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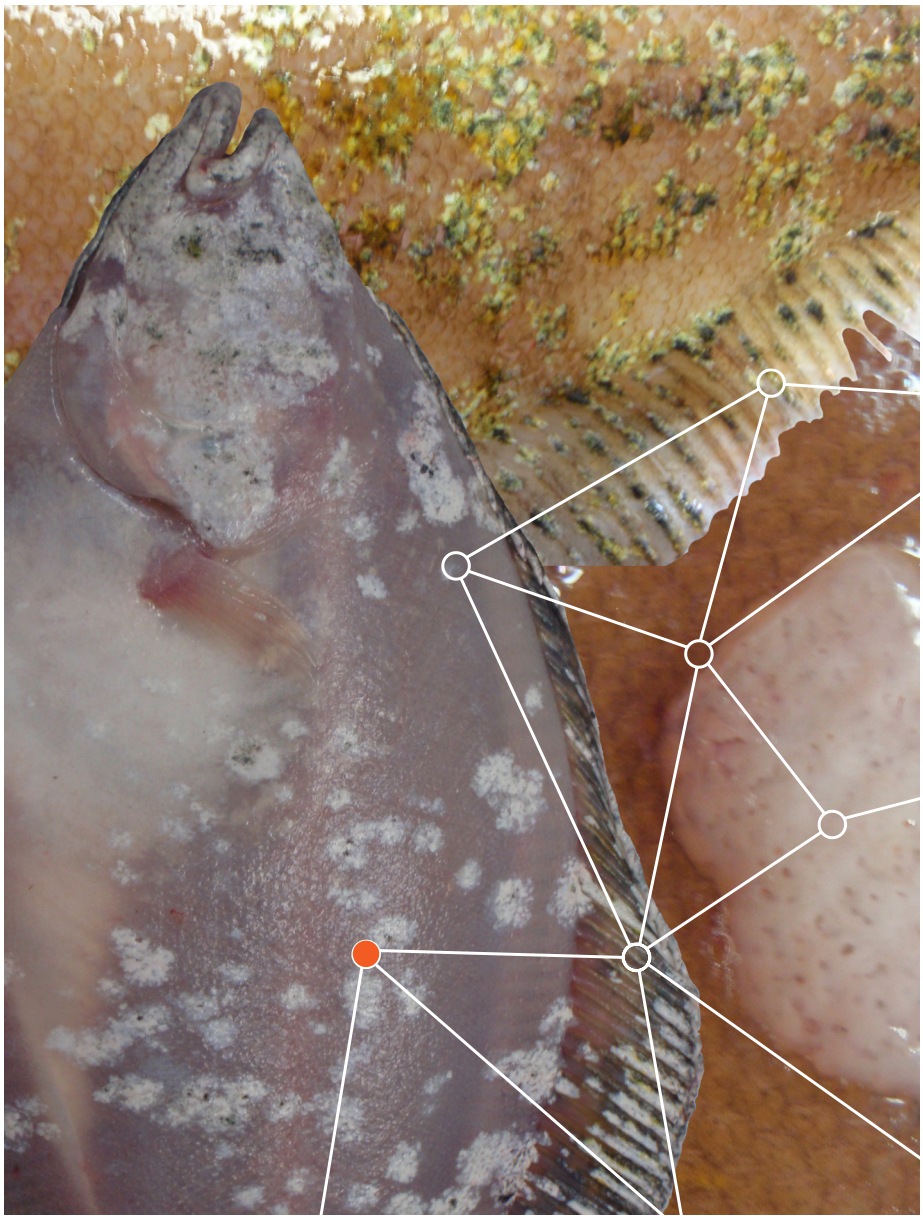

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Contents

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Christine Paillard

Susceptible species

Clams of the genus *Ruditapes*, *Tapes* and *Venerupis* in wild and reared populations (*Ruditapes philippinarum, Ruditapes decussatus, Tapes rhomboids,* and *Venerupis aurea*) are susceptible. All these species could be experimentally infected with *V. tapetis* (Maes and Paillard, 1992). The most sensitive species is the manila clam, *R. philipinarum.* The syndrome, which characterizes brown ring disease, has been reported in several other bivalve species including *Mercenaria mercenaria, Dosinia exoleta, Pecten maximus, Crassostrea virginica*; Maes and Paillard, 1992). However, the bacterium *V. tapetis* has not been found in those species.

Aetiological agent

The aetiological agent, *Vibrio tapetis*, first isolated in 1990 in Landeda (North Finistère, in France) has been previously named as VP1 (Paillard *et al.,* 1989; Paillard *et al.,* 1990; Borrego *et al.,* 1996).

The reference strain, *V. tapetis* CECT4600T is also known as CIP 104856. The plasmid sequencing has been completed (Erauso *et al.,* 2011) and 17 *V. tapetis* complete genome strains have been sequenced and molecular comparative genomic analysed (Paillard *et al.,* unpublished).

Geographical distribution

The disease was first detected in Europe, in Brittany (France) in 1987 and subsequently in Spain in 1994, England in 1997, in Ireland in 1998 and occasionally in Italy in 1990 (Paillard *et al.,* 2004; Drummond *et al.,* 2007). In Norway, in 2003, BRD has been also diagnosed in manila clams, seeded between 1988 and 1991 (Paillard *et al.,* 2008). BRD in manila clams was first reported in northern Asia (yellow sea, China Korea) in 2004 South Korea (Park *et al.,* 2006) and Hyogo prefecture in Japan in 2008 (Matsuyama *et al.,* 2010) To date, BRD has never been observed where susceptible clam species were first introduced outside their natural range to Western USA and Canada in 1936. Since 1990, in Italy, in north Adriatic lagoons, the disease has not spread, and is generally absent after the summer. In Spain, BRD is observed generally in both *R. decussatus* and *R. philippinarum* inhabiting several Rias of the Galician coast of northern Spain where environmental conditions, in particular temperature, are similar to those of the north European Atlantic coast. Considering the geographical distribution of this disease, BRD can be classified as a cold-water disease.

Associated environmental conditions

The sensitivity of *V. tapetis* to temperature must be emphasized. It does not grow at temperatures exceeding 27°C, and is rapidly killed at 30°C in seawater (Paillard *et al.,* 1997). Field and experimental studies confirm the significant effect of temperature on the development of BRD and on clam immune defence responses. Along the French

Atlantic coast, a border (River Loire) has been identified with the production sites located in the south of Loire exhibiting lower prevalence's of BRD (up to 3%) compared to northern sites with higher prevalence's (20 to 60%). In France, higher prevalence's of the disease are regularly observed in sites where manila clams were cultivated compared to wild populations (Paillard *et al.*, 2014; Paillard, 2016).

Significance

BRD provokes mass mortalities of reared juvenile and adult clams, especially in winter and spring, when maximum prevalence occurs.

Several physiological parameters such as growth, weight and condition index are subsequently affected. Diseased clams are significantly smaller, lighter and have a condition index significantly lower than healthy ones (Flye-Sainte-Marie *et al.*, 2007; Paillard, 1992). Biochemical analyses of the dried flesh of experimentally diseased clams have shown that there is a decrease in glycogen levels.

It is clear that the clams are weakened by the BRD and therefore more sensitive to various causes of mortality such as environmental stress, pollution and secondary infections but also reduced ability to cope with commercially practices such as seeding, high density, handling, storage, transport, and marketing (Jean *et al.*, 2011). Consequently, BRD has a real impact on fisheries and aquaculture of clams.

Gross clinical signs

V. tapetis perturbs initial shell growth by adhering to the periostracal lamina, organic matrix involved normally in calcium biomineralization. This disorganized periostracal lamina is not a good substrate for the biomineralization process and therefore accumulates between the pallial line and the edge of the shell, forming a brown ring, the name of this disease (Paillard *et al.*, 1989; Paillard *et al.*, 1994). The characteristic sign of BRD is an obvious abnormal brown deposit, organic in nature, which is generally located between the pallial line and the edge of the shell (Paillard, 2004) (Figure 1). Two signs allow identification of the disease. The first is an organic film made up of one or several layers, which strongly adheres to the prismatic layer. The second sign is the presence of conchiolin spots surrounded by a pale brown halo adhering to the inner layer. Clams can recover from the disease by covering the organic deposit by shell secretions. This defence process has been named nacrezation. A classification system based on disease and recovery stages have been established for use in epidemiological and experimental studies (Paillard and Maes, 1994). Deformations of the external shell, showing strong growth cessation, are very often associated with this disease.

This syndrome is a defence reaction and it is not exclusively due to *V. tapetis*, some others parasites (fungi, annelids, and trematodes) are also well known to lead pallial edge reaction, after shell boring, epithelial irritation, or because of living parasites within mantle tissue. Because of the non-specificity of the brown ring symptom, BRD diagnostic must include *V. tapetis* detection.

Light microscopy

Alterations of the periostracal lamina are observed due to the adhesion and colonization of *V. tapetis* in the first steps of infection, before brown spots deposits appear on the inner shell (Allam *et al.*, 1996; Paillard and Maes, 1995a, b). Tissue lesions are not systematically observed in diseased clams. Alterations of the digestive gland and the mantle are uniquely detected during the more severe stages of the disease (Plana *et al.*, 1991; 1996).

Physiological alterations

Alterations of immune parameters have been well described in the hemolymph and in the extrapallial fluids of wild and experimentally diseased clams (Allam *et al.,* 2000; 2002; 2006; Choquet *et al.*, 2003; Paillard, 2004). Physiological processes, such as breathing, the activities of clearance and filtration, decrease sharply in clams presenting with advanced stages of the BRD (Flye Sainte Marie *et al.*, 2007; 2009). The repair process is involved in healing and "resistance" clams facing the BRD (Trinkler *et al.,* 2010 a, b; 2011). Transcriptomic study of the mantle and haemocytes of clams after in vivo infections with *V. tapetis* have shown alterations of the immune cell cytoskeleton and enzymes involved in phenoloxydase system and biomineraliztion process (Allam *et al.,* 2014; Brulle *et al.,* 2012; Jeffroy *et al.,* 2011; 2013; Le Bris *et al.,* 2015; Richard *et al.,* 2015). A theoretical model has been recently developed (Paillard *et al.,* 2014); results of simulations illustrate the complex interaction of temperature effects on propagation and viability of the bacterium, on the phagocytic activity of the hemocytes, and on other physiological processes of the host clam.

Control measures and legislation

A significant increase in temperature reaching 27–30°C has a preventive effect on the development of the disease (Paillard *et al*., 2004). In France, hatcheries have adapted their clam cycle culture to take into account this effect. To produce disease-free clams, larvae and juveniles are usually produced at sites located south of River Loire in France. Using the model developed by Paillard *et al*. (2014) slight increases in temperature between 1°C and 2°C, generally favoured disease development, indicating that climate warming might facilitate the spread of BRD (Paillard *et al*., 2014).

Diagnostic methods

For each clam sample, the Brown Ring Disease (BRD) diagnosis requires two complementary tests.

The development and recovery of BRD is assessed by scaling macroscopic symptoms according to the classification system established by Paillard and Maes, 1994 (n = 100 minimum). The description a new shell repair stage (SRS 2.5) has been added to the classification of BRD (Paillard, 2004).

Quantification of *V. tapetis* burden in shell fluids measured by an immuno-enzymologic method (ELISA), (Noël *et al*., 1996) or by a recently developed qPCR (Bidault *et al*., 2015).

In cases where *Vibrio tapetis* is isolated at a new geographic location or within a new host, the use of replica tests is required (Paillard and Maes, 1992). Thereafter, multilocus sequence analysis (MLSA) is necessary to confirm *V. tapetis* identification of new isolates (Balboa and Romalde, 2013; Balboa *et al.,* 2013).

A clam sample will be considered as healthy under two conditions:

1) No detection of brown deposit after macroscopic and microscopic observations (binocular microscope).

2) No detection of V. *tapetis* by the ELISA technique (detection limit is 5×10^4 CFU ml^{-1} (fluids) or g^{-1} (whole clams homogenates) or by qPCR (detection limit 11.3 bacteria per mL of extrapallial fluid of clam 10^3 CFU ml^{-1} in extrapallial fluids).

Key references

Allam, B., Tanguy, A., Jeffroy, F., Le Bris, C., Pales Espinosa, E., and Paillard, C. 2014. Transcriptional changes in Manila clam (*Ruditapes philippinarum*) in response to brown ring disease. Fish and Shellfish Immunology, 41: 2–11.

Allam, B., and Auffret, M. 2000. Induction of an antibacterial activity in the hemolymph of the Manila clam, *Ruditapes philippinarum*. Journal Scientifique Libanais, 1: 3–11.

Allam, B., Paillard, C., and Auffret, M. 2000. Alterations in hemolymph and extrapallial fluid parameters in the Manila clam, *Ruditapes philippinarum*, challenged with the pathogen *Vibrio tapetis*. Journal of Invertebrate Pathology, 76: 63–69.

Allam, B., Paillard, C., Auffret, M., and Ford, S. E. 2006. Effects of the pathogenic *Vibrio tapetis* on defence factors of susceptible and non-susceptible bivalve species: II. Cellular and biochemical changes following in vivo challenge. Fish Shellfish Immunology, 20: 384–397.

Allam, B., Paillard, C., Ford, S. E. 2002b. Pathogenicity of *Vibrio tapetis*, the etiological agent of brown ring disease in clams. Diseases of Aquatic Organisms, 48: 221–231.

Allam, B., Paillard, C., and Maes, P. 1996. Localization of the pathogen *Vibrio* P1 in clams affected by Brown Ring Disease. Diseases of Aquatic Organisms, 27: 149–155.

Balboa, S., and Romalde, J. L. 2013. Multilocus sequence analysis of *Vibrio tapetis*, the causative agent of Brown Ring Disease: Description of *Vibrio tapetis* subsp britannicus subsp nov. Systematic and Applied Microbiology, 36: 183–187.

Balboa, S., Bastardo, A., and Romalde, J. L. 2013. Disentangling the Population Structure and Evolution of the Clam Pathogen *Vibrio tapetis*. Microbial Ecology 67:145-154.

Bidault, A., Richard, G. G., Le Bris, C., and Paillard, C. 2015. Development of a Taqman real-time PCR assay for rapid detection and quantification of *Vibrio tapetis* in extrapallial fluids of clams. PeerJ 3:e1484; DOI 10.7717/peerj.1484

Borrego, J. J., Castro, D., Luque, A., Paillard, C., Maes, P., Garcia, M. T., Ventosa, A. 1996. *Vibrio tapetis* sp. nov., the causative agent of the brown ring disease affecting cultured clams. International Journal of Systematic Bacteriology, 46: 480–484.

Brulle, F., Jeffroy, F., Madec, S., Nicolas, J. L., Paillard, C. 2012. Transcriptomic analysis of *Ruditapes philippinarum* hemocytes reveals cytoskeleton disruption after in vitro *Vibrio tapetis* challenge. Developmental and Comparative Immunology, 38: 368–76.

Choquet, G., Soudant, P., Lambert, C., Nicolas, J. L., and Paillard, C. 2003. Reduction of adhesion properties of R*uditapes philippinarum* hemocytes exposed to *Vibrio tapetis*. Diseases of Aquatic Organisms, 57: 109–116.

Drummond, L. C., Balboa, S., Beaz, R., Mulcahy, M. F., Barja, J. L., Culloty, S. C., and Romalde, J. L. 2007. The susceptibility of Irish-grown and Galician-grown Manila clams, *Ruditapes philippinarum*, to *Vibrio tapetis* and Brown Ring Disease. Journal of Invertebrate Pathology, 95: 1–8.

Erauso, G., Lakhal, L., Bidault-Toffin, A., Le Chevalier, P., Bouloc, P., Paillard, C., and Jacq, A. 2011. Evidence for the role of horizontal transfer in generating pVT1, a large mosaic conjugative plasmid from the clam pathogen, *Vibrio tapetis*. PLoS ONE 6(2): e16759. doi:10.1371/journal.pone.0016759.

Flye-Sainte-Marie, J., Jean, F., Paillard, C., and Kooijman, S. 2009. A quantitative estimation of the energetic cost of brown ring disease in the Manila clam using Dynamic Energy Budget theory. Journal of Sea Research, 62: 114–123.

Flye-Sainte-Marie, J., Pouvreau, S., Paillard, C., and Jean, F. 2007. Impact of brown Ring Disease on energy budget of the Manila clam *Ruditapes philippinarum*. Journal of Experimental Marine Biology and Ecology, 349(2): 378–389.

Flye-Sainte-Marie, J., Jean, F., Ford, S. E., and Paillard, C. 2008. Effect of sediment grain-size on development of brown ring disease in the Manila clam *Ruditapes philippinarum*. Aquaculture, 278: 184–187.

Jean, F., Flye-Sainte-Marie, J., Oudard, C., and Paillard, C. 2011. Handling enhances the development of signs of Brown Ring Disease in *Ruditapes philippinarum*. Journal of Shellfish Research, 30: 13–15.

Jeffroy, F., and Paillard, C. 2011. Involvement of nitric oxide in the in vitro interaction between Manila clam, *Ruditapes philippinarum*, hemocytes and the bacterium *Vibrio tapetis*. Fish and Shellfish Immunology, 31(6): 1137–41.

Jeffroy, F., Brulle, F., and Paillard, C. 2013. Differential expression of genes involved in immunity and biomineralization during Brown Ring Disease development and shell repair in the Manila clam, *Ruditapes philippinarum*. Journal of Invertebrate Pathology, 113: 129–136.

Le Bris, C., Richard, G., Paillard, C., Lambert, C., Seguineau, C., Gauthier, O., Pernet, F., *et al.* 2015. Immune responses of phenoloxidase and superoxide dismutase in the manila clam *Venerupis philippinarum* challenged with *Vibrio tapetis* – Part I: Spatio-temporal evolution of enzymes' activities post-infection. Fish and Shellfish Immunology, 42: 16–24.

Maes, P., and Paillard, C. 1992. Effet de Vibrio P1, pathogène de *Ruditapes philippinarum* sur d'autres espèces de bivalves. In: Les mollusques marins, biologie et aquaculture, Ifremer, Actes de colloques, no 14: 141–148.

Matsuyama, T., Sakai, T., Kiryu, I., Yuasa, K., Yasunobu, H., Kawamura, Y., Sano, M. 2010. First Isolation of *Vibrio tapetis*, the Etiological Agent of Brown Ring Disease (BRD), in Manila Clam *Ruditapes philippinarum* in Japan. Fish Pathology, 45: 77–79.

Nöel, D., Nicolas, J.-L., Boulo, V., Mialhe, E., and Roch, P. 1996. Development of a colony-blot ELISA assay using monoclonal antibodies to identify *Vibrio* P1 responsible for "brown ring disease" in the clam *Tapes philippinarum*. Aquaculture, 146: 171–178.

Paillard C. 2016. An ecological approach to understanding host-pathogen-environment interactions: the case of Brown Ring Disease in clams. In "Oysters and Clams: Cultivation, Habitat Threats and Ecological Impact". Nova Science Publishers, Inc. (NOVA). Chapter 7.

Paillard C., and Maes P. 1990. Etiologie de la maladie de l'anneau brun chez *Tapes philippinarum*: pathogénicité d'un *Vibrio* sp. Comptes Rendus de l'Académie des Sciences. Paris, 310, Série III: 15–20. (Prix pamaq 1992).

Paillard, C., Kjornes, K., Le Chevalier, P., Le Boulay, C., Harkestad, L., Eriksen, A. G., Willassen, *et al.* 2008. Isolation of a *Vibrio tapetis*-like strain from introduced Manila clams, *Ruditapes philippinarum*, affected by Brown Ring Disease in Norway. Diseases of Aquatic Organisms, 81(2): 153–161.

Paillard, C., Percelay, L., Le Pennec, M., and Le Picard, D. 1989. Origine pathogène de l'anneau brun" chez *Tapes philippinarum* (Mollusque, bivalve). Comptes Rendus de l'Académie des Sciences. Paris, 309, Série III: 235–241.

Paillard, C. 1992. Etiologie et Caractérisation de la Maladie de l'Anneau Brun Chez la Palourde d'Elevage, *Ruditapes philippinarum*. Université de Bretagne Occidentale, Brest, pp. Vol I, 194 pp and Vol II, 100 pp.

Paillard, C. 2004. A short review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. Aquatic Living Resources, 17: 467–475.

Paillard, C., Allam, B., and Oubella, R. 2004. Effect of temperature on defense parameters in Manila clam *Ruditapes philippinarum* challenged with *Vibrio tapetis*. Diseases of Aquatic Organisms, 59: 249–262.

Paillard, C., Jean, F., Ford, S. E., Powell, E. N., Klinck, J. M., Hofmann, E. E., and Flye-Sainte-Marie, J. 2014. A theoretical individual-based model of Brown Ring Disease in Manila clams, *Venerupis philippinarum*. Journal of Sea Research, 91: 15–34.

Paillard, C., and Maes, P. 1994. The brown ring disease symptom in the manila clam, *Ruditapes philippinarum*: establishment of a classification system. Diseases of Aquatic Organisms, 19: 137–146.

Paillard, C., and Maes, P. 1995a. The Brown Ring Disease in the Manila clam, *Ruditapes philippinarum*. I. Ultrastructural alterations of the periostracal lamina. Journal of Invertebrate Pathology, 65: 91–100.

Paillard, C., and Maes, P. 1995b. The Brown Ring Disease in the Manila clam, *Ruditapes philippinarum*. II. Microscopic study of the brown ring syndrome. Journal of Invertebrate Pathology, 65: 101–110.

Paillard, C., Maes, P., Mazurié, J., Claude, S., Marhic, A., and Le Pennec, M. 1997. Epidemiological survey of the brown ring disease in clams of Atlantic coast: role of temperature in variation of prevalence, Eighth Symposium of the International Society for Veterinary Epidemiology and Economics. (ISVEE'97). AEEMA Publications, Paris, July 8–11. Paris, France, pp. 14.03.11-14.03.13.

Paillard, C., Maes, P., and Oubella, R. 1994. Brown Ring Disease in Clams. Annual Reviews in Fish Disease, 4: 219–240.

Park, K.-I., Paillard, C., Le Chevalier, P., and Choi, K.-S. 2006. Report on the occurrence of brown ring disease (BRD) in Manila clam, *Ruditapes philippinarum*, on the west coast of Korea. Aquaculture, 255: 610–613.

Plana, S., and Le Pennec, M. 1991. Altérations de la glande digestive et conséquences nutritionelles chez la palourde *Ruditapes philippinarum* contaminée par une bactérie du genre *Vibrio*. Aquatic Living Resources, 4: 255–264.

Plana, S., Sinquin, G., Maes, P., Paillard, C., and Le Pennec, M. 1996. Variations in the biochemical composition of the juveniles of manila clam, *Ruditapes philippinarum*, infected by a *Vibrio* sp. Diseases of Aquatic Organisms, 24: 205–213.

Richard, G., Le Bris, C., Lambert, C., Guérard, F., and Paillard, C. 2015. Immune responses of phenoloxidase and superoxide dismutase in the manila clam *Venerupis philippinarum* challenged with *Vibrio tapetis* – part II: combined effect of temperature and two *Vibrio tapetis* strains. Fish and Shellfish Immunology, 44 (1): 79–87.

Trinkler, N., Bardeau, J. F., Marin, F., Labonne, M., Jolivet, A., Crassous, P., and Paillard, C. 2011. Mineral phase in shell repair of Manila clam *Venerupis philippinarum* affected by brown ring disease. Diseases of Aquatic Organisms, 93: 149–162.

Trinkler, N., Labonne, M., Marin, F., Jolivet, A., Bohn, M., Poulain, C., Bardeau, J. F., *et al*. 2010a. Clam shell repair from the brown ring disease: a study of the organic matrix using Confocal Raman micro-spectrometry and WDS microprobe. Analytical and Bioanalytical Chemistry, 396: 555–567.

Trinkler, N., Sinquin, G., Querne, J., and Paillard, C. 2010b. Resistance to Brown Ring Disease in the Manila clam, *Ruditapes philippinarum*: A study of selected stocks showing a recovery process by shell repair. Journal of Invertebrate Pathology, 104: 8–16.

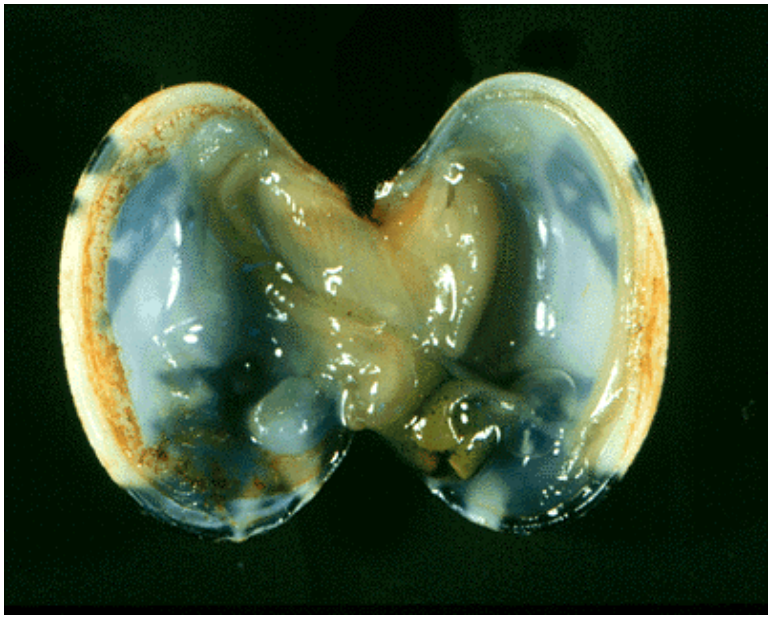

Figure 1. Clam juvenile, *Ruditapes philippinarum*, exhibiting conchiolin deposit on the inner face of the valves. Experimental transmission, four weeks after pallial *V. tapetis* inoculation.

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