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Date: 13 May, 2008 **Our reference:** **DF 08/ 05** **Samples supplied by:** Andrew Jeffs **Company :** Two Fathom Ltd **Address** **Your reference:** Vietnam Lobsters	**Sample Description:** Puerulus and juvenile *Panulirus* from Vietnam, fixed in 10% formalin then transferred into 70% ethanol. **Sample screening date:** 10-12 May 2008 **Screening method:** light microscopy of H&E stained histological sections

Disclaimer:

DigsFish Services have taken all reasonable steps to ensure the information contained herein is accurate at the time of publication, however sample sizes used were small and less than required to make statistically rigorous conclusions on absence or prevalence of disease agents or pathological conditions. Hence this report can only be offered as general advice only.

CASE REPORT: Pathology Report for Two Fathom Ltd

Sample statistics:

N = 5 *P. versicolor* puerulus
N = 4 *Panulirus* spp. puerulus
N = 4 *P. versicolor* juveniles, mean CL = 70 mm, range 60 – 80 mm.

Gross signs

Diseased puerulus and juvenile *Panulirus versicolor* and puerulus *Palinurus* spp. were lethargic and moribund. Juvenile *P. versicolor* had milky flesh and haemolymph.

Histopathology:

The 5 *P. versicolor* puerulus all had significant pathology in the digestive gland characterised by atrophy and necrosis of the digestive gland epithelium. Most of the affected epithelial cells had enlarged nuclei with marginated chromatin and cowdry-like intranuclear inclusion bodies characteristic of viral infection (Figures 1, 2). Similar inclusions were evident in the nuclei of atrophied digestive gland epithelial cells of all 4 of the *Palinurus* spp. puerulus (Figure 3). Occasional intranuclear inclusions were also observed in small numbers of haemocytes and in focal lesions within subcuticular epithelium of the gills in both species of puerulus. Other lesions observed included hyaline necrosis of nerve tissue cells in several, but not all pueruli of both species (Figure 4). A small number of bacteria were also visualized in the gills of two *P. versicolor*, one of which had gills which appeared to have suffered severe desiccation.

The juvenile *P. versicolor* were suffering from heavy systemic infections with a small, pleomorphic rickettsia like organism (RLO). The RLO was observed throughout the somatic musculature (Figure 5), connective tissues and epithelium of the digestive gland (Figure 6), haemolymph (Figure 7) and gills (Figure 8). The presence of the RLO was associated with extensive necrosis in all affected organs. In the digestive gland the epithelial cell architecture was severely disrupted, and in at least 2 of the juveniles this was also associated with the presence of enlarged cell nuclei with marginated chromatin (Figure 6), suggesting concurrent viral infection. One melanised gill filament in a juvenile lobster contained large numbers of fungal hyphae.

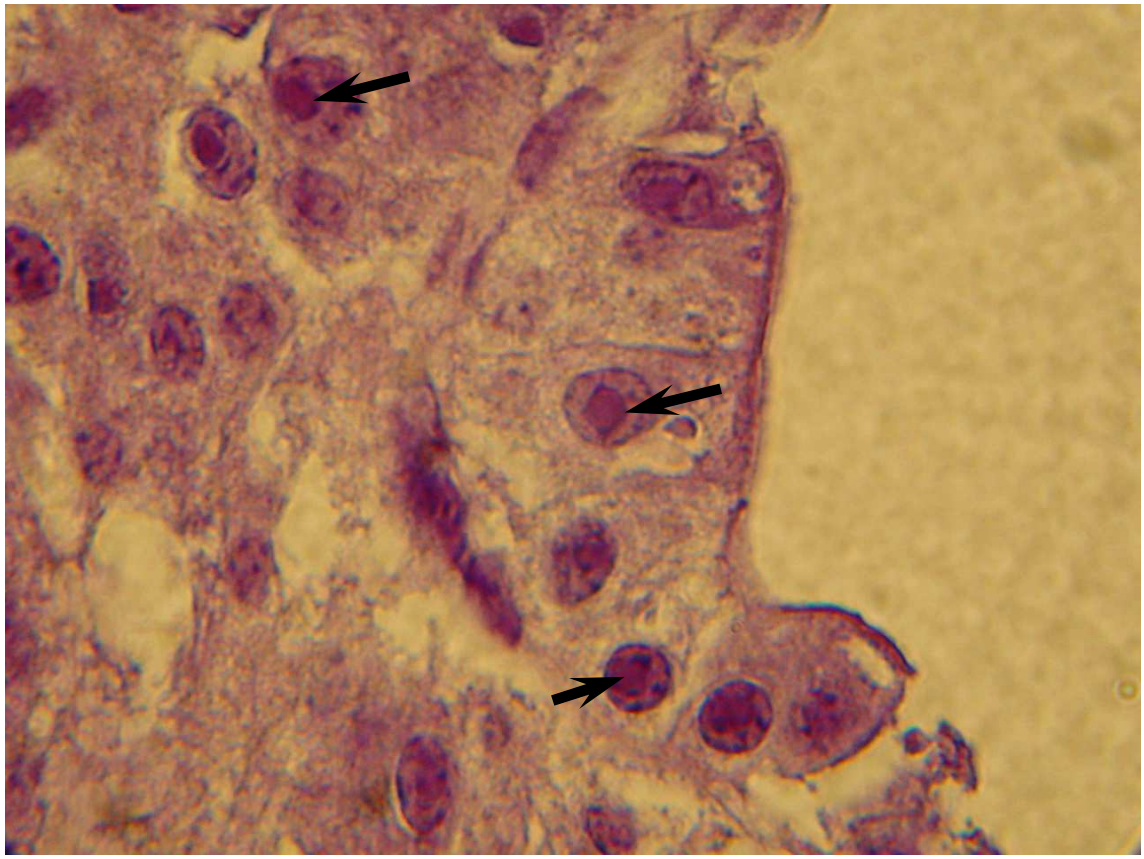

Figure 1. Digestive gland epithelium of a *Panulirus versicolor* puerulus, showing presumptive intranuclear viral inclusions (arrows) inside enlarged nuclei with marginated chromatin. 400 x magnification.

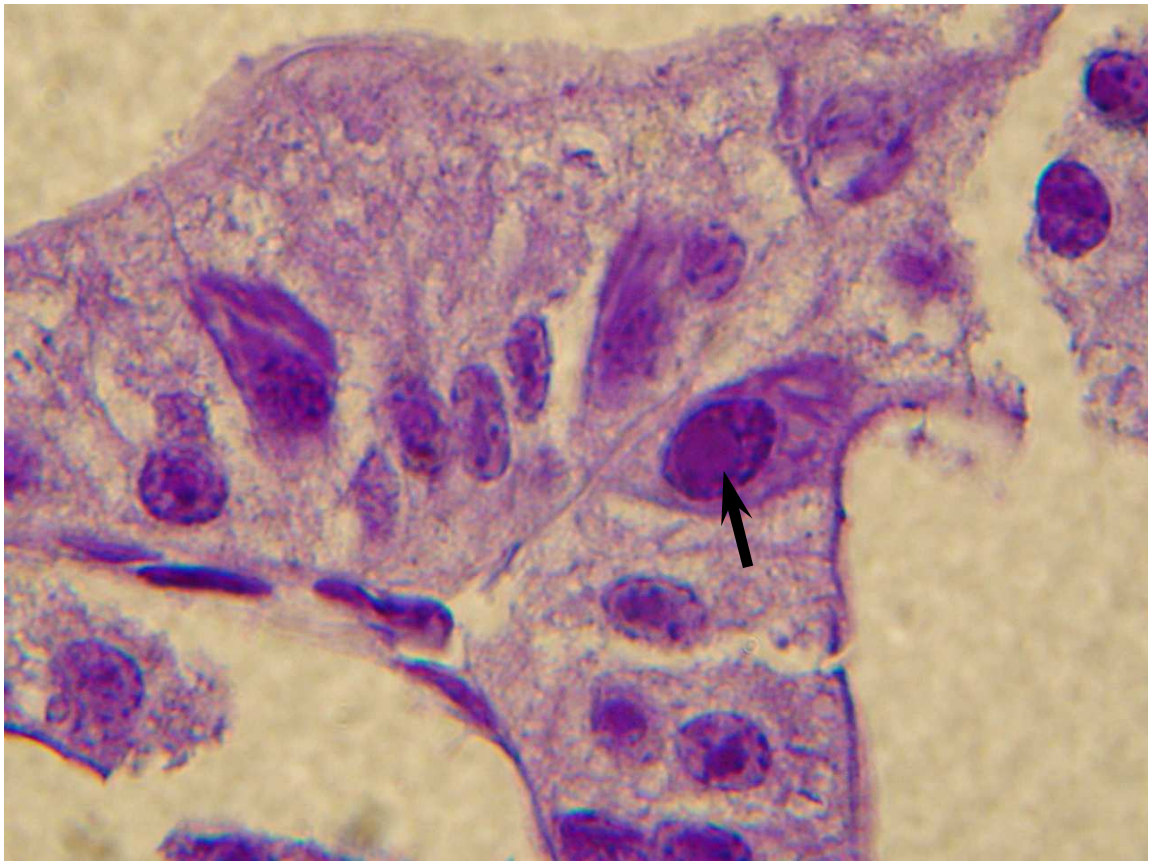

Figure 2. Digestive gland epithelium of a *Panulirus versicolor* puerulus, showing a classical presumptive intranuclear viral inclusion (arrow) inside enlarged nuclei with marginated chromatin. 500 x magnification.

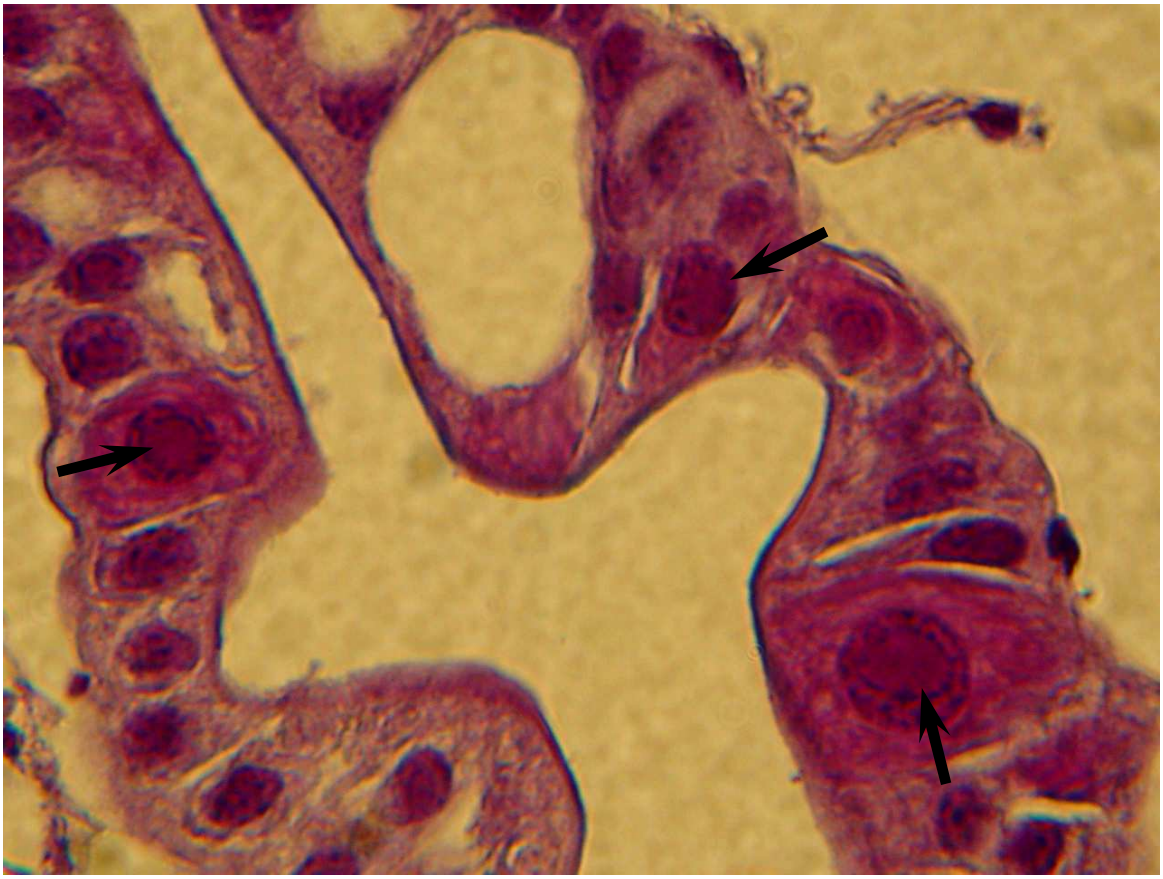

Figure 3. Digestive gland epithelium of a *Panulirus* spp. puerulus, showing several presumptive intranuclear viral inclusions (arrows) inside enlarged nuclei with marginated chromatin. 500 x magnification.

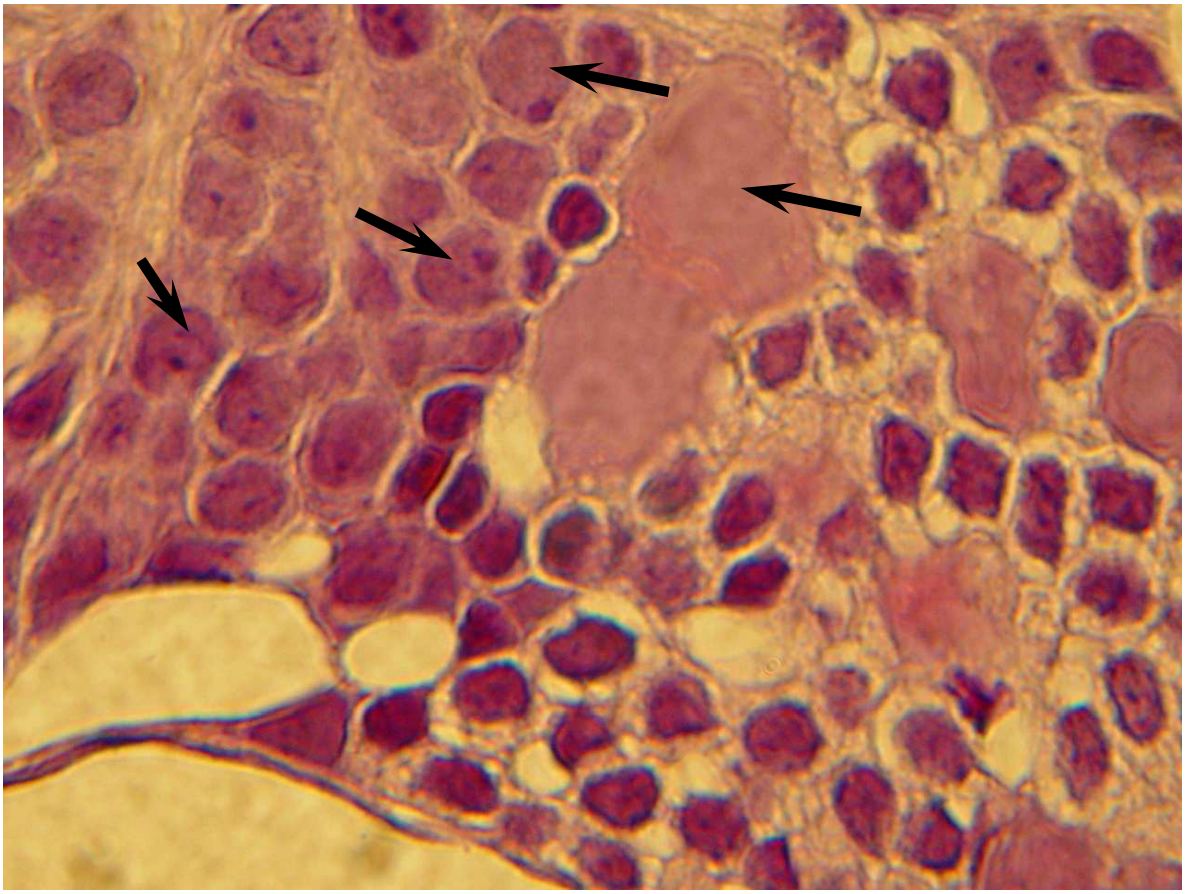

Figure 4. Nerve tissue of a *Panulirus versicolor* puerulus, showing unusual enlarged necrotic cells (arrows) with a hyaline appearance and pyknotic nuclei. 500 x magnification

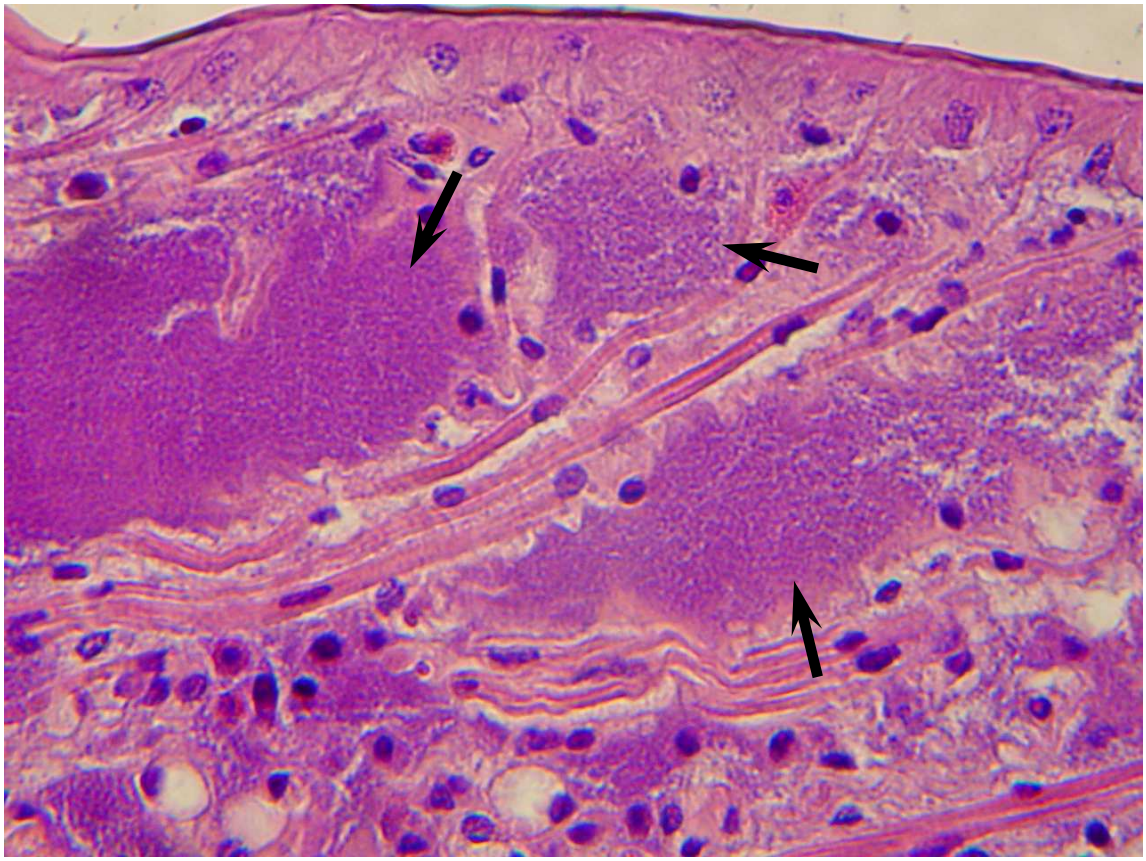

Figure 5. Extensive infiltration of rickettsia-like organisms (RLO) (arrows) within the muscle fibres of the tail muscle of a juvenile *P. versicolor*. 200 x magnification.

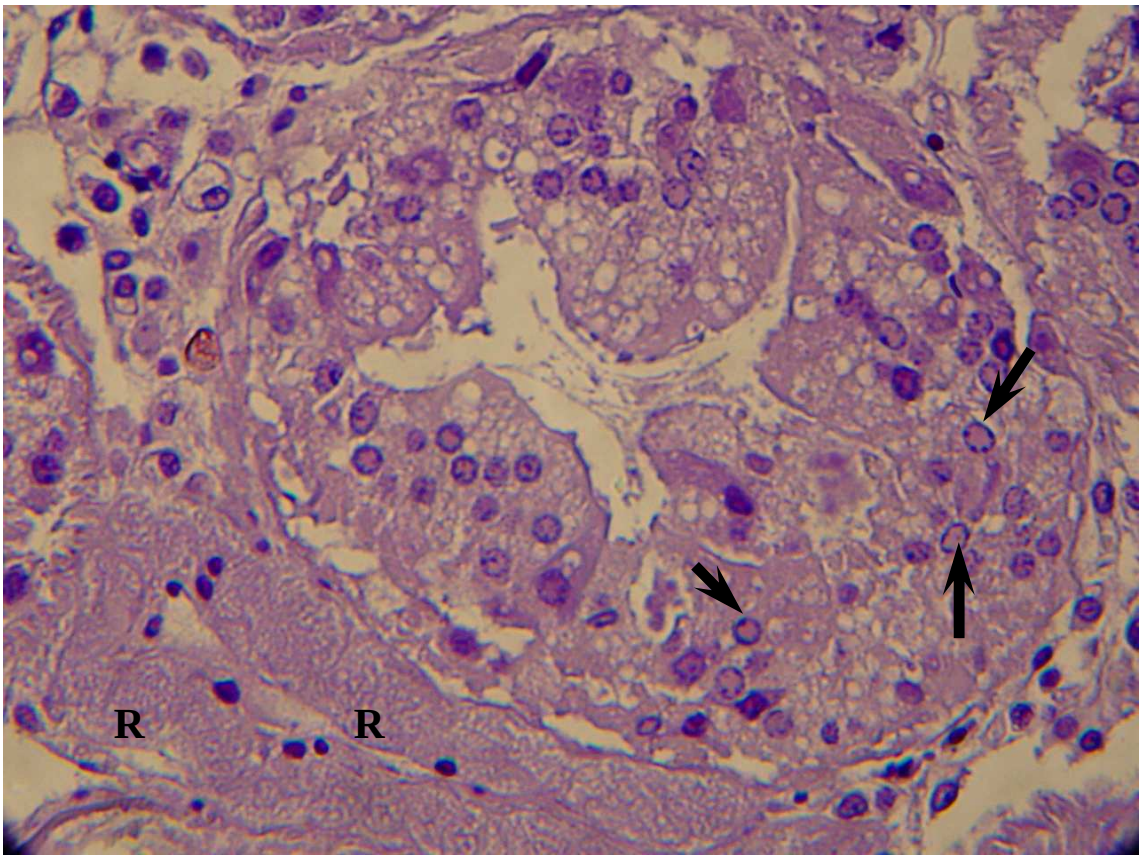

Figure 6. Digestive gland of a juvenile *P. versicolor* showing breakdown of epithelial cell architecture with cell nuclei enlarged with marginated chromatin (arrows) and infiltration of connective tissue with RLOs (R). 200 x magnification.

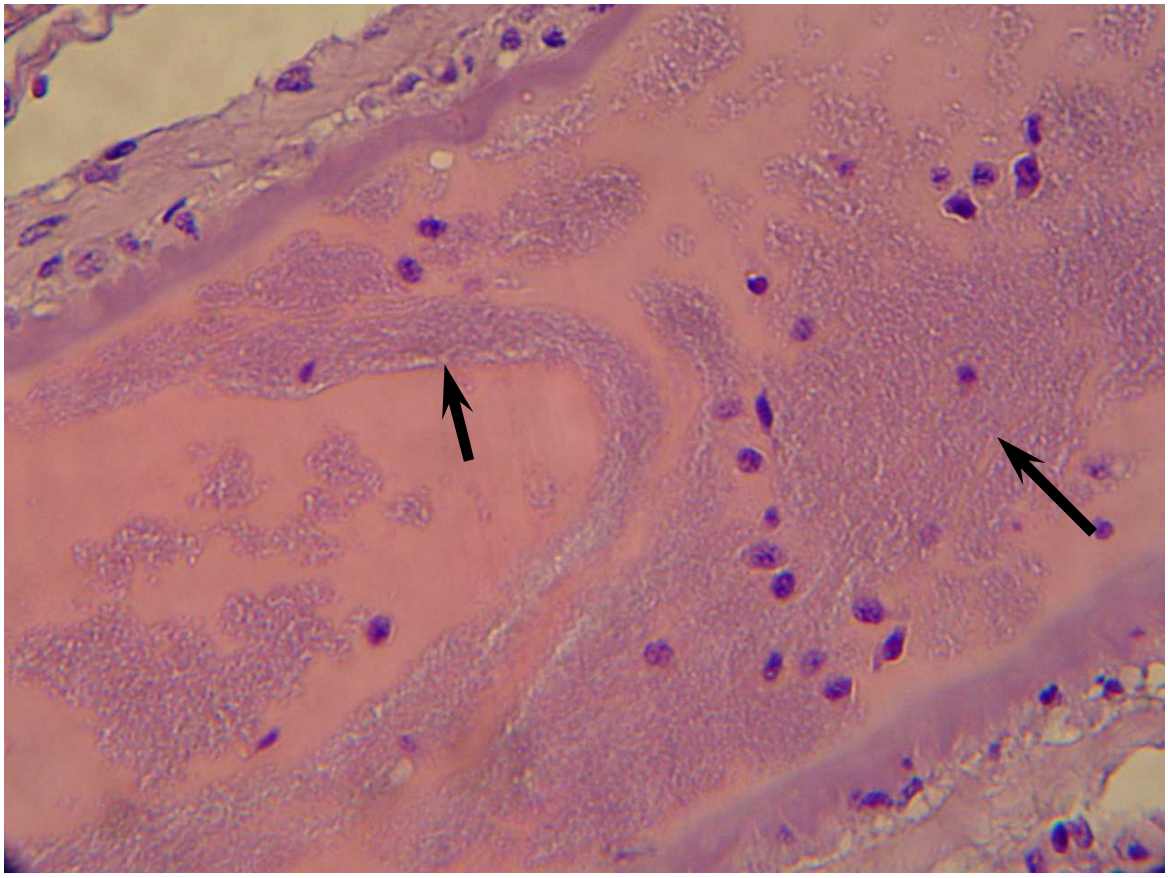

Figure 7. Blood vessel in a juvenile *P. versicolor* showing large numbers of RLOs (arrows) within the haemolymph. 200 x magnification.

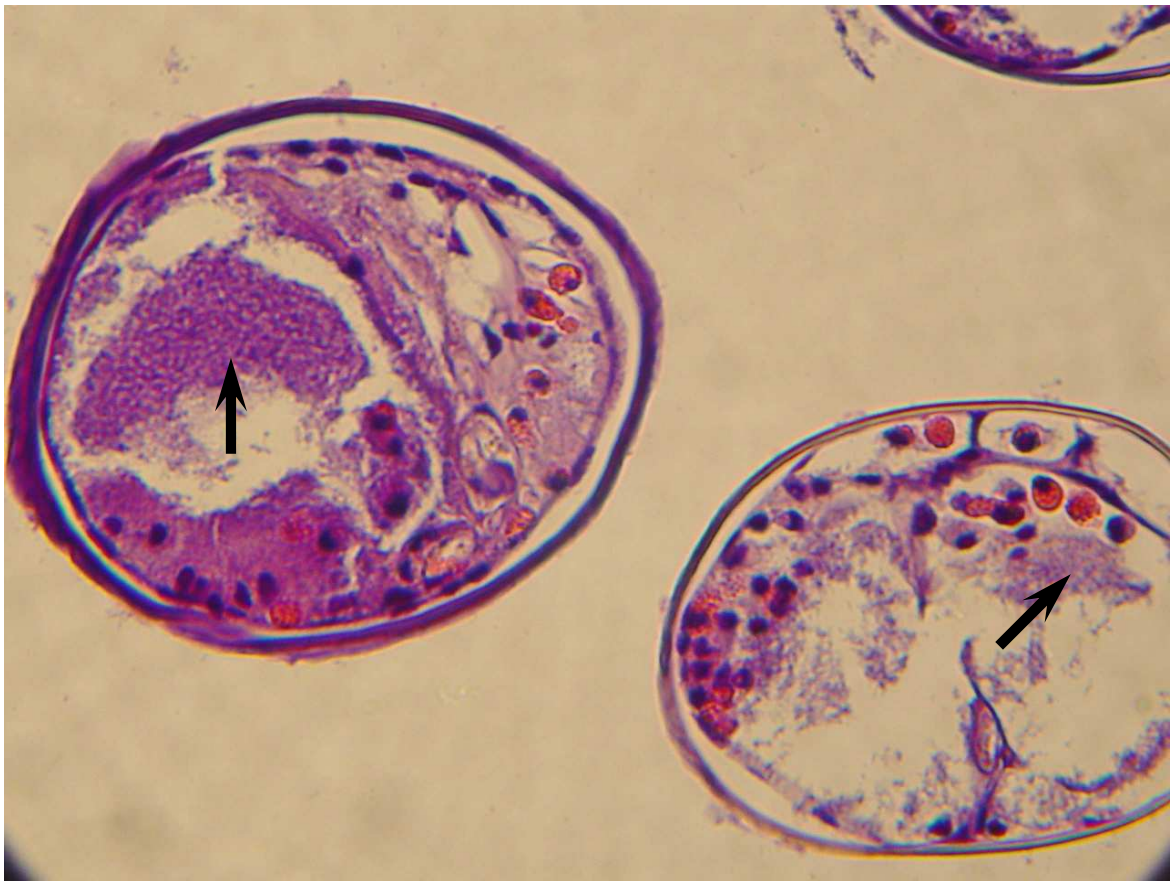

Figure 8. Gill of a juvenile *P. versicolor* showing necrosis of subcuticular epithelium associated with large numbers of bacteria (arrows) within the haemolymph sinuses. 200 x magnification.

COMMENTS:

The absence of significant bacterial infections in the pueruli and the consistent association of pathological changes in the digestive gland and other organs with the presence of intranuclear inclusions strongly suggests the mortalities of the pueruli were associated with the presence of a viral disease agent. While viral diseases are well documented in other crustaceans such as penaeid shrimp and crayfish, only two have been recorded from lobsters to date (Shields et al. 2006). One of these is White Spot Syndrome Virus (WSSV), an internationally notifiable disease agent which was found to be able to experimentally infect *Panulirus versicolor* and *P. penicillatus* without clinical signs of disease (Chang et al. 1998). The other is *Panulirus argus* Virus 1 (PAV1), which was first noticed in 1999 in *Panulirus argus* in the Caribbean (Behringer et al. 2001) and since has been recorded in an increasing number of locations in that part of the world (Shields and Behringer 2004, Montgomery-Fullerton et al. 2007, Lozano –Alvarez et al. 2008). PAV1 was the first naturally occurring pathogenic virus reported for lobsters and is considered to be a significant threat to the spiny lobster fishery and aquaculture industries in that part of the world (Shields and Behringer 2004).

The histological appearance of the inclusions observed in the *Panulirus versicolor* from Vietnam do not appear to conform with those of WSSV, however, they are superficially similar to the inclusions reported for PAV1 in *P. argus.* Also, like PAV1, the disease in *P. versicolor* appears to mainly affect very small lobsters. However, PAV1 mainly infects connective tissues and haemocytes, as well as some haematopoietic tissues and fixed phagocytes. In contrast, in Vietnamese *P. versicolor*, the main tissues affected are the hepatopancreas and, possibly, the nervous tissues.

Clearly more work is required to determine the affinities of the putative viral agent in *P. versicolor* and *Panulirus* spp. The minimum work required for confirmatory proof of viral infection, (which would be required for publication of any sort of authoritative paper on this finding) is electron microscopy of the putative inclusions in order to detect and, if they are present, describe the viral particles. However, it would also be preferable to obtain molecular information which would help determine the affinities of the putative virus with PAV1. I should be able to arrange these services through colleagues Jones and Shields who may be able to help without charge (most likely provided they are co-authors on any subsequent papers which use information they provide).

The juvenile lobsters had heavy systemic infections with a Rickettsia –like organism (RLO). This is the first record of a RLO causing disease in lobsters, though previously they have been known to cause disease in crabs and prawns (Bower et al. 1996). While the RLO appears a significant disease agent in its own right, the presence of abnormal nuclei within hepatopancreas cells of at least 2 of the 4 juveniles examined suggests the putative virus may also be involved with this disease presentation. Concurrent infections of viruses with RLOs are also common in penaeid shrimp (Bower 1996). Fungal infections in captive lobsters are usually associated with poor husbandry (Diggles 2001). The fungal infection of the gills of one of the lobsters here therefore suggests that rearing conditions were poor. Clearly attention to cleanliness of the environment in which these lobsters are held is required to reduce colonization of compromised lobsters by fungi.

Uninfected *P. argus* avoid conspecifics infected with PAV1 (Behringer et al. 2006), and it is possible that this is normal behaviour for wild lobsters. Hence, aggregating *P. versicolor* for aquaculture may significantly increase their chances of infection with disease agents, including viruses and RLOs. Clearly if lobsters are to be pursued as part of aquaculture development in Vietnam, and success is to be obtained, disease prevention and control will need to be at the forefront of future research efforts.

Ben Diggles, PhD.

References

Behringer D et al. (2001). The first viral disease reported in lobsters. *The Lobster Newsletter* Vol 14 (1):1-4.

Behringer D et al. (2006). Ecology: Avoidance of disease by social lobsters. *Nature* 441: 421

Bower, S (1996). Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: Rickettsial Infection of Penaeid Shrimp. http://www-sci.pac.dfo-mpo.gc.ca/shelldis/pages/ripsp_e.htm

Bower, S et al. (1996). Stained prawn disease (SPD) of *Pandalus platyceros* in British Columbia, Canada, caused by a rickettsial infection. *Diseases of Aquatic Organisms* 24: 41-54

Chang P et al. (1998). Detection of white spot syndrome associated baculovirus in experimentally infected wild shrimp, crabs and lobsters by insitu hybridization. Aquaculture 164: 233-242.

Diggles B (2001). A mycosis of juvenile spiny rock lobster *Jasus edwardsii* (Hutton, 1875) caused by *Haliphthoros* sp., and possible methods of chemical control. *J. Fish Dis* 24: 99-110.

Lozano-Álvarez E, et al. (2008). Presence of *Panulirus argus* Virus 1 (PaV1) in juvenile spiny lobsters Panulirus argus from the Caribbean coast of Mexico. *Diseases of Aquatic Organisms* 79: 153-156.

Montgomery-Fullerton, M et al. (2007). Detection of *Panulirus argus* virus 1 by PCR in Caribbean spiny lobsters. *Diseases of Aquatic Organisms* 76: 1-6

Shields J, Behringer D (2004). A new pathogenic virus in the Caribbean spiny lobster *Panulirus argus* from the Florida Keys. *Diseases of Aquatic Organisms* 59: 109-118.

Shields J et al. (2006). Chapter 5: Pathogens, parasites and other symbionts. In: Lobsters: biology, management, aquaculture and fisheries. (ed. B.F. Phillips) Blackwell Scientific, UK. ISBN: 10:1-4051-2657-4. pp 146-204.