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THE ECOLOGY OF *BONAMIA* AND DECLINE OF BIVALVE MOLLUSCS

Summary: *Bonamia* is a protozoan parasite of the haemocytes of oysters *(Tiostrea chilensis),* in which it has an annual developmental cycle between November and August each year. The parasite transmits directly, oyster to oyster, and therefore disease spread is related to host stock density. The Foveaux Strait oyster population experiences large mortalities every 20-30 years, and these may be attributable to *Bonamia*. The parasite appears to become less pathogenic at the end of, and probably between, mass mortalities, and some oysters appear more tolerant of infection than others. On the basis of these observations, and considering other protist pathogen:oyster models, the apparently reduced pathogenicity of *Bonamia* is discussed in terms of parasite kinetics. The population dynamics and selection of parasite tolerant host stocks, and kinetics of parasite transmission, may explain the cyclic nature of large-scale mortalities in Foveaux Strait, without change in parasite pathogenicity.

Keywords: *Bonamia* sp.; pathogenicity; parasite dynamics; resistance; tolerance.

Introduction

The protozoan parasite *Bonamia* sp. is a member of the small Phylum Haplosporidia, some members of which are serious pathogens in aquatic organisms, particularly oysters *(Ostrea, Tiostrea, Crassostrea, Saccostrea).* It has caused large mortalities among Foveaux Strait oysters since the end of 1985 (Dinamani *et al.,* 1987a; Dinamani, Hine and Jones 1987b; Hine and Jones, 1994; Doonan, Cranfield and Michael, 1994), and is the only known OIE (Office International des Epizooties; the world animal health organization) Notifiable Disease of aquatic organisms known in New Zealand. The other *Bonamia* species, *B. ostreae,* causes serious disease in oysters *(Ostrea* spp.) in Europe, and was probably introduced into Europe from North America (Elston, Farley and Kent, 1986). The *Bonamia* spp. are ultrastructurally similar, but initial genomic studies suggests that they are different species (R. Adlard, *pers. comm.;* University of Queensland), and description awaits completion of genomic investigations.

Bonamia sp. infects only the phagocytic haemocytes of flat oysters *(Tiostrea chilensis* Phillipi) (Hine and Wesney, 1994a), which is a hostile environment as the function of haemocytes is to internalise and destroy foreign organisms. Despite this, several human and other animal pathogens *(Mycobacterium, Leishmania, Toxoplasma, Trypanosoma* and microsporidians) infect and live in phagocytes, and in *T. chilensis* around New Zealand, an apicomplexan protozoan also infects, grows and divides in haemocytes (Hine, 1989a; Hine and Wesney, 1994a). The mechanisms utilised by *Bonamia* sp. to accomplish this resemble those of the mammalian pathogens listed above (Hine and Wesney, 1994a), and were foreseen by Cheng (1987). The parasite has an annual developmental cycle in its host, proliferating from November to May, becoming senescent and dying out by August, except for a few parasites that remain beneath the basement membrane of the gut (Hine, 1991a, b). Parasite proliferation is most evident in haemocytes that have entered the ovary to reabsorb unspawned ova (Hine, 1991a), and lipid appears to be the energy reserve utilised by the parasite (Hine and Wesney, 1994b). Unusual cytoplasmic structures in *Bonamia* sp. (Hine, 1992), that were once thought to be associated with possible integration of a viral genome (Hine and Wesney, 1992), may be due to abnormal lipid metabolism associated with senescence (Hine and Wesney, 1994b).

The history of bonamiasis in New Zealand flat oysters is unclear, but *Bonamia* is apparent in Foveaux Strait oyster tissues fixed in 1964 (Hine and Jones, 1994), in which the parasite is not associated with lesions or pathological changes. The cause of mass mortalities in Foveaux Strait oysters in the early 1930s is unknown, but similar mortalities in the early 1960s were attributed to sporocysts of *Bucephalus longicornutus* Manter, a digenean trematode (Howell, 1963, 1966, 1967). However, the investigator involved was a helminthologist who may have over-looked *Bonamia,* and since then *B.*

longicornutus has never been observed to be associated with mortalities in oysters.

Very large scale mortalities occurred among oysters *(Ostrea angasi* Sowerby) in south-eastern Australia at the end of the last century, resulting in large-scale movement of live oysters from New Zealand to Australia early this century (Dartnall, 1969). These oysters were re-layed live in Tasmanian and Victorian waters until needed by the restaurant trade. Epizootics among oysters *(O. angasi)* in Port Phillip Bay, Victoria in early 1991 were clearly associated with a species of *Bonamia,* indistinguishable from *Bonamia* sp. in New Zealand. Further infections were found in *O. angasi* around Tasmania and at Albany, Western Australia, soon after. Two interpretations of these facts are; a) that the large epizootics in Australia at the end of the last century were due to *Bonamia* sp., and this pathogen is enzootic in Australia and New Zealand, or b) that *Bonamia* was taken to Australia from New Zealand in live oysters earlier this century, leading to infections detected in the early 1990s. The former interpretation is favoured because it accounts for the mortalities in Australia last century, and explains the presence of *Bonamia* at Albany, in the absence of human movement of oysters westward. The recent report of *Bonamia* in *Tiostrea chilensis* in Chile (Kern, 1993) may be further evidence that *Bonamia* sp. is an ancient enzootic parasite of Southern Hemisphere flat oysters *(Ostrea, Tiostrea).*

This paper examines the population biology of the host and parasite and the interaction between *Bonamia* and *T. chilensis* haemocytes. From this, and from consideration of how hosts and parasites interact in other bivalve:protistan parasite models, mechanisms are identified that may explain the cyclic epizootics observed in Foveaux Strait oysters.

Results

Bonamia sp. in Foveaux Strait

Surveys were carried out in September 1986, January 1987, June-July 1990, February-March 1992 and in March-April 1995, to determine the distribution of *Bonamia* in Foveaux Strait oyster stocks (Dinamani *et al.,* 1987a; and in a series of unpublished reports to the New Zealand Fishing Industry Board Advisory Committee by Hine (1986 to 1990) and available from the NIWA library. Although different vessels and different techniques were used in these surveys, it is possible to deduce how *Bonamia* has spread within Foveaux Strait. The fisheries management areas used in these surveys are shown in Fig. 1.

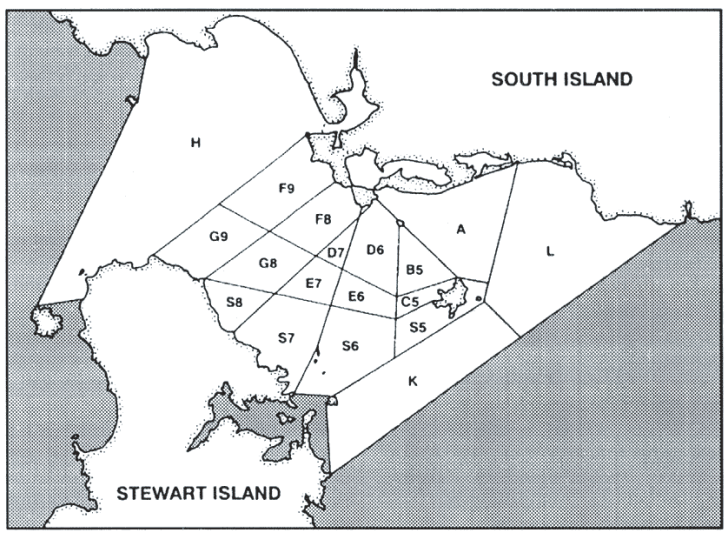

Figure 1: *Fisheries management areas surveyed/or the distribution of* Bonamia *in Foveaux Strait, 1986-1995.*

Examination of 1156 oysters in September 1986 and January 1987 (Dinamani, *et al*., 1987a), showed the distribution of infection within the strait to be similar, occurring at the boundary of areas H, F9 and G9, extending down into the northern part of G8 and E7, and eastwards as far as E6 and the northern boundary of S6. Infection was highest (>30%) at the boundary of areas H, F9 and G9 (September 1986) and in the northern half of G8 (both surveys). Large quantities of shell from freshly dead oysters ("clocks"), with hinge ligament intact and no fouling on the internal valve surfaces, showed a similar distribution, with >4.0 clocks per live oyster in the northern part of G8.

The 1990 survey (n=3433) revealed a spread of infection down through the centre of the strait with high prevalence (27-63%) in southern D6 and D7, in H near the H/F9 border (44%), and southern part of G9 (<77%). Prevalence was low (2-7%) in southern beds (S6, S7, S8), and also low (2-10%) in eastern beds (B5, C5, S5). The greatest prevalence of infection in the 1992 survey (n=1626) was seen in the south of area H (33% and 60%) off the Stewart Island coast, and in F8 (33%), with 10-15% infection north of Ruapuke Island, and lower prevalence through the centre of the strait. The 1995 survey (n=3898) showed moderate prevalence (11-25%) of infection to the north and east of Ruapuke Island and 26% infection at one site near the boundary of G8 and G9 (H.J. Cranfield, *pers. comm.)*. However, light infections with *Bonamia* were present throughout the strait. The mean prevalence for all samples/survey was 21 % in 1990, 8 % in 1992 and 4.5% in 1995. Mean prevalence could not be calculated from the data presented for the 1986 and 1987 surveys (Dinamani *et al*., 1987a).

In general there was movement from the central western beds through the centre of the strait, reaching the eastern beds in 1990-1992. The current flows in the strait are complex and change with the tides, but the overall (residual) flow is from west to east, the direction of spread of infection. Movement lateral to this direction appeared to be slower, with infection of southern beds in G9 occurring in 1990, and in area H off the Stewart Island coast in 1992, although these areas are closer to the original site of infection than the eastern beds around Ruapuke Island, which became infected at about the same time. The observed patterns are consistent with direct transmission by infective stages carried passively on currents, and direct transmission occurs by co-habitation of oysters in aquaria (P. Hine. *unpubl. data)*.

Over the period of the surveys, of incidence of infection, the degree of infection in individual oysters, also changed markedly. Although data for the 1986 and 1987 surveys are not available, the 1990, 1992 and 1995 surveys used a uniform scale from 1-5 for determining degree of infection. Category I oysters had <5 infected haemocytes per oyster, and in category 2 infection was confined to a few haemocytes in one part of the oyster, without associated tissue damage. Category 3 oysters had more widespread infection and disease (histopathological changes) was considered to be likely to develop, but was not present. Category 4 oysters were heavily infected, showed associated tissue damage and were considered diseased. In category 5 oysters, tissue damage was so extensive that the oysters were considered to be moribund. In 1986 and 1987 most oysters would have been classified as 4 or 5. In 1990,200 (28%) oysters were in grades 4 or 5, by 1992 this had reduced to 21 %, but by 1995 only 1 of 174 (0.6%) of oysters fell into grade 4. Therefore *Bonamia* is now widely distributed in Foveaux Strait, but at low prevalence and very low intensity of infection.

Host:parasite interaction

In order to grow and divide, *Bonamia* sp. must enter host haemocytes, and to do this must escape haemocyte killing mechanisms. From studies on *Bonamia ostreae* in oysters *(Ostrea edulis)* in Europe (Hervio *et al*., 1989; Chagot *et al*., 1992; Mourton *et al*., 1992), and on *Bonamia* sp. in *T. chilensis in vivo* (Hine and Wesney, 1994a), it appears that *Bonamia* suppresses the respiratory burst (Hervio *et al*., 1989), binds to the haemocyte surface and is actively phagocytosed (Chagot *et al*., 1992). Once in the phagosome it releases lipids that are inserted into the phagosome membrane which enlarges to form a parasitophorous vacuole (PV) (Hine and Wesney, 1994a). The modified phagosome membrane does not permit phagosome-lysosome fusion, and therefore haemocyte hydrolytic enzymes are prevented from entering the PV and coming into contact with the parasite (Hine and Wesney,1994a).

Interestingly, *B. ostreae* also inhibits the respiratory burst of Pacific oyster *(Crassostrea gigas)* haemocytes (Hervio *et al*., 1989), and is also phagocytosed by them (Chagot *et al*., 1992), but the haemocytes subsequently kill the parasite (Renault, Cochennec and Grizel, 1995). The reasons for this are unclear, but may be due to failure of the parasite to modify the phagosome membrane to form a PV, or the presence of more effective enzymes with which to kill *B. ostreae*.

Bonamia was widespread at low prevalence and intensity in Foveaux Strait oysters in the March-April 1995 survey. Previous studies at that time of

year have shown the parasite to be abundant and rapidly proliferating in individual oysters (Hine, 1991 a, b). It appears that this is no longer occurring, but conversely, neither is the host destroying the parasite, even though it is able to do so (Hine and Wesney, 1994b). This situation resembles that seen in oysters fixed in July 1964, when epizootics were not occurring. The parasite appears to be able to survive many years in the host population at sub-clinical levels, in contrast to the extreme pathogenicity shown during epizootics. This change in host:parasite relationships may be due to changes in the host, the parasite or both, and these in turn may be affected by the external environment.

Discussion

Host stock selection and resistance

Changes in the host population may be explained by selection and elimination of susceptible hosts during epizootics, leaving oysters with increased resistance or tolerance to infection. There is good evidence that American oysters *(Crassostrea virginica* Gmelin) are variable in their resistance to infection by *Haplosporidium nelsoni* Haskin, Stauber and Mackin, another haplosporidian, and oysters with elevated resistance to infection can be selectively bred (Haskin and Ford, 1979, 1987; Ford and Haskin, 1987; Matthiessen, Feng and Leibovitz, 1990; Barber, Ford and Littlewood, 1991). The nature of resistance to *H. nelsoni* appears complex (Ford and Haskin, 1987), and it was thought to be related to serum agglutinins (Chintala and Fisher, 1991). These agglutinins coat extracellular parasites to make them agglutinate, or to permit them to be phagocytosed by haemocytes, by altering or masking the surface receptors on such "non-self" organisms. It is now known that genotypically different stocks of C. *virginica,* showing different degrees of resistance to *H. nelsoni,* have different patterns of haemocyte receptors, as revealed by lectin studies (Cheng and Dougherty, 1994; Cheng, Dougherty and Burrell, 1993, 1994; Cheng, Manzi and Burrell, 1995). Furthermore, a saccharide on the haemocyte surface, lathyrose, is a marker for resistance to *H. nelsoni* infection, and resistance can now be related to lectin-mediated binding affinities, and receptor patterns with stock genetics (Cheng and Dougherty, 1994; Cheng *et al.,* 1994, 1995).

H. nelsoni plasmodia, unlike sporogonic stages, are extracellular and appear to evade attachment to, and phagocytosis by, haemocytes, by having surface receptors that mimic host haemocyte surface receptors, and they are therefore not recognized as "non-self" (Kanaley and Ford, 1990). The methods used by *H. nelsoni* for evading detection by C. *virginica* haemocytes are, however, the opposite to those that must be used by *Bonamia* sp., which can only feed, grow and divide if phagocytosed by a haemocyte (Cheng, 1987; Chagot *et al.,* 1992; Hine and Wesney, 1994a). Paradoxically oysters *(Tiostrea)* may not suffer from bonamiasis if they can readily detect and kill the pathogen, or if they fail to detect and phagocytose it. Their ability to detect but not effectively kill *Bonamia* is what makes them susceptible to infection and disease. Therefore oysters that produce populations of haemocytes that do not have receptors that permit attachment and phagocytosis of *Bonamia* sp. are likely to be more resistant to infection leading to disease.

Host selection is likely to favour resistant oysters, and current concepts of bivalve immunity and host:parasite dynamics suggests that such mechanisms are regulated by the role of receptors in host:parasite recognition (Cheng *et al.,* 1984; Cheng, 1987). There is circumstantial evidence for variable resistance or tolerance to *Bonamia* between *T. chilensis* stocks in New Zealand. In the summer of 1991/92 mortalities occurred among *T. chilensis* stocks that derived from Foveaux Strait, Tasman Bay and Otago harbour, that were being held under the same conditions at the MAF Fisheries aquaculture facility at Mahanga Bay, Wellington harbour. These were moved from Mahanga Bay to the Fisheries Research Centre nearby, as a source of *Bonamia* for research. Foveaux Strait oysters died within a few months, the Tasman Bay oysters a year later, and Otago harbour oysters the following year, despite being held together, presumably with equal exposure to the parasite.

Other factors, such as selection for elevated production of oxidative or hydrolytic enzymes may also be involved in resistance, but there is no evidence for this. One indeterminate factor is the role of the external environment in such situations (Cheng, 1987), and these directly or indirectly affect both host and parasite. In particular, temperature and starvation are likely to affect host susceptibility to disease, and human habitat modification this century may have created a situation favouring disease. Before oyster dredging began just over 100 years ago, the benthos of Foveaux Strait largely comprised complex communities of sponges, bryozoans, brachiopods and other invertebrates, interspersed with small discreet oyster beds. Subsequent dredging removed large areas of the spongelbryozoan fauna, known as "mullocky bottom", and greatly increased the extent of the beds. This increased oyster numbers and probably oyster densities, as well as removing

the complex communities that may have reduced dispersal stages of *Bonamia* during filter feeding.

Changes in *Bonamia* sp. pathogenicity

There is no evidence for the existence of strains varying in virulence in haplosporidians, although there have been no studies to detect such strains. Virulent strains may arise by group or clonal selection (Read and Schrag, 1991), or may vary between individual parasites without selection. In the present case, the pathogenicity of *Bonamia* appears to have declined, but this reduction may simply be related to reduced numbers of *Bonamia* in Foveaux Strait oysters, as seen in other bivalve:protist models (Cheng, 1987), rather than a decline in virulence.

This may be explicable in terms of *Bonamia* kinetics. In *B. ostreae,* which also transmits directly between flat oysters *(Ostrea edulis),* the size of the infective dose to which an oyster is exposed is directly related to the likelihood that infection will be established (Hervio, 1992). This probably also applies to *Bonamia* sp., in which case transmission of infection is likely to be dependent on host stock density and distance from susceptible hosts/beds downstream. Decrease in oyster density in a bed will reduce the likelihood of cross-infection between beds, because of the reduced release of infective stages. The effect is likely to be less marked within beds, where oysters are closer together and dilution of infective stages in the water column is less, but the density of infective stages reaching individual oysters within beds is still likely to be reduced compared with the time before the epizootics when oyster densities were greater.

Bonamia sp. may be killed by, but also may kill, phagocytic haemocytes (Hine and Wesney, 1994a). If the parasite population does not kill its host before the parasite population crashes around August, the host will carry very light infections through to November (Hine, 1991b). If the host does not then acquire further infection from upstream, it may survive to the following year with a very light parasite load. Bearing in mind that reduced oyster densities mean reduced numbers of infected particles being shed from beds, and reduced *Bonamia* burdens within individuals will also result in reduced shedding of infectious stages, the number of infectious particles to which an individual or bed is exposed will also be reduced following epizootics. The apparent cessation of epizootics would also result in the decrease of infective stage shedding from dead and decomposing oysters.

Epizootics may therefore occur when sub-clinical infections are supplemented by incoming infectious stages from individuals or beds upstream, killing the host before the August population crash. Epizootics may cease when the number of incoming particles declines to the extent that hosts, particularly those selected for resistance to infection, can tolerate the burden through to the parasite population crash in August. Such population dynamics explain the current widespread low prevalence and intensity of infection, and consequent lack or mortalities.

If *Bonamia* sp. exists at a low level for many years, a change must occur that results in increased release of infective stages between individuals and beds, causing death and release of more particles through a cascade process. Otherwise the infections would remain sub-clinical indefinitely. Two types of triggering event that may upset host:parasite relations in favour of *Bonamia* can be identified at present. One is the effect of the external environment on ectotherm hosts (Cheng 1987), which are complex and hard to identify. Bonamiasis may have been triggered in New Zealand by host starvation as the occurrence of epizootics began at a time when mutton birds, albatrosses and yellow-eyed penguins, which utilize marine resources, showed signs of starvation or population decline. This may be related to ozone depletion reducing phytoplankton productivity in Antarctic waters (Smith *et al.,* 1992), and regional fluctuations in productivity between 1985 to 1989 (Harris *et al.,* 1991). The other involves an increase in the proportion of oysters in the population that are susceptible to higher levels of infection. Following epizootics surviving oysters are likely to have elevated tolerance or resistance to infection, but this may decline in subsequent generations due to genetic drift. If the proportion of haemocytes that recognize and phagocytose *Bonamia* increased, there would be a concomitant increase in the number of the infective stages produced.

The epizootics in Foveaux Strait appear to be a natural phenomenon, and the interactions of host and parasite may be compared with the predator-prey cycles of other organisms. If *Bonarnia* is enzootic to the Southern Hemisphere, the relationship is probably long-standing and resilient, although the extension of Foveaux Strait beds by humans since the 1890s may have exacerbated the situation by creating larger beds of high density. The annual pattern of infection suggests a long-standing host:parasite association, in contrast to the the lack of an apparent infection cycle and widespread epizootic nature of *B. ostreae* infections in *O. edulis*. In the latter case there is strong circumstantial evidence that *B. ostreae* was introduced into France in *O. edulis* from California in the late 1970s (Elston *et al.,* 1986), in which case the relationship is recent

and the host immunologically naive on first contact. *H. nelsoni* also appears to be poorly adapted to C. *virginica,* and as it was first detected in Delaware Bay in 1957 and Chesapeake Bay in 1959, it may be a recent arrival in east coast C. *virginia* stocks (Andrews, 1982). It contrasts with a similar organism, *Haplosporidium costale,* also in C. *virginica,* which like *Bonamia* sp. has a well defined annual pattern of development (Andrews, 1982). The degree to which a stable relationship has developed between host and parasite is likely to be a reliable indicator of the period of time over which they have been associated.

Caution must be exercised in comparing protistan pathogen:bivalve models. *Haplosporidium* spp. and *Bonamia* spp. have features of haplosporidians and are pathogens in oysters, but *Haplosporidium* spp. are extracellular and cannot be transmitted directly between hosts, whereas *Bonamia* spp. are obligate intracellular parasites with direct life-cycles. Comparison with other protist pathogen:bivalve models may be useful when considering the spectrum of bivalve immune responses, but pathogens are better compared with other pathogens using the same *modus vivendi,* even if they infect very different host groups.

It is concluded that *T. chilensis* and *Bonamia* sp. have a longstanding association that is usually in equilibrium, but which may be upset by changes in oyster density, the proportion of susceptible oysters and environmental changes, including human habitat modification. Keeping the Foveaux Strait stocks fished to oyster densities that do not trigger *Bonamia* epizootics, but allow adequate stock recruitment, may be the best way to manage the fishery. Against this, there is evidence from the Dutch oyster fisheries that repeated trawling of oyster beds increases the prevalence and intensity of bonamiasis, possibly as a result of stress on the oysters. Only with a better understanding of the ecology of *Bonamia,* its host, their interaction with other benthic invertebrates and the influence of environment, can the fishery be managed for long-term sustainability.

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