



## Minireview

Diseases of *Nephrops* and *Metanephrops*: A reviewGrant D. Stentiford<sup>a,\*</sup>, Douglas M. Neil<sup>b</sup><sup>a</sup> European Community Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Barrack Road, Weymouth, Dorset DT4 8UB, United Kingdom<sup>b</sup> Department of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom

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## ABSTRACT

*Nephrops* and *Metanephrops* are commercially exploited genera within the family Nephropidae (clawed lobsters). Commercial fisheries for each genus exist in the Northern and Southern Hemispheres and utilise trawling or trapping for capture. Despite a relative lack of dedicated disease surveys on lobsters from these fisheries, several important symbionts and pathogens have been described. The most significant known pathogen of *Metanephrops* (*challengeri*) is a microsporidian parasite (*Myospora metanephrops*) which causes destruction of the skeletal and heart muscles of infected lobsters while the most significant known pathogen of *Nephrops* (*norvegicus*) is a dinoflagellate parasite assigned to the genus *Hematodinium*. This parasite has been responsible for an ongoing epidemic in fished populations of *N. norvegicus* in Northern Europe since at least the early 1980s and since then extensive studies on its life history and pathogenesis have occurred. Despite these research efforts significant gaps exist in our knowledge of the effects of parasites such as *Hematodinium* on the fished and non-fished portions of *Nephrops* populations and on the effect of fishery practices on the spread of infection. Furthermore, little is known about the effect of this (and other) pathogens on cohort survivability and the likelihood that early life stages will be effectively recruited to the fishery. This review summarises the available literature on diseases of these two lobster genera and provides an assessment of future research needs in this discipline.

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## 1. The Clawed lobsters

Four families comprise the crustacean group referred to as the lobsters; Nephropidae (clawed lobsters), Palinuridae (spiny lobsters), Synaxidae (furry lobsters) and the Scyllaridae (Slipper lobsters). Despite the numerous species that comprise these families, only relatively few are commercially important since most are small, do not aggregate or live in deep water – life history traits that make their exploitation less feasible (Dow, 1980; Holthuis, 1991). This review covers diseases of the clawed lobsters comprising the Nephropidae, with Palinurid lobsters covered elsewhere within this volume. The Family Nephropidae (Dana, 1852) includes the sub-family Nephropinae (Dana, 1852) which contains a number of commercially exploited genera including *Homarus*, *Nephrops* and *Metanephrops*. Two other genera, *Eunephrops* and *Thymopides* are also classified within the Nephropinae. Diseases affecting lobsters of the genus *Homarus* are covered in a separate review within this volume. Furthermore, lobsters within the genera *Eunephrops* and *Thymopides* are deep-water species with limited distribution in the Western Atlantic (*Eunephrops bairdii*, *Eunephrops cadensi* and *Eunephrops manningi*) and southern Indian Ocean (*Thymopides grobovi*, *Thymopides laurentae*), respectively. Whilst lobsters within these genera are large enough for exploitation and thus are of potential interest for fishery exploitation, lack of detailed knowledge on their distribution and life history coupled with their deep water habit (up to 1200 m) preclude a profitable fishery at present (Burukovsky and Averin, 1977; Holthuis, 1991; Poupin, 1993; Diaz et al., 2003 Segonzac and MacPherson, 2003). In light of the current review, it is perhaps not surprising that no published information exists on the pathogen fauna of these lobster genera. With the exception of the Homarids, lobsters within the genera *Nephrops* and *Metanephrops* are the only commercially exploited members of the Nephropidae.

### 1.1. Genus *Metanephrops*

The genus *Metanephrops* is comprised of a number of species, some of which are the subject of significant commercial fisheries. All described species are distributed within the Indo-Pacific region, with identified populations in Madagascar and Africa (*Metanephrops mozambicus*), Indonesia (*Metanephrops andamanicus*, *Metanephrops velutinus*, *Metanephrops sibogae*), Taiwan (*Metanephrops armatus*, *Metanephrops formosanus*), Hong Kong (*Metanephrops neptunus*), Japan (*Metanephrops japonicus*, *Metanephrops thompsoni*), Australia (*Metanephrops australensis*, *Metanephrops challengerii*, *M. sibogae*, *M. velutinus*) and New Zealand (*M. challengerii*). All species inhabit muddy substrates at depths of between 150 and 800 m and the majority of species are thought to burrow. The most readily exploited species (*M. andamanicus*, *M. challengerii*, *M. japonicus*, *M. mozambicus*, *M. thompsoni*) are captured in trawl fisheries following emergence from their burrows. The largest fishery exists in New

Zealand (up to 1000 tonnes of *M. challengerii* per annum) (Fig. 1) with the global fishery for *Metanephrops* species often exceeding 1200 tonnes per annum (source: [www.fao.org](http://www.fao.org)).

### 1.2. Genus *Nephrops*

The genus *Nephrops* contains a single species, the Norway lobster *Nephrops norvegicus* (originally *Cancer norvegicus* Linnaeus, 1758). It is found in large commercially exploited populations in the eastern Atlantic region from Iceland, the Faroes and Norway in the North of its range to the Atlantic coast of Morocco and the Mediterranean Sea in the south. Unlike *Metanephrops* species, *N. norvegicus* can be found at depths as shallow as 20 m, with maximum depths of 800 m. It is an important member of the burrowing marine benthic community on soft sediments, and over the past 50 years it has been the subject of a major fishery in the northeast Atlantic (Bell et al., 2006). In addition, due to its availability, adaptability to aquarium conditions and convenience for use as a laboratory model, it has been widely studied by the scientific community and numerous publications describe the feeding ecology (Loo et al., 1993; Cristo, 1998), reproduction (Farmer, 1974a), moult cycle (González-Gurriarán et al., 1998), behaviour (Rice and Chapman, 1971; Farmer, 1974b,c; Aréchiga and Atkinson, 1975; Atkinson and Naylor, 1976; Newland and Chapman, 1989) and fishery for this species. Considerable advances in our understanding of the life history of *N. norvegicus* in the field have assisted with the management of *N. norvegicus* as a fisheries target (Tuck et al., 1997a,b; Merella et al., 1998; Sardá, 1998), though as for most other marine



Fig. 1. *Metanephrops challengerii* captured from the fishery off Southern New Zealand. Male lobsters (right) and egg-bearing female lobsters (left). Image courtesy of Dr. Ian Tuck, National Institute of Water and Atmospheric Research (NIWA), New Zealand.

fisheries, significant gaps exist with regard to drivers of recruitment and mortality in the fishery. In recent years, *N. norvegicus* has become one of the most important shellfish species captured in the northeast Atlantic, with annual landings of around 60,000 tonnes (source: [www.fao.org](http://www.fao.org)). The bulk of landings are from trawler capture, with lobsters usually being 'tailed' at sea and landed as 'scampi'. A trapping fishery also exists for the capture of larger animals in sheltered waters or where trawling is not feasible. Trapped animals are usually landed and transported live to distant markets. Such animals fetch a higher unit price but must be of high vigour to survive the rigors of handling and transportation (Stentiford and Neil, 2000; Ridgway et al., 2006).

The natural history of *N. norvegicus* impinges upon its availability to the fishery. Lobsters are captured when present on the surface of the sediment (Farmer, 1974c). Female lobsters spend much of the winter within their burrows incubating eggs and are thus largely unavailable to the fishery during these times, causing a strong predominance of males in the catches (Farmer, 1974b). The feeding ecology of the female lobster during incubation is not well understood, though suspension feeding may play a significant role in nutrient supplementation (Loo et al., 1993). Following spawning, females emerge from the burrow to feed, moult and be mated by hard-shelled male lobsters (Farmer, 1974a). On the sediment surface, capture by trawlers is further affected by the ability of lobsters to perform escape swimming. Following disturbance by the trawling apparatus, lobsters undergo a series of rapid abdominal flexions and extensions (tail flips), which propel the animal backwards (Newland et al., 1992). Both the speed and endurance of tail flip swimming have implications for capture by trawl nets. Once within the net, the retention of captive lobsters is dependent upon the size of the lobster, the mesh size and the crowding of the net with other species.

## 2. Diseases of *Metanephrops*

Despite a significant fishery or fishery potential for members of the *Metanephrops* genus, the combination of their deep-sea habit, their limited potential for culture (Holthuis, 1991) and their localised sale as fresh or frozen product likely defines the paucity of published reports on disease in this genus. However, the aggregated distribution that allows their capture in commercially attractive quantities also suggests a rather gregarious habit similar to that of their sister genera *Nephrops*. Since dedicated disease surveys of *Metanephrops* have not been carried out, it is not surprising that descriptions of viral, bacterial, fungal or protozoan pathogens are absent from the literature. The literature does however contain a few reports of crustacean (copepod and isopod) infestations of *Metanephrops* collected incidentally through faunal surveys of the Indo-Pacific region.

*Nicothoe analata*, a parasitic copepod was described attached to the gills of specimens of *Nephrops* (= *Metanephrops*) *sinensis* captured from the South China Sea (Kabata, 1966). Later studies by the same author described a further two species of the same genera (*N. brucei* and *N. simplex*); *N. brucei* infecting *Nephrops* (= *Metanephrops*) *sagamiensis* collected from Japan and from *Nephrops* (= *Metanephrops*) *andamanacus* collected from South Africa, and *N. simplex* infecting *Nephrops* (= *Metanephrops*) *japonicus* collected from Japan. Furthermore, the host range for *N. analata* was extended to *Nephrops* (= *Metanephrops*) *boschmai* and *Nephrops* (= *Metanephrops*) *andamanacus* from Australia and Bali, *Nephrops* (= *Metanephrops*) *sagamiensis* from Japan, and *Nephrops* (= *Metanephrops*) *sibogae* from Indonesia (Kabata, 1967). The description of members of this genus infecting *Metanephrops* follows work on a similar species, *Nicothoe astaci* infecting the European lobster (*Homarus gammarus*) from Scotland (Audoin and Milne-Edwards,

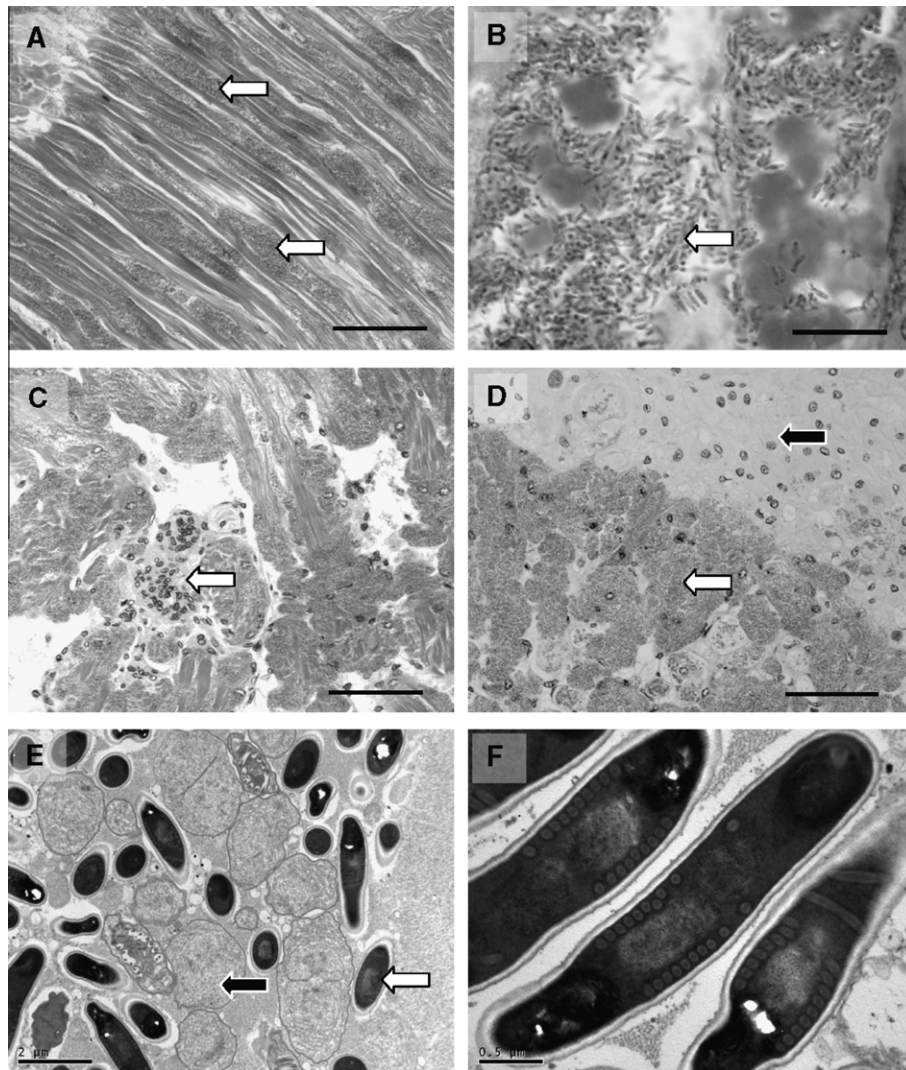
1826, in Mason, 1959). The genus is considered truly parasitic since it attaches to the gill lamellae of host lobsters and draws haemolymph via its styliform mandibles that pierce the cuticle (Mason, 1959; Shields et al., 2006). Impact of infection on the host has not been reported though prevalence is highest in soft-shelled *H. gammarus* (Mason, 1959) and parasites are likely shed during moulting. No data is available on the prevalence of these parasites in *Metanephrops* species or on their likely impact on host populations.

The velvet lobster (*M. velutinus*) supports a commercial export fishery in Western Australia (Holthuis, 1991). Additionally, *M. velutinus* has been captured from the western Indo-Pacific as part of faunal surveys of the region. As part of these surveys, *M. velutinus* has been shown to be type host for the parasitic bopyrid isopod *Pseudione nephropis* (Shiino, 1951; Markham, 1999). *Pseudione* forms a heterogeneous group of separate sex isopods containing numerous species infecting a diverse range of decapod hosts including carideans, thalassinoids, galatheoids, pagurids, lithodids and nephropids. Infection by *Pseudione* manifests as a distension of the affected gill chamber and atrophy of the gill lamellae (Paradiso et al., 2004) and has been associated with significant growth reduction and possible physiological castration in other decapod hosts (Munoz, 1997; Roccatagliata and Lovrich, 1999; Gonzalez and Acuna, 2004). Based upon the single description of *P. nephropis* infection in *M. velutinus*, no information is available on the individual or population impact of this parasite.

Perhaps the most significant known pathogen of the genus is the recently discovered microsporidian *Myospora metanephrops* (Stentiford et al., 2010). The parasite was discovered during fishing surveys of commercially significant stocks of *M. challengerii* off New Zealand. Following trawl capture, affected lobsters were clearly distinguishable from their non-affected counterparts due to lethargy and an apparent alteration in the normal pigmentation and translucency of the carapace of the cephalothorax, abdomen and limbs. When observed ventrally, the large abdominal flexor muscles of affected lobsters were clearly visible through the arthroal membranes, while muscles extending into the telson blades were imparted with a distinctive white and opaque appearance which clearly contrasted the hyperpigmented telson carapace. Histology reveals a widespread infection of the skeletal and heart musculature in addition to the longitudinal and circular muscles surrounding the hepatopancreatic tubules and the mid- and hind-gut. In heavily infected lobsters, cysts packed with merogony and sporogony stages of the parasite largely replaced muscle fibres and constituent myofibrils of the major abdominal flexor muscles of the abdomen. Transmission electron microscopy of infected muscle fibres revealed multiple stages of the microsporidian parasite with development progressing through merogony and sporogony to the production of mature spores (Fig. 2). All stages were diplokaryotic. Analysis of the SSU rDNA of the microsporidian revealed closest similarity to other muscle infecting microsporidians from marine crustacean hosts (Stentiford et al., 2010). Interestingly, *M. metanephrops* is the first example of a microsporidian parasite in the clawed lobsters. Further work is now required to investigate the commercial significance of this parasite, both in terms of population level mortality and effects on the marketability of musculature extracted from infected animals.

## 3. Diseases of *Nephrops*

The relatively replete literature concerning symbionts and pathogens of *Nephrops* compared to the other genera of clawed lobster (exclusive of *Homarus*) is likely testimony to the large numbers of studies on the ecology of this genus. Such ecological studies when coupled with those describing the fishery for *Nephrops* across



**Fig. 2.** *Myospora metanephrops* infection in musculature of *Metanephrops challengerii* (Stentiford et al., 2010). (A) Light micrograph of parasite cysts (white arrows) between intact myofibrils of abdominal musculature. Bar = 200  $\mu$ m. (B) Light micrograph of spore stages liberated from a ruptured cyst in the abdominal musculature (arrow). Bar = 25  $\mu$ m. (C) Light micrograph of infection of muscle fibres of heart myocardium with associated haemocyte infiltration (arrow). Bar = 100  $\mu$ m. (D) Light micrograph of heavily infected heart myocardium (white arrow) with minimal involvement of spongy pericardial cells (black arrow). Bar = 100  $\mu$ m. (E). Transmission electron micrograph of abdominal muscle fibre containing early (meront, sporont, sporoblast – black arrow) and late (spore – white arrow) parasite stages. Bar = 2  $\mu$ m. (F). Transmission electron micrograph of mature spores showing elongate nature, diplokaryotic nuclei and approximately 11 turns of the polar filament coil. Bar = 0.5  $\mu$ m.

its natural range have led to the description of a number of important symbionts, some of which have been shown to have a significant detrimental effect on host populations. The relatively gregarious nature of *Nephrops*, its burrowing habit and its existence in large, relatively uniform and sessile populations may contribute to its suitability as a host and may assist with the transmission of symbionts and pathogens between individuals. The remainder of this chapter is devoted to a synthetic description of known symbionts and pathogens of the genus *Nephrops*. Furthermore, an up to date analysis of the most significant pathogenic disease of *Nephrops* (the parasitic dinoflagellate *Hematodinium*) is provided.

### 3.1. Epizoic symbionts

#### 3.1.1. Symbiont pandora

*N. norvegicus* collected from the Kattegat Straits between Denmark and Sweden are host to *S. pandora*, the type species of a new genus within a newly erected phylum, the Cycliophora. The acoelomate metazoan has sessile stages that inhabit the mouth-

parts of *N. norvegicus*; these sessile stages produce separate sex, short-lived and motile stages that allow for re-colonisation of new hosts (Funch and Kristensen, 1995). The new phylum has similar morphological and life history characteristics to the Entoprocta and the Ectoprocta and has recently been shown by molecular analysis of its 18S rRNA sequence to be a sister group to a Rotifera-Acanthocephala clade (Winnepenninckx et al., 1998). Although its discovery on the mouthparts of *N. norvegicus* appears remarkable (Morris, 1995) due to the diversity of published works on this host species, it does highlight a relative lack of basic study on the symbionts of even our most commercially significant crustacean species. The presence of similar species infesting *Nephrops* from other geographical locations or from other decapod hosts has not been demonstrated to date.

#### 3.1.2. Epizoic barnacles

*Balanus crenatus* have been described causing epizoic infestations of *N. norvegicus* from the Clyde Sea Area, Scotland. Infestations had higher prevalence and were more abundant in larger animals, presumably related to the longer intermoult period of

older animals and a propensity for smaller animals to inhabit burrows (Barnes and Bagenal, 1951). While epizoic infestations such as these are unlikely to cause direct harm to host lobsters, their presence likely relates to decreased moulting frequency and hence their presence in smaller animals may indicate disruption to normal moulting by pathogens or by environmental perturbations (Stentiford and Feist, 2005).

### 3.1.3. Epizoic Foraminifera

An opportunistic study of *N. norvegicus* captured from the Irish Sea led to the discovery of epizoic Foraminiferans of the genus *Cyclogyra* attached to the pleopods of a single male lobster. The author notes this as the first example of a Foraminiferan infestation of a decapod crustacean though concedes that lack of study rather than rarity likely hampered their previous discovery (Farmer, 1977). An interesting observation from this study was the relative lack of epizoic organisms (including polychaetes, bryzoans, molluscs and cirripedes) infesting *N. norvegicus* compared to other decapod crustaceans. The basis of this lack of symbiosis is not known though may relate to the slender nature of the *N. norvegicus* chelipeds, the presence of pincers at the ends of the foremost pairs of walking legs and the role of the limbs in grooming (Mariappan et al., 2000; Bell et al., 2006).

## 3.2. Pathogens and parasites

### 3.2.1. Bacterial shell disease

Observations of commercial and survey catches of *Nephrops* report that the majority of populations are comprised of individuals with clean shells with little fouling and a low incidence of the shell disease described in several other commercially exploited decapod crustacean species (Bell et al., 2006). This finding was reinforced in a study, by Ziino et al. (2002), who assessed shell disease status in 600 *N. norvegicus* obtained from Italian fish markets. Here, just 1% of lobsters presented symptoms of shell disease, normally manifested as small erosive and melanised lesions on the chelae. Histopathology of affected regions demonstrated erosion of the cuticle and infiltration by host haemocytes, often associated with bacterial colonisation. *Pseudomonas* spp. and in one case, *Enterobacter agglomerans*, both of which have chitinase activity, were isolated from shell lesions (Ziino et al., 2002). Further analysis of lesions described by these authors suggest an aetiology consistent with host–host conflict since all lesions were described from the chelae and appeared as apparent punctures rather than as the extensive cuticular erosion reported for shell diseased *Cancer* and *Homarus* species (Bayer et al., 1989; Vogan et al., 2001; Smolowitz et al., 2005). Further studies on comparative aetiology and host susceptibility of shell disease in commercially exploited crustacean species may assist an improved definition of the condition as predominantly host or pathogen associated and furthermore may help to elucidate risk factors for its induction in specific fisheries.

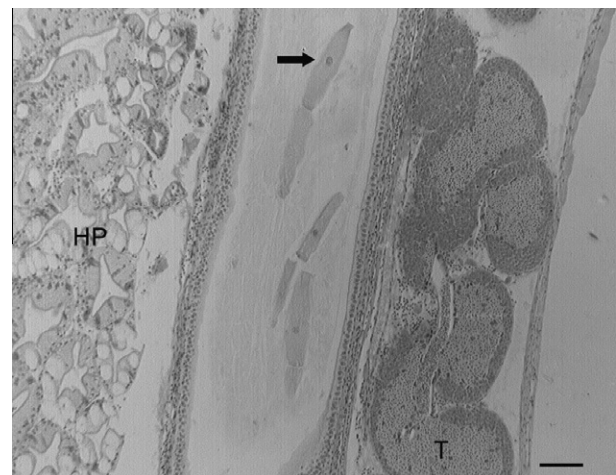
### 3.2.2. Systemic Mesanophrys-like ciliate

Systemic ciliate infections have been reported from a range of commercially exploited decapod crustaceans, some of which have gained attention due to their significant ecological and economic effect. A preliminary observation of a co-infecting *Paranophrys*-like ciliate in *Nephrops* captured from Scottish waters was provided by Field and Appleton (1996) during their studies on the culture of the dinoflagellate parasite *Hematodinium* infecting the same host. More recently, Small et al. (2005a,b) have described the parasite in detail as a scuticociliate of the genus *Mesanophrys* (= *Anophrys*, *Paranophrys*). Paradoxically, while the morphology of the parasite resembled most closely *M. carcini*, rDNA sequence data provided closer affinity with *Orchitophyra stellarum*, a scuticociliate from echinoderms. This inconsistency has prevented the naming of the

parasite from *Nephrops* and Small et al. (2005a) have suggested that for this reason, data pertaining to the number of somatic kineties may not be a robust enough measure when identifying closely related scuticociliates. As such, they suggest further work to compare the ITS region sequences for nominal *Mesanophrys* species with other known genera in order to resolve this phylogenetic issue. Morado provides a broader discussion on ciliate taxonomy elsewhere in this volume. Infection by the parasite in *Nephrops* is systemic, with proliferation of free stages within the haemal sinuses. A pronounced haemocytopenia accompanies infection, with an almost complete absence of haemocytes observed in histological section. *In vitro* culture of the pathogen has shown that it secretes several metalloproteases into the culture medium that are able to degrade specific components of the host skeletal muscle when the two are incubated. Specifically, myosin heavy chain, a critical component of the contractile apparatus in skeletal muscle is preferentially degraded (Small et al., 2005b). It follows that specific proteases secreted by such pathogens are likely implicated in the pathogenesis of this and similar diseases of crustaceans caused by ciliates (Small et al., 2005a,b).

### 3.2.3. Porospora nephropis

*P. nephropis* is a gregarine parasite (Apicomplexa) found within the alimentary canal of *N. norvegicus* captured from French waters (Léger and Duboscq, 1915; Tuzet and Ormieres, 1961). Infestations have also been observed in *N. norvegicus* captured from the Clyde Sea Area, Scotland (Field and Appleton, 1995; Stentiford, Neil and Beevers, pers. obs.), suggesting a wide geographical range for this parasite of *N. norvegicus*. A similar species, *P. gigantea* inhabits a similar niche within the hindgut of *Homarus americanus* (Montreuil, 1954; Théodoridès and Laird, 1970; Boghen, 1978; Bratley and Campbell, 1985a). The lifecycle of both species alternates between the lobster (in which spore-like gamonocyst stages develop from characteristic trophonts) and a mollusc, in which bundles of sporozoites develop, held together by a fragile membrane. Diagnosis of infection cannot be performed externally though squash or histological preparation of the gut will identify cyst like structures in the posterior intestine of infected lobsters. These spherical gamonocysts attach to the cuticular lining of the gut and may be shed at moult. The long, thread-like trophic stages inhabit the gut lumen (Bower, 1996) (see Fig. 3). Shields et al. (2006) state that *P. gigantea* is perhaps the most common parasite of Homarid lobsters, with prevalences of between 40% and 100% in different regions of the host range. Due to its complex multi-host



**Fig. 3.** Histology of trophic stages of the gregarine parasite *Porospora nephropis* inhabiting the midgut lumen of *N. norvegicus* (arrow). Adjacent hepatopancreatic (HP) and testicular (T) organs shown. Image courtesy of Dr. Nick Beevers, University of Glasgow, Scotland.

lifecycle, the availability of suitable molluscan intermediate hosts likely implicates its presence in a particular fishery (Van Engel et al., 1986). The role of *Porospora* as a disease agent in lobster populations is not well understood and no studies have been carried out on the role of this parasite either as a driver of host mortality or as to a subtler role as a modulator of host physiology or reproduction.

### 3.2.4. *Stichocotyle nephropis*

*N. norvegicus* from the Firth of Clyde, Scotland are host to the parasitic trematode *S. nephropis*. The parasite was first described by Cunningham (1887) who discovered cyst-like protuberances in the hindgut that upon further inspection were seen to contain a novel genus of Trematode. Following excystment, *S. nephropis* were observed to vary in length from less than 1 mm to 8 mm. The ventral surface of each worm contains a series of suckers along the median line, with the number varying according to the size and presumably age of the worm. Cunningham (1887) notes that more than one worm may be found within each cyst and that the wall of the cyst is cellular in nature and likely a 'pathological product' of the intestinal tissue of the host. Interestingly, the worms were discovered in an excysted (but live) state in recently dead animals and large numbers (up to 40) were found within a single host. In this early work, the prevalence of infection was estimated to be up to 25% though this varied between sampling sites. While Cunningham (1887) only described the larval form within *N. norvegicus*, later work by Odhner (1898) described what were believed to be the adult stages of *S. nephropis* in bile ducts and the spiral valve of the thornback ray, *Raja clavata* from Swedish waters. MacKenzie (1963) also reported on the prevalence of the adult parasite within *R. clavata* from Scottish waters. MacKenzie (1963) reports infection prevalence of between 1% and 48% in *N. norvegicus* collected from different locations within the Scottish fishery and his data is suggestive of a higher prevalence and intensity of infestation in larger animals captured on the west coast. In this study, of the 14 *R. clavata* assessed, only one male was shown to be infected with adult stages of *S. nephropis*. A further study of *S. nephropis* infestation of *N. norvegicus*, from the Scottish and English fishery, was carried out by Symonds (1972). Symonds reports on an increase in prevalence with size in both sexes and almost without exception, the parasite was absent from animals with a carapace length of less than 30 mm. Interestingly Symonds (1972) also reports a reduced prevalence of infection when compared to original surveys by Cunningham (1887), stating that this may indicate that this parasite is less common in British waters than reported in the late 19th century. Whether evidence for reduced prevalence of larval *S. nephropis* in *N. norvegicus* acts as a surrogate for data pertaining to reductions in stock abundance of European elasmobranch species such as *R. clavata* (Rogers and Ellis, 2000) requires further assessment but does highlight the potential importance of multi-host crustacean parasites as indicators of community integrity (Stentiford and Feist, 2005).

### 3.2.5. *Histriobdella homari*

*H. homari*, a eunicid polychaete worm has been described infecting the branchial chamber and egg mass of *N. norvegicus* from British waters. The symbiont was first described by Van Beneden (1858) and subsequently studied by Briggs et al. (1997) in Irish Sea stocks. *H. homari* has also been described infesting the gill chamber and eggs of *H. americanus* from US and Canadian waters and *H. gammarus* from Europe (Sund, 1914; Bruce et al., 1963; Uzmann, 1967; Bratney and Campbell, 1985b; Lerch and Uglem, 1996; Shields et al., 2006). Early descriptions of infestations suggested *H. homari* as an egg predator associated with poor larval production in European lobster hatcheries (Sund, 1914) though later studies by Lerch and Uglem (1996) demonstrated no correlation

between hatching success and high-intensity infestation. Shields et al. (2006) classify the likely association between *H. homari* and its lobster host as symbiotic due to the grazing habit (on bacteria and protozoans associated with the egg mass), its high prevalence (up to 100% at some sites – Uzmann, 1967), high-intensity (Bratney and Campbell, 1985a,b) and aforementioned absence of observed effect on egg mortality.

Infection prevalence and intensity in *N. norvegicus* appears to be less than that reported in populations of *H. americanus*. In a survey of Irish Sea *N. norvegicus* only isolated examples were discovered attached to the pleopod setae of male specimens (Briggs et al., 1997). Personal accounts of those working on *N. norvegicus* in Scottish waters (see Briggs et al., 1997) hint at the apparent rare and potential incidental nature of infestation within *N. norvegicus*, at least within those populations regularly assessed.

### 3.2.6. Copepod and isopod infestations

Copepod and isopod infections of *N. norvegicus* have been rarely reported in the literature. Thomson (1896) describes an *Anchorella*-like copepod from the vas deferens of *N. norvegicus* captured in Scotland. Affected individuals displayed a large and distinctive swelling on the wall of the vas deferens that caused distension of the thoracic cavity. The swelling was elicited by a large (up to 8 mm) female copepod accompanied by a morphologically dissimilar (5 mm) male. Subsequent examinations of *N. norvegicus* by Thomson (1896) defined the apparent prevalence of infection as less than 1% though no comprehensive surveys were carried out on lobsters obtained from different fishing grounds. Thomson (1896) acknowledged that at the time of writing, little was known about the life history of *Anchorella*-like copepods nor how they came to infect male hosts. No further descriptions of this parasite are apparent in the literature and as such, the intimate endoparasitic nature of this copepod infection and its close association with the male reproductive system of *N. norvegicus* remains an interesting model for furthering our understanding of copepod infestations in higher crustaceans.

One intriguing report of infestation of *N. norvegicus* by parasitic isopods exists within the current literature. During a fishing survey of the eastern Mediterranean, Ateş et al. (2006) discovered two parasitic isopod species belonging to the family Cymothoidae associated with the egg clutch of gravid female *N. norvegicus*. One specimen was identified as a male *Ceratothoa italica* (normally found infecting the buccal cavity of Mediterranean teleosts) while the other, a provisional male of *Livoneca pomatomi* (occasionally found infecting the branchial cavity of certain bathypelagic teleosts). While cymothoid isopods have been described associated with a number of non-teleost hosts previously, the authors note that in this case, there is some degree of uncertainty as to whether these parasites form true infections or whether they are accidental associations. In certain cases, such as in *Telotha henselii* infection of *Macrobrachium brasiliense*, *Macrobrachium borelli* and *Pseudopalaeomon bouveri*, studies have suggested that the association is not accidental but rather that young males of this species may utilise shrimp as intermediate hosts (Lemos de Castro, 1985; Grassini, 1994). Whether such association is true for the isopod infestation of *N. norvegicus* remains to be shown.

## 3.3. Conditions induced by capture, handling and in culture

### 3.3.1. Idiopathic muscle necrosis and systemic bacteraemia

A rapid onset abdominal muscle necrosis (termed idiopathic muscle necrosis, IMN) has been identified in Scottish *N. norvegicus* immediately following trawl capture (Stentiford and Neil, 2000). IMN has led to economic losses, particularly in the live market where affected animals succumb to initial signs of the condition within 4 h of capture, with rapid progression through the following

24 h. Affected individuals show a characteristic whitening of the abdominal muscle, firstly as individual muscle fibres, followed by progression of the lesion to adjacent muscle fibre groups, abdominal segments and eventually the whole abdomen. At this advanced stage, the commodity product (extruded abdominal muscle) has a significantly altered texture prior to and following cooking (Stentiford and Neil, pers. obs). Histopathological and ultrastructural studies of the condition reveal a progressive necrosis of affected muscle fibres, with loss of sarcomeric structure and moderate influx by host haemocytes. Ultrastructurally, sarcomeric disruption is confirmed by first a loss of structure in the region of the Z-line followed by separation and necrosis of individual myofibrils. Necrosis appears to progress longitudinally through the myofibrils, leading eventually to a homogenous matrix of cellular debris and necrotic cell products (Stentiford and Neil, 2000). The pathology, coupled with the loss of contractile proteins demonstrated using SDS-PAGE causes a loss of normal functioning of the abdomen and the characteristic 'tail flip' cannot be induced (Stentiford and Neil, 2000).

While the original description of the condition did not elucidate an aetiology (hence the designation as 'idiopathic'), the progression and pathogenesis of IMN has been studied more recently by Ridgway et al. (2006, 2007). Using a multivariate approach measuring physiological, endocrinological, immunological, microbiological and pathological indicators, Ridgway et al. (2006) demonstrated that in the crucial period of air exposure following trawl capture, *N. norvegicus* experienced large disruptions in physiological profile (increases in haemolymph L-lactate, crustacean hyperglycaemic hormone and carbohydrates and fluctuations in haemolymph pH). Furthermore, during this period, the immune competence of lobsters is impaired (indicated by reductions in circulating haemocyte titre and phenoloxidase levels). During the period immediately following capture, potential for systemic microbial infection increases (assisted by integumental damage), demonstrated by significantly increased counts of opportunistic bacterial species (Ridgway et al., 2006). Recently, Ridgway et al. (2007) have developed this principle by suggesting that IMN in *N. norvegicus* may occur as a two-stage pathogenic process, with an original lesion induced by acidosis from continuous rapid tail flipping during the capture process and a subsequent development of the condition as bacteraemia in immunosuppressed lobsters exposed to further stressors during the post-capture period. The IMN model in *N. norvegicus* serves as an excellent example of disease induction via commercial production processes and highlights how directed and applied scientific research can inform on improved practice and ultimately to assist in reducing economic impact of emergent conditions. It has been suggested by Ridgway et al. (2006) data of this type may be used to generate an internationally accepted Code of Practice for the capture, handling and transport of commercially exploited decapod crustaceans. Furthermore, it may be used to inform on best practice for disease limitation in aquaculture settings. These issues are discussed more fully by Fotedar and Evans elsewhere in this volume.

### 3.3.2. Black spot

While not considered to be a disease, 'blackspot' development in *N. norvegicus* is worthy of mention since it has been responsible for significant losses during the post-capture period when lobsters are stored on ice. Blackspot occurs as a result of polyphenol oxidase (PPO) oxidising diphenols to quinones that further undergo auto-oxidation and polymerisation to form dark pigments. Yan et al. (1989) observed a linear relationship between PPO and the rate of colour development in homogenates of *N. norvegicus*. Bartolo and Birk (1998) investigated factors that influence the degree of blackspot development in *N. norvegicus*. Due to the role of PPO in sclerotization of the exoskeleton following moulting, several stud-

ies on other decapod species have demonstrated how blackspot formation appears to be related to the moult stage of the host, with higher levels of PPO in decapods preparing to moult and in cast-off shells (Ogawa et al., 1983, 1984a,b). Further, Bartolo and Birk (1998) report that PPO activity peaked during the months coincident with the major moulting season in *N. norvegicus*. However, they also demonstrated that initial levels of PPO were not correlated with susceptibility to blackspot development post-capture; rather that changes in enzymatic activity during the post-capture and storage period may indicate likelihood of blackspot development. Their conclusion that the condition can be induced by 'traumatic' events such as capture and rough handling is further testimony to a requirement for good handling practice in fishers and those with market interests in this product. Industry experience and recent research have concluded that reducing agents based on sulphites are the most effective and practical control agents for melanosis in *N. norvegicus*, when compared to a large variety of chemical alternatives. However, due to their possible adverse effects on humans, EU regulations restrict the concentrations of sulphites to a maximum of 10 mg of SO<sub>2</sub> kg<sup>-1</sup>. Recent work has shown that alternative treatments based on 4-Hexylresorcinol, which inhibits PPO activity, can be equally effective (Martinez-Alvarez et al., 2007).

### 3.3.3. Diseases of *N. norvegicus* during culture

Since sustained attempts to grow *N. norvegicus* by aquaculture have not occurred, few reports are available on their susceptibility to disease when held in captivity. However, in a study of marine crustaceans with potential for aquaculture, Anderson and Conroy (1968) report on heavy infestations by ciliates of the genus *Zoothamnium* on the body surfaces of live *N. norvegicus* larvae hatched in the laboratory. They report that heavy infestations led to death as a result of 'trauma and interference to respiration'. A general observation regarding clawed lobsters, in particular with regard to attempts to raise them in closed culture systems is the apparent absence of viral pathogens in this group. Viral pathogens have caused massive economic impact on the culture of tropical penaeids (see the paper by Lightner in this volume) and recently the first virus has been described in spiny lobsters of the Palinuridae (Shields and Behringer, 2004 – see also the paper by Shields in this volume). Whether the absence of reports of viral pathogens in Nephropid lobsters reflects a true resistance of this group to viruses or whether insufficient field surveys have been carried out (of larvae, juveniles and adults) to describe pathogens can only be speculated. However, differential resistance of specific crustacean groups to economically significant viral pathogens may provide a fruitful research area and may directly benefit the global requirement for sustainable development of crustacean aquaculture (see Stentiford et al., 2009).

### 3.3.4. Light damage to eyes

The eyes of *N. norvegicus* are large, well pigmented and approximately kidney-shaped (the basis of their generic name '*Nephrops*' meaning 'kidney-eye'). Several studies on the behaviour of this species have shown it to spend the majority of its time within its burrow, with emergent forays to feed and mate (Chapman and Howard, 1979; Chapman, 1980; Bell et al., 2006). They have a well-described diurnal activity pattern that coincides with peak catch rates via trawling and this varies according to the depth of water of resident populations (Chapman and Rice, 1971; Chapman and Howard, 1979). While light (and the eye) is important for maintenance of this pattern, several behavioural studies have shown that other factors (such as food availability and even innate endogenous rhythms) also play a role (see Bell et al., 2006). Shields et al. (2006) have recently summarised the pathological effects of light on eye of *N. norvegicus*. Light damage to the eyes of *N. norvegicus*

was first observed by Loew (1976). Subsequent studies by Shelton et al. (1986a,b) and Gaten (1988) followed the pathogenesis of the condition that proceeded to a loss of retinula cells in the ommatidia and haemocytic infiltration of necrotic regions. In later field studies of tagged and released lobsters with light damaged eyes, Chapman et al. (2000) demonstrated that once damaged, the eyes did not recover their ability for light adaptation. However, light damage did not appear to influence mortality since recaptures occurred up to several years following release.

#### 4. *Hematodinium* sp. – a model disease

Of the known pathogens and parasites described from *N. norvegicus*, by far the most significant in terms of ecological and economic impact is the dinoflagellate parasite *Hematodinium* sp. Disease associated with this pathogen had been reported anecdotally in Scotland since the early 1980s, during which period it was termed 'post moult syndrome' due to the coincident appearance of affected lobsters during the main moulting period for *N. norvegicus*. However, it was not until the early 1990s that 'post moult syndrome' was shown to be associated with a dinoflagellate parasite of the genus *Hematodinium*, previously only known to infect crabs (Field et al., 1992). Since this description, *Hematodinium* and *Hematodinium*-like species have been discovered in an increasing array of decapod and non-decapod crustacean hosts (see elsewhere within this book), many of which are the subject of commercially important fisheries. In a recent review of this pathogen group, Stentiford and Shields (2005) suggest them to be one of the most significant (known) pathogens of wild marine crustaceans. Since the majority of studies carried out on *Hematodinium* have been in commercially exploited hosts, there is high likelihood that this parasite group is distributed widely amongst decapod (and some non-decapod) hosts. The section that follows is devoted to a description of the disease caused by *Hematodinium* sp. in *N. norvegicus*.

##### 4.1. *Hematodinium* sp. as an emergent pathogen

Routine examination of *N. norvegicus* from the west of Scotland fishery in the early 1980s led to discovery of a low prevalence on lobsters that displayed an opaque but apparently hyperpigmented carapace, milky-white haemolymph and a general moribund status following capture. The condition, termed 'post moult syndrome' was initially thought to be due to a hyperplastic increase in host haemocytes associated with the concurrent moult season of *N. norvegicus* in the region. However, by the late 1980s the increased prevalence and poor quality of animals showing these symptoms began to evoke comment from fishermen and processors and a full-scale survey was launched (Field et al., 1992). Detailed examination of affected lobsters revealed that they were infected by masses of non-motile protistan parasites that resembled dinoflagellates of the Order Syndiniales, most similar to the parasite *Hematodinium perezii*, described by Chatton and Poisson (1931) from European portunid crabs (Field et al., 1992). The parasite also resembled *Hematodinium*-like dinoflagellates that had previously been described from the crabs *Callinectes sapidus* (Newman and Johnson, 1975), *Cancer irroratus*, *Cancer borealis*, *Ovalipes ocellatus* (Maclean and Ruddell, 1978), *Necora puber* (Wilhelm and Boulo, 1988), *Cancer pagurus* (Latrouite et al., 1988), *Chionoecetes bairdi* and *Chionoecetes opilio* (Meyers et al., 1987, 1990; Eaton et al., 1991). The discovery of *Hematodinium* sp. in *N. norvegicus* was highly significant since it marked the first description of a *Hematodinium*-like pathogen in Nephropid lobsters and it appeared to show similar pathological and epizootiological features to those previously described in infections of commercially exploited crab

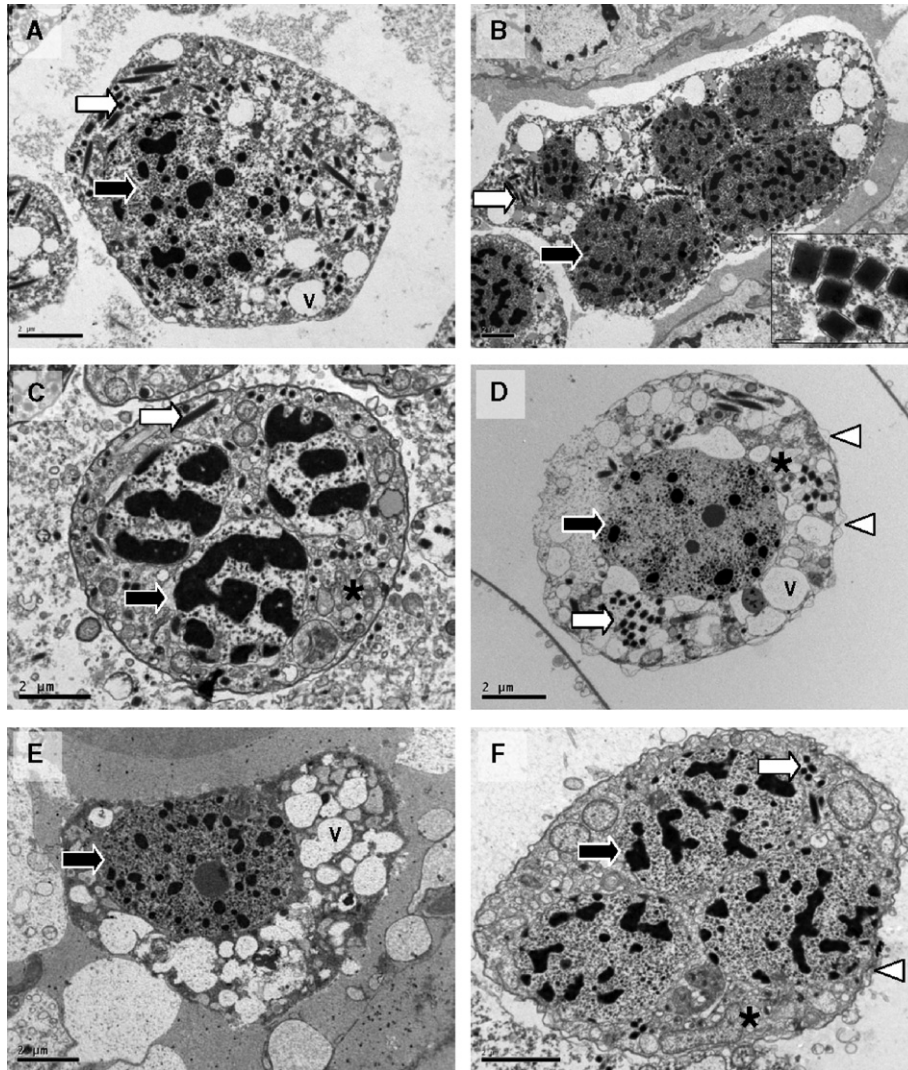
species. In the subsequent decade, a significant work program to describe and diagnose the pathogen and to investigate its effect on the life history traits and population structure of its host followed.

##### 4.2. Taxonomy

Despite its commercial significance, the taxonomy of *Hematodinium* spp. and the *Hematodinium*-like dinoflagellates remains confused with most isolates (including that from *N. norvegicus*) still only named generically. Despite significant advances in molecular diagnostic capabilities (see below), the description of isolates of *Hematodinium* spp. as specific entities in their respective hosts has been hampered by a failure to compare 'new' isolates to the type species (*H. perezii*) from type hosts (*Carcinus maenas* or *Liocarcinus depurator*) collected from the type location (English Channel, off France) as detailed by Chatton and Poisson (1931). The paucity of studies of the type host has largely been attributed to the very low prevalence of *H. perezii* in European portunid crabs (see Stentiford and Shields, 2005). However, recent studies have shown that while infection prevalence in *C. maenas* may in fact be rather low (less than 1%), with infected crabs also displaying low-level infections (Stentiford and Feist, 2005), infection prevalence in *L. depurator* may be significantly higher, with animals displaying pathologies similar to those associated with *Hematodinium* sp. infection in hosts such as *N. norvegicus*. Recent unpublished work has reported *H. perezii* in *L. depurator* at sites in the English Channel (between France and the United Kingdom) at prevalences of up to 25% (Stentiford, G.D., pers. obs.). The pathological outcome of infection appears similar to all other published accounts though the ultrastructure of known isolates is somewhat varied (Fig. 4) compared to the type species. Stentiford and Shields (2005) state that the description of *H. perezii* as type species in *C. maenas* and *L. depurator* may have been unfortuitous for taxonomic studies of other isolates due to the difficulty in obtaining type material. However, it appears that this may have been an oversimplification since infection prevalence and severity appears to differ between the two 'type' hosts. Work is currently underway to compare *Hematodinium* sp. isolates from *N. norvegicus* and commercial crab species with *H. perezii* from *L. depurator* collected from the English Channel (Small, H.J., pers. comm.). Further, due to the apparent differences in host-pathogen relationship observed in *C. maenas* and *L. depurator*, it is possible that the original 'type' description from these two hosts may need to be modified to describe two discrete species with differing pathological outcomes. Further efforts are now required to gather appropriate material from *H. perezii*-infected *C. maenas* in order to provide taxonomic clarity in the group. Only when this occurs can we investigate the global epidemiology of this important disease and calculate risk factors for its transfer to naïve geographical locations and hosts.

##### 4.3. *Hematodinium* diagnosis

Several tools are available for diagnosis of *Hematodinium* infections in *N. norvegicus*. Intriguingly, heavily-parasitised animals can be recognised externally via an increased opacity and pronounced hyperpigmentation of the carapace, particularly of the chelipeds and cephalothorax. The degree of hyperpigmentation appears to develop with increasing infection severity and was a major feature in the initial discovery of the condition in Scottish *N. norvegicus* during the early 1980s. The external appearance of the carapace has been used as a field diagnostic tool for *Hematodinium* infection of *N. norvegicus* in Scottish (Field et al., 1992; Stentiford et al., 2001c), Swedish (Tärnlund, 2000) and Irish (Briggs and McAliskey, 2002) waters. In addition, since this symptom of infection is also observed in other *Hematodinium*-infected hosts, it has been used



**Fig. 4.** Transmission electron micrographs of *Hematodinium perezii* and *Hematodinium* sp. infecting *Nephrops norvegicus*, *Cancer pagurus*, *Callinectes sapidus*, *Carcinus maenas* and *Liocarcinus depurator*. (A) Bi-nucleate stage of *Hematodinium* sp. from *N. norvegicus* containing nuclei with condensed chromatin (black arrow), abundant trichocysts (white arrow) and vacuoles (v) throughout cytoplasm. Scale = 2  $\mu$ m. (B) Multi-nucleate plasmodium of *Hematodinium* sp. from *N. norvegicus* showing similar features to A. Scale = 2  $\mu$ m. Inset: higher power image of cross section of several trichocysts. (C) Multi-nucleate plasmodium of *Hematodinium* sp. from *C. pagurus* displaying similar features to A and also, abundant mitochondria and other organelles within cytoplasm (asterisk). Scale = 2  $\mu$ m. (D) Apparently uni-nucleate stage of *Hematodinium* sp. from *C. sapidus* displaying similar features to A, B and C but with clearly visible alveolar membrane, raised from the cell surface in several regions (arrow heads). Scale = 2  $\mu$ m. (E) Uni-nucleate stage of *Hematodinium perezii* from European *C. maenas*. Note the apparently different appearance of the cytoplasm of the parasite cell compared to A–D but presence of a nucleus containing condensed chromatin profiles (arrow) and vacuole-rich cytoplasm (v). Trichocysts were not observed in any of the parasites from this preparation. Scale = 2  $\mu$ m. (F) Multi-nucleate plasmodium of *H. perezii* from European *L. depurator*. Note the presence of similar features to those depicted in A–D but a contrast to the ultrastructural appearance of the isolation from *C. maenas* (E). Scale = 2  $\mu$ m.

to diagnose *Hematodinium* sp. in *C. pagurus* (Stentiford et al., 2002), *C. bairdi* (Meyers et al., 1987, 1990) and *C. opilio* (Taylor and Khan, 1995; Dawe, 2002; Pestal et al., 2003; Shields et al., 2005). However, while this method remains useful for the detection of advanced infections, it does not detect low-level 'sub-patent' or potentially latent (low-level, tissue-based) infections in *N. norvegicus* (Tärnlund, 2000; Stentiford et al., 2001c).

Recognising the limitation of carapace colouration as an accurate diagnostic tool for *Hematodinium* infections of *N. norvegicus*, Field et al. (1992) developed a novel approach to diagnosis via visualisation of the haemolymph through the thin cuticle and epithelia of the pleopods. Not only did this technique diagnose infection *per se*, it also allowed assignment of a grade of relative severity (Field et al., 1992; Field and Appleton, 1995). A pleopod is removed from the abdomen and assessed for the presence of parasites within the haemal space using low-power microscopy. This method has

been used in field studies of *Hematodinium* infections in populations of *N. norvegicus* from the Scottish west coast (Field et al., 1992, 1998; Stentiford et al., 2001c) and allows for detection of 4–50% more infected lobsters than the carapace discolouration method (Tärnlund, 2000; Stentiford et al., 2001c). In addition to its utility as a diagnostic tool during field surveys, the technique has also been used to grade infection severity of *N. norvegicus* during numerous studies of the pathological, physiological and behavioural manifestation of *Hematodinium* infection in host lobsters. As such, despite recent advances in antibody and molecular based diagnostic methodologies, the pleopod staging technique remains a useful tool for use by field and laboratory scientists.

Several studies have used histological preparations of the haemolymph and tissues for the diagnosis of *Hematodinium* infection. Histology offers significant advantages to either carapace or pleopod based diagnosis since it allows for direct visualisation of the

pathogen and associated effects within the host. This capacity is improved further when electron microscopy is employed to provide an ultrastructural perspective. Methanol-fixed haemolymph smears stained with either Giemsa or haematoxylin and eosin provide satisfactory results (see Meyers et al., 1987; Love et al., 1993; Hudson and Shields, 1994; Messick, 1994; Taylor and Khan, 1995; Wilhelm and Mialhe, 1996; Messick and Shields, 2000) though wet smears, prepared using poly-L-lysine-coated slides, fixed in Bouins solution or 10% neutral buffered formalin (NBF), and stained with haematoxylin and eosin procedure or a modified Giemsa provide a more reliable diagnostic medium (Messick, 1994; Messick and Shields, 2000; Shields and Squyars, 2000; Pestal et al., 2003). Non-lethal methods based upon analysis of haemolymph samples are useful since they allow pathogenesis to be monitored in laboratory settings. However, since evidence exists to suggest that sub-patent and latent infections may not be visible within haemolymph smears, more accurate diagnostic assessments can be made using organ and tissue histology. While several fixatives have been used for histology of crustacean tissues, Davidson's seawater fixative (Hopwood, 1996) appears to offer consistent results (certainly for marine decapod species), though we have used 10% NBF to good effect in animals collected from estuarine habitats. For stenohaline *N. norvegicus*, tissue and organ samples (normally heart, hepatopancreas, cheliped muscle, abdominal muscle, gonad and gill) are excised and fixed in Davidson's seawater fixative for 24 h before transfer to 70% industrial methylated spirit for storage prior to processing. Fixed samples are then prepared for histology using standard protocols (for example see Stentiford et al., 2002) and stained using haematoxylin and eosin. *Hematodinium* parasites are easily diagnosed in sections due to their condensed chromatin profiles that stain densely with haematoxylin. For electron microscopy, small samples (c. 2 mm<sup>3</sup>) of hepatopancreas should be fixed in 3% glutaraldehyde in 0.1 M sodium citrate buffer (pH 7.4) with 1.75% sodium chloride for 2 h at room temperature followed by post-fixation in reduced 1% osmium tetroxide for 1 h at 4 °C (for example see Stentiford et al., 2002). Uranyl acetate staining will define uninucleate and multi-nucleate stages of *Hematodinium* based upon their characteristic nuclei with condensed chromatin profiles, cytoplasmic trichocysts and a bounding alveolar membrane. At present, ultrastructural diagnosis is considered the definitive diagnostic tool for *Hematodinium* infections of *N. norvegicus*. Stentiford and Shields (2005) note that while Neutral Red is an excellent vital stain for *Hematodinium*, at least in fresh smears of *H. perezi* in green crabs (Chatton and Poisson, 1931) and infections in blue crabs (Shields, J.D., unpubl. data), it does not stain *Hematodinium* from *N. norvegicus*, and thus is not a good indicator for *Hematodinium* infections in general. Janus Green has also been used as a vital stain (Chatton and Poisson, 1931) but its use as an indicator of infections has not been evaluated.

Due to the commercial significance of *Hematodinium* as a pathogen of *N. norvegicus*, it is perhaps not surprising that considerable attention has been afforded to the development of more specific diagnostic tests based upon immunological and molecular technologies. The successful *in vitro* culture of *Hematodinium* isolated from *N. norvegicus* by (published by Appleton and Vickerman (1998)) was critical in allowing antibodies to be raised against the parasite and further, for development of an indirect immunofluorescent antibody technique (IFAT) for diagnosis (Field and Appleton, 1996). The IFAT provided the first clue that apparently uninfected *N. norvegicus* could harbour sub-patent or even latent infections (Field and Appleton, 1996) and that the field analysis techniques based upon the carapace colour and pleopod technique may be underestimating true field prevalence. The development of a Western-blot technique using these antibodies allowed for the objective monitoring of *Hematodinium* infection in the *N. norvegicus* fishery over a complete season, providing more accurate data on

infection-associated mortality for potential use in stock assessment models (Stentiford et al., 2001d). In this study, it was shown that the technique demonstrated not only an ability to diagnose infection earlier in the season but also that the pleopod technique underestimated prevalence by as much as 25%, particularly in the early season when infection severity was lower. Using this data, it was suggested that sensitive immunological diagnostic tools could be used to predict the patent prevalence that occurs later in the same season. Whether fisheries managers can utilise the information to offset mortalities due to development of patent infection remains to be shown. The technique developed by Field and Appleton (1996) and utilised by Stentiford et al. (2001d) has been further developed into an ELISA-based diagnostic test that provides an even more rapid diagnosis of the disease in this species (Small et al., 2002). Recent work however has shown that the polyclonal antibody raised against cultured *Hematodinium* from *N. norvegicus* can cross react with epitopes found on other protozoan parasites (Bushek et al., 2002). With this in mind, care must be taken when applying such a technique, particularly where the background pathogen fauna of the host is not well known.

The first PCR-based diagnostic assay developed for the detection of *Hematodinium* in decapod hosts was reported by Hudson and Adlard (1994). A 680 bp product was amplified from the 3' end of the SSU region of the 18S ribosomal DNA and a sequence of this product was subsequently shown to be specific to *Hematodinium* (Hudson and Adlard, 1996). Comparison of sequences from several hosts indicated that *Hematodinium* in *C. sapidus* was different to that infecting *N. norvegicus*, *C. bairdi* and *C. opilio*. In a later study, additional primer sets were developed for the *Hematodinium* parasite infecting *C. sapidus* (Gruebl et al., 2002; Sheppard et al., 2003), these being used to demonstrate sub-patent infections in blue crabs. In light of concerns about the specificity of the polyclonal antibody raised against cultured *Hematodinium* from *N. norvegicus*, Small et al. (2006) have recently reported improved diagnostic tests for the disease based upon PCR. In this study, Small et al. (2006) note that the PCR primer sets used in previous studies of *Hematodinium* in decapods have been based upon conserved regions of the 18S and 5.8S regions of the rDNA. As such, the authors state that such primer sets are not specific for particular *Hematodinium* species. Using approaches based upon amplification of variable regions of the parasite genome, Small et al. (2006) report a species-specific primer set for *Hematodinium* infection of *N. norvegicus*. A diagnostic band at 380 bp allows for the definitive diagnosis of *Hematodinium* sp. from *N. norvegicus* and now opens the potential for sensitive epidemiological investigations of infection in this species and others that may host the same parasite within the fishery. Furthermore, the demonstration of parasite labelling using the *in situ* hybridization protocol described by Small et al. (2006) will now allow for parasite transmission and early pathogenesis trials to be carried out. Since transmission is significantly understudied in this parasite group, this should be seen as a promising new development.

Whilst molecular tools are clearly assisting field scientists with diagnosis of *Hematodinium* infection in decapods, the expression and sequencing of elements of the parasite's genome (via intelligent primer design and PCR) is likely to prove fruitful in establishing the taxonomic link between *H. perezi* and the *Hematodinium*-like parasites infecting other crustacean hosts, including *N. norvegicus*. As stated above, accurate species descriptions are required in order to assess the biosecurity risk of decapod movements, particularly to areas where commercial capture and culture industries exist.

#### 4.4. Current status of infection prevalence monitoring

The defining characteristics of enzootic *Hematodinium* spp. infection systems have until now been regarded to be an annual

and highly seasonal occurrence of patently infected hosts, with a mortality of epizootic proportions in the infected hosts and a subsequent 'low' season when infected hosts are not abundant and are sometimes undetectable (Briggs and McAliskey, 2002; Chualain et al., 2009; Field et al., 1992, 1998; Love et al., 1993; Messick and Shields, 2000; Meyers et al., 1990; Sheppard et al., 2003; Stentiford et al., 2001c,d; Stentiford and Shields, 2005). The infection system in the *N. norvegicus* fishery in Clyde Sea Area in Scotland has exhibited these characteristics for over 20 years, with the year-on-year peaks of patent prevalence remaining stable at 20–25% of the sampled catches (Field et al., 1992, 1998; Stentiford et al., 2001c,d; Beevers, 2010). This seasonal epizootic from an apparently parasite-free population is a feature that is consistent with the existence of mechanisms and reservoirs to perpetuate the infection from year to year. However, these methods are subjective and lack sensitivity, and thus may have overlooked low-level infection.

More recently, through the use of a sensitive high throughput enzyme linked immunosorbent assay (ELISA) (Small et al., 2002) and polymerase chain reaction assays (PCR) (Gruebl et al., 2002; Small et al., 2006) alongside the subjective measures of patent disease to monitor the presence of infected animals throughout the infection peaks and nadirs in the Clyde Sea, the limited sensitivity of the subjective measures of patent disease has been highlighted. However, use of a multiple assaying technique (using ELISA, PCR, the pleopod method and the body colour method) has revealed pre-patent levels of infection comparable to the patent disease peak throughout the year, and a level of prevalence that, although fluctuating, is consistently above the values previously reported during the apparent infection nadirs (Beevers, 2010). These results therefore challenge the classification of the patent disease peak as epizootic in nature. For this reason it may be argued that it is not appropriate to describe the *Hematodinium* sp. associated disease in *N. norvegicus* from the Clyde Sea as a seasonal disease, but rather that patent disease be described as 'seasonally apparent'.

By holding in tanks sub-patently *Hematodinium* sp. infected *N. norvegicus* caught in the patent infection nadir, it has been possible to show that patent infection development can take up to 9 months, with gross signs of the disease being concurrent with that observed in the wild fishery (Beevers, 2010). These observations provide evidence that at least some of the infected hosts found in the mortality peaks for *N. norvegicus* might be represented by animals that were infected the previous year or earlier. However this does not exclude the possibility that alternative hosts exist in the life cycle of these parasites or that infection and death due to advanced disease happen in the same year.

Seasonal peaks in patent *Hematodinium* disease have also been reported in other wild, commercially-harvested decapods (Stentiford and Shields, 2005), and it is possible that in these cases also infected hosts are present throughout the year, though not detected either due to limitations in the sampling methodology or a low sensitivity of the assays used. The application of more sensitive diagnostic methods to these infection systems should therefore be a priority for future research. One current approach to such a thorough and ongoing monitoring of parasite spread and load in fisheries and associated marine fauna is using parasite material to generate both PCR-based diagnostic tools, capable of discerning species and strain differences of parasites, and antibody-based dipstick tests that can be employed in the field without specialist training (Gornik, S., pers. comm.). Candidate genes/proteins for antibody generation can be identified using a molecular data set of highly-expressed proteins specific to the infection stage of *Hematodinium* using (1) analysis of expressed genes as mRNAs from EST data, and (2) protein data analysed by two-dimensional protein electrophoresis and mass-spectrometry. ELISA and lateral flow-type diagnostic tools can then be developed using antibodies raised against such highly expressed parasite proteins.

#### 4.5. Pathogenesis

A section on the pathogenesis of any disease should rightfully start by considering the mode of transmission of the parasite between susceptible hosts. However, despite a considerable body of knowledge on this parasite and its existence in wild populations and significant advances in our ability to diagnose infection (even before disease manifests), very little is known about its transmission and of the early stages of infection. This is likely a reflection of the difficulty in defining pathogenic processes in wild marine populations but also indicates a knowledge gap in the taxonomy, life cycle, host range and latency periods for this parasite group, particularly in relation to the discontinuous life history patterns of their hosts.

*Hematodinium* infections have been transmitted via inoculation of infected haemolymph into naïve *C. bairdi* (Meyers et al., 1987), *C. sapidus* (Shields and Squyars, 2000) and *Portunus pelagicus* (Hudson and Shields, 1994). These trials have demonstrated that filamentous trophonts and vegetative, amoeboid trophonts can establish infection (Meyers et al., 1987; Hudson and Shields, 1994; Shields and Squyars, 2000). Previously, micro- and macrospores were also shown to produce infections when inoculated into *C. bairdi* (Eaton et al., 1991) though this could not be demonstrated using a similar approach in *N. norvegicus* (Appleton and Vickerman, 1998). Stentiford and Shields (2005) have previously stated that despite its simultaneous production and active *en masse* exit from heavily infected hosts, the dinospore is not necessarily the infective stage but instead an intermediate stage preceding a resting cyst or some other non-parasitic stage (see Shields, 1994). This theory is partially supported by the report by Appleton and Vickerman (1998) that the culture of dinospores leads directly to the development of filamentous trophonts *in vitro*. While *in vitro* sporulation of *Hematodinium* from *N. norvegicus* is not synchronous, the event within infected host lobsters appears to be so with almost complete exsporulation from host gills and arthroal membranes and death occurring soon after (Appleton and Vickerman, 1998). Similar events have been recorded in *C. pagurus* (Stentiford and Shields, 2005) though in *C. sapidus* at least, there is potential for multiple sporulation events within a given host, some of which may lead to high spore densities and some of which do not (Shields and Squyars, 2000).

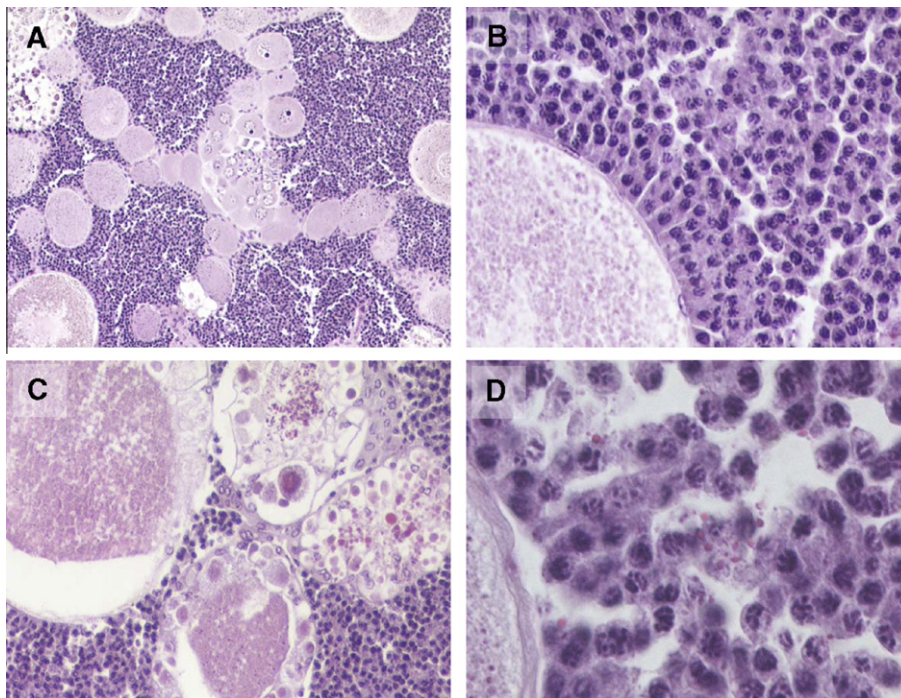
Despite the identity of the infective stage(s) within the life cycle of the parasite, some uncertainty also exists surrounding the route of entry into naïve hosts. Several studies have suggested that the moult stage is an important period for obtaining infection but data is circumstantial, particularly considering the apparently extended incubation period to patent disease and the difficulty in relating an infection event to a mortality event or season (Stentiford and Shields, 2005). There is evidence for transmission to naïve *C. sapidus* via ingestion of infected food (Sheppard et al., 2003), though this was not the case for *P. pelagicus* (Hudson and Shields, 1994). Given the potential cannibalistic tendencies and scavenging nature of many decapod crustacean species, this route for transmission appears likely (Sheppard et al., 2003). Further possibilities, at least in the case of *N. norvegicus* are that dinospores are ingested via suspension feeding (which occurs during certain seasons) (Loo et al., 1993), or that intermediate hosts, such as the benthic amphipod *Orchomene nanus* (a scavenger with preference for crustacean carrion) are intermediate or reservoir hosts for the parasite (Johnson, 1986; Stentiford and Shields, 2005; Small et al., 2006). Some studies have suggested potential for sexual transmission (Meyers et al., 1996) though this appears somewhat unlikely given the arrested development and significant pathological disruption of the ovary in infected female hosts (see Stentiford et al., 2002). Clearly, dedicated studies on the transmission of *Hematodinium* between infected and naïve *N. norvegicus* are required to clarify this issue

and to ascertain whether commercial practices (such as the discarding of disassembled catch) may contribute to the spread of this disease in the fishery. The use of emerging molecular tools, particularly PCR and ISH should assist these studies.

Field et al. (1992) provided the first description of the pathogenesis of *Hematodinium* in *N. norvegicus*, following studies of the Scottish west coast fishery. The pathological manifestation of the disease in decapods has been amply covered by several authors and is also described in detail elsewhere in this volume. A brief overview is given here. Due to the relatively open circulatory system of decapods and the systemic nature of *Hematodinium* infections, it is not surprising that all major organ systems are variously impacted during disease. Externally, patently infected lobsters display a brightly coloured but opaque ('cooked' appearance) carapace that remains following haemolymph removal and death of the host. Haemolymph drawn from patently infected lobsters is milky white with increased viscosity and opacity. Centrifugation of haemolymph from lobsters in advanced stages of infection can produce cell pellets amounting to approximately 50% of the sample by volume (Stentiford, G.D., pers. obs.), equating to the approximate 8-fold increase in circulating cells compared to uninfected lobsters reported by Field et al. (1992). The remaining plasma fraction is generally devoid of colour and has a significantly increased clotting time and often fails to form a normal clot. The haemolymph cell fraction from the majority of heavily infected lobsters consists of non-motile stages similar in size to host haemocytes (5–14 µm in diameter). Most of these are uninucleate while others contain multiple nuclei. Field and Appleton (1995) report that four stages are commonly observed within infected *N. norvegicus* – these being uninucleate forms and multinucleate plasmodia circulating freely in the haemolymph, filamentous syncytia attached to host organs and a separate syncytial network ramifying between (for instance) muscle fibres of the abdomen and heart. These forms approximately correlate to the developmental stages

described *in vitro* by Appleton and Vickerman (1998). Furthermore, infected lobsters containing masses of bi-flagellate stages corresponding to the macrospore and microspore forms (Field and Appleton, 1995; Appleton and Vickerman, 1998) are occasionally encountered. The uninucleate stages encountered free within the haemolymph likely correspond to the sporoblast stages described by Appleton and Vickerman (1998) and it is these that give rise to the flagellated spore stages encountered in some animals. Presumably the multinucleate syncytial networks (either attached to the surface of host organs or ramifying between three dimensional structures such as the abdominal musculature) give rise to the circulating stages and it is via these attached stages that infection appears to establish within the host. This is reinforced by reports on the detection of parasites (via immunological and molecular diagnostics) within organs prior to their appearance in the haemolymph (Field and Appleton, 1996; Stentiford et al., 2001d; Small et al., 2002). These attached stages also compose the 'creamy deposits' reported to occur coating the internal organs of *Hematodinium*-infected hosts (Stentiford and Shields, 2005) (see Fig. 5).

It is generally reported that crabs and lobsters infected with *Hematodinium* exhibit haemocytopenia (reduction in the haemocyte count) though this has only been demonstrated quantitatively for infected *C. sapidus* (Shields and Squyers, 2000). Studies on *N. norvegicus* have demonstrated that the combined cell count for haemocytes and parasites significantly increases during patent infections though specific counts on haemocytes were not carried out (Field and Appleton, 1995). Despite significant declines in haemocyte counts reported for *C. sapidus*, other studies have shown that although high parasite numbers are recorded during patent *Hematodinium* infections, efficient haemocyte responses can occur to other pathogens that may co-infect the host. In the case of *C. pagurus*, co-infecting yeast pathogens elicited a pronounced immunological response from crabs displaying advanced *Hematodinium* infections, suggesting that although circulating haemocyte



**Fig. 5.** *Hematodinium* sp. infestation of the ovary of *N. norvegicus*. (A) Ovarian follicle infiltrated by large numbers of uni- and multi-nucleate stages. Oocytes are apparently arrested in a pre-vitellogenic state (arrow). Bar = 200 µm. (B). Multinucleate plasmodial stages attached to the surface of a degenerate oocyte (arrow). Bar = 50 µm. (C). Necrotic vitellogenic oocytes containing remnant vitellogenin inclusions (arrow) or a fine granular matrix (asterisk). Bar = 100 µm. (D). Droplets of vitellogenin (arrow) apparently liberated from necrotic vitellogenic oocytes, among parasite plasmodial stages. Bar = 50 µm. Material used for obtaining images courtesy of Dr. Beth Leslie, Atlantic Fisheries College, Shetland.

numbers may be apparently reduced, the host can still respond to pathogenic challenge when stimulated appropriately (Stentiford et al., 2002). Field and Appleton (1995) demonstrated apparent stimulus of haemopoietic activity in heavily infected lobsters though this did not appear to manifest as increased numbers of released or circulating haemocytes. Evidence from the literature suggests a rather complex immunological relationship between *Hematodinium* and its decapod hosts and further investigations on the interaction between hosts and their parasite isolates may provide intriguing insights into the pathogenesis of this disease.

#### 4.6. Pathology and its effect on commercial products

In keeping with the scope of this volume, it is important to consider the pathological manifestations of *Hematodinium* infection, particularly in light of their effect on the commercial exploitation of *N. norvegicus* as fisheries products. Since the late 1990s, a high unit value has designated *N. norvegicus* as the most valuable fisheries species landed in the UK and Ireland. Product is sold live, fresh dead or frozen mainly from the UK, Denmark and Ireland with major exportation to Italy, France and Spain, and to countries outside of the European Union. The live market is largely dependent on trap-caught animals, while trawl fishery dominates the fresh and frozen markets (Bell et al., 2006). Health and quality of product is clearly an important consideration for the live market (where animals are shipped to market destinations via road in 'vivier' systems). For the frozen market, animals are often 'tailed' at sea, the abdominal section being processed for the production of 'scampi'. In general, smaller animals are captured via trawling and larger animals by trapping. The abdominal musculature is the major harvestable product in both cases.

Several studies have considered the effect of *Hematodinium* on the abdominal musculature of *N. norvegicus*. Field and Appleton (1995) state that despite the presence of network-like parasite syncytia between muscle fibres and the association of uninucleate forms with the sarcolemmal membrane, histology of the abdominal musculature remained largely normal, even in advanced stages of infection. Later studies demonstrated that the water content of the abdominal musculature was relatively unaltered in late stage infections and that ultrastructural damage to the muscle was generally limited to the fibre periphery (Stentiford et al., 2000b). The effect of *Hematodinium* infection on the abdominal musculature of *N. norvegicus* contrasts that seen in some other *Hematodinium*-host models. In several crab species, the gross appearance, water content, histology, mechanical structure and texture of the muscle is altered during late stage infections (Meyers et al., 1987; Field et al., 1992; Messick, 1994; Hudson, 1995; Wilhelm and Mialhe, 1996; Stentiford et al., 2002; Stentiford and Shields, 2005). This is perhaps best demonstrated in *C. pagurus* where *Hematodinium* infections cause almost complete degeneration of the claw musculature with separation of the sarcolemma from contractile myofibrils and severe disorganisation of filaments in the region of the Z-line (Stentiford et al., 2002). Unpublished results from our laboratories have shown that in contrast to the abdominal musculature, the histological structure of the cheliped muscles of infected *N. norvegicus* is significantly altered similar to that seen in infected crabs, even during early stage infections. The contrasting effects of the disease on different muscle groups of *N. norvegicus* and between *N. norvegicus* and other decapods has previously been discussed in relation to differential responses of these muscle groups to parasite-derived proteases or to the specific structural properties of the abdominal musculature of *N. norvegicus* that prevents severe infiltration or collection of the parasite in this body region (see Stentiford et al., 2000b, 2002; Stentiford and Shields, 2005). Whatever the cause, the relative lack of effect of disease on the abdominal musculature is perhaps fortuitous for the fresh dead and frozen 'tailed' market for *N. norvegicus* since at least in

terms of mechanical integrity, the product appears not to be greatly altered.

Despite the general lack of effect of *Hematodinium* on the structural integrity of *N. norvegicus* abdominal musculature, several studies have demonstrated that significant alterations in host physiology and biochemistry accompany the progression of this disease. The massive proliferation of parasite stages within the haemolymph of infected lobsters (and crabs) lead to a pronounced hypoxia and to a switch to anaerobic metabolism during the advanced stages of the disease (Taylor et al., 1996; Love et al., 1996; Shields et al., 2003). Infected lobsters display perturbations in carbohydrate handling capacity with reserves of glycogen in the muscle and hepatopancreas being significantly reduced concomitant with infection (Stentiford et al., 2000b, 2001a). Glycogen from these regions is converted to glucose in response to stress (e.g. hypoxia). The response is controlled by the release of crustacean hyperglycemic hormone (CHH) from the sinus gland. In infected lobsters, plasma concentrations of CHH are significantly elevated, probably due to a parasite-derived disruption in feedback loops that control the release of the hormone from the sinus gland (Stentiford et al., 2001a). With advancing disease status, the elevated concentration of plasma CHH causes a depletion of glycogen in the hepatopancreas and a transitory increase in plasma glucose (presumably utilised by the developing parasite population) (Stentiford et al., 2001a). *Hematodinium* infection also results in a complete depletion of glycogen in the abdominal muscles (Gornik et al., 2010). Reductions in glycogen have also been reported to occur in several other *Hematodinium*-decapod models and likely represent a causal factor for morbidity and eventual mortality in infected hosts (Stentiford et al., 2000b; Shields et al., 2003). In addition to disruptions in carbohydrate profiles, detailed studies on the free amino acid (FAA) profiles of plasma and muscle have also been carried out on *Hematodinium*-infected lobsters (Stentiford et al., 1999, 2000b) and on the ratio of nucleotides in the muscle, as expressed in the adenylate energy charge (AEC) (Gornik et al., 2010). Tissue and haemocyte degradation and the induction of a generalised host stress response were associated with an increase in the concentration of FAAs such as taurine during infection. Also, the muscle AEC values were significantly reduced compared with those of uninfected animals. Such changes, in addition to those recorded for proteins (above) and salts in the plasma of *Hematodinium*-infected crabs (Hudson, 1995; Love et al., 1996) indicate a departure from the normal electrolyte status during disease and suggest induction of a significant stress cascade in infected hosts. As expected, the effect of alterations in salt, glycogen, FAA, nucleotides and protein handling in infected hosts are reflected in the organoleptic qualities of the abdominal musculature of infected animals. In a trial with a trained sensory panel, considerable differences have been detected between samples of fresh tail meat from uninfected and from heavily-parasitised animals with the latter being described as bland in flavour and after-taste, slightly less firm and less chewy, and with an overall lower liking (Neil et al., unpubl. data). In addition to alterations in the palatability of meat harvested from infected lobsters destined for the frozen and fresh dead market, departures from the normal physiological and biochemical well-being of the host will also impact upon survival and quality of animals destined for the live market. The impact of diseases such as *Hematodinium* as drivers for post-capture mortality events in decapods destined for live markets is currently understudied. Since other organ and tissue systems are not considered as direct commercial products from *N. norvegicus*, detailed descriptions of the pathology of these organs has not been provided here. The subject matter is described in more detail by Field and Appleton (1995) and by Stentiford and Shields (2005). Furthermore, Morado covers the pathological manifestation of *Hematodinium* infection in crabs within this volume.

#### 4.7. *Hematodinium* changes host behaviour

Based on the physiological perturbations imposed on lobsters by patent *Hematodinium* infection, several studies have considered how these effects may alter the locomotory performance and life history traits of host lobsters. *N. norvegicus* populations are generally found burrowing into soft muddy sediments and their capture (by trawlers) depends on emergence from the burrow and an inability to escape (via tail flipping) from an advancing fishing net (Farmer, 1974c). Since lobsters predominantly emerge to feed, any factor that alters the requirement for food may be expected to affect this entrained diurnal emergence behaviour (Farmer, 1974a). Furthermore, once out of the burrow, any factor that impedes swimming behaviour may also impact upon catchability by predators and the fishery (Newland et al., 1992). Both the speed and endurance of tail flip swimming have implications for capture by trawl nets. Studies by Stentiford and colleagues have demonstrated how burrow emergence pattern and swimming endurance are significantly altered during patent *Hematodinium* infections in *N. norvegicus*. In controlled tank trials using time-lapse filming of burrow emergence behaviour of healthy and diseased lobsters, heavily infected lobsters were spent more than 10 times longer out of the burrow than their uninfected counterparts. Infected lobsters also showed a loss of the diurnal (dusk/dawn) emergence pattern observed in uninfected lobsters. The increased time spent out of the burrow was interpreted as a symptom of 'physiological starvation' whereby the progressive consumption of host nutrient reserves by the growing parasite population necessitated increased feeding activity by infected animals. Alternatively, relatively hypoxic conditions within the burrow may have forced emergence onto the sediment surface in disease-stressed lobsters (Stentiford et al., 2001b). One intriguing event, reported by Stentiford et al. (2001b), was the spasmodic tail flipping that occurred in two lobsters immediately prior to death during filming. Whether this event coincided with the exsporation phase of the disease was not evident from the recordings though it is interesting to speculate that some active manipulation of host behaviour by the parasite population may assist with liberation of dinospores from terminally infected lobsters.

In addition to the increased time spent out of the burrow, infected lobsters also exhibit significantly reduced swimming ability. *Hematodinium*-infected lobsters showed a reduction in the number of tail flips performed, the number of swimming 'bouts' performed and the total distance travelled, concomitant with increasing disease burden. Further, the velocity of the first (giant-fibre mediated) flip was significantly less in diseased animals (Stentiford et al., 2000a). Studies on burrowing behaviour and swimming performance in diseased lobsters demonstrate how parasites may affect key life history traits of decapods. In addition to contributing towards the efficient transmission of parasites between hosts, some changes may also affect the availability of infected animals to the fishery (Field et al., 1998) and should be considered when estimating prevalence and mortality in natural populations (Stentiford and Shields, 2005).

#### 4.8. *Hematodinium* epizootiology

Studying parasite epizootiology in wild decapod populations is often hampered by inconsistent access by scientists to the fishery and an absence of long-term monitoring programs for commercially significant pathogens. The importance of *Hematodinium* as a mortality driver in the Scottish *N. norvegicus* fishery has been recognised since the early 1990s and considerable effort has been devoted to understanding the life history of this parasite in Scottish and Irish waters. Similar recognition of the importance of this parasite in temperate water crab stocks has led to *Hematodinium* being

the perhaps the best studied parasite of commercially exploited wild decapod stocks. Comprehensive surveys of *Hematodinium* prevalence in Scottish and Irish *N. norvegicus* populations using the pleopod diagnostic technique have demonstrated a consistently expressed seasonality with maximal prevalence reaching up to 70% during the spring (February–April) and lowest levels of detectable disease during the summer and autumn (July–October) (Field et al., 1992, 1998; Field and Appleton, 1995; Stentiford et al., 2001c,d; Briggs and McAliskey, 2002). More detailed surveys that attempted to include latently and sub-patently infected animals into prevalence estimates using antibody-based diagnostics demonstrated considerable underestimation of prevalence (when using the pleopod method) in the early season, with this decreasing as infections progress to patent disease in the later season (Stentiford et al., 2001d). Recent developments in molecular diagnostic tools for *Hematodinium* may allow this issue to be re-addressed, utilising the increased sensitivity of nucleic acid detection over the antibody techniques employed by Stentiford et al. (2001d) (Small et al., 2006, 2007). By combining *in situ* hybridization technologies with controlled transmission studies researchers should be able to identify initial infection sites on the host and further to elucidate early events in the pathogenesis of disease.

When studying the epizootiology of *Hematodinium* infection in decapods, it is important to consider information on potential reservoirs or alternative hosts to the pathogen and how infection prevalence in these organisms may affect that seen in the target fishery. In studies off the north east coast of the United States, Johnson (1986) demonstrated the presence of dinoflagellate parasites of the Order Duboscquodina, family Syndinidae in several species of benthic amphipods. The parasites were morphologically most similar to the decapod pathogen *H. perezi*. As in decapods, host reaction to the presence of parasites was very rare. In some cases, a co-infection with an unidentified fungus elicited a strong host reaction suggesting that host immune ability was not degraded by *Hematodinium* infection but rather that *Hematodinium* evades the system, a feature also recognised by Stentiford et al. (2003) for *Hematodinium*-yeast co-infections of the crabs *C. pagurus* and *N. puber*.

Similar studies have been carried out on the copepods. *Calanus finmarchicus* collected from the Clyde Sea Area in Scotland (the main site for the majority of studies on *Hematodinium* infection in Scottish *N. norvegicus* described above) were host to several dinoflagellate parasites (including *Syndinium* sp. and several more haemocoelic pathogens of 'more doubtful status') (Jepps, 1937) and high mortalities of *Paracalanus indicus* due to dinoflagellate parasites were reported by Kimmerer and McKinnon (1990). Considering these studies and the apparent propensity for non-decapod hosts to harbour infections by *Hematodinium*-like pathogens, it is tempting to suggest that these hosts may play an important role in the life history of *Hematodinium* infections of commercially significant decapods. Using molecular diagnostic and pathogen typing tools and by considering diseases of commercial species at the level of the ecosystem in which these hosts exist, it should now be possible to better investigate the life cycles of these important pathogens and to understand the interactions between apparent definitive and alternative or reservoir hosts. Studies of this kind may also allow for interpretation of the seasonal peak-nadir pattern of *Hematodinium* infections in commercially exploited decapods by identifying the ecosystem compartment where the pathogen resides when outside of the target host.

#### 4.9. Direct and indirect mortality

Consensus opinion of those carrying out studies on *Hematodinium* and *Hematodinium*-like dinoflagellate infections of decapods considers that patent disease has a fatal outcome with little evidence

for recovery (see Stentiford and Shields, 2005) and this is particularly apparent for patently infected *N. norvegicus* (Field et al., 1992). Assessment of long-term field data for *N. norvegicus* burrow counts (an accepted indicator of stock density) has shown that reductions in burrow count and landings per unit effort (LPUE) coincided with the highest prevalence of *Hematodinium* in *N. norvegicus* from this fishery (Field et al., 1998). While it is not possible to solely ascribe this reduction to the parasite, the data highlights how disease may contribute a significantly higher proportion of natural mortality than traditionally allowed for in fisheries models. Interestingly, several studies have also demonstrated how *Hematodinium* prevalence is highest at sites where *N. norvegicus* populations are smallest and/or are composed of relatively size matched individuals (Field et al., 1998; Stentiford et al., 2001c). Since population size-structuring is likely to be altered by fishing pressure, it is intriguing to propose that such anthropogenic drivers for population restructuring may have an effect on the detected prevalence of pathogens within hosts from those populations. In such a way, where diseases are deemed to have 'emerged' within a fishery (as apparent for *Hematodinium* in *N. norvegicus* populations from the Clyde Sea Area, Scotland in the late 1980s), emergence may be based on movement towards a higher proportion of susceptible hosts within the population (i.e. within a given size range or age) rather than a sudden environmental change or appearance of a pathogen. Given this effect, management of *Hematodinium* epidemics within *N. norvegicus* populations may be better predicted (and potentially mitigated) by closer observation to size-structuring in host populations and management of fisheries effort in populations that appear to be reaching optimal structuring for epidemics to occur.

In addition to direct mortality effects of *Hematodinium* in *N. norvegicus*, evidence also exists for effective castration of individuals and host populations. Shields (1994) notes that while dinoflagellate infections of crustaceans are typically parasitic castrators, castration has not been examined in crabs or lobsters infected by *Hematodinium*. With this said, several authors have noted severe infiltration of the ovary of infected crabs by *Hematodinium* (Messick and Shields, 2000; Stentiford et al., 2002) with apparent cessation of oocyte maturation in infected females. In this way, castration could be effected through the disruption of the testis or ovary of infected hosts. Briggs and McAliskey (2002) have demonstrated that infected female *N. norvegicus* from the Irish Sea do not develop mature gonads and we have shown that the gonads of *N. norvegicus* are destroyed during patent infections (Fig 5). Since size at maturity and peak size for *Hematodinium* infection prevalence coincides at approximately 21–34 mm in female *N. norvegicus* (Hillis and Tully, 1993; Tuck et al., 2000), the potential for disruption to recruitment in affected populations appears to be high. Further consideration of this issue is recommended in future studies.

## 5. Conclusions and future directions

Despite the relatively replete literature on the effects of *Hematodinium* infection on *N. norvegicus*, there is a comparative dearth of information on other pathogens (and their effects) of the clawed lobster genera *Nephrops* and *Metanephrops*. A lack of field surveillance driven by a perceived inability to manage and control disease in commercially exploited lobsters (and other wild crustaceans) is likely the reason for this. The outcome is a literature that appears to fall short on the information required to properly manage this important commercial resource and one that fails to fully assess the effect that disease may have on regulation of stock size and structure. Furthermore, attempts to assess the effect of diseases (such as *Hematodinium*) on the fishery stock of lobsters is hampered by a lack of coherence between data sets related to disease prevalence and those pertaining to stock size and structure. Future

efforts to integrate data collection procedures for fisheries and disease will lead to a better understanding of natural mortality in the field and may allow managers to more accurately discriminate sites that are more heavily affected by disease from others that are not. In addition, closer surveillance of wild populations will provide early warning for impending epidemics based around anthropogenically derived shifts in the population size, age and sex structure caused by fishing pressure. An increasing reliance on crustacean fisheries, particularly in the European marine area where *Nephrops* is considered a key resource will necessitate and drive this process.

Several research areas are particularly lacking. Firstly, none of the studies described in this chapter have considered the presence and effect of diseases in juvenile lobsters. As stated elsewhere in this volume, in aquaculture scenarios, diseases of juvenile Penaeid shrimp have played a highly significant role in defining success during on-growing phases of production. While diseases are often visible in the exploited (adult) portion of the fishery, they may potentially play an even more important role in defining the success of earlier life stages, and therefore in overall recruitment success for the fishery. The *Nephrops* fishery potentially provides an excellent model for this type of research since adults and juveniles are found on the same fishing grounds (and even share the same burrows during early development) and to a certain extent can be collected with similar fishing technologies. In other lobster genera (such as *Homarus*) juveniles are often more cryptic and are potentially separated from the fishing grounds where the adults are found. While studying juveniles and adults from the same grounds will inform on the susceptibility of different life stages to particular diseases, it will also highlight potentially undiscovered pathogens in host life stages not previously considered. Such approaches may elucidate immunological reasons for the apparent lack of description of viral and other pathogens in adult clawed lobsters. This approach is also consistent with any stock assessment techniques based upon cohort-to-cohort success by providing detailed overviews of the specific pathogen profile of animals within respective cohorts.

A second major theme for future studies should involve the effect that commercial practices may have on assisting the perpetuation and spread of pathogens of commercially important crustaceans. A much-neglected potential threat to biosecurity, particularly within the European Union is the relatively free movement of live crustaceans from point of capture to point of market. In several countries (particularly the UK), following capture, wild marine crustaceans (e.g. *N. norvegicus*, *C. pagurus*, *H. gammarus*) are transported live to continental Europe for resale and consumption. With the exception of *H. gammarus* (which are subject to certain movement restrictions associated with the notifiable pathogen causing Gaffkemia), the movement of live crustaceans in this way is relatively uncontrolled with losses in transport remaining unrecorded and morbid or dead animals potentially finding their way back into the aquatic environment at the distant site. Furthermore, water used for transporting animals may be released to local waterways or drains. The significant dearth in knowledge of potential pathogens of our major commercially exploited species and their potential for transmission to other commercially exploited and reservoir species identify these as high risk practices. Live transport of animals to distant markets when coupled with the apparent high potential for parasites such as *Hematodinium* to infect multiple decapod and non-decapod hosts is cause for concern (Stentiford and Shields, 2005), especially since similar scenarios have previously caused major problems for Penaeid shrimp culture (see Lightner paper in this volume).

A final theme for future study, particularly where expensive monitoring programs are devoted to surveying of wild stocks in-

volves a shift from screening for individual pathogens of concern at the time to a more holistic screening program using tools to identify both target and non-target pathogens. Issues such as climate change are likely to progressively and unexpectedly alter the balance between host, pathogen, and environment. In such a way, low prevalence of innocuous commensals of today may become highly prevalent and significant pathogens of the future and alternatively, major pathogens of today may become less of a challenge for future stocks. Closer attention to pathogens in these wild resources, using holistic tools such as histopathology and dedicated multiplex molecular diagnostic tools not only inform on likely driving forces for mortality in wild fisheries but also pre-warn about potential problems that may exist when attempting to intensively culture these species in the future.

## References

- Anderson, J.I.W., Conroy, D.A., 1968. The significance of disease in preliminary attempts to raise Crustacea in sea water. *Bull. Off. Int. Epiz.* 69, 1239–1247.
- Appleton, P.L., Vickerman, K., 1998. In vitro cultivation and development cycle in culture of a parasitic dinoflagellate (*Hematodinium* sp.) associated with mortality of the Norway lobster (*Nephrops norvegicus*) in British waters. *Parasitology* 116, 115–131.
- Aréchiga, H., Atkinson, R.J.A., 1975. The eye and some effects of light on the locomotor activity in *Nephrops norvegicus*. *Mar. Biol.* 32, 63–76.
- Ateş, A.S., Trilles, J.-P., İşmen, A., Yiğın, C.C., 2006. New unusual associations involving parasitic isopods. *Crustaceana* 79, 375–380.
- Atkinson, R.J.A., Naylor, E., 1976. An endogenous activity rhythm and rhythmicity of catches of *Nephrops norvegicus*. *J. Exp. Mar. Biol. Ecol.* 25, 95–108.
- Barnes, H., Bagenal, T.B., 1951. Observations on *Nephrops norvegicus* (L.) and on an epizoic population of *Balanus crenatus* Brug. *J. Mar. Biol. Assoc., UK* 30, 369–380.
- Bartolo, I., Birk, E.O., 1998. Some factors affecting Norway lobster (*Nephrops norvegicus*) cuticle polyphenol oxidase activity and black spot development. *Int. J. Food Sci. Technol.* 33, 329–336.
- Bayer, R.C., Prince, D.L., Waltz, C.D., Corey, A.R., Getchell, R.G., 1989. Scanning electron microscopy and X-ray analysis of shell disease lesions in the American lobster. *J. Shellfish Res.* 8, 481–482.
- Beever, N., 2010. Life Cycle and Prevalence of a Dinoflagellate Parasite *Hematodinium* sp. in the Norway Lobster *Nephrops norvegicus* (L.). Ph.D. Thesis, University of Glasgow.
- Bell, M.C., Redant, F., Tuck, I., 2006. *Nephrops* species. In: Phillips, B.F. (Ed.), *Lobsters: Biology, Management, Aquaculture, Fisheries*. Blackwell Publishing Ltd., Oxford, UK, pp. 412–461.
- Boghen, A.D., 1978. A parasitological survey of the American lobster *Homarus americanus* from the Northumberland Strait, southern Gulf of Lawrence. *Can. J. Zool.* 56, 2460–2462.
- Bower, S.M., 1996. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: Gregarine Parasitism of Lobsters. <[http://www.pac.dfo-mpo.gc.ca/sci/shellfish/pages/gregplo\\_e.htm](http://www.pac.dfo-mpo.gc.ca/sci/shellfish/pages/gregplo_e.htm)>.
- Brattey, J., Campbell, A., 1985a. A survey of parasites of the American lobster, *Homarus americanus* (Crustacea: Decapoda), from the Canadian maritimes. *Can. J. Zool.* 64, 1998–2003.
- Brattey, J., Campbell, A., 1985b. Occurrence of *Histriobdella homari* (Annelida, Polychaeta) on the American lobster in the Canadian maritimes. *Can. J. Zool.* 63, 392–395.
- Briggs, R.P., McAliskey, M., 2002. The prevalence of *Hematodinium* in *Nephrops norvegicus* from the western Irish Sea. *J. Mar. Biol. Assoc., UK* 82, 427–433.
- Briggs, R.P., Atkinson, R.J.A., McAliskey, M., Rogerson, P.Y., 1997. *Histriobdella homari* on *Nephrops norvegicus* from the Irish Sea and Clyde Sea area. *J. Mar. Biol. Assoc., UK* 77, 557–559.
- Bruce, J.R., Colman, J.S., Jones, N.S., 1963. Marine Fauna of the Isle of Man and Its Surrounding Seas. LMBC Memoirs, second ed. no. 36, p. 307.
- Burukovsky, R.N., Averin, B.S., 1977. A replacement name, *Thymopides*, proposed for the preoccupied generic name *Bellator* (Decapoda, Nephropidae). *Crustaceana* 32, 216.
- Bushek, D., Dungan, C.F., Lewitus, A.J., 2002. Serological affinities of the oyster pathogen *Perkinsus marinus* (Apicomplexa) with some dinoflagellates (Dinophyceae). *J. Eukar. Microbiol.* 49, 11–16.
- Chapman, C.J., 1980. Ecology of juvenile and adult *Nephrops*. The Biology and Management of Lobsters, vol. 2. Academic Press (Chapter 4).
- Chapman, C.J., Howard, F.G., 1979. Field observations on the emergence rhythm of the Norway lobster, *Nephrops norvegicus* (L.) using different methods. *Mar. Biol.* 10, 157–165.
- Chapman, C.J., Rice, A.L., 1971. Some direct observations on the ecology and behaviour of the Norway lobster *Nephrops norvegicus*. *Mar. Biol.* 10, 321–329.
- Chapman, C.J., Shelton, P.M.J., Shanks, A.M., Gaten, E., 2000. Survival and growth of the Norway lobster *Nephrops norvegicus* in relation to light-induced eye damage. *Mar. Biol.* 136, 233–241.
- Chatton, E., Poisson, R., 1931. Sur l'existence, dans le sang des crabs, de peridinies parasites: *Hematodinium perezii* n.g., n.sp. (Syndinidae). *CR Seances Soc. Biol. Paris* 105, 553–557.
- Chualain, C.N., Hayes, M., Allen, B., Robinson, M., 2009. *Hematodinium* sp. in Irish *Cancer pagurus* fisheries: infection intensity as a potential fisheries management tool. *Dis. Aquat. Organ.* 83, 59–66.
- Cristo, M., 1998. Feeding ecology of *Nephrops norvegicus* (Decapoda: Nephropidae). *J. Nat. Hist.* 32, 1493–1498.
- Cunningham, J.T., 1887. On *Stichocotyle nephrops*, a new trematodes. *Trans. Roy. Soc. Edin.* 32, 273–280.
- Dana, J.D., 1852. Crustacea. In: United States Exploring Expedition during the Years 1838, 1839, 1840, 1841, 1842 Under the Command of Charles Wilkes, U.S.N., vol. 13, 1620 p.
- Dawe, E., 2002. Trends in the prevalence of bitter crab disease caused by *Hematodinium* sp. in snow crab (*Chionoecetes opilio*) through out the Newfoundland and Labrador continental shelf. In: *Crabs in Cold Water Regions: Biology, Management, and Economics*, Alaska Sea Grant College Program, AK-SG-02-01, 2002, pp. 385–400.
- Diaz, N., Gervain, P., Druault-Aubin, V., 2003. First results of experimental deep-sea Crustacean fisheries in Guadeloupe (F.W.I.). *Proc. Gulf. Caribb. Fish. Inst.* 54, 307–320.
- Dow, R.L., 1980. The clawed lobster fisheries. In: Cobb, J.S., Phillips, B.F. (Eds.), *The Biology, Management of Lobsters*, vol. II. Academic Press, New York, pp. 265–313.
- Eaton, W.D., Love, D.C., Botelho, C., Meyers, T.R., Imamura, K., Koeneman, T., 1991. Preliminary results on the seasonality and life cycle of the parasitic dinoflagellate causing Bitter Crab Disease in Alaskan Tanner crabs (*Chionoecetes bairdi*). *J. Invertebr. Pathol.* 57, 426–434.
- Farmer, A.S.D., 1974a. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). *J. Zool. Lond.* 174, 161–183.
- Farmer, A.S.D., 1974b. Burrowing behaviour of the Norway lobster, *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *Estuar. Coast. Mar. Sci.* 2, 49–58.
- Farmer, A.S.D., 1974c. Field assessments of diurnal activity in Irish sea populations of the Norway lobster, *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). *Estuar. Coast. Mar. Sci.* 2, 37–47.
- Farmer, A.S.D., 1977. Epizoic Foraminifera on *Nephrops norvegicus*. *J. Mar. Biol. Assoc., UK* 57, 877–878.
- Field, R.H., Appleton, P.L., 1995. A *Hematodinium*-like infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. *Dis. Aquat. Organ.* 22, 115–128.
- Field, R.H., Appleton, P.L., 1996. An indirect fluorescent antibody technique for the diagnosis of *Hematodinium* sp. infection of the Norway lobster *Nephrops norvegicus*. *Dis. Aquat. Organ.* 24, 199–204.
- Field, R.H., Chapman, C.J., Taylor, A.C., Neil, D.M., Vickerman, K., 1992. Infection of the Norway lobster *Nephrops norvegicus* by a *Hematodinium*-like species of dinoflagellate on the west coast of Scotland. *Dis. Aquat. Organ.* 13, 1–15.
- Field, R.H., Hills, J.M., Atkinson, R.J.A., Magill, S., Shanks, A.M., 1998. Distribution and seasonal prevalence of *Hematodinium* sp. infection of the Norway lobster (*Nephrops norvegicus*) around the west coast of Scotland. *ICES J. Mar. Sci.* 55, 846–858.
- Funch, P., Kristensen, R.M., 1995. Cyclophora is a new phylum with affinities to Entoprocta and Ectoprocta. *Nature* 378, 711–714.
- Gaten, E., 1988. Light-induced damage to the dioptric apparatus of *Nephrops norvegicus* (L.) and the quantitative assessment of damage. *Mar. Freshwater Behav. Physiol.* 13, 169–183.
- Gonzalez, M.T., Acuna, E., 2004. Infestation by *Pseudione humboldtensis* (Bopyridae) in the squat lobsters *Cervimunida johni* and *Pleuroncodes monodon* (Galatheidae) off northern Chile. *J. Crustacean Biol.* 24, 618–624.
- González-Gurriarán, E., Friere, J., Farina, A.C., Fernández, A., 1998. Growth at moult and intermoult period of the Norway lobster *Nephrops norvegicus* from Galician waters. *ICES J. Mar. Sci.* 55, 924–940.
- Gornik, S.G., Albalat, A., Atkinson, R.J.A., Coombs, G.H., Neil, D.M., 2010. The influence of defined ante-mortem stressors on the early post-mortem biochemical processes in the abdominal muscle of the Norway lobster, *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Biol. Res.* doi: 10.1080/1745100903147468.
- Grassini, C.M., 1994. Estudios preliminares de *Telotha henselii* (Crustacea: Isopoda: Cymothoidea) parásito de camarones Palemonidos. *Ann. Mus. Hist. Nat. Valparaiso* 22, 81.
- Gruebl, T., Frischer, M.E., Sheppard, M., Neumann, M., Maurer, A.N., Lee, R.F., 2002. Development of an 18S rRNA gene-targeted PCR-based diagnostic for the blue crab parasite *Hematodinium* sp. *Dis. Aquat. Organ.* 49, 61–70.
- Hillis, J.P., Tully, O., 1993. Growth Rate, Mortality and Small Mean Size in Irish Sea *Nephrops*. *ICES Council Meeting Papers*, Copenhagen. 13 pp.
- Holthuis, L.B., 1991. Marine Lobsters of the World: An Annotated and Illustrated Catalogue of Species of Interest to Fisheries Known to Date. *FAO Fisheries Synopsis*, 125, vol. 13. 292 pp.
- Hopwood, D., 1996. Fixation and fixatives. In: Bamcroft, J.D., Stevens, A. (Eds.), *Theory and Practice of Histopathological Techniques*, fourth ed. Churchill Livingstone, Hong Kong, pp. 23–46.
- Hudson, D.A., 1995. Biochemical parameters of the serum of the sand crab, *Portunus pelagicus*, with reference to the parasitic dinoflagellate, *Hematodinium australis*. *Bull. Eur. Assoc. Fish. Pathol.* 15, 202–205.
- Hudson, D.A., Adlard, R.D., 1994. PCR-techniques applied to *Hematodinium* spp. and *Hematodinium*-like dinoflagellates in decapod crustaceans. *Dis. Aquat. Organ.* 20, 203–206.
- Hudson, D.A., Adlard, R.D., 1996. Nucleotide sequence determination of the partial SSU rDNA gene and ITS1 region of *Hematodinium* cf. *perezii* and *Hematodinium*-like dinoflagellates. *Dis. Aquat. Organ.* 24, 55–60.

- Hudson, D.A., Shields, J.D., 1994. *Hematodinium australis* n. sp., a parasitic dinoflagellate of the sand crab *Portunus pelagicus* from Moreton Bay, Australia. *Dis. Aquat. Organ.* 19, 109–119.
- Jepps, M.W., 1937. On the protozoan parasites of *Calanus finmarchicus* in the Clyde Sea Area. *Quart. J. Microscop. Sci.* 79, 589–658.
- Johnson, P.T., 1986. Parasites of benthic amphipods: dinoflagellates (Duboscquodiniida: Syndinidae). *Fish. Bull.* 84, 605–614.
- Kabata, Z., 1966. *Nicothoë analata* sp. nov., a parasitic copepod from the South China Sea. *Crustaceana* 11, 10–16.
- Kabata, Z., 1967. *Nicothoë* Audouin H. Milne Edwards, 1826 (Crustacea, Copepoda), a genus parasitic on *Nephrops* Leach, 1816 (Crustacea, Decapoda). *Zool. Meded.* 42, 147–161.
- Kimmerer, W.J., McKinnon, A.D., 1990. High mortality in a copepod population caused by a parasitic dinoflagellate. *Mar. Biol.* 107, 449–452.
- Latrouite, D., Morizur, Y., Noël, P., Chagot, D., Wilhelm, G., 1988. Mortalité du tourteau *Cancer pagurus* provoquée par le dinoflagellate parasite: *Hematodinium* sp. Conseil International pour l'Exploration de la Mer, CM. 1988/K:32.
- Léger, L., Duboscq, O., 1915. *Porospora nephropsis* n. sp. *CR Soc. Biol.* 78, 368–371.
- Lemos de Castro, A., 1985. Ectoparasitism of *Telotha henselii* (Von Martens) (Isopoda, Cymothoidea) on *Macrobrachium brasiliense* (Heller) (Decapoda-Palaemonidae). *Crustaceana* 49, 200–201.
- Lerch, F., Uglem, I., 1996. High density of *Histriobdella homari* Van Beneden, 1858 (Annelida, Polychaeta) on ovigerous female European lobsters (Decapoda, Nephropidae). *Crustaceana* 69, 916–920.
- Loew, E.R., 1976. Light and photoreceptor degeneration in the Norway lobster, *Nephrops norvegicus* (L.). *Proc. Roy. Soc. Lond. B* 193, 31–44.
- Loo, L.-O., Pihl Baden, S., Ulmestrand, M., 1993. Suspension feeding in adult *Nephrops norvegicus* (L.) and *Homarus gammarus* (L.) (Decapoda). *Neth. J. Sea Res.* 31, 291–297.
- Love, D.C., Rice, S.D., Moles, D.A., Eaton, W.D., 1993. Seasonal prevalence and intensity of Bitter Crab dinoflagellate infection and host mortality in Alaskan Tanner crabs *Chionoecetes bairdi* from Auke Bay, Alaska, USA. *Dis. Aquat. Organ.* 15, 1–7.
- Love, D., Thomas, R., Moles, A., 1996. Bitter Crab Hemolymph Studies: Indications of Host Physiological Condition. Alaska Sea Grant College Program, AK-SG-96-02, 1996.
- MacKenzie, K., 1963. *Stichocotyle nephropsis* Cunningham, 1887 (Trematoda) in Scottish Waters. *Ann. Mag. Nat. Hist.* 6 (Ser. 13), 505–506.
- MacLean, S.A., Ruddell, C.L., 1978. Three new crustacean hosts for the parasitic dinoflagellate *Hematodinium perezii* (Dinoflagellata: Syndinidae). *J. Parasitol.* 64, 158–160.
- Mariappan, P., Balasundaram, C., Schmitz, B., 2000. Decapod crustacean chelipeds: an overview. *J. Biosci.* 25, 301–313.
- Markham, J.C., 1999. Crustacea Isopoda: Bopyridae in the MUSORSTOM collections from the tropical Indo-Pacific. 2. Species in subfamily Pseudioninae infesting non-anomuran hosts. *Mem. Mus. Natl. Hist. Nat.* 180, 254–265.
- Martinez-Alvarez, O., López-Caballero, M.E., Montero, P., Gómez-Guillén, M.C., 2007. Spraying of 4-hexylresorcinol based formulations to prevent enzymatic browning in Norway lobsters (*Nephrops norvegicus*) during chilled storage. *Food Chem.* 100, 147–155.
- Mason, J., 1959. The biology of *Nicothoë astaci* Audouin and Milne Edwards. *J. Mar. Biol. Assoc. UK* 38, 3–16.
- Merella, P., Alemany, F., Carbonell, A., Quetglas, A., 1998. Fishery and biology of Norway lobster *Nephrops norvegicus* (Decapoda: Nephropidae) in Mallorca (western Mediterranean). *J. Nat. Hist.* 32, 1631–1640.
- Messick, G.A., 1994. *Hematodinium perezii* infections in adult and juvenile blue crabs *Callinectes sapidus* from coastal bays of Maryland and Virginia, USA. *Dis. Aquat. Organ.* 19, 77–82.
- Messick, G.A., Shields, J.D., 2000. Epizootiology of the parasitic dinoflagellate *Hematodinium* sp. in the American blue crab *Callinectes sapidus*. *Dis. Aquat. Organ.* 43, 139–152.
- Meyers, T.R., Koeneman, T.M., Bothelho, C., Short, S., 1987. Bitter Crab Disease: a fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs *Chionoecetes bairdi*. *Dis. Aquat. Organ.* 3, 195–216.
- Meyers, T.R., Botelho, C., Koeneman, T.M., Short, S., Imamura, K., 1990. Distribution of bitter crab dinoflagellate syndrome in southeast Alaskan tanner crabs, *Chionoecetes bairdi*. *Dis. Aquat. Organ.* 9, 37–43.
- Meyers, T.R., Morado, J.F., Sparks, A.K., Bishop, G.H., Pearson, T., Urban, D., Jackson, D., 1996. Distribution of bitter crab syndrome in tanner crabs (*Chionoecetes bairdi*, *C. opilio*) from the Gulf of Alaska and the Bering Sea. *Dis. Aquat. Organ.* 26, 221–227.
- Montreuil, P., 1954. Parasitological Investigations. Rapport Annual, Station Biologique Marine Department Peches 1953, Quebec. Contribution 50, Appendix 5, pp. 69–73.
- Morris, S.C., 1995. A new phylum from the lobster's lips. *Nature* 378, 661–662.
- Munoz, G.L., 1997. First record of bopyrid isopods (Isopoda: Epicaridea) in the ghost-shrimp *Notiax brachyophthalma* (M. Edwards, 1870) and some aspects on the host-parasite relationship. *Gayana. Oceanol.* 5, 33–39.
- Newland, P.L., Chapman, C.J., 1989. The swimming and orientation behaviour of the Norway lobster, *Nephrops norvegicus* (L.) in relation to trawling. *Fish. Res.* 8, 63–80.
- Newland, P.L., Neil, D.M., Chapman, C.J., 1992. Escape swimming in the Norway Lobster. *J. Crust. Biol.* 12, 342–353.
- Newman, M.W., Johnson, C.A., 1975. A disease of blue crabs (*Callinectes sapidus*) caused by a parasitic dinoflagellate, *Hematodinium* sp. *J. Parasitol.* 63, 554–557.
- Odhner, T., 1898. Ueber die geschlechtsreife Form von *Stichocotyle nephropsis* Cunningham. *Zool. Anz.* 21, 509–513.
- Ogawa, M., Kurotsu, T., Ochiai, I., Kozima, T.T., 1983. Black colouration of spiny lobster genus *Panulirus* White. II. Mechanism of discolouration in spiny lobster tails stored in ice. *Nipp. Suis. Gakk.* 49, 1065–1075.
- Ogawa, M., Magalhaes-Neto, E., Aguiar-Junior, O., Kozima, T.T., 1984a. Black discolouration of spiny lobster genus *Panulirus* White. III. Incidence of melanosis in the integumentary tissue. *Nipp. Suis. Gakk.* 50, 471–475.
- Ogawa, M., Perdigão, N.B., Santiago, M.E., Kozima, T.T., 1984b. On physiological aspects of blackspot development in shrimp. *Nipp. Suis. Gakk.* 50, 1763–1769.
- Paradiso, M.L., Bottari, T., Marino, F., Boyko, C.B., Rinelli, P., Giannetto, S., 2004. Presence and histopathology of the parasitic isopod, *Pseudione affinis* (Epicaridea, Bopyridae) on pandalid shrimps from the central Mediterranean Sea. *Crustaceana* 77, 397–405.
- Pestal, G.P., Taylor, D.M., Hoenig, J.M., Shields, J.D., Pickavance, R., 2003. Monitoring the presence of the lethal parasite *Hematodinium* sp. in snow crabs from Newfoundland. *Dis. Aquat. Organ.* 53, 67–75.
- Poupin, J., 1993. Deep-Sea Fauna of the French West Indies: Fishing Surveys on-board R.V. Polka Carried out in 1993. Institut Français de Recherche Scientifique pour le Développement en Coopération. Etudes et Thèses, 1993.
- Rice, A.L., Chapman, C.J., 1971. Observations on the burrows and burrowing behaviour of two mud-dwelling Decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. *Mar. Biol.* 10, 330–342.
- Ridgway, I.D., Taylor, A.C., Atkinson, R.J.A., Stentiford, G.D., Chang, E.S., Chang, S.A., Neil, D.M., 2006. Morbidity and mortality in Norway lobsters, *Nephrops norvegicus*: physiological, immunological and pathological effects of aerial exposure. *J. Exp. Mar. Biol. Ecol.* 328, 251–264.
- Ridgway, I.D., Stentiford, G.D., Taylor, A.C., Atkinson, R.J.A., Neil, D.M., 2007. Idiopathic muscle necrosis in the Norway lobster *Nephrops norvegicus*: aetiology, pathology and progression to bacteraemia. *J. Fish Dis.* 29, 1–14.
- Roccatagliata, D., Lovrich, G.A., 1999. Infestation of the false king crab *Paralomis granulosa* (Decapoda: Lithididae) by *Pseudione tuberculata* (Isopoda: Bopyridae) in the Beagle Channel, Argentina. *J. Crust. Biol.* 19, 720–729.
- Rogers, S.I., Ellis, J.R., 2000. Changes in the demersal fish assemblages of British coastal waters during the 20th century. *ICES J. Mar. Sci.* 57, 866–881.
- Sardá, F., 1998. Symptoms of overexploitation in the stock of the Norway lobster (*Nephrops norvegicus*) on the “Serola bank” (western Mediterranean sea off Barcelona). *Sci. Mar.* 62, 295–299.
- Segonzac, M., MacPherson, E., 2003. A new deep-sea lobster of the genus *Thymopides* (Crustacea: Decapoda: Nephropidae) collected near the hydrothermal vent Snake Pit, Mid-Atlantic Ridge. *Can. Biol. Mar.* 44, 361–367.
- Shelton, M.J., Gatén, E., Chapman, C.J., 1986a. Light and retinal damage in *Nephrops norvegicus* (L.) (Crustacea). *Proc. Roy. Soc. Lond. B* 226, 217–236.
- Shelton, M.J., Gatén, E., Chapman, C.J., 1986b. Accessory pigment distribution in the compound eye of *Nephrops norvegicus* (L.) (Crustacea: Decapoda). *J. Exp. Mar. Biol. Ecol.* 98, 185–198.
- Sheppard, M., Walker, A., Frischer, M.E., Lee, R.F., 2003. Histopathology and prevalence of the parasitic dinoflagellate *Hematodinium* sp. in crabs (*Callinectes sapidus*, *Callinectes similis*, *Neopanope sayi*, *Libinia emarginata*, *Menippe mercenaria*) from a Georgia estuary. *J. Shellfish Res.* 22, 873–880.
- Shields, J.D., 1994. The parasitic dinoflagellates of marine crustaceans. *Ann. Rev. Fish. Dis.* 4, 241–271.
- Shields, J.D., Behringer, D.C., 2004. A new pathogenic virus in the Caribbean spiny lobster *Panulirus argus* from the Florida Keys. *Dis. Aquat. Organ.* 59, 109–118.
- Shields, J.D., Squyars, C.M., 2000. Mortality and hematology of blue crabs, *Callinectes sapidus*, experimentally infected with the parasitic dinoflagellate *Hematodinium perezii*. *Fish. Bull.* 98, 139–152.
- Shields, J.D., Scanlon, C., Volety, A., 2003. Aspects of the pathophysiology of blue crabs, *Callinectes sapidus*, infected with the parasitic dinoflagellate *Hematodinium perezii*. *Bull. Mar. Sci.* 72, 519–535.
- Shields, J.D., Taylor, D.M., Sutton, S.G., O'Keefe, P.O., Collins, P.W., Ings, D.W., Pardy, A.L., 2005. Epizootology of bitter crab disease (*Hematodinium* sp.) in snow crabs, *Chionoecetes opilio*, from Newfoundland, Canada. *Dis. Aquat. Organ.* 64, 253–264.
- Shields, J.D., Stephens, F.J., Jones, B., 2006. Pathogens, parasites and other symbionts. In: Phillips, B.F. (Ed.), *Lobsters: Biology, Management, Aquaculture, Fisheries*. Blackwell Publishing Ltd., Oxford, UK, pp. 146–204.
- Shiino, S.M., 1951. Some bopyrid parasites found on the decapod crustaceans from the waters along the Mie Prefecture. *Rep. Fac. Fish., Pref. Univ. Mie* 1, 26–40.
- Small, H.J., Wilson, S., Neil, D.M., Hagan, P., Coombs, G.H., 2002. Detection of the parasitic dinoflagellate *Hematodinium* in the Norway lobster *Nephrops norvegicus* by ELISA. *Dis. Aquat. Organ.* 52, 175–177.
- Small, H.J., Neil, D.M., Taylor, A.C., Bateman, K., Coombs, G.H., 2005a. A parasitic scuticociliate infection in the Norway lobster (*Nephrops norvegicus*). *J. Invertebr. Pathol.* 90, 108–117.
- Small, H.J., Neil, D.M., Taylor, A.C., Coombs, G.H., 2005b. Identification and partial characterisation of metalloproteases secreted by a *Mesanoophrys*-like ciliate parasite of the Norway lobster *Nephrops norvegicus*. *Dis. Aquat. Organ.* 67, 225–231.
- Small, H.J., Neil, D.M., Taylor, A.C., Atkinson, R.J.A., Coombs, G.H., 2006. Molecular detection of *Hematodinium* spp. in Norway lobster *Nephrops norvegicus* and other crustaceans. *Dis. Aquat. Organ.* 69, 185–195.
- Small, H.J., Shields, J.D., Moss, J.A., Reece, K.S., 2007. Conservation in the first internal transcribed spacer region (ITS1) in *Hematodinium* species infecting crustacean hosts found in the UK and Newfoundland. *Dis. Aquat. Organ.* 75, 251–258.
- Smolowitz, R., Chistoserdov, A.Y., Hsu, A., 2005. A description of the pathology of epizootic shell disease in the American lobster, *Homarus americanus*, H. Milne Edwards 1837. *J. Shell. Res.* 24, 749–756.

- Stentiford, G.D., Feist, S.W., 2005. A histopathological survey of shore crab (*Carcinus maenas*) and brown shrimp (*Crangon crangon*) from six UK estuaries. *J. Invertebr. Pathol.* 88, 136–146.
- Stentiford, G.D., Neil, D.M., 2000. A rapid onset, post-capture muscle necrosis in the Norway lobster, *Nephrops norvegicus* L. from the West Coast of Scotland, United Kingdom. *J. Fish Dis.* 23, 251–264.
- Stentiford, G.D., Shields, J.D., 2005. A review of the parasitic dinoflagellates *Hematodinium* species and *Hematodinium*-like infections in marine crustaceans. *Dis. Aquat. Organ.* 66, 47–70.
- Stentiford, G.D., Neil, D.M., Coombs, G.H., 1999. Changes in the plasma free amino acid profile of the Norway lobster *Nephrops norvegicus* at different stages of infection by a parasitic dinoflagellate (genus *Hematodinium*). *Dis. Aquat. Organ.* 38, 151–157.
- Stentiford, G.D., Neil, D.M., Atkinson, R.J.A., Bailey, N., 2000a. An analysis of swimming performance in the Norway lobster, *Nephrops norvegicus* L. infected by a parasitic dinoflagellate of the genus *Hematodinium*. *J. Exp. Mar. Biol. Ecol.* 247, 169–181.
- Stentiford, G.D., Neil, D.M., Coombs, G.H., 2000b. Alterations in the biochemistry and ultrastructure of the deep abdominal flexor muscle of the Norway lobster, *Nephrops norvegicus* L. during infection by a parasitic dinoflagellate of the genus *Hematodinium*. *Dis. Aquat. Organ.* 42, 133–141.
- Stentiford, G.D., Chang, E.S., Chang, S.A., Neil, D.M., 2001a. Carbohydrate dynamics and the crustacean hyperglycaemic hormone (CHH): effects of parasitic infection in Norway lobsters (*Nephrops norvegicus*). *Gen. Comp. Endocrinol.* 121, 13–22.
- Stentiford, G.D., Neil, D.M., Atkinson, R.J.A., 2001b. Alteration of burrow-related behaviour of the Norway lobster, *Nephrops norvegicus* during infection by the parasitic dinoflagellate *Hematodinium*. *Mar. Freshwater Behav. Physiol.* 34, 139–156.
- Stentiford, G.D., Neil, D.M., Atkinson, R.J.A., 2001c. The relationship of *Hematodinium* infection prevalence in a Scottish *Nephrops norvegicus* population to seasonality, moulting and sex. *ICES J. Mar. Sci.* 58, 814–823.
- Stentiford, G.D., Neil, D.M., Coombs, G.H., 2001d. Development and application of an immunoassay diagnostic technique for studying *Hematodinium* infections in *Nephrops norvegicus* populations. *Dis. Aquat. Organ.* 46, 223–229.
- Stentiford, G.D., Green, M., Bateman, K., Small, H.J., Neil, D.M., Feist, S.W., 2002. Infection by a *Hematodinium*-like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab *Cancer pagurus*. *J. Invertebr. Pathol.* 79, 179–191.
- Stentiford, G.D., Evans, M., Bateman, K., Feist, S.W., 2003. Co-infection by a yeast-like organism in *Hematodinium*-infected European edible crabs (*Cancer pagurus*) and velvet swimming crabs (*Necora puber*) from the English Channel. *Dis. Aquat. Organ.* 54, 195–202.
- Stentiford, G.D., Bonami, J.R., Alday-Sanz, V., 2009. A critical review of susceptibility of crustaceans to Taura syndrome, yellowhead disease and white spot disease and implications of inclusion of these diseases in European legislation. *Aquaculture* 291, 1–17.
- Stentiford, G.D., Bateman, K.S., Small, H.J., Moss, J., Shields, J.D., Reece, K.S., Tuck, I., 2010. *Myospora metanephrops* (n. gn., n. sp.) from marine lobsters and a proposal for erection of a new Order and Family (Crustaceacida; Myosporidae) in the Class Marinosporidia (Phylum Microsporidia). *Int. J. Parasitol.* 40, 1433–1446.
- Sund, O., 1914. Beretning om anlæg av statens hummeravlsstation og driften i 1913. *Arseberetning Vedkommende Norges Fiskerier* 4, 525–532.
- Symonds, D.J., 1972. Infestation of *Nephrops norvegicus* (L.) by *Stichocotyle nephrops* Cunningham in British waters. *J. Nat. Hist.* 6, 423–426.
- Tärnlund, S., 2000. A Comparison of Two Methods for Identifying and Assessing the Parasitic Dinoflagellate *Hematodinium* sp. in Norway Lobster (*Nephrops norvegicus*). MSc Thesis, University of Göteborg, Sweden.
- Taylor, D.M., Khan, R.A., 1995. Observations on the occurrence of *Hematodinium* sp. (Dinoflagellata: Syndinidae): the causative agent of Bitter Crab Disease in the Newfoundland snow crab (*Chionoecetes opilio*). *J. Invertebr. Pathol.* 65, 283–288.
- Taylor, A.C., Field, R.H., Parslow-Williams, P.J., 1996. The effects of *Hematodinium* sp.-infection on aspects of the respiratory physiology of the Norway lobster, *Nephrops norvegicus* (L.). *J. Exp. Mar. Biol. Ecol.* 207, 217–228.
- Théodoridès, J., Laird, M., 1970. Quelques Eugrégaires parasites d'invertébrés marins de St. Andrews (Nouveau Brunswick). *Can. J. Zool.* 48, 1013–1013-16.
- Thomson, J.S., 1896. A copepod parasite of *Nephrops norvegicus*. *Proc. Roy. Soc. Edin.* 13, 246–250.
- Tuck, I.D., Chapman, C.J., Atkinson, R.J.A., 1997a. Population biology of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland – I: growth and density. *ICES J. Mar. Sci.* 54, 125–135.
- Tuck, I.D., Chapman, C.J., Atkinson, R.J.A., Bailey, N., Smith, R.S.M., 1997b. A comparison of methods for stock assessment of the Norway lobster, *Nephrops norvegicus*, in the Firth of Clyde. *Fish. Res.* 32, 89–100.
- Tuck, I.D., Atkinson, R.J.A., Chapman, C.J., 2000. Population biology of the Norway lobster, *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland II: fecundity and size at onset of sexual maturity. *ICES J. Mar. Sci.* 57, 1227–1239.
- Tuzet, O., Ormieres, R., 1961. Sue quelques gregarines parasites de crustacés décapodes. *Ann. Sci. Nat. Zool. Biol. Anim.* 3, 773–783.
- Uzmann, J.R., 1967. *Histriobdella homari* (Annelida: Polychaeta) in the American lobster, *Homarus americanus*. *J. Parasitol.* 53, 210–211.
- Van Beneden, P.-J., 1858. Histoire naturelle d'un animal nouveau, désigné sous le nom d'*Histriobdella*. *Bull. l'Acad. R. Belg.*, 2me série, 9–10, 270–303.
- Van Engel, W.A., Harris, R.E.J., Zwerner, D.E., 1986. Occurrence of some parasites and a commensal in the American lobster, *Homarus americanus*, from the Mid-Atlantic Bight. *Fish. Bull.* 84, 197–200.
- Vogan, C.L., Costa-Ramos, C., Rowley, A.F., 2001. A histological study of shell disease syndrome in the edible crab, *Cancer pagurus*. *Dis. Aquat. Organ.* 47, 209–217.
- Wilhelm, G., Boulo, V., 1988. Infection de l'étrille *Liocarcinus puber* (L.) par un dinoflagellate parasite: *Hematodinium* sp.. *Con. Int. Expl. Mer, Ser. CM, K 32 (E)*, 1–10.
- Wilhelm, G., Mialhe, E., 1996. Dinoflagellate infection associated with the decline of *Necora puber* crab populations in France. *Dis. Aquat. Organ.* 26, 213–219.
- Winnepenninckx, B., Backeljau, T., Kristensen, R.M., 1998. Relations of the new phylum Cycliophora. *Nature* 393, 636–638.
- Yan, X., Taylor, K.D.A., Hanson, S.W., 1989. Studies on the mechanism of blackspot development in Norway lobster (*Nephrops norvegicus*). *Food Chem.* 34, 273–283.
- Ziino, G., Giuffrida, A., Stancanelli, A., Panebianco, A., 2002. Shell disease in *Nephrops norvegicus* from the Mediterranean Sea. Morphological and hygienic remarks. *Arch. Lebensmittelhyg.* 53, 134–136.