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The effects of stressors on the dynamics of *Bonamia exitiosus* Hine, Cochennec-Laureau & Berthe, infections in flat oysters *Ostrea chilensis* (Philippi)

P M Hine, B K Diggles, M J D Parsons, A Pringle and B Bull

National Institute of Water and Atmospheric Research, Kilbirnie, Wellington, New Zealand

Abstract

Oysters, *Ostrea chilensis*, infected with *Bonamia exitiosus* were held under stressful conditions for 14 days, and then under normal conditions for 3.5 months, to determine the affects of stress on *B. exitiosus* infection levels. The stressors used were (1) air exposure for 8 h daily; (2) hot water (25–26 °C), or (3) cold water (7 °C) for 1 h daily; (4) hyposaline (15‰), or (5) hypersaline (39–40‰) water; (6) starvation in filtered sea water; and (7) vigorous stirring four times a day. A control tank held oysters in static sea water, which was changed daily. Oysters were also (8) kept in a trough among heavily infected oysters (trough exposure), or (9) in a trough without other oysters (control), for 4 months. Oysters in hyposaline conditions all died within 3 weeks, but this was apparently unrelated to *Bonamia* infection. Otherwise, cumulative mortalities were highest in trough exposure, cold, hypersaline and hot treatments. There was a significant difference in prevalence between treatments, and the stir, hot, cold, hypersalinity and trough exposure treatments had significantly higher intensities of infection than controls. The mean intensity of *Bonamia* infection was significantly higher among female and spent oysters than in male and hermaphrodite oysters.

Keywords: Bonamia exitiosus, infection dynamics, *Ostrea*, stressors, salinity, temperature.

Correspondence *Dr M Hine, National Centre for Disease Investigation, MAF Operations, PO Box 40-742, Upper Hutt, New Zealand.*
(e-mail: hinem@maf.govt.nz)

Introduction

Bonamiosis is an Office International des Epizooties (OIE) listed notifiable disease of flat oysters, *Ostrea* spp., caused by two pathogens, *Bonamia ostreae* in Europe and the United States, and *B. exitiosus* in Australasia. Since the initial reports of *B. ostreae* by Pichot, Comps, Tigé, Grizel & Rabouin (1979), and *B. exitiosus* (= *Bonamia* sp.) by Dinamani, Hine & Jones (1987), studies have concentrated on ultrastructure (Brehélin, Bonami, Cousserans & Vivarès 1982; Hine 1991a; Hine & Wesney 1994a,b; Hine, Cochennec-Laureau & Berthe 2001), life or developmental cycles (Hine 1991a,b; Montes, Anadon & Azevedo 1994), experimental infections (Hervio, Bachère, Boulo, Cochennec, Vuillemin, Le Coguic, Cailletaux, Mazurie & Mialhe 1995), pathology (Balouet, Poder & Cahour 1983), host:parasite interactions (Chagot, Boulo, Hervio, Mialhe, Mourton & Grizel 1992; Hine & Wesney 1994b), diagnostic techniques (Carnegie, Barber, Culloty, Figueras & Distel 2000; Cochennec, Le Roux, Berthe & Gerard 2000), resistance of selected stocks to infection (Baud, Gérard & Naciri-Graven 1997; Naciri-Graven, Martin, Baud, Renault & Gérard 1998), and impact on wild and cultured oyster stocks (Montes, Villalba, López, Carballal & Mourelle 1991; Robert, Borel, Pichot & Trut 1991; Cáceres-Martinez, Robledo & Figueras 1995). These primarily diagnostic and aetiological studies have provided much scientific information, but in order to manage the disease in wild and cultured stocks, other, often basic, information is required.

Epizootics of bonamiosis, caused by *B. exitiosus*, destroyed 90% of wild commercial-sized oysters, *Ostrea chilensis* (Philipi), in beds in the south of the

South Island of New Zealand between 1985 and 1993 (Doonan, Cranfield & Michael 1994). In order to effectively manage the recovering oyster beds, it is necessary to develop a mathematical model of the effect of *B. exitiosus* on the fishery. To achieve this, basic epidemiological information is required, such as how oyster density affects the distance over which transmission occurs, including the infectious dose, and how long *B. exitiosus* can survive outside its host. The resistance to, or tolerance of, infection by host oysters is also important, and this may be determined by stock genetics, and the degree to which oysters are stressed. It has long been thought that stress lowers the resistance of oysters to infection, by suppressing the immune system allowing endogenous parasites to proliferate and cause disease. Handling and transport elevate mortality levels among infected oysters, however, there is little information on the degree to which stress increases susceptibility of oysters to *Bonamia* infection. Experimental dredging of *B. ostreae*-infected oysters, *O. edulis,* in the Gravelingen inland sea in the Netherlands showed that when one of two comparably infected beds was dredged, oysters in the dredged bed had higher levels of *Bonamia* infection than in the undredged bed (van Banning 1991).

This study was carried out to determine the effects of mechanical and physiological stress on the dynamics of *B. exitiosus* infecting *O. chilensis.* Physiological stress was imposed by prolonged exposure to air, warm and cold water, hypo- and hyper-saline water, and starvation, while mechanical stress, corresponding to dredging, was simulated by vigorously stirring oysters four times a day.

Materials and methods

Samples comprising 580 *O. chilensis*, >58 mm shell width, were collected from stations in Foveaux Strait that just previously had been shown to have a similar low prevalence (6%) and intensity (grades 1 and 2 in Hine 1991b) of *B. exitiosus* infection. Of these, 480 oysters were divided into eight groups of 60 oysters which, daily for 14 days, were treated as follows:

Group 1 (air exposure) were kept on a mesh tray in a large trough (~135 L) with flow-through sea water. Daily the tray was lifted from the trough and set above the water. Oysters were returned to the tank 8 h later.

Group 2 (hot water) were kept in a 50-L tank with flow-through sea water, and daily water flow was stopped for 1·5 h. During this time ~20 L of water was siphoned from, and 10 L of 75 °C sea water was added to the tank. The tank was left for 1 h at 25–26 °C before water flow was recommenced.

Group 3 (cold water) were kept in a 50-L tank with flow-through sea water, and daily water flow was stopped for 1·5 h. During this time ~20 L of water was siphoned from the tank and frozen sea water was added and stirred to accelerate cooling. The tank was left for 1 h after the tank had reached 7 °C (usually 10 min) before water flow was recommenced.

Group 4 (hyposaline) were kept in a 40-L static tank with aeration. The salinity was maintained at 15‰ by adding 18 L of distilled water to the tank. The water was changed daily.

Group 5 (hypersaline) were kept in a 40-L static tank with aeration. The salinity was maintained at 39–40‰ by adding dissolved table salt to the tank. The water was changed daily.

Group 6 (filter) were kept in a 40-L static tank with aeration, and daily the water in the tank was replaced with 0.22 μm filtered sea water to starve the oysters by removing their food source.

Group 7 (stir) were kept in a 50-L tank with flow-through sea water. Four times a day (09.00, 11.00, 14.00, 16.30) the oysters were stirred vigorously with a stiff piece of plastic hosing.

Group 8 (static control) were kept in a 40-L static tank with aeration, and daily the water in the tank was replaced with raw sea water.

The other 100 oysters were maintained in aquaria and fibreglass raceways as follows.

Group 9 (trough exposure): 45 oysters were kept in a mesh bag in a large trough (~250 L) with flow-through sea water, among bags of oysters infected with *Bonamia*. The oysters were cohabited with the infected oysters for 4 months.

Group 10 (flow control): 55 oysters were kept in a large trough (~115 L) with flow-through sea water without any other oysters for 4 months.

After their 14 day treatments, the oysters in groups 1–8 were maintained in flow-through aquaria in Wellington Harbour sea water (9–19 °C and 32–36‰) for 4 months. During this time, they and the oysters in groups 9 and 10 were inspected daily and all gapers and dead oysters were

removed and fixed for histology in 10% formalin in filtered sea water, processed using standard histological techniques, stained with haematoxylin and eosin (H&E) and examined by light microscopy. After 3 months half of the surviving oysters in each treatment were removed and fixed as described above. After 4 months, all of the remaining surviving oysters were removed and fixed as described above. The mortality rates and intensity of *Bonamia* in dead and surviving oysters were then compared between treatments. Replicate experiments could not be carried out because of lack of space and one of us (P.M.H.) leaving the laboratory during the course of the experiment.

Intensity of *B. exitiosus* was graded as 1–5, as in Hine (1991b), and intensity of apicomplexan infection as in Hine (2002).

Statistics

The cumulative mortalities for each treatment were plotted with Kaplan–Meier mortality curves which are designed for censored survival data (Cox 1984). In this case the censoring was the removal of live oysters at week 15. The mortality at any given week was calculated as:

$$M(t) = 1 - \prod_{\text{weeks} \le t} \left(1 - \frac{\text{no. fatalities during week}}{\text{no. oysters at start of week}}\right)$$

To determine whether significant differences in the prevalence of *Bonamia* occurred between treatments and controls after the 4-month observation period, we compared *Bonamia* prevalence at 4 months, using a mortality-adjusted prevalence defined as:

$$\frac{\text{number of oysters with } \textit{Bonamia}\text{, including those which had already died with } \textit{Bonamia}}{\text{original number of oysters, excluding those which had already died without } \textit{Bonamia}}$$

Oysters which died but were negative for *Bonamia* (including those sampled after 3 months) were excluded because they may still have contracted *Bonamia* if they had survived the entire 4 months. Statistically, this procedure is an approximation to a survival analysis in which oysters dying from causes other than *Bonamia* are censored. The statistical significance of the differences in mortality-adjusted prevalence after 4 months, between treatments and the pooled static and flow controls, was assessed using a log-linear model (Agresti 1990):

$$\begin{aligned}\text{number of oysters} \sim \text{Poisson} * (\text{mean} = \textit{Bonamia} \\ + \text{sex} + \text{treatment} + \textit{Bonamia} * \text{sex} + \\ \textit{Bonamia} * \text{treatment} + \text{sex} * \text{treatment})\end{aligned}$$

This model also excluded oysters which had already died without contracting *Bonamia*.

These log-linear models were fitted as General Linear Models (GLMs) in S-PLUS. The effect of each treatment was tested for significance at the $\alpha = 0.05$ level, using a *t*-test on the corresponding *Bonamia**treatment interaction. A significant result indicated a statistically significant difference in mortality-adjusted prevalence between that treatment and the pooled controls, allowing for the effect of sex.

The significance of the overall difference between treatments was also tested, using a chi-squared test of residual deviance on the *Bonamia**treatment term. A significant result indicated overall significant differences in mortality-adjusted prevalence between treatments, allowing for the effect of sex.

Treatment effects on the intensity of *Bonamia* infections were tested using a linear regression model, using sex as a covariate:

$$\begin{aligned}\textit{Bonamia} \text{ intensity} \sim \text{normal } (\text{sex effect} \\ + \text{treatment effect } \sigma^2)\end{aligned}$$

All oysters, i.e. those sampled alive and those sampled dead, were included. Treatment effects were tested for significance at the $\alpha = 0.05$ level. Sex effects were also examined.

Results

Nearly half of all oysters (49.8%) died during the course of the 4-month experiment (Table 1). The highest three mortality rates were in the hyposalinity, trough exposure and cold treatments, with 100%, 61.3% and 58.3% mortality, respectively (Fig. 1, Table 1). Mortality rates of oysters exposed to hyposalinity (15‰) were 30% after 1 week, 96.6% after 2 weeks and 100% after 3 weeks (Fig. 1). The prevalence (8.3%) and mean intensity (2.6) of *Bonamia* in the oysters which died from the hyposaline treatment were the lowest of all treatments, however, indicating that mortality was the result of the treatment, not *Bonamia* infection. Histopathological examination of these oysters showed oedema of the adductor muscle, gonad, digestive tubules and gills, and digestive tubule necrosis, sloughing of the digestive

Table 1 Results of experiments after 134 days observation. Figures in bold denote the three treatments with the highest values

Treatment	All oysters	Expose	Stir	Hot	Cold	Hypo-salinity	Hyper-salinity	Filter (starved)	Static control	Trough exposure	Flow control
No. of oysters	580	60	60	60	60	60	60	60	60	45	55
Per cent male	17.9	22	20	22.9	13.3	10.2	20	22	11.8	13.6	22.6
Per cent female	49.8	50.8	45	52.5	56.6	64.4	51.7	45.7	55.9	29.5	39.6
Per cent hermaphrodite	12.2	11.9	18.3	6.5	11.6	5.1	6.7	13.6	15.3	13.6	20.7
Per cent spent/no sex	20	15.2	16.7	18	18.3	20.3	21.7	18.6	16.9	43.2	17
Total mortality (%)	49.8	40.1	33	44.3	**58.3**	**100**	53.3	33	23.7	**61.3**	33
Per cent mortality male oysters	26.2	**38.5**	16.6	7.2	**37.5**	**100**	33.3	0	28.6	33.3	16.6
Per cent mortality female oysters	57.3	53.3	40.7	56.2	**67.7**	**100**	51.7	48.2	30.3	**84.7**	38.1
Per cent mortality hermaphrodite oysters	14.3	0	**27.3**	0	0	**100**	**25**	0	0	16.6	18.2
Per cent mortality spent/no sex oysters	64.3	33.3	40	72.7	**81.8**	**180**	**84.7**	63.7	20	68.5	55.6
Prevalence *Bonamia* – all oysters (%)	75.6	74.6	**91.6**	**96.7**	85	8.3	86.7	64.4	81.4	**93.2**	72.2
Prevalence *Bonamia* surviving oysters (%)	79.3	60	**92.5**	**97.1**	76	–	89.3	53.8	77.8	**94.1**	83.3
Prevalence *Bonamia* dead oysters (%)	72	**95.8**	90	**96.3**	91.4	8.3	84.4	85	**92.9**	92.6	70.6
Mean intensity *Bonamia* all oysters[a]	3.3	**3.6**	**3.5**	3.4	3.4	2.6	**3.6**	2.9	3.0	**3.7**	2.6
Mean intensity *Bonamia* live oysters[a]	2.5	**2.9**	**2.8**	2.5	2.4	–	2.6	2.4	2.4	**2.7**	1.8
Mean intensity *Bonamia* dead oysters[a]	4.3	4.3	**5**	**4.7**	4.1	2.6	**4.6**	3.6	4.4	4.3	4.3
Mean intensity apicomplexan live oysters[a]	1.9	2	1.5	2	**2.1**	**–**	**2.2**	**2.1**	1.9	**2.7**	1.6
Mean intensity apicomplexan dead oysters[a]	1.9	2.2	1.5	1.5	1.8	1	**2.4**	1.8	1.7	**2.5**	**2.4**

[a] Denotes based on a semiquantitative logarithmic scale for which: 0, not infected; 1, light infection; 2, light/moderate infection; 3, moderate infection; 4, moderate/heavy infection; 5, heavy infection.

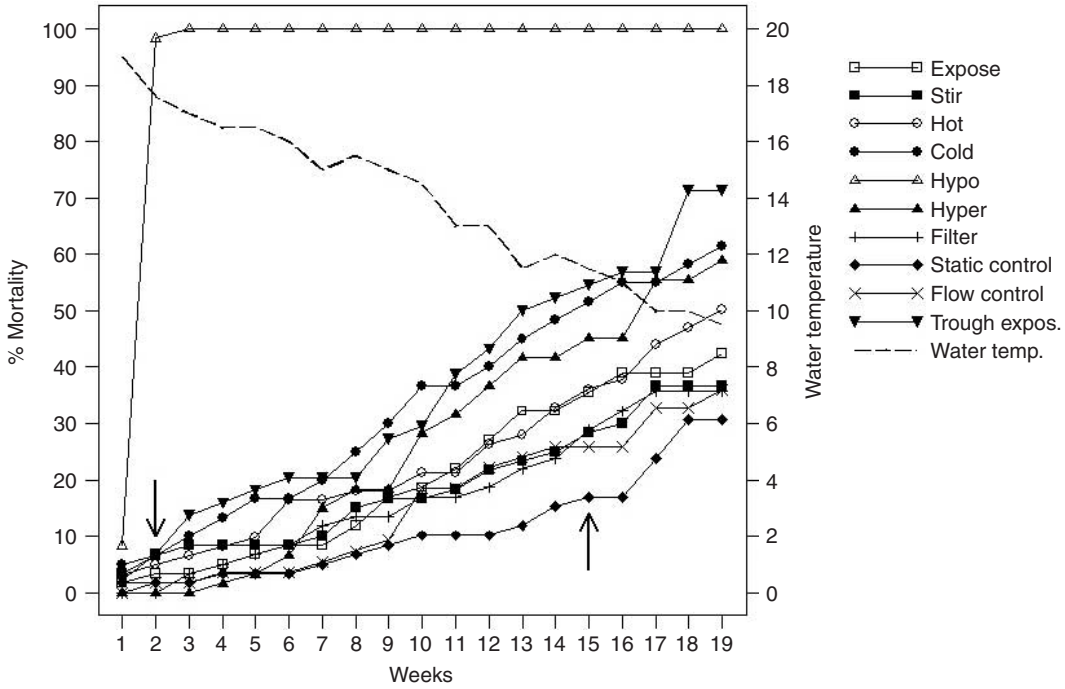

Figure 1 Kaplan–Meier mortality curves for oysters in relation to treatments. Treatments were conducted until the end of the second week (arrow) and oysters were monitored for 4 months post-treatment. Half of the surviving oysters were sampled for *Bonamia* in week 15 (arrow).

epithelium, and necrosis in most organs, including kidney.

The treatments which resulted in the top three overall prevalences of *Bonamia* in both dead and surviving oysters were hot water (96.7%), trough exposure (93.2%) and stir treatments (91.6%) (Table 1). Statistical analysis did not indicate a statistically significant difference between any individual treatment and the pooled controls in the prevalence of *Bonamia* (Table 2). However, there was a significant ($P = 0.009$) overall difference in *Bonamia* prevalence between the treatments. The prevalence of *Bonamia* in dead oysters was highest in the hot water treatment (96.3%), followed by the air exposure (95.8%) and static control treatments (92.9%). The highest prevalence of *Bonamia* in surviving oysters was again found in the hot water treatment (97.1%), followed by the trough exposure (94.1%) and stir treatments (92.5%) (Table 1). The lowest prevalence of *Bonamia* was in the hyposalinity and filter treatments.

The highest mean intensity of *Bonamia* infections in both dead and surviving oysters was found in the trough exposure (3.7), air exposure and hypersalinity (3.6) and stir treatment (3.5). The highest mean intensities of infection in dead oysters were found in stir treatment (5), followed by the hot water (4.7) and hypersaline treatments (4.6). The highest mean intensity of infections recorded in surviving oysters were from the air exposure (2.9), stir (2.8) and trough exposure (2.7)

Table 2 Results of statistical analyses comparing the prevalence of *Bonamia* in oysters from different treatments with that of control oysters. There was no significant difference between any individual treatment and the pooled controls, however, there was an overall significant difference ($P < 0.009$) in prevalence between treatments

Treatment	Mortality-adjusted prevalence after 4 months	*P*
Controls	0.93	–
Expose	0.86	0.22
Stir	0.98	0.19
Hot	1.00	0.86
Cold	0.93	0.85
Hypo	1.00	0.89
Hyper	0.95	0.70
Filter	0.86	0.27
Trough control	0.98	0.28

treatments (Table 1). Statistical analysis showed that the stir, hot, cold, hypersalinity and trough exposure treatments had significantly higher mean *Bonamia* intensity than the pooled controls, while the hyposalinity treatment had significantly lower mean *Bonamia* intensity (Table 3, Fig. 2). The oysters in the filter treatment also had lower intensity *Bonamia* infections than controls, but this difference was not significant (Table 3, Fig. 2).

The intensity of *Bonamia* infections of dead oysters was significantly higher (Mann–Whitney rank sum test, $P < 0.0001$) than those of surviving oysters (Fig. 2), suggesting that an increase in intensity of *Bonamia* was associated with mortality of the oysters that died during the experiments.

Table 3 Results of statistical analyses comparing the mean intensity of *Bonamia* in treated oysters with that of pooled controls, allowing for the effects of sex

Treatment	Relative difference from pooled controls in mean *Bonamia* intensity	*P*
Controls	0	–
Expose	0.46	0.08
Stir	1.04	<0.001
Hot	1.04	<0.001
Cold	0.58	0.03
Hypo	– 2.23	<0.001
Hyper	0.84	0.002
Filter	– 0.33	0.21
Trough exposure	1.24	<0.001

Female and spent oysters appeared more likely to die than did male and hermaphrodite oysters (Table 1). The regression model found that the prevalence of *Bonamia* in oysters pooled from all treatments did not vary significantly between sexes (Table 4). However, significant differences in the mean intensity of *Bonamia* infections were apparent between sexes, after allowing for the treatment effects (Table 5). The mean intensity of *Bonamia* infection was significantly higher among female ($P < 0.001$) and spent oysters ($P = 0.002$) than in male and hermaphrodite oysters.

A concurrent infection with an undescribed apicomplexan parasite occurred in all oysters examined. The intensity of the apicomplexan did not vary significantly between dead (mean intensity 1.9) and surviving oysters (1.9) (Table 1) (Mann–Whitney rank sum test, $P = 0.956$), indicating that it was not directly associated with mortalities.

Table 4 Prevalence of *Bonamia* after 4 months by oyster sex (all treatments pooled)

Sex	Relative mortality-adjusted *Bonamia* prevalence after 4 months
Male	0.88
Female	0.97
Hermaphrodite	0.89
No sex/spent	0.94

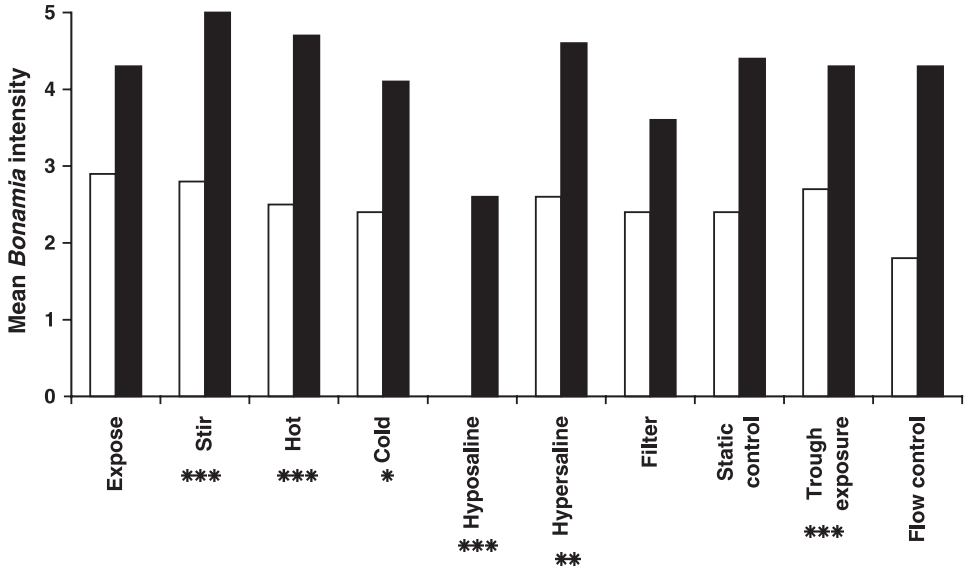

Figure 2 Comparison of the mean intensity of *Bonamia* in dead oysters (black columns) and surviving oysters (white columns). Treatments significantly different from controls are marked with either $^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$.

Table 5 Relative intensity of *Bonamia* by oyster sex, allowing for treatment effects

Sex	Relative difference from males in mean *Bonamia* intensity	*P*
Male	0	–
Female	1.14	< 0.001
Hermaphrodite	–0.14	0.59
No sex/spent	0.69	0.002

Discussion

It is recognized that there is a genetic basis to disease resistance (Gaffney & Bushek 1996) or tolerance (Baud *et al.* 1997; Naciri-Graven *et al.* 1998) in oysters, and that whether disease develops may depend on the genotypes of both host and parasite (Bushek & Allen 1996). It is also apparent that oyster species differ in their tolerance to environmental factors (Child & Laing 1998; Friedman, Cherr, Clegg, Hamdoun, Jacobsen, Jackson & Uhlinger 1998, 1999), and that even within a species, the genotypes that exist within a particular environment are determined by that environment (Bushek & Allen 1996). This study utilized wild infected oysters from one site to minimize the influence that genetics might have on the results obtained.

Over the duration of these experiments there was a large increase in the prevalence of *Bonamia* in most treatments and also in control oysters. After 4 months, the prevalence of *Bonamia* in control oysters (70–80%) and the trough exposure treatment (93.2%) was much higher than the 6% prevalence at the start of the experiments. This suggests that captive holding of infected oysters at high density was a major factor predisposing oysters to infection with *Bonamia*. This is probably because of direct oyster to oyster transmission during cohabitation (Hine 1996). The fact that dead oysters had significantly higher intensity *Bonamia* infections than surviving oysters strongly suggests that most of the mortalities observed were due to oysters dying from bonamiosis.

The salinities chosen for this study (15–40‰) were less hyposaline, but more hypersaline, than the salinity range of *O. chilensis* (8–33‰) reported by Buroker, Chanley, Cranfield & Dinamani (1983). However, mortalities were highest among oysters kept at the hyposaline 15‰, with 100% mortality after 3 weeks. Although parasites such as the protozoan *Perkinsus marinus* may reduce the tolerance of oysters to salinity changes (Paynter, Pierce & Burreson 1995), the prevalence of *B. exitiosus* at the beginning of the experiment was low (6%), and therefore parasite-induced salinity intolerance does not explain the mortalities. Similarly, below 18‰, the filtration rate of *O. chilensis* decreases (Toro 1996) and therefore oysters grown in low salinity may experience reduced nutrition (Winstead 1995). Starvation could be a factor, but this is unlikely as oysters in the filtered water group had the lowest mortality and the lowest *B. exitiosus* prevalence among surviving oysters. It appears more likely that high mortality at 15‰ was due to the rapidity with which oysters had to adapt to sudden salinity changes when, first, full strength sea water and then distilled water was added to the tank each day. This is supported by the observed histopathology. Oysters in the hypersaline group had the third highest mean intensity of infection in dead oysters (4.6), possibly because increase in salinity to above the normal range of *O. chilensis* causes stress resulting in suppression of immune defences.

The temperatures chosen for this study (7–26 °C) were about the range that *O. chilensis* may normally tolerate (6–23 °C) (Buroker *et al.* 1983), although slightly above the maximum temperature. Foveaux Strait oysters normally live at 8–15 °C (Cranfield 1968). Oysters kept at elevated temperatures had the highest *B. exitiosus* prevalence among dead and surviving oysters and the second highest mean intensity of infection. In Pacific oysters, *Crassostrea gigas*, mortalities are higher at neap tides when air and water temperatures are higher than at other times (Cheney, MacDonald & Elston 2000). *Crassostrea gigas* adapted to chronic heat stress may lose their ability to mount a rapid stress response to high temperatures (Hamdoun, Cheney, Elston, MacDonald & Cherr 2000) and the temperature needed to mount such a response may be near the upper thermal limit of the oyster (Friedman *et al.* 1998). Induction of temperature tolerance is accompanied by production of heat shock proteins (HSPs) (Friedman *et al.* 1998), which may in turn reduce chemotaxis and phagocytosis by oyster haemocytes (Friedman *et al.* 1999). Similar mechanisms may favour *Bonamia* in oysters held at elevated temperatures, but there are basic differences in the way flat oysters and Pacific oysters adapt to cold water (Child & Laing 1998) and it would be premature to conclude that mechanisms known for *C. gigas* can be extrapolated to *O. chilensis.*

The oysters used in this study were the survivors from a larger sample, 15–20% of which died during

air-transport ~800 km from the oyster beds to the laboratory. Examination of those that died in transit or in the 4 days following transport showed them all to be very heavily infected (grades 4 or 5, see Hine 1991b). In such cases, where infection levels are high, such mortalities are common and are usually attributed to stress. While such occurrences are common, there is little published evidence that dredging, handling or transporting oysters, predisposes them to infection. However, in a study in which replicate areas of *B. ostreae*-infected *O. edulis* in the Gravelingen (the Netherlands) were dredged or left undredged, from June to November 1989, at the end of the study, the oysters in the undredged beds had a *B. ostreae* prevalence of 26–38%, with 33% mortalities, compared with 42–44% prevalence and 40% mortalities in the dredged beds (van Banning 1991). In this study, the third highest overall prevalence of *Bonamia*, and the highest *Bonamia* intensity among dead oysters, were in the stir treatment group.

The other stressor on the oysters was *B. exitiosus.* The only other prevalent parasite that may have stressed the oysters, an apicomplexan (Hine 2002), only occurred at very low levels and is unlikely to have affected the results. *Bonamia exitiosus* may affect the host directly, causing tissue damage and disease. It may also impose a metabolic burden on the host. Oysters, *C. virginica*, infected with another haplosporidian, *Haplosporidium nelsoni*, require 70% more oxygen under conditions of rapid temperature increase compared with uninfected oysters (Littlewood & Ford 1990). Like other stressors, *Bonamia* may induce HSP production by the oyster, as in *P. marinus* infections (Brown, Bradley & Paynter 1993), or it may reduce induced thermotolerance, as in oysters with *Nocardia* infections (Friedman *et al.* 1999).

During summer, haemocytes enter the gonad to absorb unspent ova in $<25\%$ of female oysters (Jeffs & Hickman 2000). Intrahaemocytic *Bonamia* also enter the gonad, where they proliferate rapidly (Hine 1991a,b), using lipid from absorbed host eggs for energy (Hine & Wesney 1994a,b). Female oysters lose their energy supply to *B. exitiosus,* or from spawning, exhausting their energy reserves, at a time when energy is needed to produce haemocytes to contain and eradicate *Bonamia* infection (Hine 1991a,b). The data from the present study supports these observations, as female and spent oysters had significantly higher intensity *Bonamia* infections than male and hermaphrodite oysters. Female and spent oysters were also more likely to die from bonamiosis than were male or hermaphrodite oysters. Concurrent apicomplexan infections during the epizootic of 1986–93 may have exacerbated the problem, as the apicomplexans utilize host glycogen, denying the oyster its other energy reserve (Hine 2002).

The prevalence of *Bonamia* in treated oysters was not significantly different from that of control oysters, however, many of the treatments caused significantly higher intensity *Bonamia* infections than were observed in the control oysters. In particular, the increased intensity of *Bonamia* in oysters in the trough exposure, stir and hot water treatments were highly significant ($P < 0.001$). The trough exposure result may be because, of the ease of direct transmission when infected oysters are in close proximity, or that *B. exitiosus* proliferated within the oysters because of increased stress associated with crowding. The stir result suggests that oysters have higher disease levels after physical manipulation, and therefore, dredging, handling and transport probably also increase disease levels. Research suggests that to consider each stressor in isolation is too simplistic in relation to natural beds, as there are complex interactions between environmental factors and the host, and between the host and parasite (Lenihan, Micheli, Shelton & Peterson 1999). Despite that, the results obtained here suggest that extreme environmental factors, and physical manipulation, increase disease levels, as does crowding among *B. exitiosus*-infected oysters.

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