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The prevalence of *Hematodinium* in *Nephrops norvegicus* from the western Irish Sea

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Infection of *Nephrops norvegicus* by a dinoflagellate parasite belonging to the genus *Hematodinium*, is reported in populations of *Nephrops* from the Irish Sea. Diseased animals are recognized by an opaque vivid body colour and high densities of parasites in the haemolymph. Infection causes a general morbidity of the host along with a reduction in swimming performance, which eventually leads to the death of the host lobster. Research vessel cruises performed over the period 1994–2001 have shown *Hematodinium* to occur in populations of *Nephrops* from the Irish Sea throughout this period. High infection prevalence during the month of April and negligible levels during October agree with published data on seasonal infection levels in Scottish *Nephrops* stocks. Data on spatial and temporal infection prevalence are presented for the first time for the Irish Sea and show variation between stations and between years. Mean infection prevalence peaked at 18% of captured *Nephrops* during April 1996 and was followed by a downward trend to 2001. Infection predominates in small *Nephrops* (<30 mm carapace length) and in females is normally associated with immature animals. Although a positive correlation with seawater salinity was noted, preliminary analysis did not show a relationship between prevalence and other environmental factors.

INTRODUCTION

Crustaceans are hosts for a wide range of parasitic microorganisms (reviewed by Stentiford, 2000). These include species of virus, bacteria, fungi and protozoa (mainly microsporidians). It is likely that all decapod crustaceans act as hosts for protistan parasites, but have yet to be described. Sprague & Couch (1971) provide a review of the early literature. In addition to their importance to the phytoplankton dinoflagellates occur as symbionts in a wide range of marine invertebrates (Trench, 1987). Dinoflagellate parasites of the genus *Hematodinium* have been reported infecting a number of decapod crustacean hosts. *Hematodinium perezi*, the type species, occurs in the portunid crabs *Carcinus maenus* and *Liocarcinus depurator* from European waters (Chatton & Poisson, 1931). More recently a description of *H. australis* by Hudson & Shields (1994) suggest there to be higher species diversity and host specificity by the parasite than once thought (Pouchet, 1885 in Coats, 1999). *Hematodinium* has now been described from a number of crab hosts from around the world. It has also been described from several species of pandalid shrimp (Bower et al., 1993) and in benthic amphipods (Johnson, 1986).

Field (1992), who described infected animals from the Clyde Sea area, Scotland, first documented the occurrence of *Hematodinium* in *Nephrops*. Further investigation demonstrated the presence of infected *Nephrops* from other Scottish sites, both on the east and west coasts (Field et al., 1992; Field & Appleton, 1995; Stentiford et al., 2001c). In addition *Hematodinium* has been reported in *Nephrops* captured from the German Bight (F. Redant, personal communication), the Skaggerak and Kattegat (M. Ulmestrand, personal communication) and from the Irish Sea (Briggs & McAliskey, 1996).

Research on *Hematodinium* infection in *Nephrops* from Scottish waters has demonstrated a seasonal pattern in infection prevalence, peaking during April or May (Appleton et al., 1997; Stentiford et al., 2001b,c). In addition infection appeared to be virtually absent in animals caught during the summer and autumn months (Stentiford et al., 2001b). Laboratory studies have succeeded in maintaining isolated *Hematodinium* parasites *in vitro*, enabling the progression of the parasite through a series of characteristic developmental stages to be studied (Appleton & Vickerman, 1998). Although the mode of infection has not been discovered, the possibility of an intermediate host has not been ruled out (Stentiford, 2000; Stentiford et al., 2001b,c).

Infected animals are recognized by an opaque vivid body coloration, thought to be due to high densities of parasites in the haemolymph. Infection causes a general morbidity of the host along with a reduction in swimming performance (Stentiford et al., 2000a) which eventually leads to death. Anecdotal evidence suggests that the muscle from infected animals has a bitter taste, this being the basis of the term 'Bitter Crab Disease' applied to infection of several crab species by *Hematodinium*-like dinoflagellates. It has been shown (Stentiford et al., 2001a) that *Hematodinium* places a heavy metabolic load on the host by acting as a carbohydrate sink. Here, parasites are thought to remove glucose from the haemolymph, thereby disrupting the release of the crustacean hyperglycaemic hormone (CHH). Infection also changes the tissue amino acid profile and causes alterations in muscle

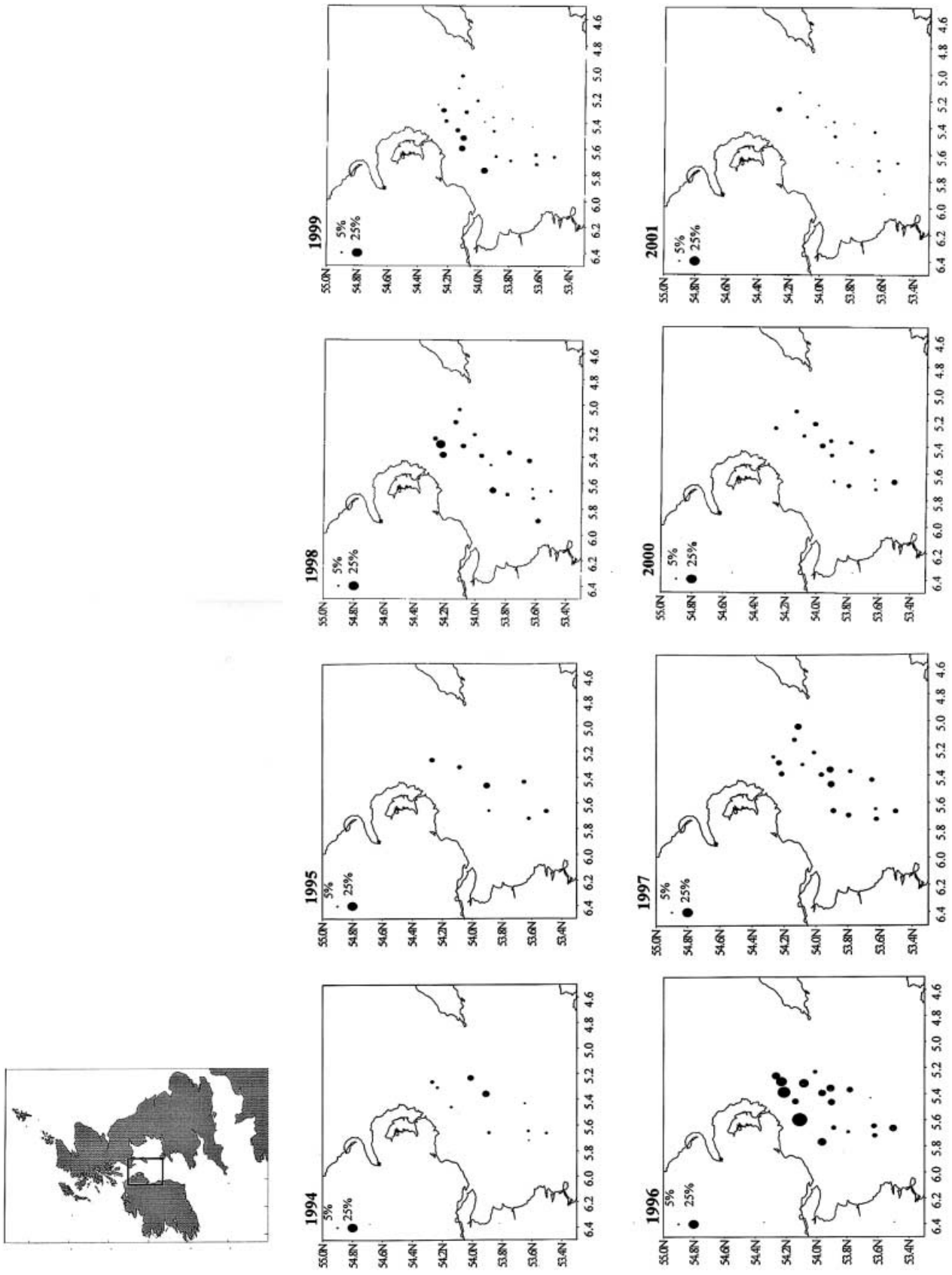

Figure 1. Spatial distribution of advanced *Nephrops* infected by *Hematodinium* during spring (mainly April) surveys over the period 1994–2001.

sarcolemmal structure along with localized disruption of myofibrillar bundles around the fibre periphery (Stentiford et al., 2000b).

Nephrops are captured either by trawling or potting (creeling) and form one of the most important fisheries in the north-east Atlantic. The United Kingdom catch is over 30,000 tn annum^{-1} and is thus one of the most commercially valuable species with a first sale value of over £100 m (Shellfish Association of UK Statistics). Irish Sea stocks are exploited mainly in waters to the west of the Isle of Man by vessels from Northern Ireland and the Republic of Ireland. Combined mean annual landing of over 9000 tn are common in the Irish Sea fishery for recent years.

This paper presents data on the spatial and temporal prevalence of *Hematodinium* infection of *Nephrops* captured from the western Irish Sea during surveys performed by the Department of Agriculture and Rural Development (DARD) over the period 1994–2001. As it is possible that peaks of infection prevalence and the subsequent progression of the disease to advanced stages within the host is triggered by one or more environmental factors the relationship between depth, temperature and salinity were investigated.

MATERIALS AND METHODS

Research vessel surveys (RV 'Lough Foyle') of the western Irish Sea *Nephrops* grounds were completed during spring (mainly April) and summer (mainly August) over the period of 1994–2001. In addition to population data on *Nephrop*s and associated species, data were collected on the prevalence of *Hematodinium* infection in *Nephrops*. Hauls of 60 min duration were completed at each station. These stations were spread over the western Irish Sea *Nephrops* grounds and have a depth range of 45–125 m, as indicated in Figure 1. The gear, a custom made 20-fathom *Nephrops* trawl net of nominal mesh size 50-mm throughout, was used in all cruises. Total catch bulk was quantified by counting baskets filled from the catch and sample baskets were sorted to provide an assessment of species composition. The *Nephrops* in each sub-sample were divided into male and female components, carapace length–frequency was measured using vernier callipers and the ovary maturity stage noted according to an arbitrary scale described by Briggs (1988) as detailed in Table 1. Initially during this study a range of diagnostic procedures were used to assess the prevalence of *Hematodinium* in *Nephrops* samples. In view of the large number of individual lobsters to be screened from each station however, the body coloration method was adopted for routine assessment of *Hematodinium* prevalence and provided a rapid assessment of the prevalence of advanced disease stages. Because the body coloration method is only reliable for detecting advanced stages of infection it is likely that some animals deemed 'clean' by this method had low levels of infection. Seawater samples were taken for analysis from a continuous sampling device moored over the western Irish Sea *Nephrops* grounds. Salinity was measured by a profiling

Table 1. *Scale for classifying ovary development in* Nephrops norvegicus *(Briggs, 1988 and after Bailey, 1984).*

Stage	Description of ovary
I	Immature; ovaries as thin white threads
II	Ovaries thicker than in 'I' with a tinge of pale green colour
III	Ovaries larger than 'II' and dark green in colour
IV	Ovaries much larger than 'III' and clearly visible through the carapace. Dark green/black
V	Ovaries pale green with dark green specks. Represents reabsorption of ovarian material
Berried	Ovigerous females with eggs attached to the pleopods

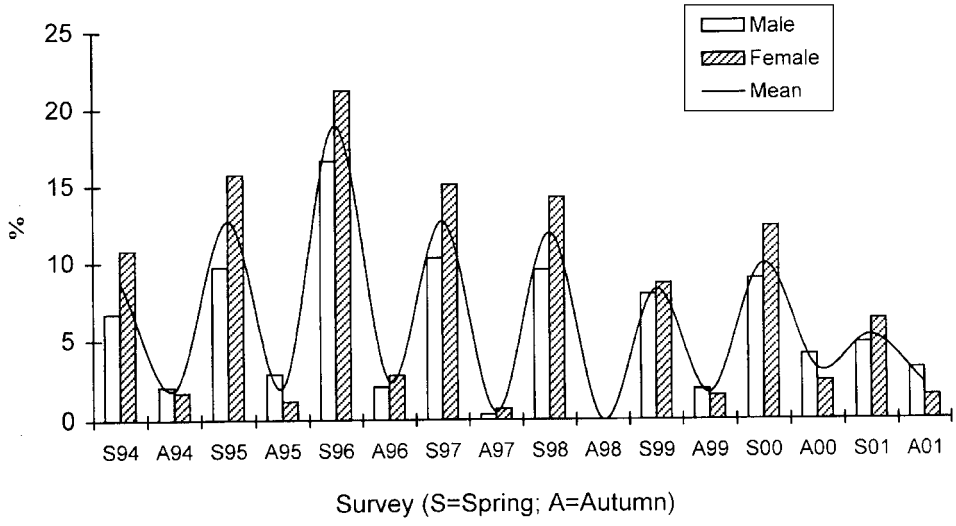

Figure 2. Temporal variation in *Hematodinium* prevalence (%) during *Nephrops* surveys over the period 1994–2001.

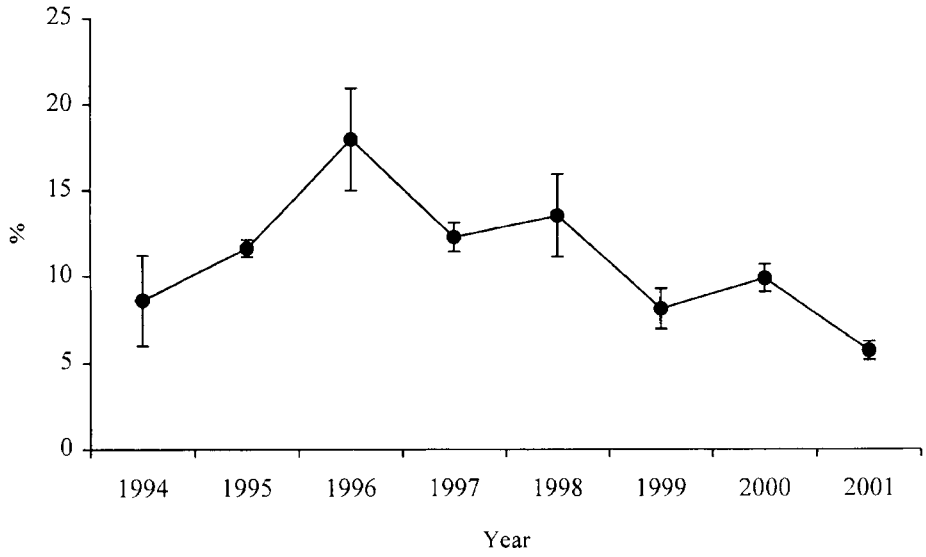

Figure 3. Average prevalence (%) of *Nephrops* (sexes combined) at advanced stages of *Hematodinium* infection over the period 1994–2001. (Vertical bars are standard errors.)

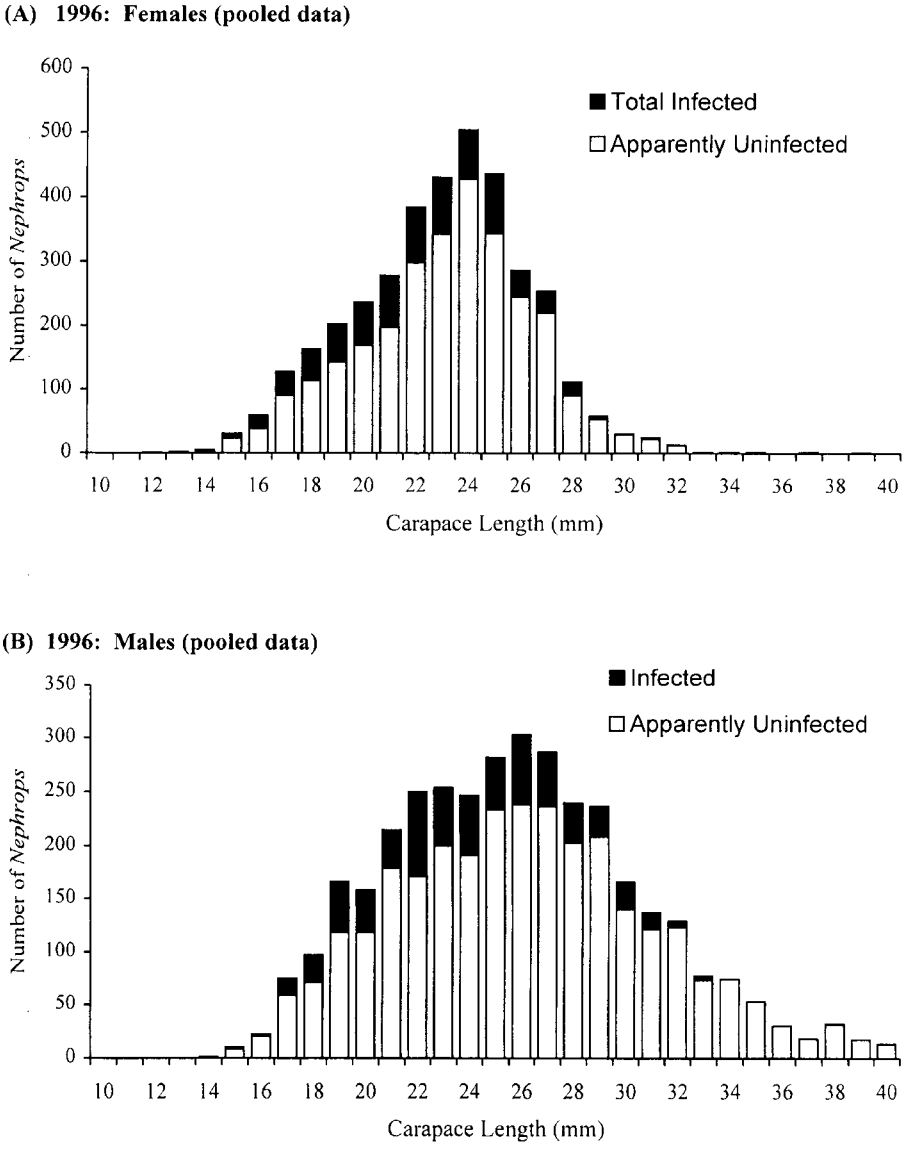

Figure 4. Size composition (carapace length in mm) of *Nephrops* captured during RV 'Lough Foyle' survey completed during April 1996 showing number of animals with advanced stages of *Hematodinium* infection (shaded black) at length.

conductivity/temperature/depth package and checked by a bench salinometer at an independent reference laboratory (B. Stewart, personal communication). These data have been collected since 1996 and were used to explore the relationship between environmental factors and *Hematodinium* infection.

RESULTS

Results of surveys of Irish Sea *Nephrops* fishing grounds over the period 1994–2000 demonstrated a seasonal pattern in the prevalence of *Hematodinium* infection, with peaks in the spring (April/May). Each sub-sample of *Nephrops* examined during spring surveys contained individuals with advanced stages of the infection in both sexes. Although infection was widespread with a peak in prevalence during the spring of each year, there was a marked spatial variability in infection (see Figure 1). Temporal data demonstrated an infection peak during spring 1996 (Figures 2 & 3) with infection reaching over 35% at some stations (mean level 18%). Examination of both male and female *Nephrops* size composition over this period showed that although animals of >30 mm carapace length can be infected, *Hematodinium* infection prevalence is highest in smaller animals (Figure 4A,B). Detailed study of infected female *Nephrops* at different stages of maturity during the peak infection period of April 1996 (Figure 5) showed the infection to occur in 33% of female *Nephrops* with stage I ovaries, but in only 1.4% of animals with ovaries beginning to mature (stage II). Advanced stages of *Hematodinium* infection were not seen in mature ($\geqslant$stage III) female *Nephrops*.

Investigation into the relationship between environmental factors and *Hematodinium* infection indicated no apparent correlation between average infection levels and temporal trends in temperature or water depth. A positive correlation was observed between seawater salinity and *Hematodinium* infection (Figure 6). Although the salinity range reported is relatively small the observation agrees with those of Messick (2000), who described high infections of *Hematodinium* in the American blue crab, *Callinectes sapidus*, during periods of elevated salinity.

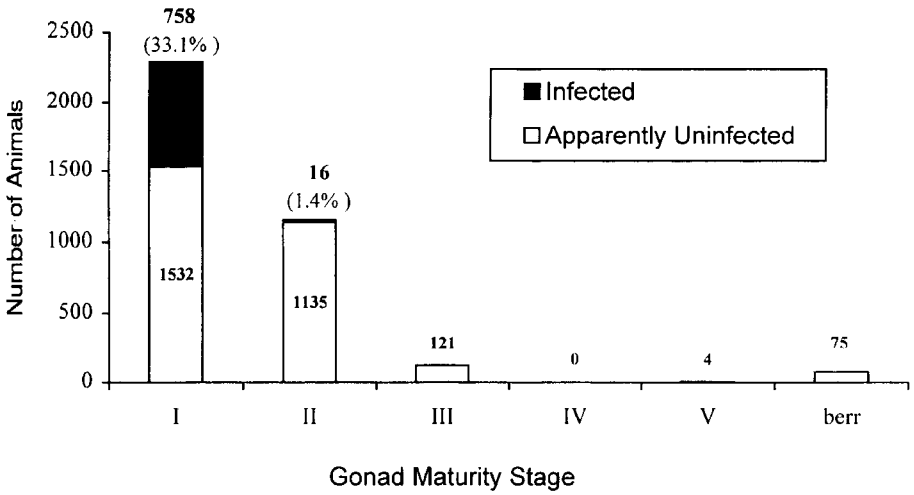

Figure 5. Number of female *Nephrops* at each gonad maturity stage with advanced stages of infection by *Hematodinium*.

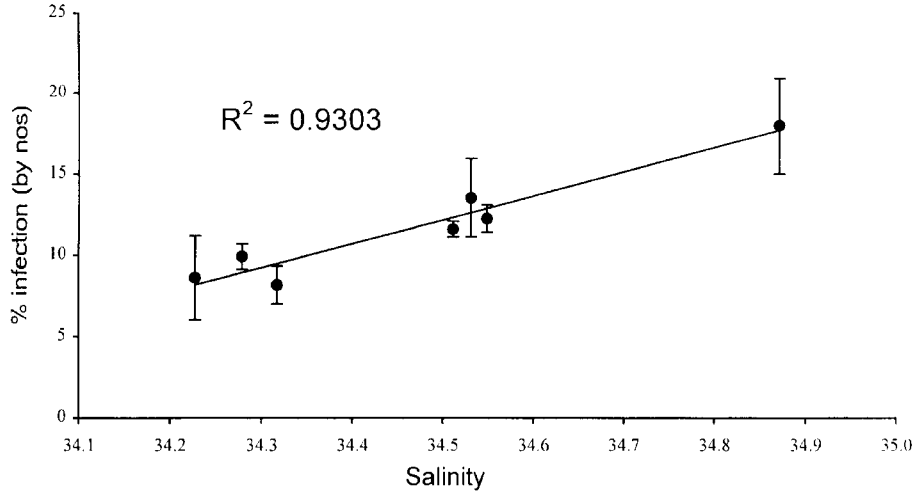

Figure 6. Linear regression of percentage of *Nephrops* (sexes combined) with advanced stages of *Hematodinium* infection and mean seawater salinity over the period 1994–2000. (Vertical bars are standard errors.)

DISCUSSION

Although the body coloration method has been shown to be an imprecise method for *Hematodinium* diagnosis because of uncertainties in the recognition in lightly infected animals (Stentiford, 2001c), the method does allow for rapid diagnosis of advanced stages of infection. It is therefore a useful tool for screening large numbers of *Nephrops* from several stations spread over a wide geographic range, as in this study.

Seasonal variability in the prevalence of advanced stages of *Hematodinium* infection in Irish Sea *Nephrops*, follows similar seasonal trends to those described for Scottish *Nephrops* stocks (Field, 1992; Field & Appleton, 1995, 1996; Stentiford, 2001c). It has been proposed (Field et al. 1992) that the spring peak in *Hematodinium* prevalence is synchronized to the moult cycle of *Nephrops*, which is known to undergo ecdysis at this time. Stentiford et al., (2001c) disputes the view that infection occurs during this time via trauma sites in the soft cuticle of females caused during mating, as males are also infected during spring (Figure 2) despite moulting later than females (Farmer, 1974). It is thus more likely that infection occurs by the ingestion of motile or encysted forms of the parasite during the period of food scarcity when females spend a prolonged period in their burrows and suspension feeding is known to occur (Loo et al., 1993). It is also possible that males become infected at the same time by ingestion of parasite forms (e.g. dinospores). It has been reported that the period of peak *Hematodinium* infection prevalence in Scandinavian waters is autumn (Tärnlund, 2000) which does not coincide with the mating cycle.

The advanced stage of infection observed using the body coloration method occurred in a range of host sizes, though highest infection prevalence was seen to dominate in small *Nephrops*. Our results suggest that infection does not occur in sexually mature female *Nephrop*s. It is not known why immature animals are more susceptible to infection. Perhaps mature females produce some antimicrobial compound that provides immunity. It is also possible that physiological changes associated with the onset of maturity provide some form of immunity. Perhaps dietary changes with maturity are a factor or maybe larger animals are generally more robust and can withstand infection. As advanced stages of *Hematodinium* infection are thought to always result in death of the host (Field, 1992) there is likely to be an annual disease-inflicted mortality in immature *Nephrops*. Infection prevalence was as high as 37% of *Nephrops* caught at one station during the 1996 survey, with the annual mean infection prevalence in that year being 18%. It is likely however that these high mortalities are an artefact brought about by the increased catchability of infected *Nephrops*. Studies by Stentiford et al. (2000a) and Stentiford (2000) for example, examined various components of swimming performance in uninfected and infected *Nephrops*. Swimming performance declined with increasing severity of the disease. This is likely to result in the escape reaction, studied in healthy animals by Newland et al. (1992), to be less pronounced. It is proposed that reduced swimming performance of *Nephrops* at different stages of infection may lead to infection stage-specific alteration in the probability of trawl capture (Stentiford et al., 2000a). Increased catchability of infected *Nephrops* is also caused by extended periods of emergence from their burrows compared to uninfected animals (Stentiford et al., 2001d). Attempts to incorporate disease induced juvenile mortality into *Nephrops* analytical stock assessment for the Clyde Sea area (ICES, 1997) gave unrealistic estimates of recruitment to the fishery due to an over estimate of juvenile mortality, based on disease prevalence in catches. It is also likely that infected animals are more susceptible to predation, suggesting that the disease may not necessarily add to overall natural mortality but replace some of it (Stentiford et al., 2001d).

It should be emphasized that the body coloration method for assessing *Hematodinium* infection used here is only reliable for diagnosing the most advanced stages of infection. The method has a very low sensitivity, compared with other morphological methods such as the pleopod index and with sensitive blood tests. These methods would provide a more reliable estimate of disease inflicted mortality. The pleopod method, for example, involves examination of *Nephrops* pleopods for the presence of agglutinated parasite and haemocyte material under low-power microscopy (Field & Appleton, 1995). Diagnosis can also be performed by direct observation of 0.1 ml of haemolymph, from the base of a leg using a 1-ml syringe. A haemolymph smear is then air dried on glass slides, stained with Leishman's stain and examined under a microscope for the presence of *Hematodinium* cells. Another more specialized technique is immunostaining, using polyclonal antibody specific to *Hematodinium* (Field, 1992), which provides unequivocal diagnosis of infecting cells.

The juvenile mortality inflicted on the host stock by *Hematodinium* must be separated from the artefactual mortality estimates derived from *Nephrops* catch rates. If significant, these mortality rates should be incorporated into the analytical models used to assess *Nephrops* stocks and improve the quality of these assessments and the scientific advice to managers. In order to assess *Hematodinium* inflicted *Nephrops* mortality these more sophisticated diagnostic procedures should be developed and used rather than the method used in this paper.

The data presented however does provide an overview of relative fluctuations in spatial and temporal prevalence of *Hematodinium* in populations of *Nephrops* from the Irish Sea. It would appear that the disease has been present at least since 1994 and despite inflicting juvenile mortality on the *Nephrops* stock, recent assessments indicate a stable situation with a *status quo* fisheries management recommendations (ICES, 2001). Although a correlation of temporal prevalence and salinity was noted, this and the interrelationships of other environmental factors impinging on the *Nephrops* stock needs to be further investigated. In addition to using more precise diagnostic methods to estimate mortality future research should investigate the variability of *Hematodinium* prevalence in *Nephrops* stocks throughout the north-east Atlantic and further explore the relationship between infection and environmental factors. Thus providing a means for understanding of the impact of *Hematodinium* on *Nephrops* population dynamics.

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