Received: 29 August 2019 | Revised: 21 October 2019 | Accepted: 22 October 2019

DOI: 10.1111/jfd.13116

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ORIGINAL ARTICLE

Journal of Fish Diseases | WILEY

Rapid transmission of *Bonamia exitiosa* by cohabitation causes mortality in *Ostrea angasi*

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Funding information
Flinders University; South Australian Research and Development Institute; Fisheries Research and Development Corporation, Grant/Award Number: 2015–001 Bonamiasis in farmed Native Oysters (Os)

Abstract

The haplosporidian *Bonamia* was first detected in Australian shellfish in 1991. Australian isolates in *Ostrea angasi* Sowerby, 1871 were identified as *Bonamia exitiosa* Hine, Cochennac and Berthe, 2001, which threatens development of an *O. angasi* aquaculture industry. European field data suggest that *Bonamia ostreae* Pichot, Comps, Tigé, Grizel and Rabouin, 1980 infections in *Ostrea edulis* Linnaeus, 1758 build slowly, but infection dynamics of *B. exitiosa* in *O. angasi* are unknown. We investigated *B. exitiosa* infection in *O. angasi* by cohabiting uninfected juvenile *O. angasi* with adults infected with *B. exitiosa*. Oysters were sampled at 10, 21 and 40 days after cohabitation, and *B. exitiosa* prevalence and intensity were assessed. *Bonamia exitiosa* rapidly infected and caused disease in *O. angasi*. Mortalities began at 12 days, with ~50% mortality by day 21 and >85% mortality by day 40. Mortalities displayed pathology consistent with clinical *B. exitiosa* infection. Time to first infection is likely influenced by a combination of parasite infectivity, host exposure and host immune capacity. Host death is not required for transmission, but probably facilitates release of parasites from decaying tissue. Understanding *B. exitiosa* transmission informs design and interpretation of field studies and aids development of management strategies for oyster aquaculture.

KEYWORDS

Bonamia exitiosa, cohabitation, infection dynamics, *Ostrea angasi*

1 | INTRODUCTION

Native Oysters (*Ostrea angasi* Sowerby, 1871) have been an important resource in Australia since before European settlement, with *O. angasi* being a common food source for coastal Aboriginal people (O'Sullivan, 1980). Commercial fishing of wild *O. angasi* stocks in South Australia (SA) began shortly after European colonization, but the SA fishery was closed in 1945 (Olsen, 1994) due to resource depletion caused by overfishing (Alleway & Connell, 2015). An oyster industry has established in SA based on farming the introduced Pacific oyster (*Crassostrea gigas* Thunberg, 1793) (Olsen, 1994). The threat posed to *C. gigas* aquaculture by ostreid herpes virus-1 (OsHV-1) microvariant, however, has increased interest in cultivating *O. angasi*, which are not susceptible to OsHV-1 disease (Kirkland, Hick, & Gu, 2015). Restoration of bivalve reefs has further prompted interest in *O. angasi* cultivation (Gillies, Crawford, & Hancock, 2017). Disease caused by *Bonamia exitiosa* Hine, Diggles, Parsons, Pringle, & Bull, 2002, however, remains a significant hurdle for development of *O. angasi* aquaculture (Nell, 2001; O'Connor & Dove, 2009).

Bonamia spp. are haplosporidian parasites of oysters (Morga et al., 2017; Sierra et al., 2016). *Bonamia* spp. infect the phagocytic haemocytes of oysters, in which *Bonamia* spp. cells spread to host gills, digestive gland and mantle (Sweet & Bateman, 2015). *Bonamia* spp. can infect hosts directly (Arzul & Carnegie, 2015; Culloty et al.,

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1999; Engelsma, Culloty, Lynch, Arzul, & Carnegie, 2014), but the mechanisms of infection and parasite release are poorly described. Host death may facilitate release of infective cells (Arzul & Carnegie, 2015; Hine, 1996; Hine & Jones, 1994), and consumption of cells when filter feeding is a likely mode of infection (Flannery, Lynch, & Culloty, 2016; Hine & Jones, 1994). Direct transmission means that farming with high oyster densities creates an environment that is favourable for transmission and parasitaemia (Owens, 2012).

Parasite–host interactions of *Bonamia* spp. have been investigated worldwide including susceptibility and genetic resistance to *B. ostreae* of farmed *O. edulis* populations (Martin, Gérard, Cochennec, & Langlade, 1993; Montes, Ferro-Soto, Conchas, & Guerra, 2003), age-related susceptibility of farmed (Arzul et al., 2011; Culloty & Mulcahy, 1996) and wild *O. edulis* to *B. ostreae* infection (Lallias et al., 2008), *B. ostreae* bivalve host range (Culloty et al., 1999), environmental influences of *B. exitiosa* infection in *Ostrea chilensis* Küster, 1844 wild populations (Hine et al., 2002) and *B. exitiosa* dynamics in farmed *Crassostrea ariakensis* Fujita, 1913 populations (Audemard, Carnegie, Bishop, Peterson, & Burreson, 2008; Audemard, Carnegie, Stokes, et al., 2008).

Bonamia spp. occur in both hemispheres (Carnegie & Engelsma, 2014), and a *Bonamia* sp. infection was first reported in Australia in *O. angasi* in 1991 (Hine & Jones, 1994). Australian *Bonamia* sp. infection is usually associated with poor oyster condition, but infection can also occur in oysters which appear healthy. Focal lesions from *Bonamia* sp. are most common within *O. angasi* digestive gland and gills and are less common in *O. angasi* mantle and gonad. Systemic clinical infection in *O. angasi* is rare, except for populations that are showing mortalities (Corbeil, Handlinger, & Crane, 2009). Within Australia, clinical disease and mortality due to *Bonamia* sp. have been recorded in *O. angasi* in Victoria (VIC) and Western Australia (WA), and *Bonamia* sp. infection has been confirmed in *O. angasi* in Tasmania (TAS) and New South Wales (NSW) (Corbeil et al.., 2009). A *Bonamia* sp. was identified in *O. angasi* in South Australia (SA) by Buss, Wiltshire, Prowse, Harris, and Deveney (2019). The genome of *Bonamia* isolates from SA, VIC and NSW supports that southern Australian *Bonamia* isolates from *O. angasi* are *B. exitiosa* (see Bradley, 2019).

Bonamia exitiosa infection dynamics in *O. angasi* are unknown. We aimed to begin to understand *B. exitiosa* infection in *O. angasi* by determining time to first infection, and prevalence, intensity and mortality over time in a laboratory cohabitation trial.

2 | METHODS

2.1 | Experimental animals

Juvenile *O. angasi* were sourced from the South Australian Research and Development Institute (SARDI) SA Aquatic Sciences Centre (SAASC) Mollusc Hatchery (West Beach, Adelaide, SA). Juvenile oysters were tested using real-time PCR (Corbeil et al., 2006) (n = 150) and histology (n = 150), which did not detect *B. exitiosa* (mean Bayesian estimated prevalence, 95% credible intervals: 0.017, 0.000–0.05; Buss et al., 2019). Adult *O. angasi* were collected from Coffin Bay, SA, from a site shown to have 0.90 (0.78–0.99) *B. exitiosa* prevalence (Buss et al., 2019, mean Bayesian estimated prevalence, 95% credible intervals). Oysters were maintained separately in floating baskets at the South Australian Aquatic Biosecurity Centre (SAABC), Roseworthy Campus, SA, in 500-L fibreglass tanks with aeration and a canister filter (Aqua One Nautilus 2700UVC) until use in experiments.

2.2 | Experimental system and design

The experimental system comprised eight 52-L plastic tanks containing aerated sea water. Every two to three days, the water was exchanged. Oysters were fed 1.25 L (2.0×10^6 cells/ml) of a mixed culture of *Chaetoceros muelleri* Lemmermann, 1898, *Skeletonema costatum* (Greville) Cleve, 1873 and *Pavlova lutheri* (Droop) Green, 1975 per tank following water exchange. Tank placement, maintenance and operation of the system were designed to prevent cross-contamination. Water quality was within normal parameters in all tanks for the duration of the experiment: water temperature was maintained at 16.83 ± 2.27°C (mean ± *SD*), salinity was maintained at 38 psu, and dissolved oxygen was 97.88 ± 1.53% or 7.74 ± 0.40 mg/L (mean ± *SD*). Temperature and salinity ranges for recipients were based on autumn or spring oceanographic data for Coffin Bay (see Kämpf & Ellis, 2015).

A total of 2,400 juvenile *O. angasi* (weight: 1.61 ± 0.81 g, shell length: 22.75 ± 4.54 mm, 14 months old) (mean ± *SD*) were randomly assigned to eight tanks (n = 300 per tank). Forty adult (donor) *O. angasi* (weight: 72.03 ± 15.02 g, shell length: 71.97 ± 3.76 mm) (n = 10 donors per tank) were assigned to four of the tanks as a source of *B. exitiosa* infection with juvenile recipients. The remaining four tanks held juvenile controls without donors. On days 10, 21 and 40 post-cohabitation, 44 recipients and 44 controls were sampled for heart smear and histology (Table 1). Mortalities were preferentially selected for collection on sampling days to assess if oysters were dying due to *B. exitiosa*, with live oysters comprising the remaining samples when fewer than 44 mortalities were available on sampling days. Samples with highest heart smear score were chosen for histology. Ten samples were tested for both histology and heart smear for every exposure and time treatment (Table 1). All donor animals remaining in the exposure tanks were collected at the end of the experiment (Table 1). All live donors were sampled for heart smear and 10 selected for histology (Table 1).

Oysters (controls, recipients and donors) were inspected every two to three days, and mortalities that occurred between sampling times were removed, weighed, measured and sampled for diagnostic testing (see Table 1 for sample numbers). All mortalities were sampled for heart smear (Table 1). From these mortalities, 21 oysters (recipients and donors) with highest heart smear intensities were also sampled for histology (Table 1). All oysters that were collected

TABLE 1 Total number of live and dead recipient/control and donor oysters sampled for heart smear and histology on each dedicated sampling day (days 10, 21 and 40) and mortalities that were sampled in the periods between these: days 0–10, 11–21 or 22–40

			Number of oysters sampled for heart smear and/or (histology)[a]					
			Dedicated sampling days			Mortality sampling periods[c]		
Oyster code	Oyster age	Oyster status	Day 10	Day 21	Day 40	Days 0–10	Days 10–21	Days 22–40
Recipient	Juvenile	Live	**44** (10)	**0**	**44** (10)	n/a	n/a	n/a
Recipient	Juvenile	Dead	**0**	**44** (10)	**0**	**0**	**65** (7)	**98** (6)
Control	Juvenile	Live	**44** (10)	**43** (9)	**44** (10)	n/a	n/a	n/a
Control	Juvenile	Dead	0	**1** (1)	**0**	**0**	**0**	**0**
Donor	Adult	Live	**0**	**0**	**30**[b] (10)	n/a	n/a	n/a
Donor	Adult	Dead	**0**	**0**	**0**	**1** (1)	**3** (3)	**7** (4)

[a]The numbers of oysters sampled for heart smear are indicated in bold, and the numbers of oysters sampled for histology and heart smear are within brackets.

[b]All remaining donor oysters were sampled only on day 40.

[c]n/a = not applicable.

were replaced with oysters in labelled mesh pouches separate to the experimental animals to maintain tank biomass.

2.3 | Diagnostic sampling—heart smear and histology

Histology and heart smears were prepared as described by Buss et al. (2019). Histology and heart smears were examined with a compound light microscope (Brightfield Olympus BX53) with *B. exitiosa* cell intensity graded using the scale in Buss et al. (2019).

2.4 | Terminology and statistical analyses

Parasitology terminology is consistent with Bush, Lafferty, Lotz, and Shostak (1997).

Survival of recipient and control oysters was assessed over 40 days using the Kaplan–Meier analysis with log-rank and Breslow tests in IBM SPSS version 23 for Macintosh (IBM SPSS Inc., Chicago, IL).

Intensity of *B. exitiosa* from histology and heart smears was analysed using Quantitative Parasitology version 1.0.14 (Reiczigel, Marozzi, Fábián, & Rózsa, 2019) using 95% bootstrap confidence intervals for mean intensities with 2,000 replicates. Data were assessed as being different when confidence intervals did not overlap. Intensity data used the subset of oysters that were tested by both histology and heart smear.

A Bayesian latent class model (LCM) was used to calculate estimated prevalence with credible intervals in all time periods using JAGS code modified from the prevalence R package (Devleesschauwer et al., 2015) to allow simultaneous estimation of prevalence for multiple treatments and time points. This model used results from oysters tested by both histology and heart smear. Markov chain Monte Carlo (MCMC) simulations were obtained by running the model in JAGS v. 4.3.0 (Plummer, 2017) using three chains for 10,000 iterations, thinned at a rate of 10, following 2,000 iterations for adaptation and 10,000 iterations for burn-in. JAGS was run using the R2jags package (Su & Yajima, 2015) in R (R Core Team, 2017). Convergence was assessed using the Gelman–Rubin convergence statistic and confirmed by visual inspection of trace, density and autocorrelation plots generated using the MCMCvis package (Youngflesh, 2018). Posterior predictions of diagnostic sensitivity (DSe), diagnostic specificity (DSp) and conditional covariance for positive or negative disease status from Buss et al. (2019) were used to inform priors for the latent class model. Beta prior parameters used for DSe and DSp for heart smear and histology are specified in Table 2. These beta priors reflected 95% confidence that each of these parameters falls within the credible interval with the mean specified in Table 2.

Generalized linear models (GLMs) were used to separately assess patterns in histology and heart smear intensity to account for

TABLE 2 The beta priors, associated mean and 95% credible intervals for diagnostic sensitivity (DSe) and diagnostic specificity (DSp) for heart smear and histology, used to calculate estimated prevalence, plus the posterior predictions for DSe and DSp of each test[a]

Test	DSp or DSe	Beta priors	Mean, 95% credible interval	Posterior predications (mean, credible interval)
Heart smear	DSe	(113, 72)	0.61 (0.54–0.68)	0.70 (0.64–0.72)
Heart smear	DSp	(27, 18)	0.60 (0.45–0.73)	0.68 (0.57–0.72)
Histology	DSe	(75, 23)	0.76 (0.68–0.85)	0.82 (0.74–0.84)
Histology	DSp	(40, 3)	0.93 (0.84–0.99)	0.95 (0.90–0.97)

[a]All priors were derived from Buss et al. (2019).

different samples sizes (Table 1). Analyses of heart smear intensity included data from the oysters that were not tested by histology. Due to the control oysters being *B. exitiosa* negative across sampling times, analyses of intensity were performed only for recipient and donor oysters. GLM compared intensities from histology in live recipient oysters between day 10 and day 40; day 21 was excluded as all sampled recipients were mortalities on this day (Table 1). GLM also compared intensities from heart smear between recipient and donor oysters; data from mortalities and live oysters collected in the time period days 22–40 were included to test the effect of status (dead or alive) as well as age (adult donor or juvenile recipient). GLM was also used to compare intensities from heart smear in live and dead recipients in the time periods days 0–10 and days 22–40 to see the effect of status and time; the time period days 11–21 was excluded as all recipients sampled were mortalities in this period.

A negative binomial distribution was used for all intensity analyses, due to overdispersion of data relative to a Poisson distribution. Negative binomial GLMs used the MASS package (Venables & Ripley, 2002).

For all analyses, time periods included live oysters and mortalities that were sampled on the dedicated sampling day at the end of that period and mortalities that occurred subsequent to the previous dedicated sampling day.

3 | RESULTS

3.1 | Survival

There was a significant decrease in survival of recipient oysters compared to control oysters at day 40 ($p < .001$, Kaplan–Meier; Figure 1).

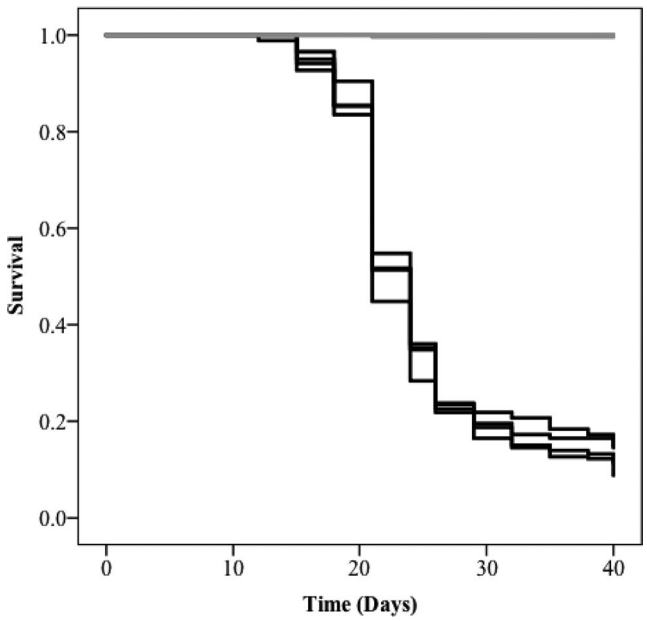

FIGURE 1 Kaplan–Meier survival curve for *Ostrea angasi* juveniles in recipient *Bonamia exitiosa* tanks (black lines) and control tanks (grey lines) for 40 days. $N = 300$ for each line. Survival for all control tanks > recipient tanks, $p < .05$

One control oyster died over the duration of the experiment. At day 40, survival of recipient oysters was 12.43% while survival of control oysters was 99.9% (Figure 1). The first recipient oyster mortality occurred on day 12, and by day 21, survival decreased to 44.8%–54.8% (Figure 1). Because mortalities were sampled preferentially, during the mortality event on day 21, all recipient oysters sampled on day 21 were mortalities (see Table 1).

3.2 | Prevalence and diagnostic performance

Estimated *B. exitiosa* prevalence increased over time, with higher estimated prevalence for recipient oysters sampled in the time period days 11–21 or days 22–40, than recipient oysters sampled in the time period days 0–10 or control oysters sampled in any time period (Table 3). Donors had higher estimated prevalence than recipients sampled in the time period days 0–10 or controls sampled in any time period (Table 3).

The posterior predicted mean DSe and DSp were higher than the means from Buss et al. (2019) used as priors in the Bayesian latent class model (Table 2), but their 95% credible intervals overlapped, indicating that DSe and DSp for histology and heart smear were similar between this study and Buss et al. (2019).

3.3 | Intensity

Recipient oysters sampled in the period days 0–10 had lower *B. exitiosa* intensity from both heart smears and histology than recipient oysters sampled in the period days 11–21 or days 22–40 or donor oysters (Table 3). Two control oysters contained cells in heart smears that were identified as likely to be *B. exitiosa*, but these animals were both negative by histology and, using the AND-rule to maximize DSp for prevalence, were classified as negative.

GLM showed live recipient oysters had significantly higher *B. exitiosa* histology intensities on day 40 than on day 10 (LRT: $\chi^2(1) = 22.38$, $p < .001$) (Figure 2). Donor oysters (dead and alive) sampled in the period days 22–40 had significantly higher *B. exitiosa* intensities from heart smears than recipient oysters (dead and alive) from the same time period (LRT: $\chi^2(1) = 166.65$, $p < .001$) (Figure 2). Recipient oysters (dead and alive) sampled in the period days 22–40 had significantly higher intensities from heart smear than recipients sampled in the period days 0–10 (LRT: $\chi^2(2) = 7.15$, $p < .001$) (Figure 2). There was no significant difference in heart smear intensity between dead and live recipient oysters (LRT: $\chi^2(1) = 0.511$, $p = .475$). Mean *B. exitiosa* intensities from heart smear and histology, for donors and recipients per time period, are summarized in Figure 2.

3.4 | Pathology

In donor and recipient oysters, *B. exitiosa* cells were observed in the gill, mantle and gonad and particularly in the connective tissue of

TABLE 3 Size data, apparent prevalence, Bayesian estimated prevalence with credible intervals and mean intensity with confidence intervals (calculated through Quantitative Parasitology) of *Bonamia exitiosa* in *Ostrea angasi* from heart smears and histology sampled in the time periods: days 0–10, 11–21 or 22–40. Confidence intervals could not be calculated when values were constant[b]

		Size data					Bayesian	Quantitative Parasitology (bootstrap, 95%)	
Treatment sampling periods	Treatment exposure[b]	Weight (g) (mean ± *SD*)	Shell length (mm) (mean ± *SD*)	Meat:shell ratio (%) (mean ± *SD*)	*n*	Apparent prevalence[c]	Estimated prevalence (95% credible intervals)[a,c,d]	Mean heart smear intensity cell count (confidence intervals)[a]	Mean histology intensity cell count (confidence intervals)[a]
Days 0–10	Control	1.54 ± 0.63	22.98 ± 4.78	22.70 ± 5.21	10	0	0.11 (0.00–0.15) [c]	0	0
Days 11–21	Control	1.74 ± 0.94	23.69 ± 5.16	26.53 ± 7.77	10	0	0.11 (0.00–0.15) [c]	0	0
Days 22–40	Control	1.89 ± 0.55	25.57 ± 2.15	29.49 ± 5.17	10	0	0.11 (0.00–0.15) [c]	1.50 (1.00–1.55)[c]	0
Days 0–10	Recipient	1.32 ± 0.88	20.61 ± 4.75	25.06 ± 8.30	10	0.40	0.51 (0.18–0.64) [b]	5.30 (4.00–6.90) [b]	5.50 (2.75–8.00) [b]
Days 11–21	Recipient	1.12 ± 0.41	21.71 ± 3.38	13.75 ± 5.13	17	0.94	0.93 (0.78–0.98) [a]	15.40 (11.40–20.30) [a]	19.20 (15.00–23.40) [a]
Days 22–40	Recipient	1.70 ± 0.95	22.75 ± 5.76	23.43 ± 11.86	16	1.00	0.94 (0.79–0.98) [a]	16.20 (12.70–20.70) [a]	19.70 (15.00–24.30) [a]
Days 0–40	Adult	72.20 ± 16.52	74.65 ± 4.75	60.50 ± 12.80	18	1.00	0.95 (0.82–0.98) [a]	24.20 (18.40–27.70)[a]	14.10 (10.20–18.30)[a]

[a]Different superscripts denote differences at a 5% level, with "a" representing the highest value.

[b]Control and recipient treatments included alive and dead juveniles sampled per time period and only included oysters that were assessed for two tests (both histology and heart smear). The adult treatment included live adults sampled on day 40 and any adult mortalities that occurred throughout the trial.

[c]Prevalence values were calculated using the AND-rule case definition (sample positive, if both heart smear and histology were positive).

[d]Priors for the heart smear and histology tests were derived from Buss et al. (2019). *SD*: standard deviation; *n*: sample number per treatment/time period.

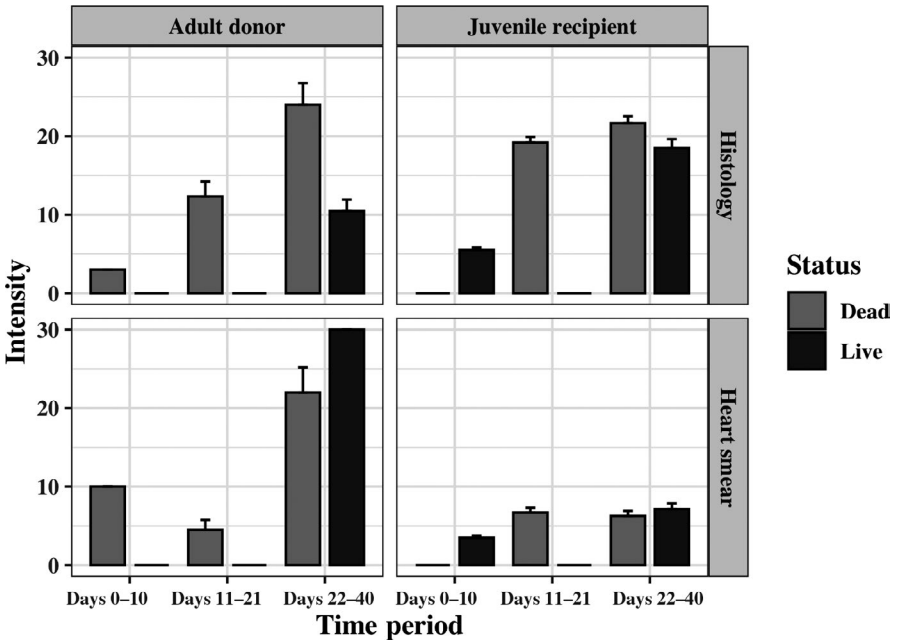

FIGURE 2 Mean *Bonamia exitiosa* intensity from heart smear and histology for dead and alive donor and recipient *Ostrea angasi* sampled in the time periods: days 0–10, days 11–21 and days 22–40. Mean ± SE. Recipients: n = 295 for heart smear and n = 43 for histology; donors: n = 40 for heart smear and n = 18 for histology

the digestive gland (Figure 3). Intracellular and extracellular infection was observed, and concentrated aggregations of *B. exitiosa* cells were common (Figures 3 and 4). For recipient oysters, after 11 days of exposure grade 3 infections (moderate) were most common in heart smear and histology, but grade 4 (heavy) and grade 5 (systemic) infections were also observed (Table 4). Donors varied in heart smear and histology infection from grade 2 (light) to grade 5 (systemic) infection (Table 4).

4 | DISCUSSION

Ostrea angasi became infected rapidly after exposure to *B. exitiosa*, with estimated prevalence reaching > 0.5 by day 10 and > 0.9 by day 40 (Table 3). Transmission of *B. exitiosa* in *C. ariakensis* also occurred rapidly in Bogue Sound, USA, but apparent prevalence after 14 days of field exposure was 0.03, and at 21 days was 0.3 (Audemard, Carnegie, Hill, Peterson, & Burreson, 2014). Our infection model places high infection pressure on recipient oysters by confining them with several large donors in a small static system, whereas Audemard et al. (2014) exposed their recipients to water from an infected estuary with much higher volume and flow which is likely to provide a lower infection pressure. In the Northern Hemisphere, time to first *B. ostreae* infection in *O. edulis* is >2 months in field exposures in enzootic areas, and in laboratory trials, the first *B. ostreae* mortalities occurred after >4 months of cohabitation (Lallias et al., 2008) (Table 5). Our data suggest that time to first infection in recipients reflects complicated influences of extrinsic factors and innate aspects of the parasite–host system.

Time to first infection and changes in prevalence and intensity of recipients are influenced by the immunocompetence of the individual hosts and the average immunological capacity of the host population. Gervais, Chollet, Renault, and Arzul (2016) and Comesaña et al. (2012) found that *O. edulis* haemocytes are more susceptible to *B. ostreae* infection than *C. gigas* haemocytes. The short time from recipient *O. angasi* exposure to first *B. exitiosa* infection may be caused by *O. angasi* having a lesser capacity to mount an effective immune response against *B. exitiosa* than other oyster species. Oysters lack immune memory homologous to vertebrates (Wang, Song, & Song, 2018) but display immune priming after exposure to a pathogen (Contreras-Garduño et al., 2016; Little & Kraaijeveld, 2004), and the offspring of primed individuals can display increased immune capacity (Green & Speck, 2018). *Ostrea edulis* from *B. ostreae*-endemic areas have lower susceptibility to *B. ostreae* than *O. edulis* from *B. ostreae*-free areas (Culloty, Cronin, & Mulcahy, 2004), but it is unclear if this was due to immune priming or mass selection in wild populations for immune competence when exposed to *B. ostreae*. The parents of our recipient animals were from farms in Coffin Bay, where *B. exitiosa* occurs at high prevalence (Buss et al., 2019), but our *O. angasi* recipients tested negative for *B. exitiosa* and appear to have been naïve to infection. This suggests that the parents of our recipient stock have not been selected for a heritable capacity to resist *B. exitiosa* infection and/or that offspring of individuals primed by exposure to *B. exitiosa* may not display markedly increased capacity to mount an immune response to *B. exitiosa* challenge.

In our experiment, *B. exitiosa* prevalence increased with ongoing exposure, which was also observed in *O. edulis* exposed to *B. ostreae* infection (Culloty et al., 1999; Montes, 1991) and *C. ariakensis* exposed to *B. exitiosa* (see Audemard et al., 2014). SA oyster culture systems are intertidal, which may reduce infection by limiting immersion compared to subtidal culture systems. Intertidal farms, however, are in shallow water with little volume and bidirectional tidal currents which may increase oyster exposure to *B. exitiosa*, rather than deep water with unidirectional currents which would

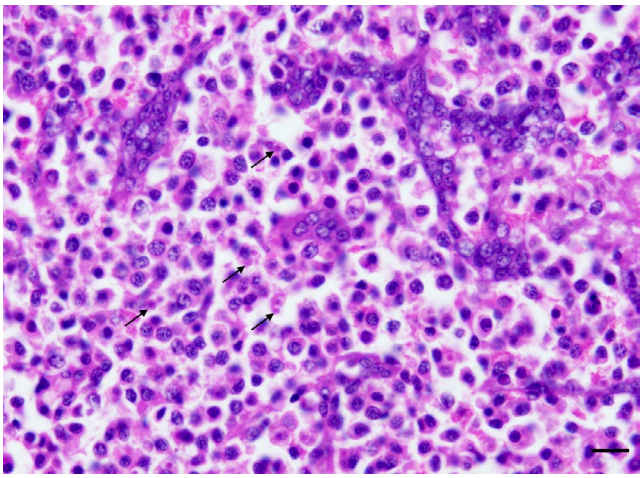

FIGURE 3 Histological section of a recipient *Ostrea angasi* with a heavy (grade 4) *Bonamia exitiosa* infection (see Buss et al., 2019) sampled on day 21. Arrows point to *B. exitiosa* cells infecting haemocytes in the connective tissue surrounding the digestive gland. Haematoxylin and eosin stain. Scale bar represents 10 µm

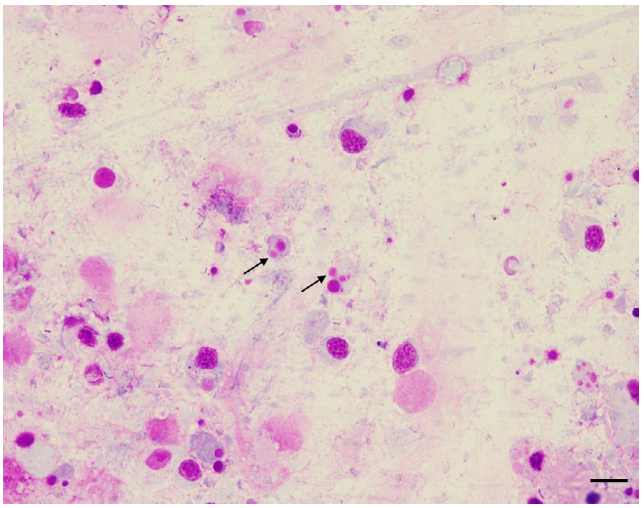

FIGURE 4 Heart smear of a recipient *Ostrea angasi* with moderate (grade 3) *Bonamia exitiosa* infection (see Buss et al., 2019) sampled on day 21. Arrows point to *B. exitiosa* cells within haemocytes. Hemacolor® stain. Scale bar represents 10 µm

TABLE 4 Grading of *Bonamia exitiosa* infection in *Ostrea angasi* by heart smear and histology sampled in the time periods: days 0–10, 11–21 or 22–40

Treatment sampling periods	Treatment exposure[a]	*n*	Heart smear grade of infection[b]						Histology grade of infection[b]					
			0	1	2	3	4	5	0	1	2	3	4	5
Days 0–10	Control	10	10	0	0	0	0	0	10	0	0	0	0	0
Days 11–21	Control	10	10	0	0	0	0	0	10	0	0	0	0	0
Days 22–40	Control	10	8	1	1	0	0	0	10	0	0	0	0	0
Days 0–10	Recipient	10	0	0	10	0	0	0	6	0	4	0	0	0
Days 11–21	Recipient	17	0	0	7	9	1	0	1	0	3	8	4	1
Days 22–40	Recipient	16	0	0	6	9	1	0	0	0	2	12	1	1
Days 0–40	Adult	18	0	0	4	7	7	0	0	0	8	8	0	2

[a]Control and recipient treatments included alive and dead juveniles sampled per time period. The adult treatment included live adults sampled on day 40 and any adult mortalities that occurred throughout the trial. *n*: sample number per treatment/time period.

[b]Grading system is described in Buss et al. (2019): grade 0: not infected; grade 1: very light infection; grade 2: light infection; grade 3: moderate infection; grade 4: heavy infection; and grade 5: systemic infection.

dilute *B. exitiosa* cells and decrease exposure. A detailed examination of *O. angasi* culture systems and their influence on *B. exitiosa* infection is lacking and warrants investigation.

Estimates of prevalence are influenced by diagnostic tests, but diagnoses of *B. exitiosa* in *O. angasi* are well characterized (Buss et al., 2019) and this understanding facilitates a broader range of analytical approaches to data. The overlap in 95% credible intervals of posterior and prior means (Table 2) increases confidence in our estimated prevalences (Table 3). The higher posterior predicted means of each test (histology and heart smear) than the estimated prior means (Table 2) imply the tests performed better in our study than in Buss et al. (2019), probably because oysters in this study had higher parasite intensity than most oysters in Buss et al. (2019).

Bonamia exitiosa intensity increased from day 10, and after day 21, there was evidence of overwhelming parasitaemia. The histological findings in recipient oysters were consistent with the description by Corbeil et al. (2009) of *O. angasi* clinically affected by *B. exitiosa*, supporting that oysters in this study were dying of *B. exitiosa* infection. The *B. exitiosa* intensities we observed were comparable to *B. ostreae* intensities observed on farms showing clinical disease in Europe (Culloty et al., 2004) and were higher than average intensities on farms in SA (Buss et al., 2019). We found no significant difference in *B. exitiosa* intensities between live or dead recipients, but Diggles and Hine (2002) found dead *O. chilensis* had higher *B. exitiosa* intensities than live *O. chilensis*. Diggles and Hine (2002) sampled their animals daily, whereas we sampled every 2–3 days, which may have led to underestimates of the parasite intensity of dead oysters through loss of *B. exitiosa* cells and infected haemocytes as tissue decayed.

The mechanism by which living oysters shed *Bonamia* spp. is undescribed. Host death may facilitate *Bonamia* spp. transmission (Hine, 1996; Hine & Jones, 1994) by releasing *Bonamia* spp. cells from

TABLE 5 The time to first *Bonamia exitiosa* or *B. ostreae* infection and time to first mortality, positive for *Bonamia* spp. infection in *Ostrea edulis* and *Crassostrea ariakensis* cohabitation trials

Parasite	Host	Factor	Time	Experiment location	Notes	Reference
B. ostreae	*O. edulis*	Time to first infection	6 months	Cork Harbour, Ireland	Field trial, cohabitation	Culloty and Mulcahy, (1996)
B. ostreae	*O. edulis*	Time to first infection	2–4 months	Cork Harbour and Galway Bay, Ireland	Field trial, cohabitation	Lynch et al. (2005)
B. ostreae	*O. edulis*	Time to first infection	3–6 months	Arosa, Aldan and Vigo estuaries, Galicia, Spain	Field trial, cohabitation	Montes (1991)
B. ostreae	*O. edulis*	Time to first infection	12–24 months	Cambados and Bueu, Galicia, Spain	Field trial, cohabitation	Montes et al. (2003)
B. ostreae	*O. edulis*	Time to first positive mortality	7 months	Western North America	Laboratory trial, cohabitation	Elston, Farley, and Kent (1986)
B. ostreae	*O. edulis*	Time to first positive mortality	4 months	La Tremblade, France	Laboratory trial, cohabitation	Lallias et al. (2008)
B. exitiosa	*C. ariakensis*	Time to first infection	3–4 weeks	Bogue Sound & Masonboro Sound, North Carolina, USA	Field trial, cohabitation	Carnegie et al. (2008)
B. exitiosa	*C. ariakensis*	Time to first infection	28 days	Virginia Institute of Marine Science (VIMS), Virginia, USA	Laboratory trial, cohabitation	Audemard et al. (2014)
B. exitiosa	*C. ariakensis*	Time to first infection	14 days	Bogue Sound, North Carolina, USA	Field trial, cohabitation	Audemard et al. (2014)

decaying oyster tissue, but we observed transmission before the first mortalities, indicating that *B. exitiosa* can be transmitted from living oysters. Stauber (1950) described phagocytic haemocytes throughout the body of oysters. These are particularly common in the epithelium of the gut (Jones, 2011), but they also cross epithelial borders to the exterior of the body (Cheng, 1996). This process is termed diapedesis (Onstad et al., 2006) and is the most likely mechanism by which haemocytes infected with *Bonamia* spp. are shed from live oysters. The continual loss of haemocytes via diapedesis is normal (Galtsoff, 1964), but increases in the presence of pathogens (Burge et al., 2007; Friedman et al., 2005; Friedman & Perkins, 1994; Heasman et al., 2004). The rate at which haemocytes and therefore *Bonamia* spp. cells are shed through diapedesis, and the intrinsic and extrinsic influences on this process, however, remain unknown.

Aspects of the *Bonamia* spp. life cycle are unknown; the lack of an obligate intermediate host is demonstrated, but it is unclear if facultative intermediate hosts exist or are epidemiologically significant. Lynch, Armitage, Coughlan, Mulcahy, and Culloty (2007) detected *B. ostreae* by PCR in pooled zooplankton. This finding needs clarification, however, because PCR cannot differentiate *B. ostreae* cells in the water column or adhering to plankton from those infecting planktonic hosts. If intermediate hosts exist in the *Bonamia* spp. life cycle, they would have an important role in transmission and environmental persistence.

Oysters of all ages are susceptible to *Bonamia* spp. infection (Arzul et al., 2011); larger and older (>20 months) *Ostrea* oysters have higher prevalence, greater mortality (Cáceres-Martínez, Robledo, & Figueras, 1995; Culloty & Mulcahy, 1996; Engelsma et al., 2010; Kroeck & Montes, 2005) and higher *Bonamia* spp. intensities (Buss et al., 2019; Culloty & Mulcahy, 1996), but younger (~12 months) *O. edulis* can still have high *B. ostreae* prevalence (Lallias et al., 2008; Lynch, Armitage, Wylde, Mulcahy, & Culloty, 2005). In *C. ariakensis*, *B. exitiosa* (see Hill et al., 2014) infected younger oysters more rapidly (Carnegie et al., 2008) which also had higher prevalence and displayed greater mortality than older oysters (Bishop, Carnegie, Stokes, Peterson, & Burreson, 2006). Our study confirmed that juvenile *O. angasi* are susceptible to *B. exitiosa* infection, and Buss et al. (2019) have found that adult *O. angasi* are also susceptible. *Ostrea edulis* larvae can acquire *B. ostreae* infection (Arzul et al., 2011), but susceptibility of *O. angasi* larvae to *B. exitiosa* is not established. No studies have compared infection dynamics of a *Bonamia* species between different hosts. It is likely that all life history stages of susceptible oysters can be infected with *Bonamia* spp., but each host–parasite combination is likely to have different infection dynamics. Understanding which life history stages of which hosts can be infected is relevant for surveillance design, translocation assessment and protection of *Bonamia* spp.-free areas.

Our experiment caused rapid infection and progression to clinical disease which provides a basis for testing *O. angasi* for susceptibility to *B. exitiosa*. Selective breeding of family lines for *Bonamia* spp. resistance is a long-term strategy for the management of farmed oysters threatened by *Bonamia* spp. In Ireland, ongoing breeding from *O. edulis* survivors of *B. ostreae* infection provided oyster stock that could be grown in *B. ostreae*-endemic

areas with consistently low prevalence and negligible *Bonamia*-associated mortality (Lynch, Flannery, Hugh-Jones, Hugh-Jones, & Culloty, 2014). Application of molecular approaches to family line selection (Cao, Fuentes, Comesaña, Casas, & Villalba, 2009; Martín-Gómez, Villalba, & Abollo, 2012) would facilitate faster selection of resistant stock than random selection of survivors through parasite challenge. Investment in a breeding programme to select *O. angasi* for resistance to *B. exitiosa* in Australia is justified if substantial industry expansion is anticipated or desired. *Bonamia ostreae* was detected in the Southern Hemisphere for the first time in New Zealand in 2015 (Lane, Webb, & Duncan, 2016), and *B. ostreae* poses a substantial threat to susceptible oysters in Australia (Animal Health Committee, 2018). The impact of *B. ostreae* in Australia is assessed as severe, but the susceptibility of *O. angasi* to *B. ostreae* and whether selectively reared *B. exitiosa*-resistant oysters would also be resistant to *B. ostreae* are unknown. If *B. exitiosa*-resistant oysters are also resistant to *B. ostreae*, a breeding programme would provide additional insurance against the threat posed by *B. ostreae*.

Cohabitation provides an informative way to mimic natural infection by *Bonamia* spp. and study transmission. We demonstrated rapid infection, with increasing *B. exitiosa* prevalence, intensity and mortalities in *O. angasi* exposed to an infection source. Given that *B. exitiosa* is widely distributed in southern Australia, this knowledge is important for management and decisions about species diversification for the edible oyster industry in Australia.

ACKNOWLEDGEMENTS

This work was funded by the Fisheries Research and Development Corporation (FRDC) project "2015–001 Bonamiasis in farmed Native Oysters (Ostrea angasi)" on behalf of the Australian government and its Department of Agriculture and Water Resources, with additional support from Flinders University and the South Australian Research and Development Institute (SARDI). We thank all participating oyster farmers, the South Australian Oyster Growers Association (SAOGA) and Prof. Xiaoxu Li (SARDI, Aquatic Sciences) for their cooperation and supply of oyster stock. We are grateful to Dr Brian Jones and Dr Henry Lane (Ministry for Primary Industries, New Zealand Animal Health Laboratory, Wallaceville), who demonstrated specific diagnostic techniques for Bonamia. Thanks are also due to Dr Mark Crane, Dr Nick Moody and Dr David Cummins (CSIRO Australian Animal Health Laboratory, Geelong) who provided training in qPCR diagnostics and Tracey Bradley (Department of Economic Development, Jobs, Transport and Resources, Victoria) who provided Victorian Bonamia identification material. Histological sections were made by Cheryl Day at the Veterinary Diagnostic Laboratory, School of Animal and Veterinary Sciences, The University of Adelaide Roseworthy Campus. Yvette Hannig and Pat Vilimas at the Flinders Microscopy Suite at Flinders University assisted with further microscope training.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Buss JJ, Harris JO, Elliot Tanner J, Helen Wiltshire K, Deveney MR. Rapid transmission of *Bonamia exitiosa* by cohabitation causes mortality in *Ostrea angasi*. *J Fish Dis*. 2019;00:1–11. https://doi.org/10.1111/jfd.13116