

Fig. 1. Light micrographs of basophilic, Gram-negative inclusions (arrows) in the cytoplasm of hepatopancreatocytes in *Cherax quadricarinatus* H&E. (A) Almost all hepatopancreatocytes in the tubules are infected. Scale bar = 42 μm . (B) Inclusions often contain cleared areas. Scale bar = 10 μm

lesions were not observed in the remaining hepatopancreatic tissues stored in Bouin's fixative. The crayfish also had early stage eye necrosis (Edgerton et al. 1995), bacteraemia, and moderate *Cherax quadricarinatus* bacilliform virus infection.

Electron microscopy indicated that the cytoplasmic inclusions were microcolonies of a RLO within membrane-bounded vacuoles (Fig. 2). The RLO had both a cell wall and plasma membrane and was pleomorphic, with rounded forms more common than rod-shaped forms. The RLO ranged in length from 0.2 to 0.4 μm but further morphological and cytopathological descriptions were not possible due to suboptimal fixation for electron microscopy.

Discussion. The basophilic inclusions were similar to rickettsia-like microcolonies which have been observed systemically in *Cherax quadricarinatus* (Ketterer et al. 1992, Owens et al. 1992), and which were observed in approximately one third of moribund crayfish examined in the study of the chronic mortality at this farm (Edgerton et al. 1995, Edgerton 1996). The same, or very similar, systemic RLO has been associated with mortality in *C. quadricarinatus* farms in Ecuador (Jiménez & Romero 1997). The systemic RLO infects the spongy connective tissues, neural tissues, nephridial canal epithelium, myocardium, cuticular epithelium, haemopoietic and mandibular organ cells (Edgerton 1996). The systemic RLO in *C. quadricarinatus* has never been observed in endodermal enteric tissues even in very intense fulminating infections (Edgerton et al. 1995, Edgerton 1996, unpubl. data). In

this report the RLO was observed only in hepatopancreatocytes. This distinct differentiation of tissue tropism is consistent with other crustacean-infecting RLOs (Bower et al. 1996). Furthermore, the cytopathology also differed in that the systemic rickettsia-like microcolonies do not usually contain central clear zones as was observed with this hepatopancreatic RLO. It therefore appears that at least 2 distinct RLOs infect *C. quadricarinatus*; one a systemic agent, and the other exclusively infecting hepatopancreatocytes.

The fact that the hepatopancreatic RLO has been observed in only 1 *Cherax quadricarinatus* may suggest that it is relatively rare. Farming of *C. quadricarinatus* commenced approximately 10 yr ago in Australia and the industry is still in its infancy. *C. quadricarinatus* aquaculture in Australia is unsophisticated, and many farmers are hobbyists. Low numbers of sick crayfish are detected by most farmers, but are not reported as they are considered to be normal or insignificant. Survival as low as 30% is not uncommon in some ponds without obvious explanation; however, disease is rarely considered as the cause. Research on disease remains a low priority as there have not been major widespread epizootics reported in the industry to this point. Edgerton (1996) conducted histopathological surveys on farmed *C. quadricarinatus* in north Queensland to determine the prevalence of potential pathogens. However, the examination of 32 moribund crayfish during the chronic mortality which occurred at one of the surveyed farms (Edgerton et al. 1995, Edgerton 1996) revealed a high incidence for some pathogens

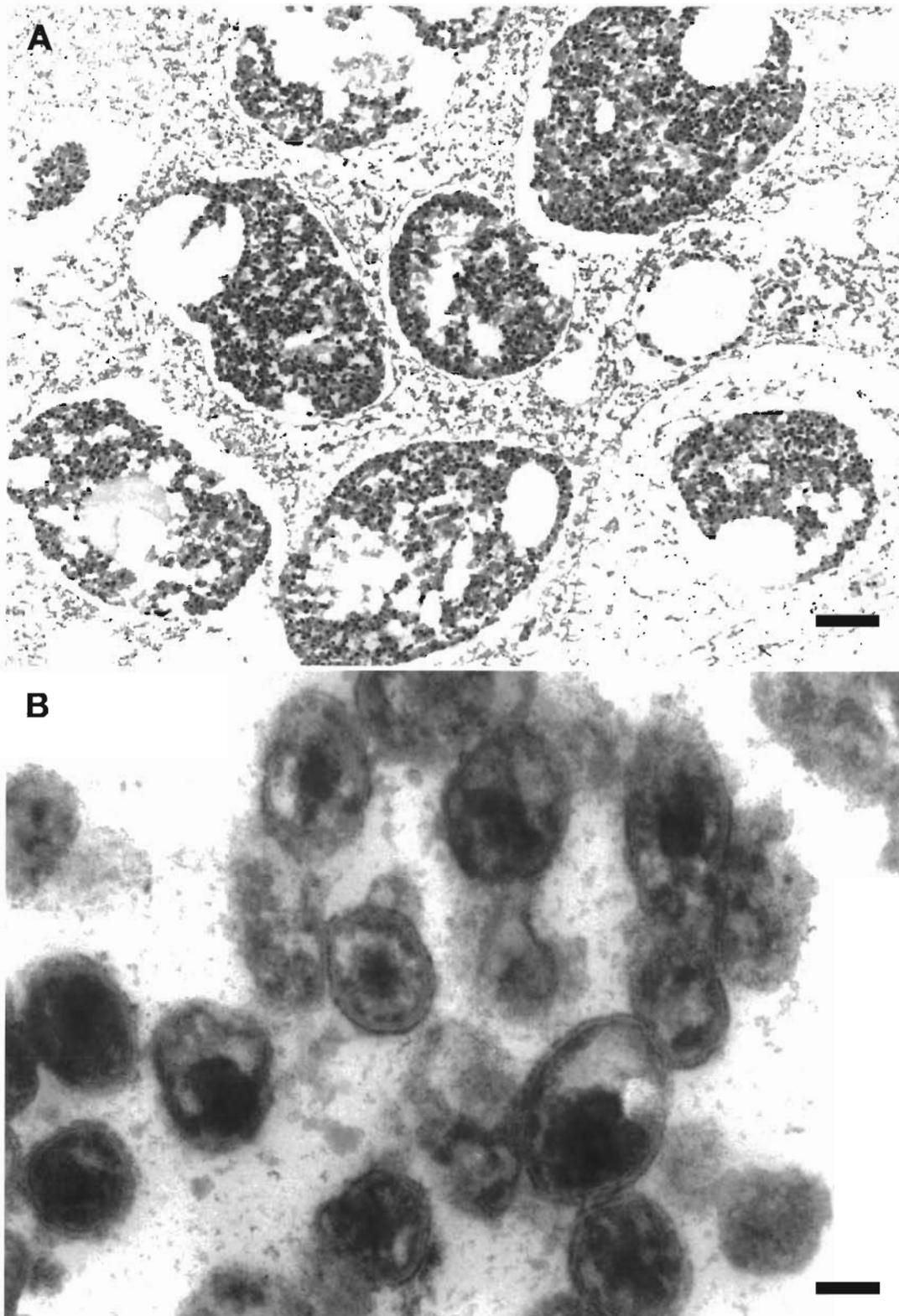


Fig. 2. Electron micrographs of rickettsia-like microorganisms in *Cherax quadricarinatus* hepatopancreocytes. Uranyl acetate and lead citrate. (A) Several rickettsia-like microcolonies in cytoplasmic vacuoles. Scale bar = 3 μ m. (B) Rickettsia-like microorganisms had both a cell wall and a plasma membrane and were pleomorphic, with rounded forms most common. Scale bar = 128 nm

which were not detected in the survey, such as the systemic RLO. Closer surveillance of ponds, and submission of sick animals for disease diagnosis, will improve knowledge of diseases in cultured *C. quadricarinatus*. To this point, there have been insufficient studies done to determine the full significance of pathogens in *C. quadricarinatus* aquaculture, and further studies may show that the hepatopancreatic RLO is more common than these initial studies suggest.

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