerman for his valued opinion on the interpretation of histological material.

References

Akiyama D.M., Brock J.A. & Haley S.R. (1982) Idiopathic muscle necrosis in the cultured freshwater prawn (*Macrobrachium rosenbergii*). *Veterinary Medicine and Small Animal Clinician* **77,** 1119–1121.

Anderson I.G., Nash G. & Shariff M. (1990) Mass larval mortalities in giant freshwater prawn, *Macrobrachium rosenbergii* de Man, cultured in Malaysian modified static 'green water' systems. *Journal of Fish Diseases* **13,** 127–134.

Arcier J.-M., Herman F., Lightner D.V., Redman R.M., Mari J. & Bonami J.R. (1999) A viral disease associated with mortalities of hatchery–reared postlarvae of the giant freshwater prawn *Macrobrachium rosenbergii. Diseases of Aquatic Organisms* **38,** 177–181.

Breed G.M. & Olson R.E. (1977) Biology of the microsporidian parasite *Pleistophora crangoni* n. sp. in three species of crangonid sand shrimps. *Journal of Invertebrate Pathology* **30,** 387–405.

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Cawthorn R.J. (1997) Overview of 'bumper car' disease – impact on the North American lobster fishery. *International Journal of Parasitology* **27,** 167–172.

Chang E.S., Chang S.A., Keller R., Sreenivasula Reddy P., Snyder M.J. & Spees J.L. (1999) Quantification of stress in lobsters: crustacean hyperglycaemic hormone, stress proteins and gene expression. *American Zoologist* **39,** 487–495.

Cheng W. & Chen J.-C. (1998) Isolation and characterization of an Enterococcus–like bacterium causing muscle necrosis and mortality in *Macrobrachium rosenbergii* in Taiwan. *Diseases of Aquatic Organisms* **34,** 93–101.

Childers R.K., Reno P.W. & Olson R.E. (1996) Prevalence and geographic range of *Nadelspora canceri* (Microspora) in Dungeness crab *Cancer magister*. *Diseases of Aquatic Organisms* **24,** 135–142.

Delves-Broughton J. & Poupard C.W. (1976) Disease problems of prawns in recirculation systems in the UK. *Aquaculture* **7,** 201–217.

Dennis D.M. & Munday B.L. (1994) Microsporidiosis of palinurid lobsters from Australian waters. *Bulletin of the European Association of Fish Pathologists* **14,** 16–18.

Di Giulio R.T., Washburn P.C., Wenning R.J., Winston G.W. & Jewell C.S. (1989) Biochemical responses in aquatic animals: a review of determinants of oxidative stress. *Environmental Toxicology & Chemistry* **8,** 1103–1123.

Evans L.H., Fotedar S., Fan A. & Jones B. (1999) Investigation of idiopathic muscle necrosis and circulating hemocytes in the freshwater crayfish *Cherax tenuimanus* exposed to acute and chronic stressors. *Freshwater Crayfish* **12** (in press).

Field R.H. & Appleton P.L. (1995) A *Hematodinium*-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. *Diseases of Aquatic Organisms* **22,** 115–128.

Findley A.M., Blakeney Jr. E.W. & Weidner E.H. (1981) *Ameson michaelis* (Microsporidia) in the blue crab, *Callinectes sapidus*: parasite induced alterations in the biochemical composition of host tissues. *Biological Bulletin* **161,** 115–125.

Fridovich I. (1978) The biology of oxygen radicals. *Science* **201,** 875–880.

FRS (1999) *Nephrops stocks around Scotland*, Fisheries Research Service (FRS) fish stocks information leaflet; December 1999..

Holloway Jr. H.L. & Smith C.E. (1982) A myopathy in North Dakota walleye, *Stizostedion vitreum* (Mitchill). *Journal of Fish Diseases* **5,** 527–530.

Hulland T.J. (1985) Muscles and tendons. In: *Pathology of Domestic Animals* (ed. by K.V.F. Jubb, P.C. Kennedy & N. Palmer), vol. 1, pp. 139–199. Academic Press, London.

Jussila J., Jago J., Tsuetnenko E., Dunstan B. & Evans L.H. (1997) Total and differential haemocyte counts in western rock lobsters (*Panulirus cygnus* George) under post-harvest stress. *Marine & Freshwater Research* **48,** 863–867.

Kabre G. (1992) Effects of the microsporidian *Thelohania contejeani* on the metabolism of total muscular and hepatopancreatic phospholipids in the crayfish *Austropotamobius pallipes*, Le Reboulet, 1858. *Annales des Sciences Naturelles Zoologie* **13,** 97–102.

Kumudavalli Reddy M., Etlinger J.D., Rabinowitz M. & Fischman D.A. (1975) Removal of Z-lines and α-actinin from isolated myofibrils by a calcium-activated neutral protease. *Journal of Biological Chemistry* **250,** 4278–4284.

Laemmli U.K. (1970) Cleavage of structural proteins during assembly of the head of the bacteriophage T4. *Nature* **227,** 180–185.

Lakshmi G.J., Venkataramiah A. & Howse H.D. (1978) Effect of salinity and temperature changes on spontaneous muscle necrosis in *Penaeus aztecus* Ives. *Aquaculture* **13,** 35–43.

Langdon J.S. (1991) Microsporidiosis due to a pleistophorid in marron, *Cherax tenuimanus* (Smith) (Decapoda: Parastacidae). *Journal of Fish Diseases* **14,** 33–44.

Lindqvist O.V. & Mikkola H. (1978) On the etiology of the muscle wasting disease in *Procambarus clarkii* in Kenya. *Freshwater Crayfish* **4,** 363–372.

Momoyama K. & Matsuzato T. (1987) Muscle necrosis of culture kuruma shrimp (*Penaeus japonicus*). *Fish Pathology* **22,** 69–75.

Mykles D.L. & Skinner D.M. (1990a) Calcium-dependent proteinases in crustaceans. In: *Intracellular Calcium-Dependent Proteolysis* (ed. by R.L. Mellgren & T. Murachi), pp. 139–154. CRC Press, Boca Raton.

Mykles D.L. & Skinner D.M. (1990b) Atrophy of crustacean somatic muscle and the proteinases that do the job. A review. *Journal of Crustacean Biology* **10,** 577–594.

Nash G., Chinabut S. & Limsuwan C. (1987) Idiopathic muscle necrosis in the freshwater prawn, *Macrobrachium rosenbergii* de Man, cultured in Thailand. *Journal of Fish Diseases* **10,** 109–120.

Neil D.M., Fowler W.S. & Tobasnick G. (1993) Myofibrillar protein composition correlates with histochemistry in fibres of the abdominal flexor muscles of the Norway lobster *Nephrops norvegicus*. *Journal of Experimental Biology* **183,** 185–201.

Olson R.E. & Laman C.N. (1984) Prevalence of microsporidian infection in commercially caught pink shrimp, *Pandalus jordani*. *Journal of Invertebrate Pathology* **43,** 407–413.

Paterson B.D. & Spanoghe P.T. (1997) Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters (*Panulirus cygnus*) during commercial handling. *Marine & Freshwater Research* **48,** 829–834.

Rigdon R.H. & Baxter K.N. (1970) Spontaneous necroses in muscles of brown shrimp *Penaeus aztecus* Ives. *Transactions of the American Fisheries Society* **3,** 583–587.

Shields J.D. (1994) The parasitic dinoflagellates of marine crustaceans. *Annual Review of Fish Diseases* **4,** 241–271.

Spurr A.R. (1969) A low viscosity epoxy embedding resin for electron microscopy. *Journal of Ultrastuctural Research* **26,** 31–43.

Stentiford G.D., Neil D.M. & Coombs G.H. (2000) Alterations in the biochemistry and ultrastructure of the deep abdominal flexor muscle of the Norway lobster, *Nephrops*

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norvegicus L. during infection by a parasitic dinoflagellate of the genus *Hematodinium*. *Diseases of Aquatic Organisms* **42,** 133–141.

Stewart J.E. (1980) Diseases. In: *The Biology and Management of Lobsters* (ed. by J.S. Cobb & B.F. Phillips), pp. 301–342. Academic Press, New York.

Ulmestrand M., Valentinsson D., Sangster G.I., Bova D., Kynoch R.J., Breen M., Graham G.N., Soldal A.V., Cruikshank O., Moth-Poulson T. & Lowry N. (1998) *Nephrops survival after escape from commercial fishing gear or discarded from deck*, ICES Nephrops life history study group meeting, La Coruña, Spain.

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