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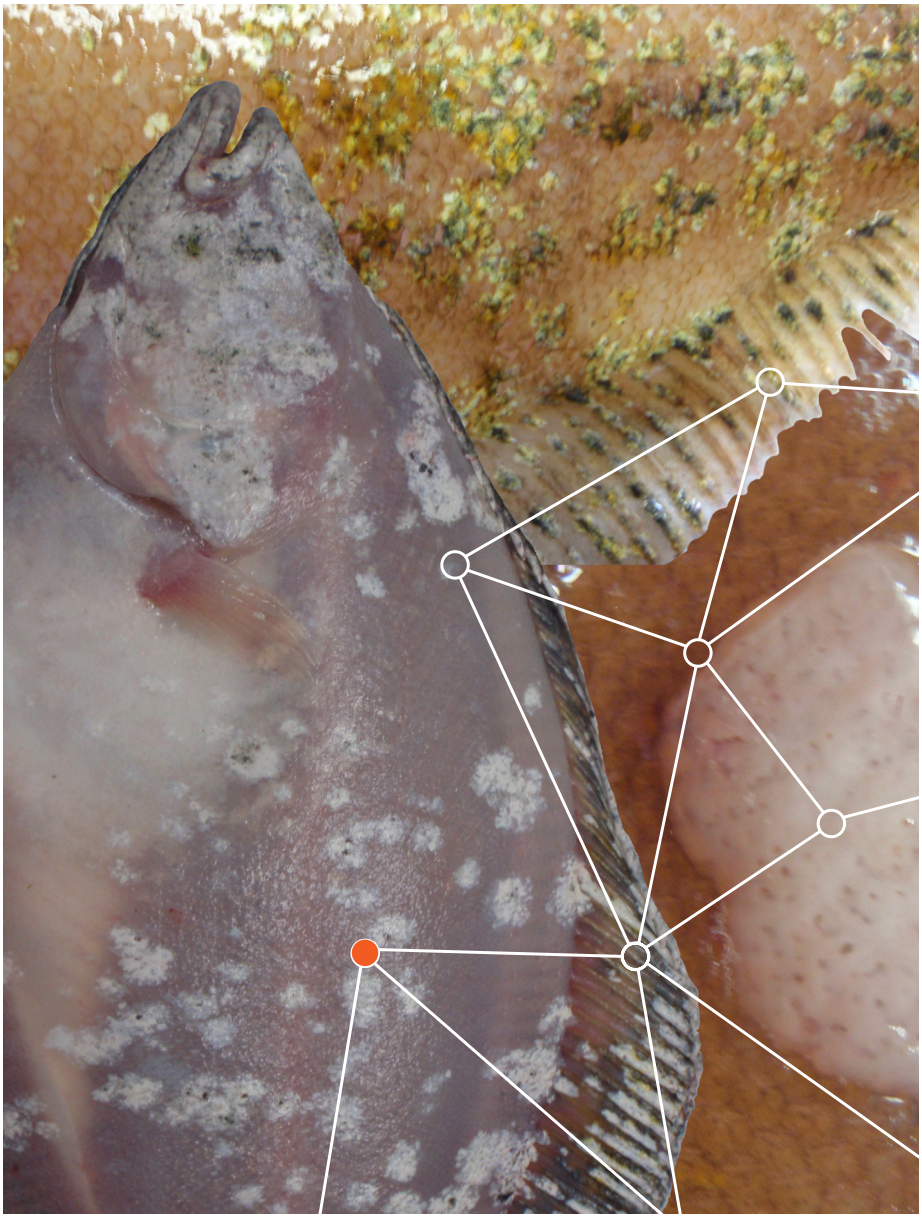

ICES INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CIEM CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

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Originals by Alderman, D. J., Green, M. (No. 11) and Balouet, G. (No. 12)
Revised by Tristan Renault and Susan Ford

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Susceptible species

Disseminated neoplasia has been reported in at least 15 bivalve species around the world, but three species, all in Europe and North America, seem especially susceptible: softshell clams, *Mya arenaria*; cockles, *Cerastoderma edule*; and mussels *Mytilus trossolus* (including those originally described as *M. edulis* in North America). Reports of disseminated neoplasia are rare in the other species, including the Pacific oyster, *Crassostrea gigas,* which is distributed worldwide.

Disease name

Disseminated neoplasia; disseminated sarcoma; systemic neoplasia; haemic neoplasia; haematopoetic neoplasia; leukaemia.

Aetiological agent

Although numerous studies have been conducted to identify the causative factor or factors involved in the development of neoplasia in molluscs, no clear answer has been obtained. Disseminated neoplasia has been induced through proximity experiments and by injection of intact neoplastic cells and cell homogenates, suggesting that an infectious agent may be involved in the disease. A retroviral aetiology has been suspected in several bivalve species based on transmission trials, detection of a reverse transcriptase activity and viral induction using 5-bromodeoxyuridine. However, no obvious viral agent has yet been identified and no definitive viral particles have been observed in transmission electron micrographs of neoplastic cells.

Geographical distribution

Disseminated neoplasia is found worldwide, but most reports are from Europe and North America.

Associated environmental conditions

Although high prevalence of disseminated neoplasia has sometimes been associated with polluted sites, there is little direct evidence supporting environmental carcinogens or other contaminants as aetiological agents. However, some authors suggest that pollutants may act as stressors inducing an increased susceptibility to infectious agents, particularly viruses. Prevalence of the condition varies seasonally, but peaks differ according to species.

Significance

Disseminated neoplasia in bivalves is generally considered to be a sarcoma (neoplasia of mesoderm-derived tissues). Although a haematopoietic origin and a gonadal origin have been proposed, a recent study showed that in soft-shell clams (*Mya arenaria*) the genotypes of neoplastic cells do not match those of the host animal. This result suggests that the disseminated neoplasia is spreading between animals in the marine

environment as a clonal transmissible cell derived from a single original clam. Moreover, *Venerupis corruga*ta DNA sequences have been detected in golden carpet shell clams (*Polititapes aureus*), presenting neoplastic cells and sharing the same habitat with diseased pullet shell clams, *V. corrugata*. This report suggests a possible interspecies transmission of disseminated neoplasia. As disseminated neoplasia progresses, normal haemocytes are replaced by neoplastic cells. Abnormal cells do not possess haemocyte functions such as phagocytosis and are not able to mediate defence functions. Affected individuals could be immuno-compromised and more susceptible to pathogenic agents. Mussels with advanced disseminated neoplasia presented a reduced bacterial clearance. Moreover, because haemocytes are also involved in digestion, absorption and transport of nutrients, these physiological functions may be impaired by disseminated neoplasia. Pathological changes include fibrosis, necrosis, compressions of epithelial and connective tissue cells, and inhibition of gametogenesis. Most individuals diagnosed with the disease eventually die; however, remission is possible if the condition does not become severe.

Gross clinical signs

No signs are specifically associated with the condition. Severely affected bivalves have watery meats and pale digestive glands.

Control measures and legislation

Avoid introducing neoplastic stocks as an infective agent may well be involved. Not notifiable to OIE.

Diagnostic methods

Molluscan neoplasia can be diagnosed using a variety of techniques including histology (Figures 1 and 2) and haemocytology (Figure 3), flow-cytometry (Figure 4) including DNA quantitation, specific antibody staining, and cytogenetics. Histopathological examination of fixed tissue, which was first used to describe neoplasia in molluscs, provides information not only on the abundance of abnormal cells, but on their location in the tissues.

For haemocytology, haemolymph is withdrawn from an adductor muscle, placed on a slide, and viewed either fresh, or fixed and stained. Because neoplastic cells do no adhere well, slides must first be coasted with poly-L-lysine before fixation. Haemocytology facilitates the counting of both neoplastic cells and normal haemocytes. Because it is non-destructive, it also permits repeated sampling of individual bivalves over time, and thus the temporal monitoring of the disease process. Several authors have assigned stages of disease severity using both histology and haemocytology. The key diagnostic feature of disseminated neoplasia consists of large (2–4 times the diameter of normal haemocytes) cells with a high nucleus-to-cytoplasm ratio, which are present in the haemolymph vessels and sinuses, and between vesicular connective tissue cells. The nuclei of the neoplastic cells are pleomorphic with one or more large nucleoli and conspicuous chromatin. In advanced cases, these cells are prevalent in all tissues and freshly drawn haemolymph is cloudy due to the abundance of the large, neoplastic cells. Since neoplastic cells usually contain more DNA than normal cells, flow cytometry has successfully been used to investigate neoplasia in bivalves. This technique provides a rapid method for the detection of neoplastic cells and the discrimination of aneuploid cells. Advantages to this approach include the ability to measure the DNA content of thousands of cells per individual in a very short time.

Key references

Barber, B. J., 2004. Neoplastic diseases of commercially important bivalves. Aquat. Liv. Res., 17, 449-466.

Elston, R. A., Moore, J. D., Brooks, K., 1992. Disseminated neoplasia of bivalve molluscs. Rev Aquat Sci 65:405-466

Gee, A., Specht, J. M., Kerk, D., Moore, J. D., Drum, A. S., Elston, R. A. 1994. Disseminated neoplastic cells in *Mytilus trossulus*: verification of host species origin by 16S-like rRNA sequence comparison. Mol Mar Biol Biot 3:7-12

House, M. L., Kim, C. H., Reno, P. W., 1998. Soft shell clams *Mya arenaria* with disseminated neoplasia demonstrate reverse transcriptase activity. Dis. Aquat. Org. 34, 187-192

Kelley, M. L., Winge, P., Heaney, J. D., Stephens, R. E., Farell, J. H., Van Beneden, R. J., Reinisch, C. L., *et al.*, 2001. Expression of homologues for p53 and p73 in the softshell clam (*Mya arenaria*), a naturally-occurring model for human cancer. Oncogen 20, 748-758

Krishnakumar, P. K., Casillas, E., Snider, R. G., Kaglay, A. N., Varanasi, U., 1999. Environmental contaminants and the prevalence of hemic neoplasia (leukaemia) in the common mussel (Mytilus edulis complex) from Puget Sound, Washington, USA. J. Invertebr. Pathol. 73, 135-146

Metzger, M. J., Reinisch, C., Sherry, J., Goff, S.P., 2015. Horizontal transmission of clonal cancer cells causes leukemia in soft-shell clams. Cell, 161(2), 255-263

Metzger, M. J., Villalba, A., Carballal, M. J., Iglesias, D., Sherry, J., Reinisch, C., Muttray, A. F., Baldwin, S. A., Goff, S. P., 2016. Widespread transmission of independent cancer lineages within multiple bivalve species. Nature 534, 705-709

Smolarz, K., Renault, T., Soletchnik, P., Wolowicz, M., 2005. Neoplasia detection in *Macoma balthica* from the Gulf of Gdansk: comparison of flow cytometry, histology and chromosomes analysis. Dis. Aquat. Org. 65, 187-195

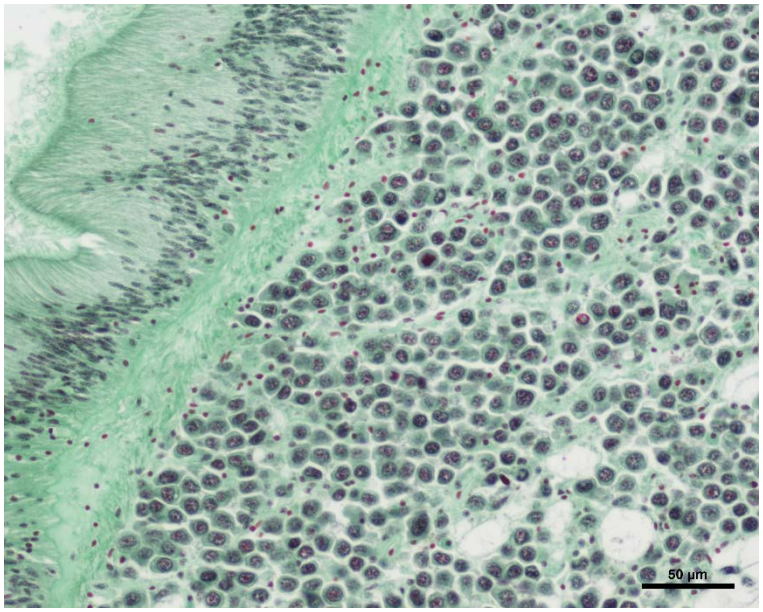

Figure 1. Histological section showing neoplastic cells in the digestive gland of a blue mussel (*Mytilus edulis*). AAENG stain. Bar = 50 µm. (Image courtesy of S W Feist, Cefas Registry of Aquatic Pathology (RAP) Acc. No. 319).

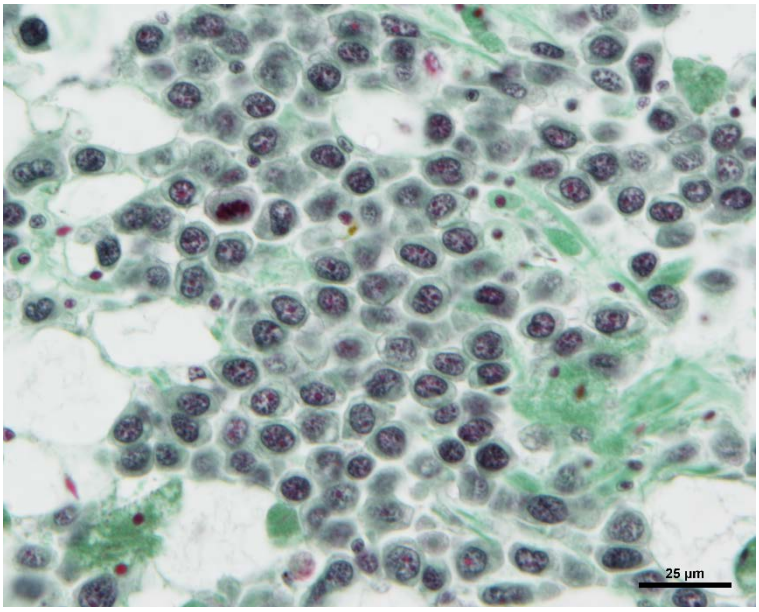

Figure 2. High power view showing neoplastic cells with characteristic large pleomorphic nuclei. AAENG stain. Bar = 25 µm. (Image courtesy of S W Feist, Cefas Registry of Aquatic Pathology (RAP) Acc. No. 319).

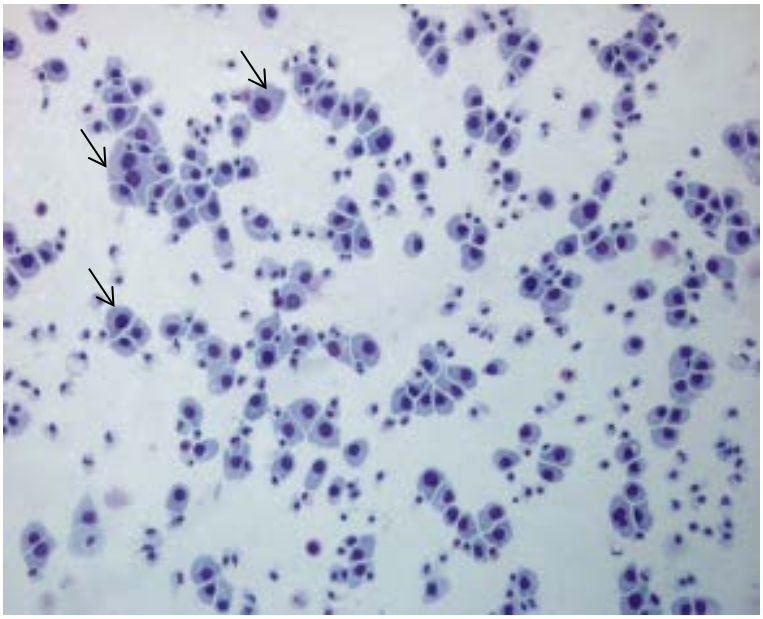

Figure 3. Neoplastic cells (arrows) from *Cerastoderma edule* (haemocytology, Hemacolor®, x100).

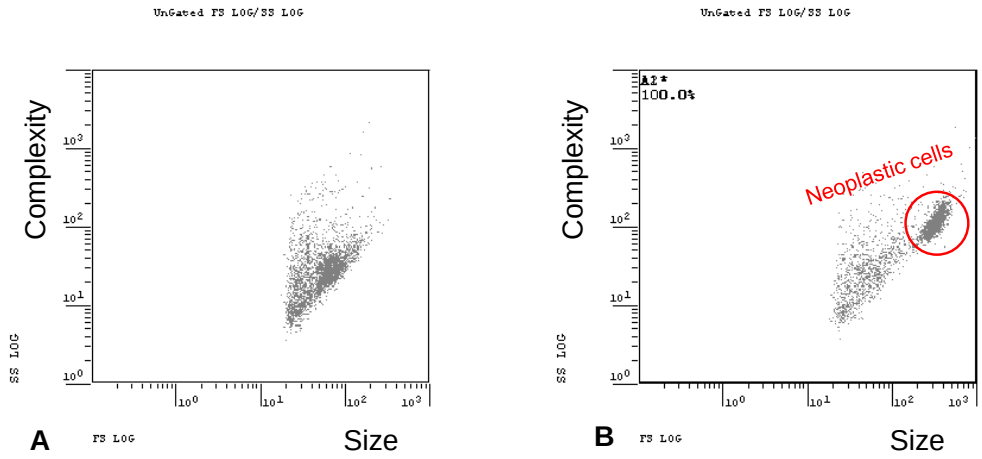

Figure 4. Neoplastic cells (red circle) from *Cerastoderma edule* (flow cytometry analysis). A: haemolymph collected from healthy animals; B: haemolymph collected from affected animals (a homogenous cell population was recorded).

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