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A survey of the Foveaux Strait oyster (*Ostrea chilensis*) population (OYU5) in commercial fishery areas and the status of bonamia (*Bonamia exitiosa*) in February 2015.

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K.P. Michael,
J. Forman,
D. Hulston.

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Ministry for Primary Industries
PO Box 2526
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The February 2015 Foveaux Strait oyster and bonamia survey was the second in a new time-series of bonamia and oyster surveys that incorporated a fully randomised, two-phase sampling design and a standard bonamia survey area to make these surveys comparable from year to year. This area represents the commercial fishery that has persisted through the fluctuations in relative oyster abundance driven by bonamia mortality. In 2014 fourteen of the 26 stock assessment survey strata from 2012 were identified as core strata, representing 75% of the recruit-sized oyster population and 46% of the stock assessment survey area. There was also some limited sampling in background strata so that the bonamia survey data could provide oyster density estimates for the whole stock assessment survey area and therefore allow these data to be incorporated into the planned five-yearly stock assessments. Simulations using the 2014 survey data predicted a CV of 11–12% from 55 sample stations.

This survey was undertaken in collaboration with the Bluff Oyster Management Company who provided a vessel, the survey dredge, and crews for the survey. We sampled 55 first phase and 5 second phase random stations, and 12 target stations in February 2015. Sampling began on the 10th of February 2015 and was completed on the 14th of February. Rougher sea conditions than usual, and a change in the winch used to deploy the sample dredge were not expected to have an effect on sampling efficiency over the survey. The abundance of kaeos (*Pyura pachydermatina*) greatly reduced dredge efficiency for tows in strata C5a and E4 and oyster density estimates for these strata are likely to be grossly underestimated.

Densities of recruit-sized oysters (58 mm in diameter and larger) showed the effects of continuing mortality from bonamia. Strata with relatively high oyster density in 2014 had lower densities in 2015. Oyster densities in some strata (C7, B3, and B6) increased, however, oyster densities there were relatively low. Pre-recruit-sized oysters generally showed a similar pattern of decline. Densities of small oysters were generally similar across the fishery between 2014 and 2015 with some large decreases and significant increases.

The density and population size of recruit-sized oysters in core strata declined between 2014 and 2015. The mean density in core strata declined from 1.40 oysters.m^{-2} in 2012 to 1.09 oysters.m^{-2} in 2014 and further declined to 0.71 oysters.m^{-2} in 2015, declining 50% since 2012 and 35% since 2014. The population size of recruit-sized oysters in core strata declined from 688.1 million oysters in 2012 to 538.0 million oysters in 2014, and further declined to 351.4 million oysters (8% CV) in 2015; declining 49% since 2012 and 35% since 2014.

Pre-recruit (smaller than 58 mm in diameter and larger than 50 mm) mean oyster densities declined from 0.60 oysters.m^{-2} in 2012, to 0.30 oysters.m^{-2} in 2014, and 0.18 oysters.m^{-2} in 2015. The population size declined from 297.4 million oysters in 2012 to 148.4 million oysters in 2014 and 89.2 million oysters in 2015; an overall decline of 70% since 2012 and 40% since 2014. Small oysters (smaller than 50 mm in diameter and larger than 10 mm) declined markedly (65%) from 451.3 million oysters in 2012 to 156.3 million oysters in 2014, but have remained similar between 2014 and 2015 with 158.5 million oysters in the most recent survey.

Pre-survey mortality estimated from recruit-sized new clocks continued to be widespread, as in previous surveys, but reflected changes in the distribution of oysters from bonamia mortality. The population

size of recruit-sized new clocks in core strata was lower in 2015 (13.5 million, 95% CI 2.0–20.7) than in 2014 (39.4 million, 95% CI 24.7–61.4) and in 2012 (22.4 million, 95% CI 12.8–36.6). Pre-survey mortality of recruit-sized oysters in core strata was lower in 2015 (3.7%) than in 2014 (6.8%), but higher than in 2012 (3.2%). There were no strata with recruit-sized new clock densities above 0.1 m^{-2} in 2015, whereas in 2014 there were six (B3, C3, C5, C7a, C8, and C9) and in 2012, two (B3 and C7). This decrease reflects the approximately 50% reduction in the recruit-sized oyster population since 2012, as well as decreased pre-survey mortality.

Quality control of qPCR reagents and procedures was undertaken before the analysis of samples in March 2015. A new synthetic standard was designed incorporating the primer and probe sequences. Improvements in the qPCR method resulted in a reduced number of samples that didn't amplify or amplified too early in the cycles, and therefore a reduction in the qPCR data omitted from the analysis. Only 2.3% of heart and 1.9% of gill tissues failed to amplify in 2015, compared with 5.7% of heart tissues and 6.4% of gill tissues in 2014. There were no false negative results from the qPCR.

The prevalence of infection in 2015 was widespread but variable at small spatial scales, with high levels of fatal infection, especially in areas with relatively high density. Areas with no detectable infection were interspersed amongst areas with high levels of infection. The prevalence in core strata declined from 89.5 million (95%CI 50.8–146.1) in 2014 to 49.8 million (95%CI 29.7–78.2) recruit-sized oysters in 2015, and this decline of 44% is higher than that for the recruit-sized oyster population (35%) suggesting that prevalence of the infection may be waning. Fatal infections were 63% of all infections and were predicted to further reduce the recruit-sized oyster population by 31–34 million (95%CI 18.8–48.7). Pre-survey mortality in core strata of 13.5 million oysters (95%CI 8.7–20.9) and post-survey mortality of 34.4 million oysters combined gives a summer mortality of 47.9 million oysters or 13.6% of the recruit-sized population.

The change in summer mortality from bonamia (18.3% in 2014 and 13.1% in 2015), and the continued low recruitment to the oyster population is expected to result in a downward trend in the oyster population. Further, both the population sizes of pre-recruit and small oyster are low, and will provide little recruitment to the fishery in the short to medium term. A significant increase in recruitment could have a major restorative effect, but there will be a 4–6 year lag before recruitment to the population flows through into recruitment to the fishery.

1. INTRODUCTION

The Foveaux Strait oyster fishery (OYU 5) is a high value, iconic fishery that has been fished for over 140 years. Oysters (*Ostrea chilensis*) are an important customary (taonga), recreational, and commercial species, and are important to the socioeconomics of Bluff and Invercargill. The OYU 5 stock is part of the Group 1 stocks in the Ministry for Primary Industries (MPI) draft National Fisheries Plan for Inshore Shellfish which recognises the relatively high biological vulnerability of Group 1 stocks (including OYU 5) and prescribes a close monitoring approach. Achieving maximum value from Group 1 stocks is considered to be best achieved through accurate and frequent monitoring to support responsive management (see MPI research specifications for OYS2013). Additionally, there is an approved collaborative fisheries plan for the management of the fishery, the Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries 2009). This plan was collaboratively developed by the Foveaux Strait Oyster Fisheries Plan Management Committee (FSOFPMC) which included representatives from the Bluff Oyster Management Company (BOMC), customary and recreational fishers, and the then Ministry of Fisheries, now Ministry for Primary Industries (MPI).

The haplosporidian parasite of flat oysters *Bonamia exitiosa* (bonamia) is thought to be an endemic disease of Foveaux Strait oysters and it is likely that periodic events of disease mortality (epizootics) have been a recurrent feature of the oyster population. Two recent bonamia epizootics in 1985–92 and from 2000 to the present (2015) have shown that bonamia mortality is a recurrent feature of the oyster population, and this mortality is the principal driver of oyster population abundance during epizootics. These events suggest that bonamia epizootics can be expected in the future. Management of the fishery recognises that recruit-sized stock abundance and future benefits from the fishery (harvest levels) are mainly determined by the levels of bonamia mortality, and that the current harvest levels and any effects of fishing on either oyster production or on exacerbating bonamia mortality are not detectable. A summary of bonamia and its effects on the fishery are given in Michael et al. (2015b).

Since 2000, research for the fishery has been directed by strategic research plans (Andrew et al. 2000, Michael & Dunn 2005, Michael 2010). In 2010, a strategic research plan (SRP) for OYU 5 was revised for five years from 2010 to 2015 (Michael 2010). This plan was collaboratively developed by the FSOFPMC. The 2010 SRP provides a broad range of research programmes aimed at maximising production from the oyster fishery and meeting the Foveaux Strait Oyster Fisheries Plan (Ministry of Fisheries 2009) goals and objectives, see Michael (2010) for details. Developing a better understanding of bonamia and monitoring its effect in the fishery are rated as the highest priorities in the Foveaux Strait Oyster Fisheries Plan and SRP.

Since 1999 there have been regular surveys of the oyster population for stock assessment, and in the years between stock assessments, annual surveys of the status of bonamia and changes to the oyster population in commercial fishery areas (see Michael et al. (2015b) for details). Surveys of the oyster population have sampled a consistent survey area: the 1999 survey area (1054 km^2) and stratum B1a, an additional stratum (16 km^2) that was introduced by oyster skippers for the survey in 2007. Since 2007, the size of the Foveaux Strait oyster survey area has remained at 1070 km^2. The original stratum boundaries have also remained similar since 1999, however some of the 1999 strata were subdivided at different times to better define the areas with commercial densities of oysters. In 2012, 26 strata were sampled.

In years between stock assessments, smaller focused surveys of bonamia prevalence and intensity (bonamia surveys) have been used to monitor the status of bonamia infection. These surveys also estimated short-term (summer) mortality from bonamia in designated commercial areas that were likely to be important to fishers in the following oyster season, see Michael et al. (2015b) for details. The combinations of strata surveyed during bonamia surveys differed as the distribution of oyster density changed with changing patterns of disease mortality. The information required from these surveys has also changed since 2000 (see Michael et al. 2013 for details).

The introduction of five-yearly stock assessments in 2012 has placed greater onus on the annual bonamia surveys to monitor changes in the oyster population in commercial fishery areas as well as the status of bonamia. These changes constitute a new time series of surveys with emphasis on both the status of the oyster population and bonamia mortality. The first of this new time series of bonamia surveys was undertaken in February 2014 (Michael et al. 2015b).

This new time-series of surveys incorporated a fully randomised, two-phase sampling design aimed at better estimating oyster densities and population sizes of oysters and new clocks. To make these surveys comparable from year to year, a standard bonamia survey area was established primarily using fishery independent survey data and fishers' logbook data. This area represents the core commercial fishery that has been consistent through the fluctuations in relative oyster abundance driven by bonamia mortality. Core strata made up 14 of the 26 stock assessment survey strata from 2012 that represented 75% of the recruit-sized oyster population and 46% of the stock assessment survey area. Some limited sampling in background strata was also undertaken to allow these data to be incorporated into stock assessments. This survey design and sampling effort predicts a coefficient of variation (CV) for survey estimates of about 11%. The 2014 survey achieved a CV of 11.2% for recruit-sized oysters in core strata, and a CV of 11.7% for the whole population from an additional 5 stations in the background stratum (Michael et al. 2015a).

This report provides a summary of information from the second of the new series of Foveaux Strait oyster surveys in the bonamia survey area undertaken in February 2015. This survey estimated the oyster population size and the status of infection by bonamia, and outlines the implications for the future stock status. This survey was undertaken as part of the research for MPI project OYS2013/01.

2. OBJECTIVES

1. To evaluate the current abundance and biomass of oysters in the OYU 5 fishery and to evaluate current and expected oyster mortality from Bonamia infection for the 2014, 2015 and 2016 fishing years.

2. To evaluate the current status of the prevalence and intensity of Bonamia in the OYU 5 fishery for the 2014, 2015 and 2016 years.

Specific Objective 3 (2014/15)

Using a stratified random sampling design estimate the current recruited abundance and biomass in the area of the commercial Foveaux Strait oyster fishery (for the 2015 fishing years), with a target CV of ≤20%.

Specific Objective 4 (2014/15)

Using a stratified random sampling design estimate the annual mortality from Bonamia in the area of the commercial Foveaux Strait oyster fishery for the 2015 fishing year.

Specific Objective 5 (2014/15)

Using a stratified random sampling design estimate the prevalence and intensity of Bonamia in the area covering the commercial Foveaux Strait oyster fishery for the 2014, 2015 and 2016 fishing year.

Specific Objective 6 (2014/15)

Evaluate which of the biomass projections, as assessed by the stock assessment model, is the most likely reflection of the current status of the OYU 5 fishery, for the 2015 fishing year.

Specific Objective 7 (2014/15)

Review all qPCR procedures prior to undertaking any analysis of tissue samples for the 2015 fishing year.

3. METHODS

Detailed methods for annual oyster surveys are given in Michael et al. (2015b). Variation to these standard methods are given below.

3.1 Methods for objective 3

Survey strata for the February 2015 survey were the same as for the February 2014 survey (Figure 1). The inclusion of a single large background stratum (Figure 1) ensures that the entire 2007 stock assessment survey area is sampled, and data from these annual surveys can be included in stock assessments for OYU 5.

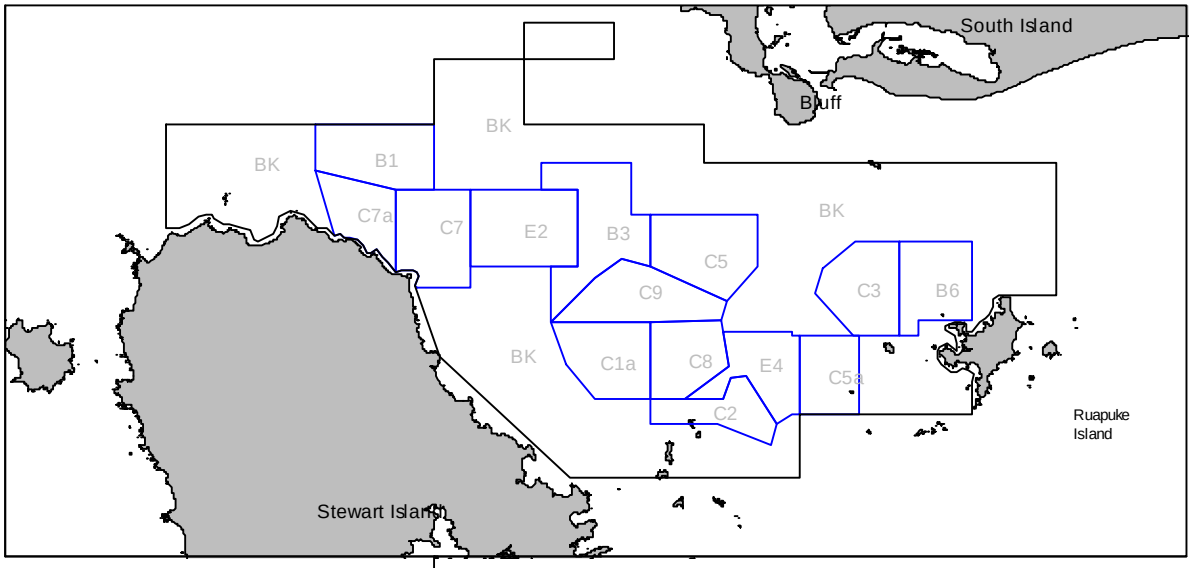

Figure 1: The 2015 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2015 survey strata representing the core commercial fishery area shown as blue lines. Strata are labelled with grey text. The remaining stock assessment survey strata which do not represent the core commercial fishery area were merged into a single, large background stratum (BK).

Simulations were undertaken in 2014 to determine the optimal stratification and the numbers of stations required to give a survey coefficient of variation (CV) for the recruit-sized population estimate in the range of 8–12% (see Michael et al. 2015a, objective 2 of OYS2013/01). Simulations predicted that 55 stations in the core strata would produce a CV of about 11%. ALLOCATE (NIWA survey software running in R), was used to allocate the numbers of stations to strata in 2015 (Table 1). Rand_Stn (NIWA random station generator) was used to generate the location of 50 random first-phase (Figure 2) and sufficient stations in each stratum to sample 5 second-phase stations to core strata, and 5 stations into the background stratum (Figure 2). Stations were generated with an exclusion zone of 0.75 nautical miles to spread stations within strata to ensure good spatial coverage and to prevent the overlap of sample tows. The 12 fixed stations were also sampled in February 2015 (Table 1, Figure 2) to provide a time series of changes in oyster density and bonamia status in localised areas. The Ministry for Primary Industries Shellfish Working Group agreed that they add value to the information obtained from these surveys.

Table 1: The numbers of first-phase stations allocated to each stratum for the February 2015 bonamia survey, the numbers of targeted sample stations in each stratum, total number of stations in each stratum, and the area of each stratum. A single, large background stratum (BK) represents the merged stock assessment survey strata outside the bonamia survey area.

Stratum	First-phase	Target	Area (km^2)
B1	3	1	78.2
B3	3	0	44.7
B6	3	0	30
B6a	0	2	*
BK	5	0	578.3
C1a	3	0	31.3
C2	3	1	21.9
C3	4	1	32.7
C5	3	1	37.7
C5a	3	1	23.5
C7	3	0	36.1
C7a	3	0	23.6
C8	3	1	26.8
C9	9	0	34.5
E2	4	2	42.8
E4	3	2	28
Totals	**55**	**12**	**1070.3**

* The allocation of random stations in the background stratum (BK) included stratum B6a. Two of the target stations are located within B6a.

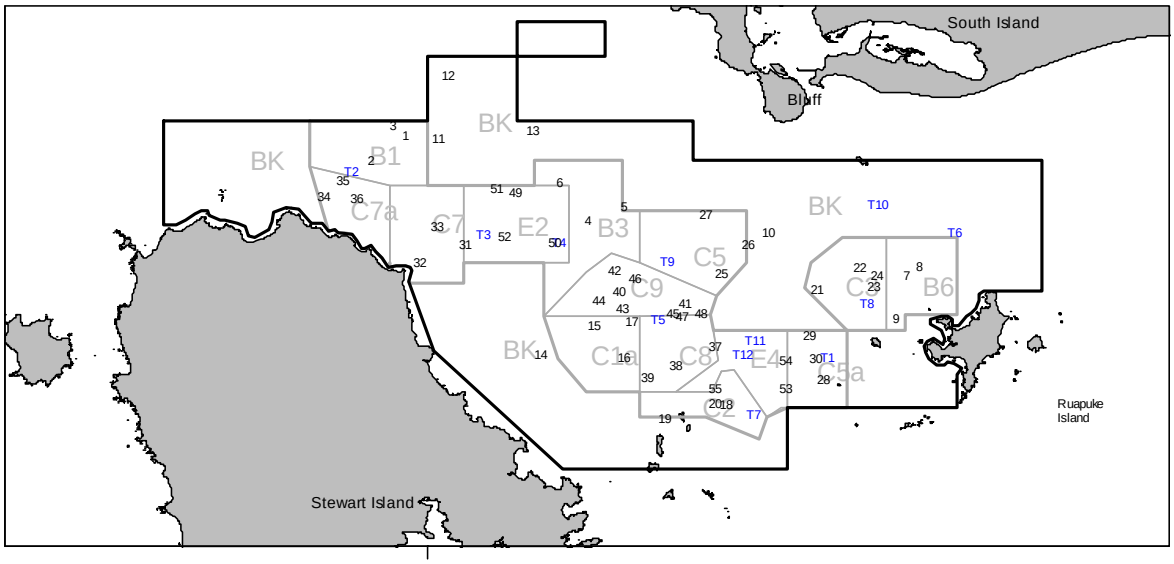

Figure 2: The 2015 survey area with the 2007 survey boundary shown as a heavy, black outer line, and the 2015 survey strata shown as grey lines and the core commercial fishery area shown by heavy grey lines. Strata are labelled with grey text. The remaining stock assessment survey strata which do not represent the core commercial fishery area were merged into a single, large background stratum (BK). First-phase station numbers shown in black text and fixed station numbers in blue text.

Catch sampling

Dredge sampling followed standard procedures for stock assessment and bonamia surveys between October 2002 and February 2014 (Michael et al. 2015b). The same commercial oyster vessel and skipper (F.V. Golden Quest skippered by Stephen Hawke) that have been used since 2011 was used to undertake the survey. Survey stations were sampled with the standard survey dredge (commercial dredge 3.35 m wide and weighing 430 kg) used since 1993 and rebuilt in 2014 to the same specifications. In February 2015, dredges were deployed using a new hydraulic winch system that replaced the traditional friction winch used for all previous surveys. Standard dredge sampling methods, standard methods for sorting the catch and recording data (data forms are shown in Appendix 1), and standard methods for sampling oysters to determine the status of bonamia were used (see Michael et al. 2015a for details).

In 2015, all the catch was sorted into live oysters, gapers (live, but moribund oysters containing the whole oyster and valves remaining apart after the adductor muscle has lost its ability to contract), and clocks (the articulated shells of recently dead oysters with the ligament attaching the two valves intact) to estimate mortality. The catch was further sorted into two size groups: recruit-sized (unable to pass through a 58 mm internal diameter ring), and pre-recruits (able to pass through a 58 mm internal diameter ring, but unable to pass through a 50 mm ring). Live oysters were sorted into a third size group, small oysters (able to pass through a 50 mm internal diameter ring and down to 10 mm in length). The station data recorded are shown in Appendix 1, see Michael et al. (2015b) for details.

Estimates of oyster densities and population size

Oyster densities and population sizes for the three size groups of live oysters were estimated for strata where three or more randomly selected stations were sampled in February 2015 and these were compared with the estimates from strata sampled in the 2012 (Michael et al. 2013) and 2014 (Michael et al. 2015a) surveys. These estimates are presented separately. The absolute population size of each size group of oysters was estimated using the combined population sizes in each stratum. Estimates of the commercial population size (Michael et al. 2015a) are given for comparison.

Estimates of absolute abundance and variance were calculated using standard stratified random sampling theory (Francis 1984, Jolly & Hampton 1990). We used an estimate of dredge efficiency from Dunn (2005), 0.17 (95% confidence intervals 0.13–0.22) re-estimated from the 1990 data of Doonan et al. (1992), and hence calculated the absolute population size of recruit, pre-recruit, and small oysters, and clocks using the combined population sizes in each stratum as,

$$\bar{x} = \sum W_i \bar{x}_i$$

where $\bar{x}$ is the estimated population size (numbers of oysters) for each size group, W_i is the area (m^2), and $\bar{x}_i$ is the mean oyster density corrected for dredge efficiency in stratum *i*. Estimates of population sizes are also presented by stratum separately.

The coefficient of variation (CV) for each stratum is calculated from the standard deviation and mean oyster density alone, and the same calculation is used for the total survey area:

$$s(\bar{x}) = \left(\sum W_i^2 s(\bar{x}_i)^2 \right)^{1/2}$$

where $s(\bar{x})$ is the standard deviation for the estimated population size and $s(\bar{x}_i)$ is the standard deviation for the mean density in stratum *i*.

The 95% confidence intervals of the population means for each stratum and the total population are estimated by resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. The total error of the estimates of the population mean has two sources:

one is the sampling error from the survey. The survey estimate of population size follows a normal distribution and this is based on standard survey sampling theory. The other source is error associated with dredge efficiency, which is assumed to be normally distributed (there are only three data points). If the two sources of error are independent, then the error can be estimated by simply adding the two variance components.

Recruitment to the fishery was summarized using plots of changes in the population estimates of pre-recruit and small oysters, and from changes in the patterns of distribution of small oyster densities, between the February 2012, February 2014 and February 2015 surveys.

3.2 Methods for objective 4: estimate the annual mortality from bonamia

Although significant winter mortality from bonamia has occurred previously (Hine 1991), we estimated summer mortality from bonamia only, and for recruit-sized oysters only. Summer mortality comprises the aggregate of two different estimates: 1. Pre-survey mortality estimated from the population size of recruit-sized new clocks and gapers that had died after the last summer, and 2. projections of future (within about two months) disease mortality from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population (objective 5). Although pre- and post- survey mortality measure different variables and pre-survey mortality may include heightened natural (non-disease related) mortality, the sum of pre- and post-survey totals gives the best estimate of summer mortality.

Pre-survey mortality, the absolute population size of recruit-sized new clocks and gapers was estimated using the same methods as for live oysters (see Section 3.3 and Michael et al 2015a for details). Post-survey mortality used the mean proportion of oysters with fatal infections (category 3−5 infections, from Diggles et al. 2003) in each stratum as a correction factor, i.e. 1 - mean proportion of category 3−5 infections. Population estimates for each stratum and the total survey area were recalculated to account for the projected mortality. Total projected mortality is the difference between the total population size at the time of the survey and the population corrected for projected bonamia mortality (at the end of summer). A second estimate of post-survey mortality uses the prevalence of oysters with non-fatal infections as a scalar to the prevalence in the dredge catch. Stratum and population estimates of fatally infected oysters were made using the method in Section 3.3 and the scaled up numbers of fatally infected oysters in each station sample.

3.3 Methods for objective 5: estimate the prevalence and intensity of bonamia infection

The numbers of infected recruit-sized oysters in the commercial population (defined by the core survey strata) and by stratum were estimated from the numbers of infected oysters determined from heart imprints and the qPCR assays. Estimates of prevalence from heart imprints assumed that oysters that tested negative for bonamia using qPCR for heart tissue analysis were also negative for heart imprints. The numbers of non-fatally and fatally infected oysters were estimated from bonamia intensity scores derived from histology and scaled-up to the size of the recruit-sized oyster population by strata and for the commercial fishery area.

Samples of up to 30 randomly selected recruit-sized oysters from each station were collected for heart imprints, histology, and molecular (qPCR) analysis to estimate levels of bonamia infection. When there were insufficient recruit-sized oysters in the catch, pre-recruit and small oysters were used to fill the sample size, or the whole catch was retained for processing. Samples were flown to NIWA, Wellington, for processing, see Michael et al. (2015b) for details.

Histology and qPCR sampling methods

Station and sample data were recorded on bonamia sampling forms (Appendix 2), and the total numbers of live and dead oysters in the samples noted. A subsample of up to 25 recruit-sized oysters from each station was taken for heart imprints and qPCR and each oyster in the sample was assigned a unique number from 1 to 25, a size category using oyster size rings, and measured for length and height (see Michael et al. (2015b) for details). Recruit-size oysters were denoted with an R, pre-recruit oysters with P, and small oysters with an O. Gaping oysters with valves of the shell apart, but which closed when tapped, were marked with an asterisk alongside the corresponding oyster number. Oysters were recorded as either incubating white (early-stage) larvae, grey (late-stage) larvae, yellow (almost ready to settle) larvae; or with no larvae present.

Heart imprints were made using standard methods. Histological samples were taken from the first five oysters processed for heart imprints, see Michael et al. (2015b) for details. The first 24 oysters sampled for heart imprints were also sampled for qPCR. Laboratory work sheets recorded sampling data including: date, name of sampler, plate number and station number and the date and time the sample was collected. The prevalence of infection (the percentage of oysters with detectable infections in each sample) was first determined by quantitative polymerase chain reaction (qPCR) methods. Samples of oysters to be scored for histology were determined from the results of the qPCR testing using standard methods (Michael et al. 2015b).

A detailed account of the qPCR method and testing is given in Maas et al. (2013) and Michael et al (2015a). This method includes a duplex qPCR assay (the co-amplification of the *Bonamia* target (ITS region of the ribosomal genes) and *Ostrea chilensis* β-actin gene as an internal control), a new master mix that is able to cope with inhibitors often found in crude extracts, and a system to delay the amplification of the internal control to prevent a low level Bonamia ITS amplification being outcompeted by the stronger internal control (β-actin) reaction. This method uses a 96 well-plate format, and analysis undertaken with a BIORAD-CFX96 qPCR instrument.

qPCR Quality control

Quality control of reagents and methods is undertaken before the samples from each survey are analysed. A synthetic standard for Bonamia was designed (dnature LTD) incorporating the primer and probe sequences. It differed slightly to the actual Bonamia sequence to allow differentiation (via high resolution melting or sequencing) from a true positive. The standard was synthesised by overlapping PCR, sequenced and provided as 10E6 copies/µl. This standard was serially diluted in an oyster lysate diluent (oyster DNA extracted using the same method as used for testing free of Bonamia) down to 1 copy per µl. These dilutions (2 µl) were tested with the Bonamia duplex assay and two copies could be reliably detected.

Aliquots of the 10E3 copies/ µl dilution were used as inter-plate calibrators to permit collation of data among multiple runs. 20X Bonamia qPCR primer/probe mix incorporated primers and probes for the Bonamia target and internal control as well as the BLOCK system to prevent the high level endogenous internal control outcompeting a low level Bonamia target. Resulting lots of this mix were tested on the synthetic template standard dilutions to ensure that the same sensitivity was maintained (i.e. detection of the 1 copy/ µl dilution).

The qPCR data were analysed using BioRad CFX Manager™ software (Version 3.0), and if needed, qPCR assays were repeated based on the criteria given in Mass et al. (2013).

The cycle of quantification (Cq) cut-off to determine positives from false positives was set at Cq 35 and derived from a standard curve analysis of serial dilutions of *Bonamia exitiosa* positive standard to extinction. All matching heart imprint slides for those samples that tested positive for bonamia infection in either heart or gill samples were examined. At least three samples that were qPCR negative were randomly selected from the remaining samples from each station, and all samples for the 25th slide from each station (for which there was no qPCR data) were also examined. Repeated samples that gave

anomalous results such as flat-liners where no reaction was detected or early ampers (very low Cq values) were also screened with histology.

Heart imprints were examined based on the methods of Michael et al. (2015a), and imprints scored based on a categorical scale (Diggles et al. 2003). In 2015, heart imprints were examined by a single experienced reader, and a review of scoring protocols was undertaken before screening samples. Three good heart imprints containing oyster haemocytes were located and examined on each slide, and the number of bonamia cells counted for each. If no bonamia cells were found, further imprints were examined to confirm the absence of bonamia.

For each station, prevalence is calculated as the proportion of the total number of oysters in the sample with bonamia infection. Each sample consisted of up to 24 qPCR heart tissue samples in which infection was indicated by a Cq of 35 or less, plus the 25th slide (which was not tested by qPCR) in which infection was indicated by the observation of at least one bonamia cell on the heart imprint slide. Mean intensity is defined from histology samples only as the mean frequency of stages 1–5 oysters (i.e., the mean stage of all oysters examined that had at least one bonamia cell observed). Exact 95% confidence intervals are given for prevalence and for the proportion of new clocks, determined from the F-distribution, i.e., for a proportion π, where $\pi = r/n$ (where r is the number of oysters infected with bonamia and n the number of oysters in the sample), the 95% confidence interval is determined by:

$$\pi_{0.025} = \frac{r}{r + (n - r + 1)F_{0.025,2n-2r+2,2r}}$$

$$\pi_{0.975} = \frac{r + 1}{r + 1 + (n - r)F_{1-0.975,2r+2,2n-2r}}$$

Population estimates of bonamia infection

We used the same two methods to estimate scaled population estimates of fatal and non-fatal infections as those given in Section 3.5. Method 1 used a correction factor from strata with three or more randomly selected stations only i.e., we did not include target stations. Method 2 used the total numbers of oysters in each bonamia infection category (1–5) based on the estimated proportion of oysters in each infection category in the sample, and scaled to the total catch for each station. The overall intensity was calculated as the average bonamia level in the population. Variance for prevalence and intensity was estimated using standard methods as for population estimates.

3.4 Methods for objective 6: evaluate the best future stock projection from the 2012 OYU 5 assessment

Under the new management plan for OYU 5, stock assessments will be carried out five-yearly with annual population and bonamia surveys between assessments. The last assessment was completed in 2012 (Fu 2013) updating the stock assessment models with data on recruitment, harvest, catch rates, population size, and mortality (mostly mortality from bonamia during epizootics). Three projections of future stock status were based on 0%, 10%, and 20% disease mortality. The MPI Shellfish Working Group recommended that no "turn handle" updates of these stock assessment models are required for annual surveys.

Projections from the 2009 stock assessment based on a TACC of 15 million oysters and with no mortality of oysters from bonamia, predicted an increase in recruit-sized stock abundance of 29% by 2012; however with a bonamia mortality of 10%, the population size would only increase by 11% over the same period (Fu & Dunn 2009). Bonamia mortality was about 10% between 2009 and 2012; and

the estimated numbers of recruit-sized oysters killed between the 2009 survey and the 2012 survey was about 198 million oysters. The population size of recruit-sized oysters increased by 21.1% between the 2009 and 2012 surveys. If the estimated post-survey mortality in 2012 (81 million oysters) is taken into account, the population size of recruit-sized oysters increased by 13.5%, consistent with the 2009 stock assessment.

It is proposed that selecting the most appropriate projection for future stock status is determined by expert opinion based on the level of summer mortality from bonamia and trends in the population sizes of small and pre-recruit oysters. When these simplistic indicators were used previously to select the most appropriate projection, the population estimates predicted were similar to the estimates of population size estimated from subsequent surveys.

3.5 Methods for objective 7: review qPCR procedures prior to testing

The qPCR method requires strict adherence to aseptic and molecular biology techniques due to the sensitivity of the method. Quality control of reagents and methods is undertaken before the samples from each survey are analysed. A synthetic standard for Bonamia was designed (dnature LTD) incorporating the primer and probe sequences. It differed slightly to the actual Bonamia sequence to allow differentiation (via high resolution melting or sequencing) from a true positive. The standard was synthesised by overlapping PCR, sequenced and provided as 10E6 copies/µl. This standard was serially diluted in an oyster lysate diluent (oyster DNA extracted using the same method as used for testing and testing free of Bonamia) down to 1 copy per µl. These dilutions (2 µl) were tested with the Bonamia duplex assay and 2 copies could be reliably detected.

Aliquots of the 10^3 copies/µl dilution were used as interpolate calibrators to permit collation of data among multiple runs. 20X Bonamia qPCR primer/probe mix incorporated primers and probes for the Bonamia target and internal control as well as the BLOCK system to prevent the high level endogenous internal control outcompeting a low level Bonamia target. Resulting lots of this mix were tested on the synthetic template standard dilutions to ensure that the same sensitivity was maintained (i.e. detection of the 1 copy/µl dilution).

In 2015, new reagents were tested at NIWA Greta Point to ensure consistency of reaction and Cq values Reagents were checked using both positive and negative controls and Cq values were checked to ensure that they were within an acceptable range. Further testing was carried out to ensure that serial dilution of the positive control produced the standard cut off value for negatives (Cq35).

In addition to the pre-analysis testing, positive and negative controls were included on every 96 well qPCR plate to ensure the validity of data from each run.

4. RESULTS

4.1 Survey operational detail

The oyster vessel *F. V.* Golden Quest skippered by Stephen Hawke, her crew, and two NIWA staff, successfully sampled 55 first phase and 5 second phase random stations, and 12 target stations in February 2015. Several allocated stations couldn't be sampled because of rough ground, stations 34, 32, and 19 were replaced by stations 95, 94, and 75 respectively. Sampling began on the 10th of February 2015 and was completed on the 14th of February, sampling on 4 days (10th–12th and 14th) over this period. Survey tows completed are shown in Figure 3, and the numbers of stations sampled in each stratum are shown in Table 2.

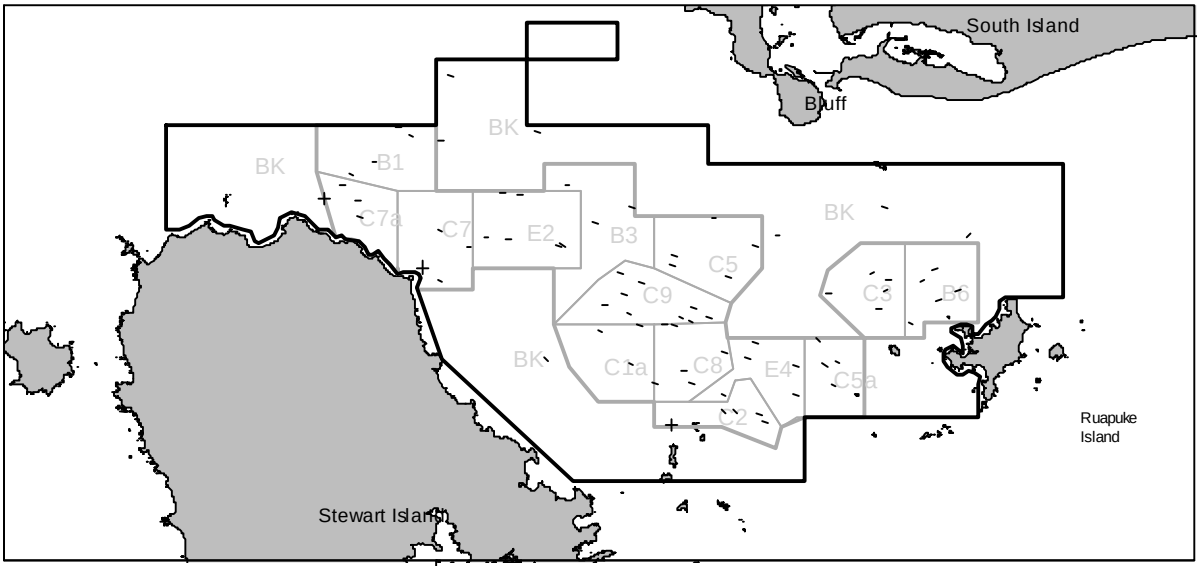

Figure 3: The survey tows (black lines) sampled in February 2015 to determine the status of bonamia infection and oyster density. The stations that could not be dredged because of foul ground are shown as crosses. The 2007 survey area is bound by the outer black line and the February 2015 survey strata are bound by the grey lines. The 2015 survey stratum labels are shown in grey text. Stock assessment survey strata outside the bonamia survey area are merged into a single stratum (BK). Bonamia survey stratum labels have been retained from previous surveys for consistency.

Samples of 30 oysters were collected from 60 of the 72 stations to determine the status of bonamia infection. Minimum sample sizes for histology were made up of pre-recruit and small oysters from stations 39, 20, 115, 23, 2, 37, 43, 48, 82, T7, and stations 53 and 54 only caught 2 and 6 oysters respectively. Oyster samples were couriered to NIWA, Greta Point (Wellington) where they were processed for heart imprints and qPCR. Oyster tissues were also taken for histology and these were archived for future research.

Table 2: The numbers of first-phase, second-phase, and target stations sampled in February 2015 by stratum.

Stratum	First-phase	Second-phase	Target	Total
B1	3	0	1	4
B3	3	0	0	3
B6	3	2		5
Bk	5	0	2	7
C1a	3	0	0	3
C2	3	0	1	4
C3	4	0	1	5
C5	3	1	1	5
C5a	3	0	1	4
C7	3	0	0	3
C7a	3	0	0	3
C8	3	2	1	6
C9	9	0	0	9
E2	4	0	2	6
E4	3	0	2	5
Total	55	5	12	72

Survey comparability

Dredge tow lengths were almost all about 0.2 nautical miles (371 m) in length (Figure 4). All oyster and clock densities were standardised to the 0.2 nautical mile standard tow length for analysis. Most of the survey stations were sampled in sea conditions less than 15 knots (Figure 5). The median wind force was 3 on the Beaufort scale (7−10 knots), with 5 and 95 percentiles of Beaufort scale 2 (3−6 knots) and 5 (16−20 knots) respectively. Maximum wind during sampling was about 20 knots. Dredge sampling during the February 2015 survey was undertaken in windier conditions than the February 2014 survey which had a median wind of 2 Beaufort scale (3−6 knots), and 5 and 95 percentiles of Beaufort scale 0 (i.e. less than 1 knot) and 3 (7−10 knots) respectively. This wind range and resulting sea conditions were mostly below the level likely to affect dredge efficiency, but there may be some survey variation due to weather.

Oyster dredges are considered saturated and cease fishing before the end of tow when they are more than 80% full on landing (Cranfield pers. comm.). Dredge saturation may lead to an underestimate of oyster density. No dredge was landed more than 80% full. Dredge fullness ranged from 1% to 80% with a median fullness of 40%, which is lower than in 2014 (50%) and higher than in 2013 (30%). Differences in dredge fullness are in part related to levels of pre-survey mortality from bonamia. Dredge saturation is not likely to have had a large effect on sampling effectiveness in 2015 and on the survey (Figure 6). Observations and anecdotal evidence from video data recorded during dredge trials suggest that dredge saturation may occur in dredges landed less than 80% full, but that when this occurs, the dredge contents were unevenly, but symmetrically, spread with contents lower in the middle of the dredge than at the edges of the dredge ring bag. This was not recorded in the 2015 survey data; future surveys will identify stations with this pattern in the distribution of catch.

Dredge efficiency is greatly reduced in areas densely populated with kaeos (*Pyura pachydermatina*) as the dredge skims above the seabed with little or no contact. Large numbers of kaeos and very few oysters were caught in stratum E4 (stations 53 and 54) and, stratum C5a (stations 28, 29, 30, T1). Oyster density was most likely underestimated at these sites.

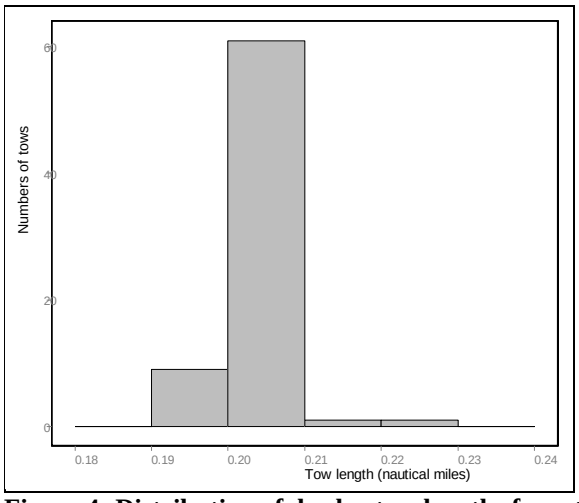

Figure 4: Distribution of dredge tow lengths from the February 2015 survey. The standard tow length was 0.2 nautical mile (371 m).

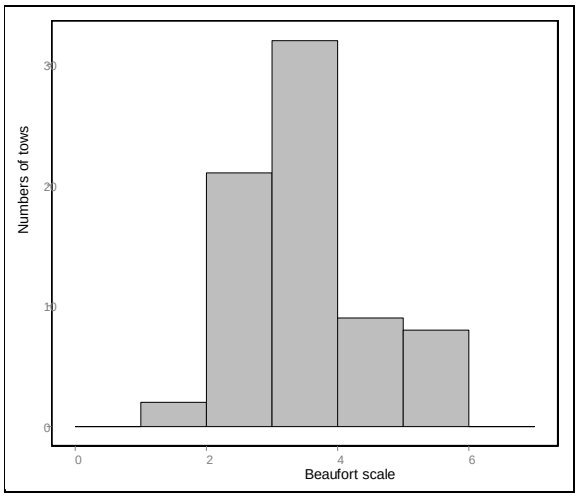

Figure 5: Distribution of sea state (Beaufort scale) recorded during survey tows in February 2015. Beaufort scale: 0, < 1 knot; 1, 1−2 knots; 2, 3−6 knots; 3, 7−10 knots; 4, 11−15 knots; 5, 16−20 knots; and 6, 21−26 knots. Sea states over a Beaufort scale of 5 may reduce dredge efficiency.

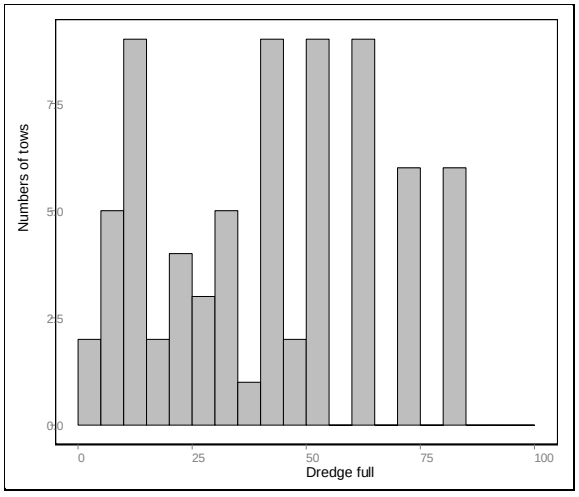

Figure 6: Distribution of dredge fullness recorded for survey tows in February 2015. One tow was landed with a dredge fullness of greater than 80%, suggesting that it may have saturated before the end of the tow leading to an underestimate of oyster density. Unpublished video data suggests that dredge saturation may occur below 80% full.

Observations from sampling

There were indications of continuing bonamia mortality from the presence of new clocks (the shells of oysters that had recently died) and gapers (moribund oysters). The number of gapers was lower than in recent years, suggesting that there was little mortality at the time of sampling. These observations suggest detectable levels of bonamia mortality before and during the February 2015 survey.

Recruitment to the commercial fishery areas surveyed appeared to be relatively low in February 2015. Most of the catches had relatively low numbers of pre-recruit and small oysters relative to the numbers of recruit-sized oysters in the catch, and spat and wings (small attached oysters) were rare in the catch.

4.2 Oyster abundance (objective 3)

Changes in oyster densities between 2014 and 2015

Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized, pre-recruit and small oysters, their means and 95% confidence intervals by stratum sampled during the 2014 and 2015 surveys are shown in Figures 7–9 respectively. Strata are arranged west to east with northern strata at similar longitudes shown before those to the south. Catches of recruit-sized oysters generally show the effects of continuing heightened mortality from bonamia. Strata with relatively high oyster density in 2014 had lower densities in 2015 (Figure 7). There are some exceptions where recruit-sized oyster densities in some strata (C7, B3, and B6) increased, however, oyster densities were relatively low in these strata.

Pre-recruit-sized oysters generally showed a similar pattern of decline (Figure 8) with significant declines in some western and central strata. Catches in strata C7 and B3 remained similar or increased. Catches of small oysters were generally similar across the fishery between 2014 and 2015 with some large decreases and significant increases (Figure 9). Catches increased the most in western strata B1 and C7, but declined most in the central fishery strata (Figure 9).

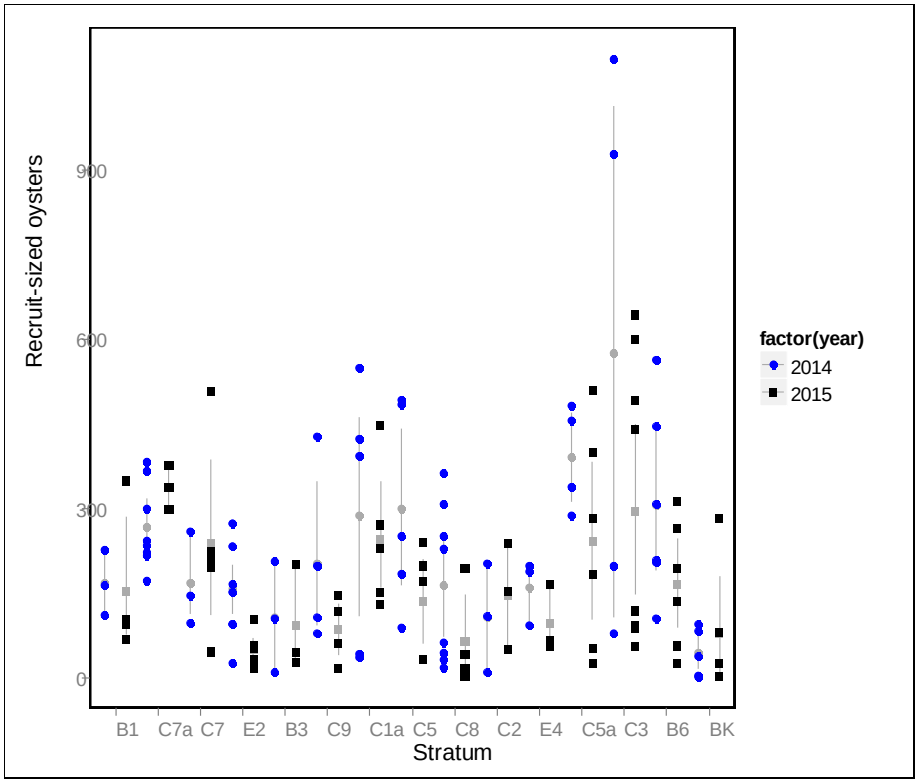

Figure 7: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized oysters, their means and 95% confidence intervals (grey) by stratum sampled during the 2014 (blue) and 2015 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

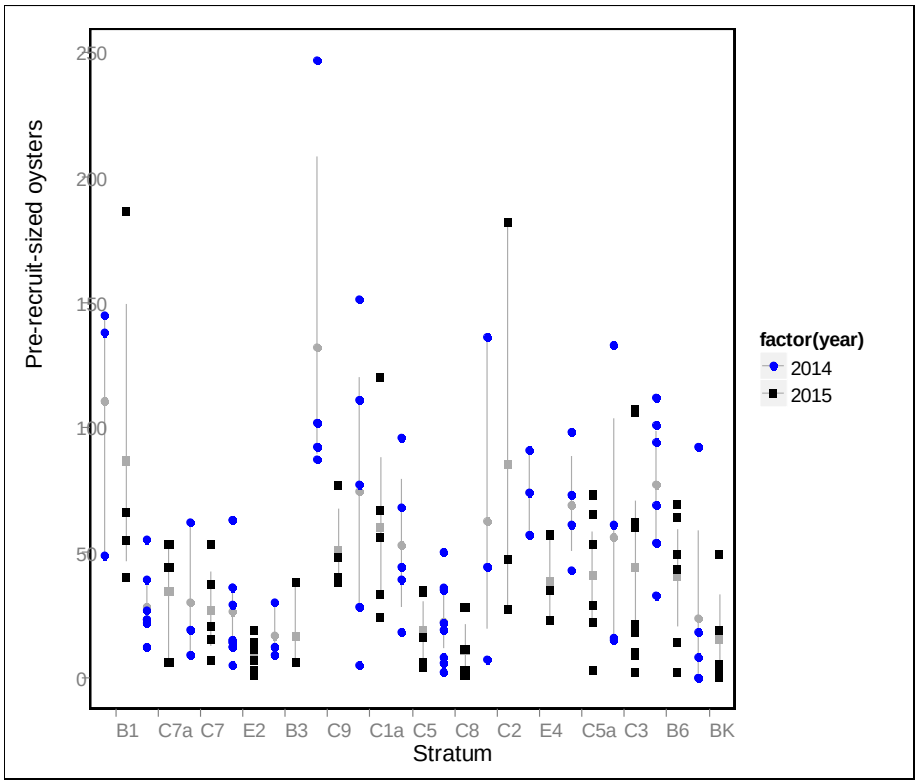

Figure 8: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for pre-recruit-sized oysters, their means and 95% confidence intervals (grey) by stratum sampled during the 2014 (blue) and 2015 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

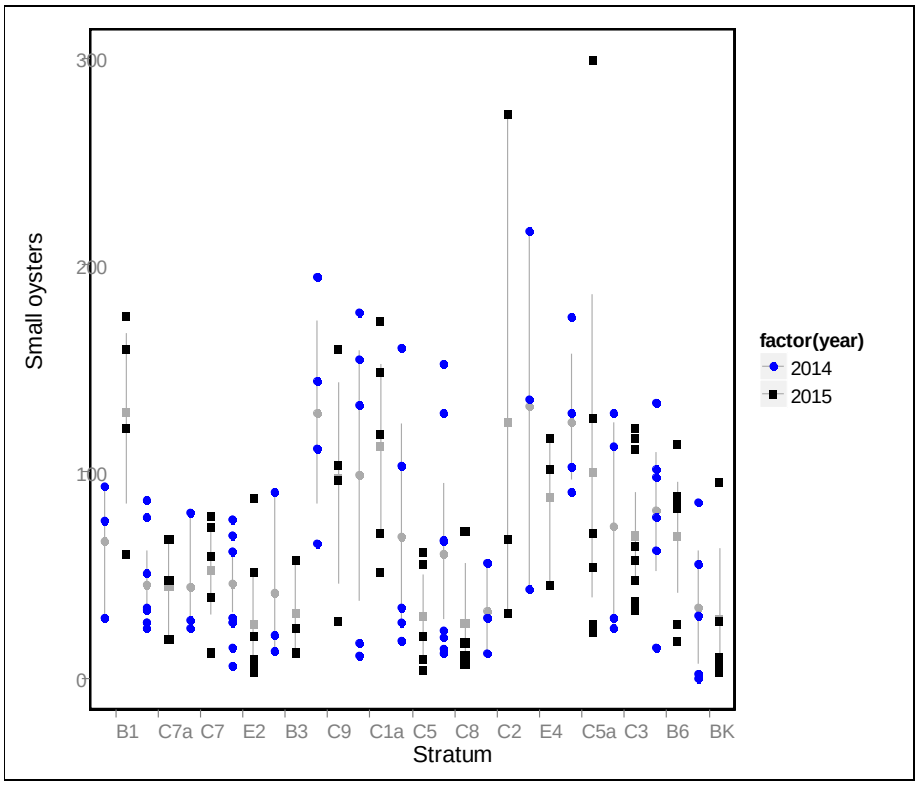

Figure 9: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for small oysters, their means and 95% confidence intervals (grey) by stratum sampled during the 2014 (blue) and 2015 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

Survey estimates of population size

Estimates of absolute population size for recruit-sized, pre-recruit, and small oysters from the February 2015 survey are shown in Tables 3–5. These tables show population estimates for the core strata (N = 14: B1, B3, B6, C1a, C2, C3, C5, C5a, C7, C7a, C8, C9, E2, and E4), all core strata combined, all background strata combined (N = 12: B1a, B1b, B2, B2a, B2b, B4, B5, B6b, B7, C4, C6, and C6a), and for the whole 2007 stock assessment survey area sampled. Two 95% confidence intervals are given: S.lower and S.upper being the sampling confidence limits calculated from the mean, standard deviation and sample size alone, and bootstrapped estimates (B.lower and B.upper) from resampling a normal distribution whose variance is based on a CV and the error of the estimated dredge efficiency. We refer to bootstrapped estimates in the text as they are likely to better represent the true range of the population size.

The population estimates for recruit-sized, pre-recruit, and small oysters from the 2012 and 2014 surveys are shown in Appendix 3 (Tables A3.1–A3.6) for comparison. Comparisons between the population estimates for all background strata combined in 2012 and 2014 should be made with caution as there were only 5 background stations sampled in total in 2014.

The density and population size of recruit-sized oysters in core strata (commercial fishery areas) declined between 2014 and 2015 (Table 3). The mean density in core strata declined from 1.40 oysters.m^{-2} in 2012 to 1.09 oysters.m^{-2} in 2014 and further declined to 0.71 oysters.m^{-2} in 2015, a 50% decline since 2012 and 35% since 2014. The population size of recruit-sized oysters in core strata declined from 688.1 million oysters in 2012 to 538.0 million oysters in 2014, and further declined to 351.4 million oysters in 2015; a 49% decline since 2012 and 35% since 2014. The coefficients of variation (CV) for all core strata combined were lower in 2015, 8.0% from 55 stations, compared with

9.2% CV (N = 84) in 2012 and 11.2% CV (N = 55) in 2014. The CV predicted for all core strata combined before the survey using the 2014 survey data was less than 10.0%.

The density and population size of recruit-sized oysters for all background strata combined are probably not well estimated by the surveys since 2012 due to the low numbers of stations sampled (N=5) over a large area (578.4 km^2). Population size increased from 230.3 million oysters in 2012 to 482.9 million oysters in 2014, and declined to 158.5 million oysters in 2015. The CVs have increased from 19.7% in 2012 (stations sampled, N=62), to 21.3% (N=5) in 2014 and 23.4% (N=5) in 2015. It is not known how well the nominal five stations sample the size of the oyster population in the background stratum.

The comparability of population estimates for recruit-sized oysters from the 2007 stock assessment survey area is unknown. The coefficients of variation for estimates between 2012 and 2015 are relatively low: 8.5% in 2012 (N = 146), 11.7% in 2014 (N = 60), and 9.1% in 2015 (N = 60). The overall decline in population size of 45% between 2012 (Table A3.2) and 2015 (Table 3) is consistent with the decline in all core strata combined. It is likely that the estimate of population size for all the background strata combined in 2014 (482.9 million oysters) was by chance high; a result of low sample numbers and random sampling, and this high estimate clearly contributed to the increase in total population size for the 2007 stock assessment survey area between 2012 and 2014, 918.4 million oysters to 1020.9 million oysters respectively. The 2015 estimate for the background stratum was 158.5 million oysters (Table 3), which was lower than in 2012 (Table A3.2).

Recruit-sized oyster population sizes (and mean densities) declined in all but three (B3, C7, and B6) of the fourteen core strata. Declines ranged from 15.3% (E4) to 86.1% (C5a) of the 2014 estimates. The greatest decline in stratum C5a is attributed to the low efficiency of sampling due to the abundance of kaeos. Several other strata also declined by more than 50% including: C2 (69.6%), E2 (63.1%), and C5 (58.0%) (Table 3).

Pre-recruit mean oyster densities in all core strata combined declined from 0.60 in 2012, to 0.30 oysters m^{-2} in 2014, and 0.18 oysters m^{-2} in 2015 (Table 4). The population size declined from 297.4 million oysters in 2012 to 148.4 million oysters in 2014 and 89.2 million oysters in 2015; an overall decline of 70% since 2012 and 40% since 2014 (Table 4). The population size for all the background strata combined and for the 2007 stock assessment survey area showed a similar downward trend. The population size of pre-recruits for the 2007 stock assessment area declined from 414.3 million oysters in 2012 to 226.2 million oysters in 2014 and 122.1 million oysters in 2015. The population size declined in all but two (B3 and C7) of the fourteen core strata. Declines ranged from 4.3% (C1a) to 75.4% (E4). Six strata declined by more than 50% E4, C5a (75.3%), C5 (69.6%), C2 (64.4%), C8 (55.9%), and B1 (51.9%) (Table 4).

The mean densities and population sizes of small oysters for all the core strata combined declined markedly (65%) from 451.3 million oysters in 2012 to 156.3 million oysters in 2014, but have remained similar in 2015 with 158.5 million oysters (Table 5). The population for all the background strata combined remained similar between 2012 (160.9 million oysters) and 2014 (156.3 million oysters), but declined 38% in 2015 to 90.5 million oysters (Table 5). Overall, the population size of small oysters declined by about 50% from 612.2 million oysters in 2012 to 302.6 million oysters in 2014, and further declined 19% to 249.0 million oysters in 2015 (Table 5). Mean small oyster density in all the core strata combined was low (0.32 oysters.m^{-2}), and low in all the core strata (ranging from 0.07 oysters.m^{-2} to 0.60 oysters.m^{-2}). Mean small oyster density for all the background strata combined was 0.16 oysters.m^{-2} and for the stock assessment survey area 0.23 oysters.m^{-2} (Table 5).

Table 6 compares survey estimates for recruit-sized, pre-recruit, and small oysters from all core strata combined and for the 2007 stock assessment survey area from 2012, 2014 and 2015, and gives the percentage change in population size between 2012 and 2014, 2014 and 2015, and 2012 and 2015.

Table 3: **Absolute population estimates from randomly allocated stations only for recruit-sized oysters in core strata (Stratum, N = 14), all background strata combined (BK, N = 12), and for the whole 2007 stock assessment survey area (All) sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) is shaded green for increases in population size and red for decreases.**

2015 Stratum	No. stns	Mean density	Density s.d.	CV	2015 Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.43	0.05	0.11	33.5	29.2	37.9	21.4	51.3	78.2	52.5
B3	3	1.65	0.11	0.07	73.6	67.9	79.4	48.9	108.8	44.7	125.5
B6	5	1.15	0.36	0.32	34.5	24.9	44.1	12.0	63.7	30.0	140.3
C1a	3	0.43	0.26	0.61	13.5	4.3	22.8	0.0	32.9	31.3	82.1
C2	3	0.37	0.19	0.50	8.0	3.4	12.6	0.2	17.8	21.9	30.4
C3	4	0.95	0.17	0.17	31.1	25.8	36.4	17.6	49.7	32.7	76.0
C5	4	0.52	0.21	0.41	19.7	11.9	27.6	3.6	40.3	37.7	42.0
C5a	3	0.10	0.05	0.56	2.3	0.8	3.7	0.0	5.3	23.5	13.9
C7	3	0.72	0.27	0.37	25.8	14.9	36.8	6.7	50.7	36.1	136.0
C7a	3	0.46	0.17	0.36	10.9	6.4	15.4	2.9	21.1	23.6	59.0
C8	5	0.90	0.33	0.37	24.1	16.2	31.9	6.2	47.2	26.8	50.6
C9	9	1.43	0.40	0.28	49.3	40.4	58.2	20.8	88.1	34.5	50.6
E2	4	0.49	0.19	0.38	21.0	13.1	28.8	5.0	41.8	42.8	36.9
E4	3	0.14	0.13	0.91	3.9	0.0	7.9	0.0	11.9	28.0	84.7
Core	55	0.71	0.06	0.08	351.4	344.0	358.8	232.1	528.8	491.8	65.3
BK	5	0.27	0.06	0.23	158.5	126.1	191.0	77.9	270.1	578.4	32.8
All	60	0.48	0.04	0.09	509.9	498.2	521.7	327.4	761.8	1070.2	50.0

Table 4: **Absolute population estimates from randomly allocated stations only for pre-recruit-sized oysters in core strata (Stratum, N = 14), all background strata combined (BK, N = 12), and for the whole 2007 stock assessment survey area (All) sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) is shaded green for increases in population size and red for decreases.**

2015 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.26	0.04	0.14	20.4	17.2	23.6	12.5	31.8	78.2	48.1
B3	3	0.17	0.07	0.42	7.5	3.9	11.1	1.4	15.2	44.7	125.2
B6	5	0.13	0.04	0.31	3.8	2.8	4.9	1.4	7.1	30.0	87.5
C1a	3	0.08	0.05	0.63	2.5	0.7	4.3	0.0	6.2	31.3	95.7
C2	3	0.25	0.06	0.25	5.5	4.0	7.1	2.6	9.6	21.9	35.6
C3	4	0.22	0.05	0.22	7.2	5.6	8.7	3.6	12.0	32.7	68.2
C5	4	0.07	0.03	0.45	2.7	1.5	4.0	0.3	5.8	37.7	30.4
C5a	3	0.02	0.01	0.62	0.6	0.2	1.0	0.0	1.4	23.5	24.7
C7	3	0.42	0.24	0.57	15.1	5.3	24.8	0.0	35.3	36.1	139.5
C7a	3	0.19	0.05	0.26	4.4	3.1	5.7	1.9	7.7	23.6	53.5
C8	5	0.16	0.05	0.32	4.4	3.2	5.6	1.5	8.2	26.8	44.1
C9	9	0.21	0.07	0.31	7.4	5.9	8.9	2.6	13.7	34.5	79.2
E2	4	0.16	0.07	0.46	6.7	3.7	9.7	0.6	14.3	42.8	50.5
E4	3	0.04	0.03	0.80	1.0	0.1	1.9	0.0	2.9	28.0	24.6
Core	55	0.18	0.02	0.12	89.2	86.2	92.1	55.8	139.2	491.8	60.1
BK	5	0.06	0.01	0.22	32.9	26.5	39.3	16.7	55.5	578.4	42.2
All	60	0.11	0.01	0.11	122.1	118.7	125.4	76.7	184.7	1070.2	54.0

Table 5: **Absolute population estimates from randomly allocated stations only for small oysters in core strata (Stratum, N = 14), all background strata combined (BK, N = 12), and for the whole 2007 stock assessment survey area (All) sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) is shaded green for increases in population size and red for decreases.**

2015 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.55	0.14	0.25	43.0	30.8	55.2	19.9	74.4	78.2	169.9
B3	3	0.22	0.07	0.32	9.7	6.2	13.2	3.6	17.8	44.7	99.9
B6	5	0.25	0.06	0.23	7.6	6.1	9.2	3.7	12.9	30.0	117.0
C1a	3	0.15	0.06	0.43	4.6	2.4	6.9	0.7	9.6	31.3	73.6
C2	3	0.37	0.12	0.33	8.1	5.1	11.0	2.8	15.1	21.9	50.8
C3	4	0.50	0.13	0.27	16.4	12.1	20.6	7.2	28.6	32.7	136.4
C5	4	0.11	0.06	0.52	4.0	2.0	6.1	0.0	9.0	37.7	36.8
C5a	3	0.06	0.01	0.25	1.3	0.9	1.7	0.6	2.3	23.5	19.5
C7	3	0.60	0.37	0.61	21.8	6.7	37.0	0.0	52.6	36.1	389.9
C7a	3	0.42	0.10	0.25	10.0	7.2	12.8	4.7	17.3	23.6	68.4
C8	5	0.28	0.09	0.31	7.6	5.5	9.7	2.8	14.1	26.8	55.3
C9	9	0.34	0.06	0.18	11.6	10.2	12.9	6.5	18.9	34.5	97.4
E2	4	0.26	0.09	0.34	10.9	7.3	14.6	3.3	20.9	42.8	82.7
E4	3	0.07	0.04	0.53	1.8	0.7	2.9	0.0	4.2	28.0	47.1
Core	55	0.32	0.04	0.12	158.5	153.4	163.6	99.6	247.1	491.8	101.4
BK	5	0.16	0.08	0.51	90.5	49.8	131.2	0.0	202.6	578.4	61.9
All	60	0.23	0.05	0.20	249.0	236.3	261.7	130.8	412.4	1070.2	82.3

Table 6: Percentage changes in the absolute population estimates from randomly allocated stations only for recruit-sized, pre-recruit, and small oysters for the 2014 core strata (N = 14), and for the whole 2007 stock assessment survey area (26 strata) sampled in 2012, 2014 and 2015. The mean oyster density per m^2 (Mean density), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), bootstrapped upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the percentage change in population size.

2012					
Core Strata	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**
Recruit	1.40	0.09	688.1	449.2	1046.7
Pre-recruit	0.60	0.10	297.4	192.6	454.4
Small	0.92	0.16	451.3	261.5	731.7
Survey total					
Recruit	0.86	0.08	918.4	600.1	1383.7
Pre-recruit	0.39	0.10	414.3	267.8	629.0
Small	0.57	0.14	612.2	370.3	967.9

2014						
Core Strata	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**	**% change 2012-2014**
Recruit	1.09	0.11	538.0	343.6	832.2	-21.8
Pre-recruit	0.30	0.12	148.4	93.7	230.7	-50.1
Small	0.32	0.10	156.3	101.1	239.4	-65.4
Survey total						
Recruit	0.95	0.12	1020.9	635.1	1554.2	11.2
Pre-recruit	0.21	0.14	226.2	135.1	352.1	-45.4
Small	0.28	0.11	302.6	189.2	459.2	-50.6

2015							
Core Strata	**Mean density**	**CV**	**Pop.n**	**B.lower 95%CI**	**B.upper 95%CI**	**% change 2014-2015**	**% change 2012-2015**
Recruit	0.71	0.08	351.4	232.1	528.8	-34.7	-48.9
Pre-recruit	0.18	0.12	89.2	55.8	139.2	-39.9	-70.0
Small	0.32	0.12	158.5	99.6	247.1	1.4	-64.9
Survey total							
Recruit	0.48	0.09	509.9	327.4	761.8	-50.0	-44.5
Pre-recruit	0.11	0.11	122.1	76.7	184.7	-46.0	-70.5
Small	0.23	0.20	249.0	130.8	412.4	-17.7	-59.3

In 1995 and 1997, the commercial population used to estimate yield was estimated as the percentage of the entire population above a density of 400 oysters per tow (equivalent to about 6–8 sacks per hour during commercial dredging). This threshold was based on an historical, economic catch rate, and when the catch rate dropped below 6 sacks per hour, fishers would move to new fishery areas. Although this method is no longer used for stock assessments, estimates of commercial population size allow some comparison with previous years; so the Shellfish Working Group requested that these estimates be included in this report.

Estimates of commercial population size (using the catch of recruit-sized oysters at each station minus 400 oysters) for the 2014 core strata (N = 14) to provide stratum by stratum comparisons, all core strata combined, all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015 are shown in Table 7, and estimates for the 2014 and 2012 surveys in Appendix 3 (Tables A3.7 and A3.8 respectively).

Ten core strata supported commercial densities in 2012, six in 2014, and only two in 2015. The mean density in the combined background strata was 0.11 oysters.m^{-2} in 2012, none in 2014 and 2015 (Tables 7, A3.7, and A3.8). The commercial population size declined from 473.9 million oysters in 2012 to 211.3 million oysters in 2014, and further declined to 55.4 million oysters in 2015. The declines in the oyster populations are consistent with the levels of bonamia mortality observed in the fishery and the prolonged period of relatively low recruitment.

Table 7: **Absolute population estimates from randomly allocated stations only for the size of the recruit-sized oyster population above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) in the 2014 core strata (N = 14), all background strata (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) is shaded red for decreases in population size and NC for no change.**

2015 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	78.2	NC
B3	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	44.7	NC
B6	5	0.49	0.49	1.00	14.8	1.8	27.8	0.0	47.5	30.0	0.0
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	31.3	NC
C2	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	21.9	0.0
C3	4	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	32.7	0.0
C5	4	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	37.7	0.0
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	23.5	NC
C7	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	36.1	NC
C7a	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	23.6	NC
C8	5	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	26.8	0.0
C9	9	1.18	0.47	0.40	40.6	29.9	51.2	8.8	81.0	34.5	47.3
E2	4	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	42.8	0.0
E4	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	28.0	NC
Core	55	0.11	0.04	0.40	55.4	49.5	61.2	12.0	109.4	491.8	26.2
BK	5	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	578.4	NC
All	60	0.05	0.02	0.40	55.4	49.8	61.0	12.0	109.4	1070.2	26.2

Changes in the distribution of live oysters

Stratified random surveys are generally not as good as grid design surveys at estimating the distribution of oysters in Foveaux Strait, especially because of the patchy distribution of oysters. The February 2015 survey sampled 60 first and second-phase random stations generated with a 0.75 nautical mile exclusion that spread sampling effort, and 12 fixed stations. All 72 stations were used to describe oyster distribution. Sampling effort was focused in core strata with background strata receiving only 5 stations for 51.4% of the survey area. The sampling was therefore insufficient to provide a consistent or complete coverage of the fishery area in 2015, and hence the survey is not likely to have estimated the distributions of oyster density well for live recruit, pre-recruit, and small oysters outside of core strata. These distributions of oysters are compared with the last stock assessment survey in 2012 which sampled 158 stations in total and provided more complete coverage of the fishery and 71 stations from the 2014 survey, which provided similar sampling effort and coverage to the 2015 survey.

The distributions of recruit-sized, pre-recruit, and small oyster density in 2015, 2014 and 2012 are shown in Figures 10–15. The distribution of oyster density is widespread, covering most of the fishery area with the highest densities in core fishery strata (Figures 10, 12, and 14). Densities of all three size groups of oysters were lower in 2015 than in 2012 and in 2014, except for small oysters which were similarly low between 2014 and 2015. The numbers and sizes of localised areas of relatively high density of recruit-sized oysters decreased between 2012 and 2015 (Figures 10 and 11), most likely the result of ongoing, low to moderate level bonamia mortality and reduced recruitment to the fishery. Densities of recruit-sized oysters decreased in western areas where there was virtually no fishing. They also decreased in northern and central areas, and were generally static in southern and eastern areas (Figures 10 and 11).

The density estimates of pre-recruit oysters decreased markedly between 2012 and 2015 (Figure 12), to become very low and patchy in 2015 (Figure 13). Pre-recruit-sized oysters are as vulnerable to bonamia mortality as recruit-sized oysters, and the low densities also reflect the low settlement of oyster spat and low survival of juveniles (small oysters) in recent years. There are a few low density, isolated patches of pre-recruits throughout most of the core strata (Figure 13).

The distributions of small oyster densities (Figures 14 and 15) showed similar patterns to pre-recruit sized oyster densities. Relatively high densities of small oysters were widespread throughout the fishery in 2012, except for in the central fishery area (Figure 14). Densities decreased markedly across the entire fishery by 2014, and further declined by 2015 with the distribution becoming very patchy. Small oysters are not vulnerable to bonamia mortality. The low densities reflect low recruitment to the oyster population which is consistent with the low spat settlement since 2009.

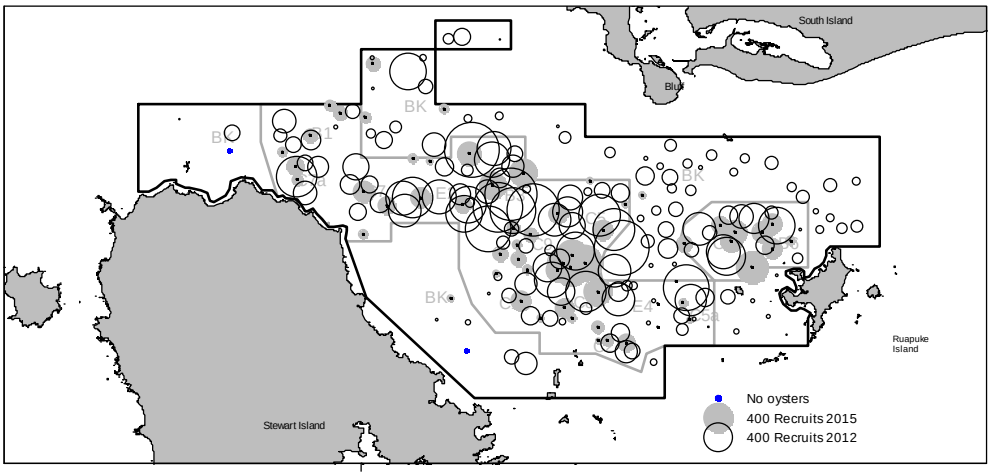

Figure 10: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during the February surveys in 2015 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

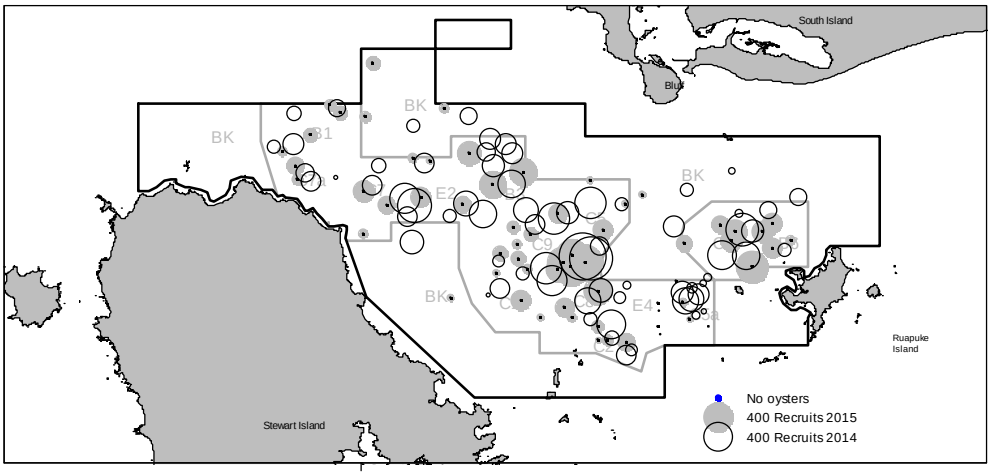

Figure 11: The densities (numbers of oysters per standard tow, 1221 m^2) of recruit-sized oysters sampled during the February surveys in 2015 (filled grey circles) and in 2014 (open black circles). Blue filled circles denote no oysters caught.

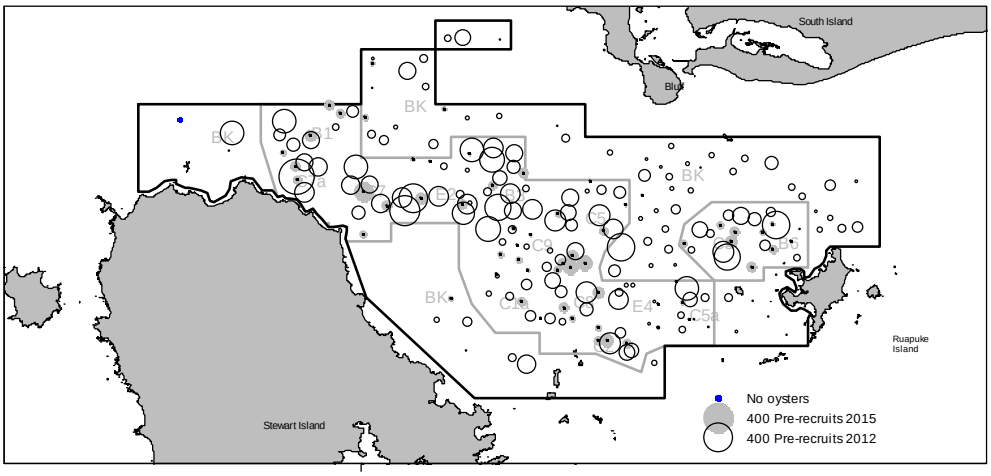

Figure 12: The densities (numbers of oysters per standard tow, 1221 m^2) of pre-recruit-sized oysters sampled during the February surveys in 2015 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

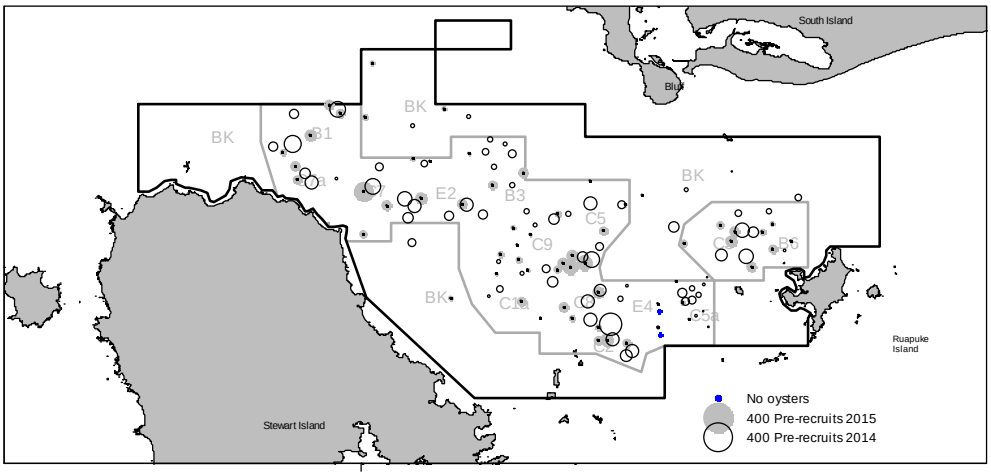

Figure 13: The densities (numbers of oysters per standard tow, 1221 m^2) of pre-recruit-sized oysters sampled during the February surveys in 2015 (filled grey circles) and in 2014 (open black circles). Blue filled circles denote no oysters caught.

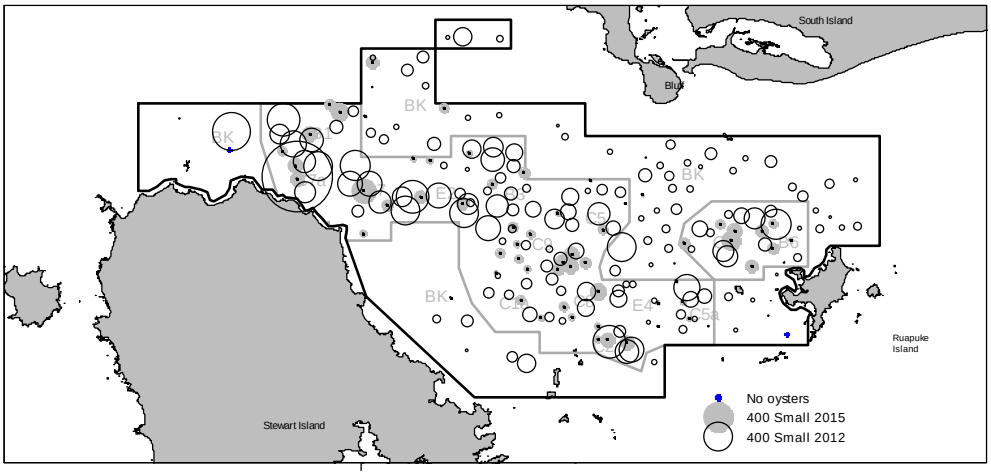

Figure 14: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled during the February surveys in 2015 (filled grey circles) and in 2012 (open black circles). Blue filled circles denote no oysters caught.

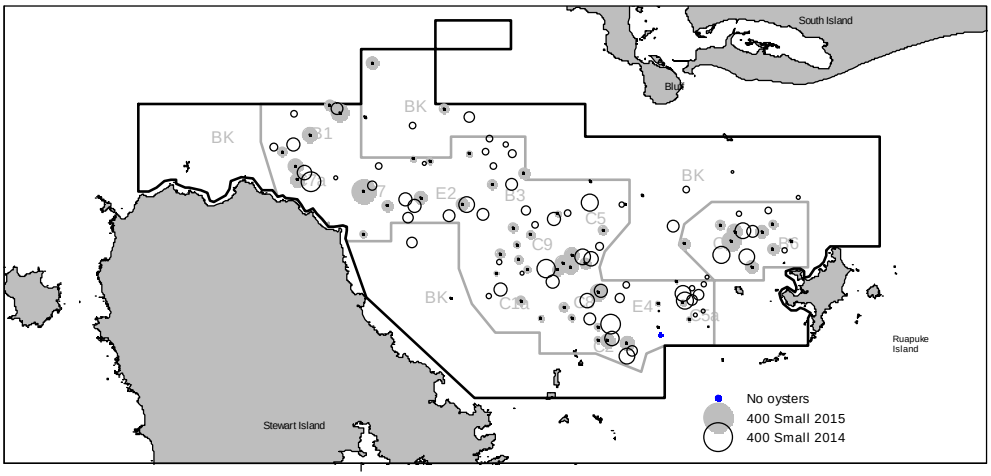

Figure 15: The densities (numbers of oysters per standard tow, 1221 m^2) of small oysters sampled during the February surveys in 2015 (filled grey circles) and in 2014 (open black circles). Blue filled circles denote no oysters caught.

Recruitment

Small oysters settle and remain attached to settlement surfaces up to a size of about 40 mm in length. Most small oysters are found on live oysters, possibly because survival of juveniles is better on large live oysters. Relatively few small oysters are found on other settlement surfaces. The median numbers of small oysters per recruited oyster can be used as an index of recruitment to the population.

The numbers of small oysters per recruit showed high fluctuations in a broadly cyclic trend between 1993 and 2015 (Figure 16). The numbers of small oysters per recruit were generally low between 1995 and 2001, suggesting reduced recruitment to the population at a time when the numbers of recruit-sized oysters were increasing and relatively high compared to 1993 (and earlier) (Figure 16). Small oysters per recruit were relatively high between 2002 and 2005 when the recruit-sized oyster population was declining rapidly from bonamia mortality. From 2009, the numbers of small oysters per recruit declined to low levels and have remained low in 2015 (Figure 16). This is consistent with the trend of declining numbers of small oysters sampled from the commercial catch between 2009 and 2012 (Fu et al. 2013), and the decreasing numbers of small oysters from stock assessment surveys (889 million oysters (574–1351) in 2009 and 607 million oysters (369–952) in 2012 (Michael et al. 2013).

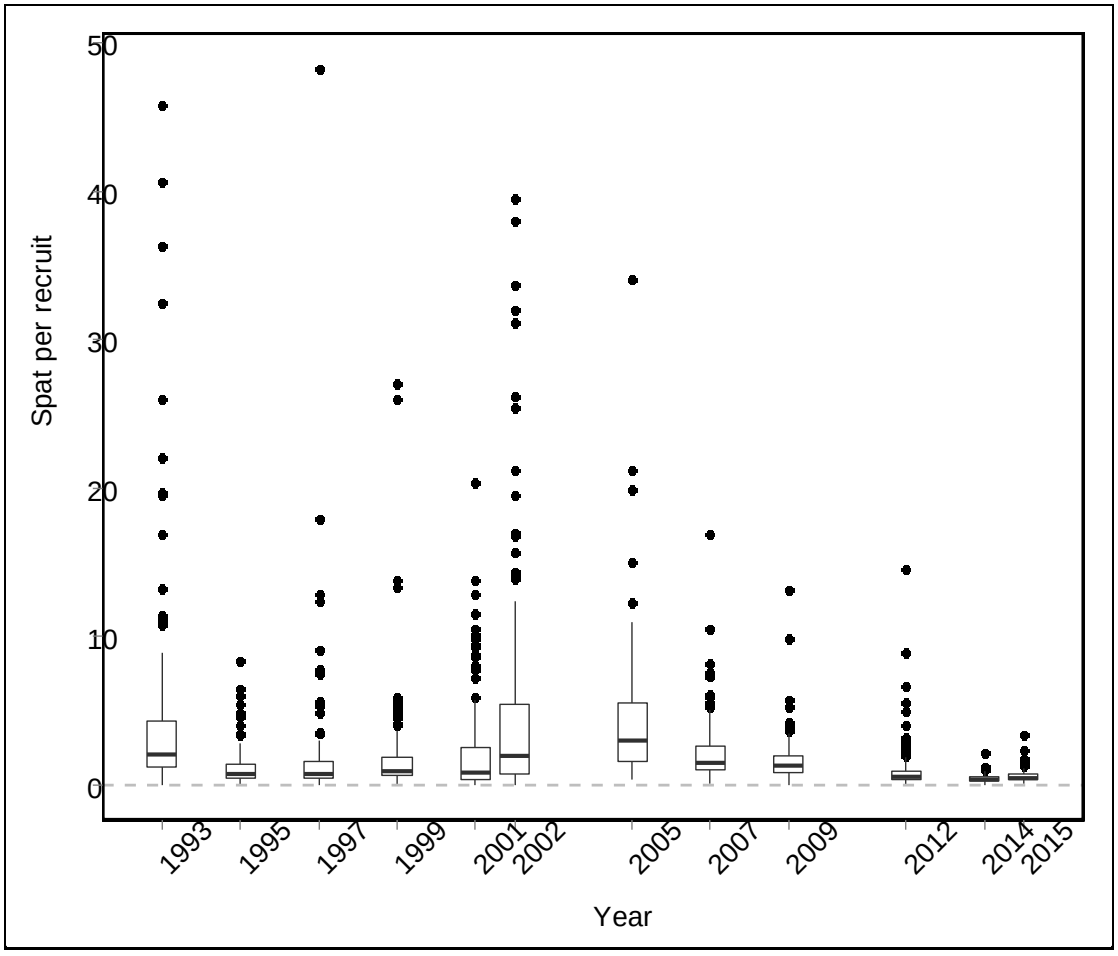

Figure 16: The numbers of small oysters per recruited oyster sampled between 1993 and 2015. The numbers of stations sampled each year varies. Medians shown as solid lines, boxes represent 50 percentiles (25–75%) and whiskers 90 percentiles (5–95%), and outliers smaller than 5% and greater than 95% as filled circles.

4.3 Estimates of oyster mortality before and during the February 2015 survey (objective 4)

Descriptive statistics for the percentages of recruit-sized and pre-recruit new clocks and gapers sampled in 2015 are given in Table 8 along with statistics for the 2012 and 2014 surveys for comparison (Table 8). Decreases in these statistics for recruit sized new clocks and gapers suggest that pre-survey mortality was lower in 2015 than in the previous two years. Pre-survey mortality for pre-recruits showed a similar trend, but the percentages are in part influenced by the low and decreasing population size.

Table 8: Descriptive statistics for the percentages of new clocks and gapers for two size groups, recruit and pre-recruit. Percentages are new clocks and gapers to new clocks, gapers and oysters combined, sampled from survey tows with more than 50 live recruit-sized or pre-recruit oysters in 2012, 2014, and 2015.

Percentage new clocks and gapers	Recruit sized			Pre-recruits		
Year	2012	2014	2015	2012	2014	2015
No. stations	112	50	54	78	30	20
Median	3.3	7.8	4.0	2.6	2.5	1.6
Minimum	0.0	1.7	0.0	0.0	0.0	0.0
Maximum	28.9	15.1	14.3	12.5	8.1	5.2
Lower 5th percentile	0.3	2.5	0.0	0.0	0.0	0.0
Upper 95th percentile	7.2	14.0	11.4	10.1	7.8	4.3
No. stations with no new clocks	5	0	5	11	8	9

The number of stations sampled in 2012 (159) was much higher than in 2014 (71) and in 2015 (72) as the 2012 survey was a stock assessment survey and the subsequent bonamia surveys sampled a smaller numbers of stations in mainly commercial fishery areas.

There were few gapers observed during the February 2015 survey, 6.9% of stations had one recruit-sized gaper and 1.4% of stations had either two or three gapers. There were no pre-recruit-sized gapers sampled in 2015. Fewer gapers were observed in 2014 (14% of stations) than in 2012, 26% of the stations sampled recorded up to 5 recruit-sized gapers per station and 4% of stations had pre-recruit gapers in 2012. The numbers of new clocks sampled from survey tows with more than 50 live oysters in 2014 and 2015 generally showed lower pre-survey mortality for recruit-sized oysters in most strata (Figure 17).

The distribution of recruit-sized oysters, new clocks and gapers combined, and recruit-sized new clocks and gaper densities showing levels of pre-survey mortality for the 2012, 2014, and 2015 surveys are shown in Figures 18–20 respectively.

The 2012 stock assessment survey showed widespread and variable distribution of recruit-sized new clock and gaper densities, and the numbers of new clocks and gapers were related to higher recruit-sized oyster densities found in strata mostly designated as commercial (E2, B3, C3, CB6, C7, and C7a) (Figure 18). The densities of new clocks and gapers were generally low with a couple of high density stations in the west. The 2014 survey found a similar pattern in the distribution of new clocks and gapers densities to the 2012 surveys, but the densities of new clocks and gapers were generally much higher, while the densities of recruit-sized oysters were lower (Figure 19). The 2015 survey showed that bonamia mortality had greatly reduced the numbers and sizes of areas with relatively high oyster density, especially west of a line from Bluff Hill to Port William, and that the densities of new clocks and gapers had greatly declined across the fishery.

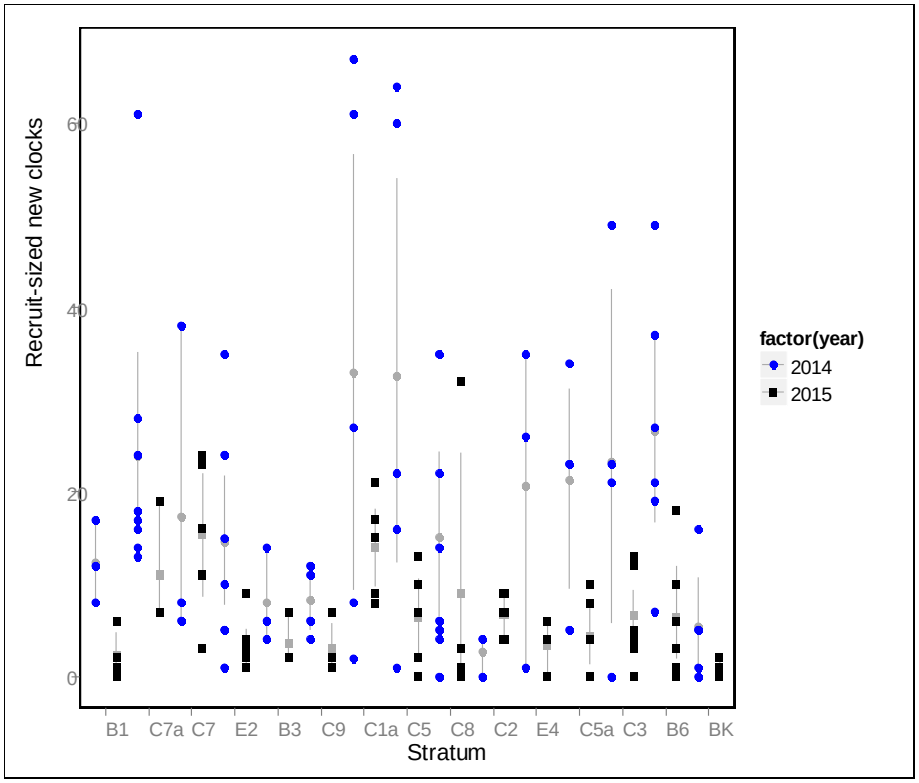

Figure 17: Plots of catches adjusted to the standard tow length (0.2 nautical miles) for recruit-sized new clocks, their means and 95% confidence intervals (grey) by stratum sampled during the 2014 (blue) and 2015 (black) surveys. Strata are arranged west to east with northern strata at similar longitudes shown first.

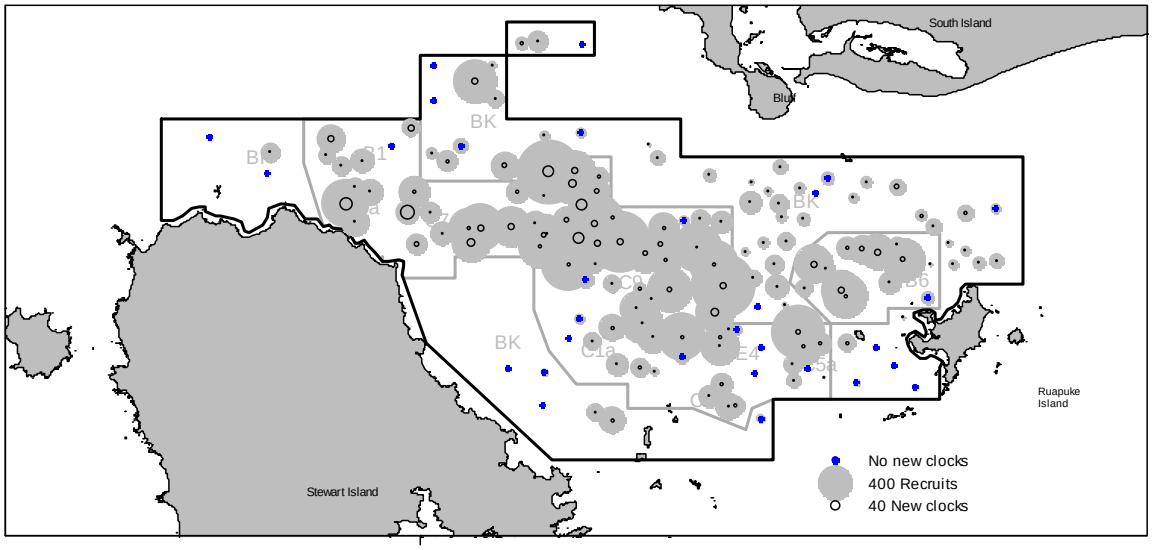

Figure 18: The distribution of recruit-sized oysters, new clocks and gaper densities combined ("Recruits", filled grey circles) and the densities of recruit-sized new clocks and gapers combined ("New clocks", black circles) showing the pre-survey mortality in February 2012. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

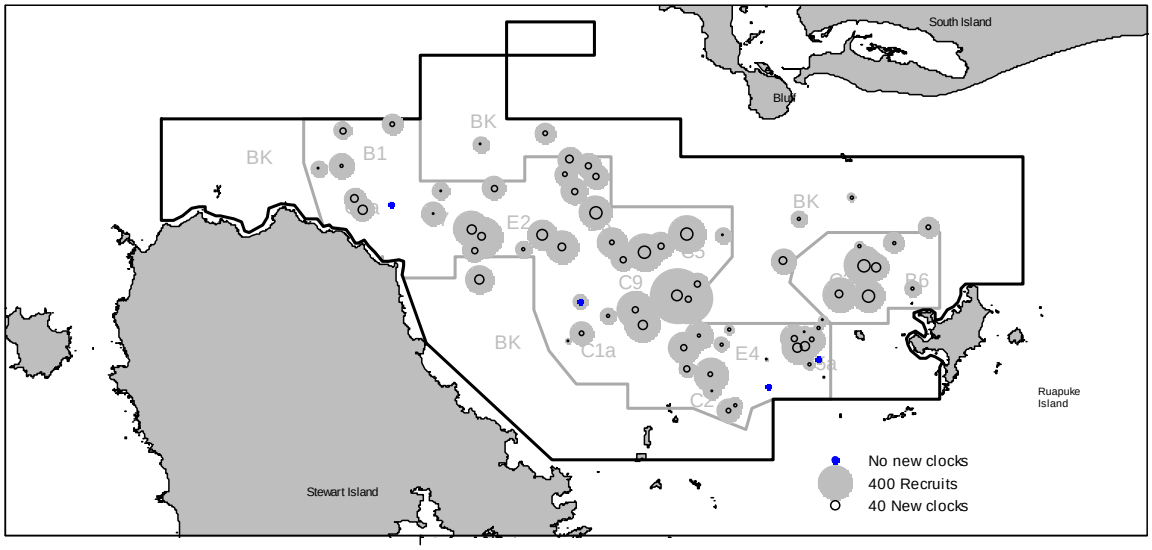

Figure 19: The distribution of recruit-sized oysters, new clocks and gaper densities combined ("Recruits", filled grey circles) and the densities of recruit-sized new clocks and gapers combined ("New clocks", black circles) showing the pre-survey mortality in February 2014. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

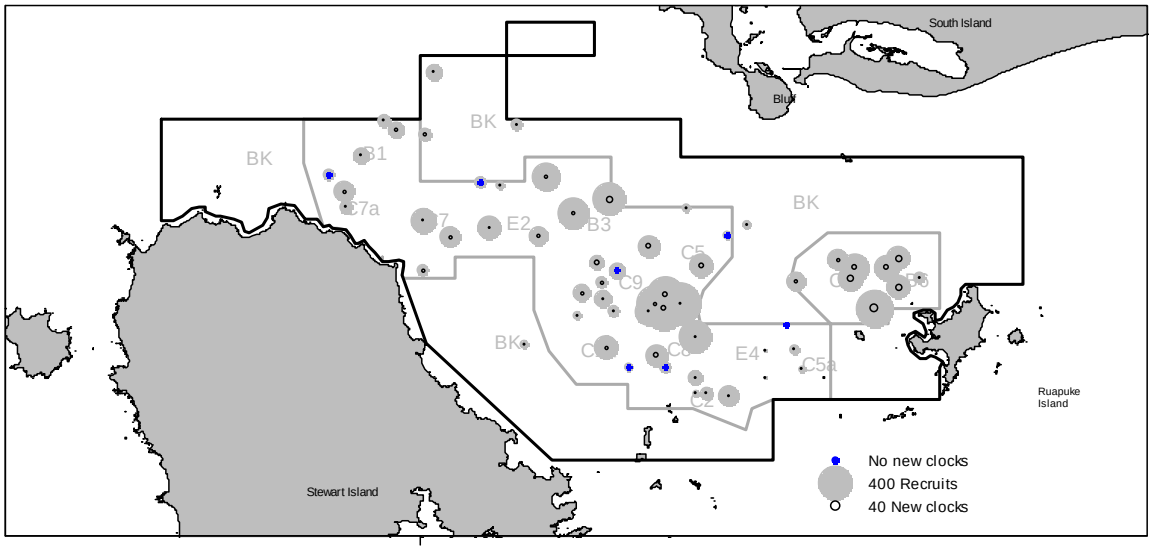

Figure 20: The distribution of recruit-sized oysters, new clocks and gaper densities combined ("Recruits", filled grey circles) and the densities of recruit-sized new clocks and gapers combined ("New clocks", black circles) showing the pre-survey mortality in February 2015. Stations with no recruit-sized new clocks and gapers are shown as filled blue circles.

New clock densities allow pre-survey mortality to be estimated. Estimates of the population sizes for recruit-sized and pre-recruit new clocks in core strata, all background strata combined, and for the whole 2007 stock assessment survey area sampled at random stations in 2015 are shown in Tables 9 and 10 respectively; and for 2012 and 2014 in Appendix 3 (Tables A3.9–A3.12).

Table 9: Recruit-sized new clocks estimated from randomly selected stations from the 2015 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) shaded green for decreases in new clock population size and red for increases.

2015 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.01	0.01	0.51	1.1	0.5	1.8	0.0	2.5	78.2	29.2
B3	3	0.05	0.02	0.37	2.4	1.4	3.4	0.7	4.6	44.7	51.2
B6	5	0.07	0.02	0.25	2.2	1.7	2.7	1.0	3.9	30.0	249.5
C1a	3	0.02	0.01	0.45	0.5	0.3	0.8	0.1	1.2	31.3	137.3
C2	3	0.01	0.00	0.20	0.2	0.1	0.2	0.1	0.3	21.9	35.5
C3	4	0.06	0.01	0.18	1.9	1.6	2.3	1.1	3.1	32.7	121.7
C5	4	0.03	0.02	0.50	1.1	0.6	1.7	0.0	2.5	37.7	142.6
C5a	3	0.01	0.00	0.66	0.1	0.0	0.3	0.0	0.4	23.5	30.0
C7	3	0.03	0.01	0.22	1.2	0.9	1.5	0.6	2.0	36.1	33.5
C7a	3	0.02	0.01	0.53	0.4	0.2	0.6	0.0	0.9	23.6	19.1
C8	5	0.02	0.01	0.51	0.5	0.3	0.7	0.0	1.0	26.8	115.9
C9	9	0.03	0.01	0.24	1.1	0.9	1.3	0.5	1.9	34.5	157.5
E2	4	0.01	0.01	0.53	0.5	0.3	0.8	0.0	1.2	42.8	21.6
E4	3	0.00	0.00	0.00	0.1	0.1	0.1	0.1	0.2	28.0	68.7
Core	55	0.03	0.00	0.11	13.5	13.1	13.9	8.7	20.9	491.8	60.4
BK	5	0.02	0.01	0.41	10.2	6.6	13.8	2.0	20.7	578.4	134.1
All	60	0.02	0.00	0.19	23.7	22.6	24.8	13.0	38.6	1070.2	79.1

Table 10: Pre-recruit-sized new clocks estimated from randomly selected stations from the 2015 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2015 Foveaux Strait oyster survey. The percentage of the 2014 estimate (% of 2014) shaded green for decreases in new clock population size and red for increases.

2015 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2	% of 2014 pop.n
B1	3	0.00	0.00	0.01	0.4	0.4	0.4	0.3	0.6	78.2	29.3
B3	3	0.00	0.00	1.00	0.1	0.0	0.2	0.0	0.2	44.7	36.1
B6	5	0.00	0.00	0.55	0.1	0.1	0.2	0.0	0.3	30.0	36.6
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	31.3	0.0
C2	3	0.01	0.00	0.26	0.1	0.1	0.2	0.1	0.2	21.9	142.5
C3	4	0.00	0.00	0.71	0.2	0.0	0.3	0.0	0.4	32.7	157.8
C5	4	0.00	0.00	0.58	0.1	0.0	0.1	0.0	0.2	37.7	45.6
C5a	3	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	23.5	0.0
C7	3	0.02	0.01	0.66	0.8	0.2	1.3	0.0	1.9	36.1	765.4
C7a	3	0.01	0.01	1.00	0.2	0.0	0.3	0.0	0.5	23.6	37.9
C8	5	0.00	0.00	1.00	0.1	0.0	0.1	0.0	0.2	26.8	51.7
C9	9	0.00	0.00	0.54	0.1	0.0	0.1	0.0	0.2	34.5	74.3
E2	4	0.00	0.00	0.64	0.2	0.1	0.3	0.0	0.4	42.8	77.6
E4	3	0.00	0.00	1.00	0.0	0.0	0.1	0.0	0.1	28.0	45.9
Core	55	0.00	0.00	0.25	2.2	2.1	2.4	1.0	3.9	491.8	62.1
BK	5	0.00	0.00	0.73	2.3	0.8	3.7	0.0	6.1	578.4	133.1
All	60	0.00	0.00	0.39	4.5	4.1	4.9	1.0	9.1	1070.2	84.9

The population size of recruit-sized new clocks in core strata was lower in 2015 (13.5 million, 95% CI 2.0–20.7) than in 2014 (39.4 million, 95% CI 24.7–61.4) and in 2012 (22.4 million, 95% CI 12.8–36.6). Pre-survey mortality of recruit-sized oysters in core strata (Table 11) was lower in 2015 (3.7%) than in 2014 (6.8%), but higher than in 2012 (3.2%). Differences in the population sizes of recruit-sized new clocks in the background strata are less comparable because of the differences in sampling effort between 2012, and 2014 and 2015. Estimates of new clock population size from background strata has a large influence on the population size for the entire 2007 stock assessment survey area. The trend in pre-survey mortality for the entire 2007 stock assessment survey area is similar to that of core strata (Table 11).

There were no strata with recruit-sized new clock densities above $0.1.m^{-2}$ in 2015 (Table 11), fewer than in 2014 (B3, C3, C5, C7a, C8, and C9) and in 2012 (B3 and C7) (Appendix 3). This decline reflects the about 50% reduction in the recruit-sized oyster population since 2012, and decreased pre-survey mortality (Table 11). The proportion of the total summer mortality occurring before and during the survey is likely to change from year to year, hence the level of post survey mortality may reflect the timing of mortality events and may not reflect increases or decreases in total mortality.

The population size of pre-recruit new clocks in core strata in 2015 (2.2 million, 95% CI 1.0–3.9) was lower than in 2014 (3.6 million, 95% CI 2.2–5.7) and in 2012 (8.9 million, 95% CI 5.1–14.4). Pre-survey mortality of pre-recruit oysters in core strata was similar between 2015, 2014 and 2012 (2.4%, 2.4%, and 2.9% respectively, Table 11).

Table 11: Estimates of pre-survey mortality for core strata (Core strata) and the entire 2007 stock assessment area (All area) for recruit-sized and pre-recruit new clocks for the 2012, 2014, and 2015 surveys. Estimates are from randomly selected stations only. Pre-survey mortality (% PS.mort) calculated as the percentage of new clocks (millions) over new clocks and oysters combined (millions).

	Recruit-sized			Pre-recruit		
Core strata						
Year	**Oysters**	**New clocks**	**% PS.mort**	**Oysters**	**New clocks**	**% PS.mort**
2012	688.1	22.4	3.2	297.7	8.9	2.9
2014	538	39.4	6.8	148.4	3.6	2.4
2015	351.4	13.5	3.7	89.2	2.2	2.4
All area						
Year	**Oysters**	**New clocks**	**% PS.mort**	**Oysters**	**New clocks**	**% PS.mort**
2012	918.4	30	3.2	414.3	12	2.8
2014	1020.9	84.1	7.6	226.2	5.3	2.3
2015	509.9	23.7	4.4	122.1	4.5	3.6

4.4 A summary of checks made to ensure consistency amongst qPCR assays between surveys (objective 7)

Quality control of reagents and procedures were undertaken before the analysis of samples in March 2015. A new synthetic standard was designed (by John Mackay, dnature Ltd) incorporating the primer and probe sequences. It differed slightly to the actual Bonamia sequence to allow differentiation (via high resolution melting or sequencing) from a true positive. The standard was synthesised by overlapping PCR, sequenced and provided as 10^6 copies / µl. This standard was serially diluted down to 1 copy per µl in an oyster lysate diluent (oyster DNA extracted using the same method as used for testing and testing free of Bonamia). The duplex assay could reliably detected Bonamia in 2 µl of the lowest dilutions representing an average of two gene copies.

The standard was tested by dnature and on the NIWA BioRad CFX96 to ensure that the internal control was not affecting the sensitivity of bonamia detection. The standard was diluted in water and in diluent (1:15 dilution) of pooled negative oyster tissue which had the internal control amplifying and competing for reagents. Both dilution schemes went out to the same dilution before flatlining showing that the internal control is not affecting the sensitivity of detection.

Aliquots of the 10^3 copies/µl dilution were used as interplate calibrators to permit collation of data among multiple runs. A dilution of 10^3 copies/µl gave cycles of quantification (Cq) of about 26.7 on the BioRad CFX96 used to run the qPCR assays; which equates to an intensity of infection of 2–3 from heart imprints. Quality control of reagent batches was undertaken by dnature: 20X Bonamia qPCR primer/probe mix incorporated primers and probes for the Bonamia target and internal control as well as the BLOCK system to prevent the high level endogenous internal control outcompeting a low level Bonamia target. Resulting lots of this mix were tested on the synthetic template at standard dilutions to ensure that the same sensitivity was maintained (i.e., detection of the 1 copy/µl dilution). Batches of reagents were tested with the synthetic standard to ensure consistency on the NIWA BioRad CFX96.

Thirty five tests of positive controls for both bonamia and β-actin, and corresponding negative controls were undertaken during the qPCR assay run to analyse the 2015 samples. None of the negative controls tested positive. The ranges of positive control values for the FAM (6-carboxyfluorescein) fluorophore used to detect bonamia and the TR (Texas-red, sulforhodamine 101 acid chloride) fluorophore used as a cross check to ensure that the qPCR reaction occurred by detecting DNA from oyster tissue (β-actin) in the sample, were tightly distributed around the expected mean (Figure 21), Cq 26.7 (95%CI 26.6–26.8). Differences in Cq values between the bonamia positive control and β-actin positive control are due to the internal control (IC) block that allows the FAM fluorophore to fluoresce before the TR fluorophore. The IC block causes some variation in the fluorescence levels of the TR fluorophore (Figure 21).

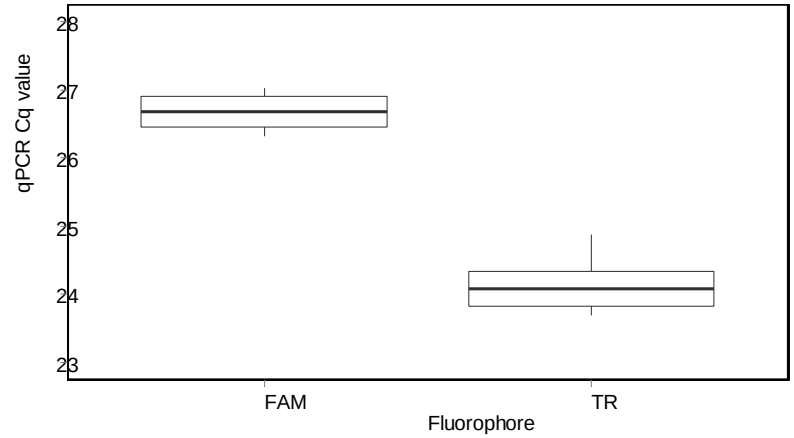

Figure 21: The range of Cq values for positive controls for bonamia FAM (6-carboxyfluorescein) flurophore used to detect bonamia and TR (Texas-red, sulforhodamine 101 acid chloride) flurophore used as a cross check to ensure the qPCR reaction occurred by detecting DNA from oyster tissues in the samples. Differences in CQ values for FAM and TR are caused by an internal control block that allows the FAM to fluoresce before the TR.

4.5 Estimates of the prevalence and intensity of Bonamia in commercial fishery areas (objective 5)

Sampling effectiveness for the prevalence and intensity of infection by bonamia

Samples of 25 recruit and pre-recruit sized oysters were collected from all but six stations in 2015. In all, 1738 samples of heart imprint slides were sampled and archived. This sample comprised 1690 recruit-sized oysters, 25 pre-recruits, and 23 small oysters. Almost all of the samples (97.2%) were of recruit-sized oysters, the same as for 2014 and similar to previous surveys. Only a subsample of these were screened (N = 857). Stations with fewer than 15 recruit and pre-recruit sized oysters (53, N = 2; 29, N = 8; and 54, N = 12) were not used in the analysis of infection.

Matching heart and gill tissue samples were taken for qPCR. Replicate gill tissue samples were also taken and archived for future reference.

Changes to the standard sampling method for the detection of bonamia in oyster tissues

The oyster samples were tested for the presence of bonamia infection using the qPCR method established in 2013 (Maas et al. 2013). The 96 well plate format and the need to run controls only allowed 24 heart tissue samples and 23 gill tissue samples from each station to be run in the initial analysis. Samples that showed anomalies in the qPCR data were rerun. The repeat scores were used in the analysis for presence/absence. Samples that failed a second assay were omitted from the qPCR data analysis, and the corresponding heart imprint slides examined (Table 12). qPCR plate wells H6 and H12 were allocated to positive and negative controls respectively and these data removed from analysis.

After the initial qPCR screening for prevalence of bonamia infection a subset of heart imprint slides were selected (Table 12) to estimate prevalence from heart imprints and the intensity of infection. These slides included:

1. All corresponding heart imprint slides for those qPCR samples that tested positive for bonamia infection in either heart or gill samples.
2. At least three heart imprint samples randomly selected from each station that were qPCR negative.
3. All 25th heart imprint slides were included in the samples screened by histology (for which there are no qPCR data).
4. All corresponding heart imprint slides for heart qPCR samples that did not amplify both fluorophores (flatliners): FAM (6-carboxyfluorescein) used to detect bonamia, and TR (Texas-red, sulforhodamine 101 acid chloride) used as a cross check to ensure that the qPCR reaction occurred by detecting DNA from oyster tissue in the sample.
5. All corresponding heart imprint slides for heart qPCR samples that were “early ampers” , i.e., the samples where either or both fluorophores amplified very early in the cycling (Cq less than 10 cycles).

Improvements in the qPCR method resulted in a reduced number of samples that didn’t amplify or amplified too early in the cycles, and therefore a reduction in the qPCR data omitted from the analysis. There were no heart or gill tissue samples amplifying early in 2015 compared with 0.4% heart tissues and 4.1% gill tissues in 2014. Thirty nine heart tissues (2.3%) and 31 gill tissues (1.9%) didn’t amplify and were omitted in 2015 (Table 12), compared with 5.7% of heart tissues and 6.4% of gill tissues in 2014 (not shown).

There was no bonamia infection detected by heart imprints in the 206 random samples selected from qPCR negative samples, i.e., there were no false negatives.

Table 12: The numbers of samples screened for bonamia using qPCR (heart and gill tissues) and heart imprints in 2015. qPCR samples were run first on the first 24 heart and first 23 gill samples from the 25 oysters in heart imprint samples taken from each station. The summary of qPCR samples gives the total numbers of heart and gill samples tested (Sample (N)), the numbers of samples omitted because they failed inclusion criteria after repeat sampling (Omitted), the numbers of valid samples (qPCR.N), and those that tested positive (Positive (<35Cq)) and those where no bonamia DNA was detected (Negative (>35 Cq)). The number of heart imprint slides screened that included qPCR positives, randomly selected negatives, and the 25th slide in each sample (Number of slides). The summary statistics for qPCR infection give the numbers of qPCR positive and negative samples (heart tissue only positives, gill tissue only positives, heart and gill tissue only positives, the numbers of randomly selected qPCR negatives, and all qPCR positive samples (heart and or gill tissues positives)) and the numbers of corresponding heart imprint samples that scored positive for bonamia infection. There were no qPCR false negatives.

qPCR samples

Bonamia infection	**Sample (N)**	**Omitted**	**qPCR.N**	**Positive <35Cq)**	**Negative (>35 Cq)**
Heart	1 672	39	1 633	380	1 253
Gill	1 605	31	1 574	432	1 142
Both H&G				324	

Histology samples

Number of slides read	857

qPCR infection	**Sample (N)**	**Histo+ve**
Heart & gill qPCR +ve	324	238
Heart qPCR +ve	380	248
Gill qPCR +ve	432	240
Selection qPCR -ve	206	0
all qPCR +ve	482	251

Comparison of qPCR and heart imprint methods.

The qPCR method shows higher sensitivity in the detection of bonamia than heart imprints (Maas et al. 2013). A large number of histological samples in which bonamia could not be detected scored positive using qPCR (Figure 22). Similar numbers of heart and gill tissue samples tested positive for bonamia, but gill tissues generally produced lower Cq values than heart tissues (Figure 22), which may either mean that they are more sensitive (provide for better amplification) or they are amplifying external contamination of gill tissue by water-borne bonamia particles. Heart tissues may provide better estimates of oyster infection and gill tissues better estimates of pathogen presence in the environment.

The quantification of bonamia cannot be directly compared between qPCR and histology as the qPCR Cq values estimate numbers of Bonamia ITS region copies while histology scores categorise the average numbers of bonamia cells in oyster haemocytes. A small number of samples with anomalous qPCR results were included in the subset of heart imprint samples examined for infection. Of the heart imprint samples that scored positive for bonamia infection, one corresponding heart and one gill sample were negative, i.e. Cq>35 (Figure 22). There may have been some inhibition of the qPCR reaction in these samples, possibly because of the small tissue sample sizes (from small oysters), incomplete digestion of tissue, or significant loss of blood during the heart imprint process.

The numbers of outliers are generally small. Boxplots of Cq values for both heart and gill tissues showed a decreasing trend with increasing intensity of bonamia infection estimated from heart imprints i.e.,

bonamia scores increasing from 1 to 5, but some overlap in the Cq values of samples with fatal and non-fatal infections (Figure 22).

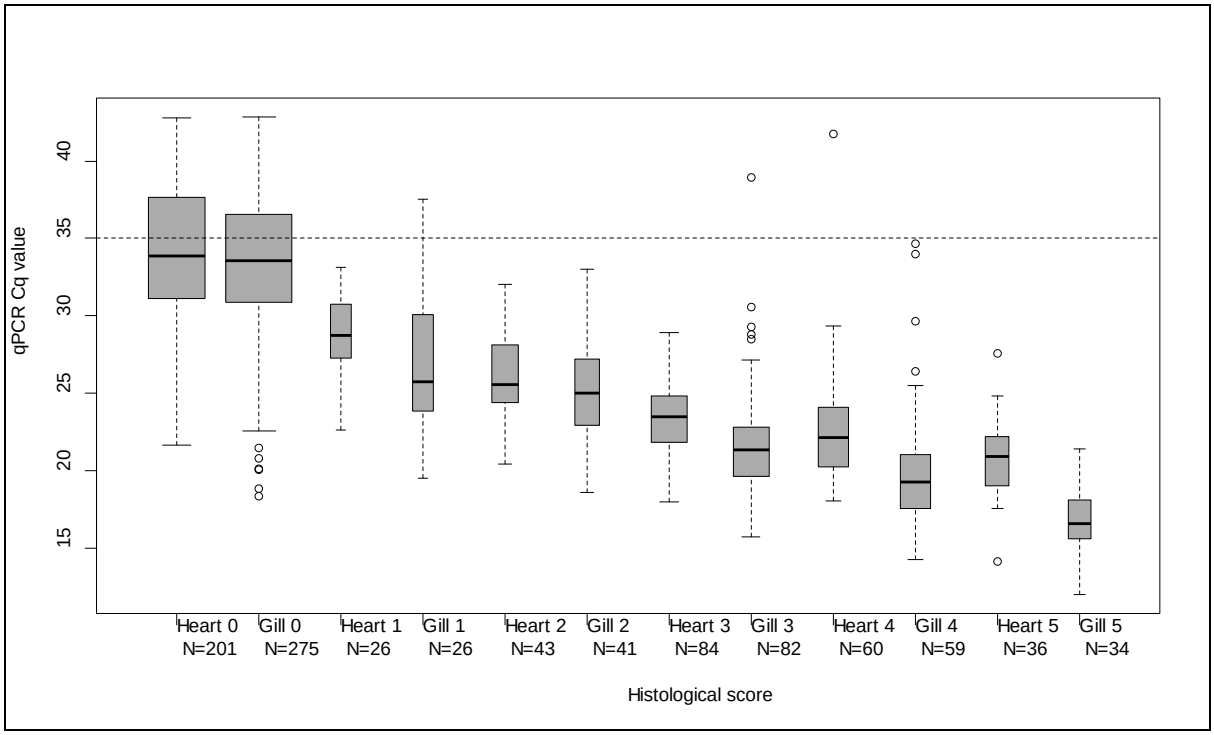

Figure 22: Boxplots of Cq values from qPCR analysis of the bonamia ITS region for paired samples of heart and gill tissues by histological score from the February 2015 survey. Cut-off levels set at 35 Cq (dashed line). Outliers for heart and gill tissues denote generally weak reactions most likely caused by inhibitors in the crude samples. Box plots show medians (solid lines), boxes 25 and 75 percentiles, whiskers at 95 percentiles, and outliers shown as black circles above and below whiskers.

Prevalence and intensity of infection in oysters by bonamia

Of the 857 heart imprint slides examined for bonamia infection, 55 slides were for the 25th oyster for which there were no qPCR data and these data were excluded from comparisons. Because there were no qPCR false negatives detected and qPCR is more likely to detect bonamia infections, we assumed that those heart imprint slides that were not examined, but were qPCR negative were also negative for heart imprints.

Estimates of prevalence at each random station (N = 57) differed with the sampling method (histology and qPCR) and amongst the heart and gill tissues (Table 13). Infection intensity was estimated from histology using the categorical score of Diggles et al. (2003).

Table 13: Mean and median prevalence (Prev (%)) and intensity estimated by histology (heart imprints), and prevalence from heart (qPCR.heart) and gill (qPCR.gill) tissues using qPCR from random stations sampled for bonamia with more than 15 recruit and pre-recruit oysters in the sample (N=57).

	Histology Prev (%)	Histology Intensity	qPCR.heart Prev.H (%)	qPCR.gill Prev.G (%)
N	57	52	57	
mean	15.3	3.2	22.4	25.6
median	12.0	3.1	20.8	25.0
s.d.	11.6	0.7	15.4	16.5
L95%CI	12.3	3.0	18.4	21.3
U95%CI	18.3	3.5	26.4	29.9

Heart imprints underestimate the true prevalence of bonamia infection, and are lower than the qPCR estimates (Table 13). The mean prevalence from heart imprints in February 2015 (15.3%) was similar to 2014 (15.2%), but higher than for other recent February surveys (2009–2012, 8–12%). qPCR analysis of heart tissues was more sensitive than heart imprints, but less sensitive than qPCR analysis of gill tissues (Table 13). Mean prevalence from qPCR analysis of heart tissues was 22.4% (Table 13), lower than in February 2014 (25.0%), but higher than for 2013 (19.6 %). The mean prevalence from qPCR analysis of gill tissues was 25.6% (Table 13), lower than in 2014 (28.9%) and in 2013 (30.5 %). We cannot rule out external contamination of gill tissues by water borne bonamia particles, especially at this time of year when disease mortality is highest. Details of recruit-sized oysters and densities by station, and bonamia infection status from histology and heart and gill tissue qPCR samples are shown in Table 14.

Of the 1423 slides taken from random stations with more than 15 recruit and pre-recruit sized oysters in 2015, a subset of 674 heart imprint slides were examined for bonamia. The remaining 749 slides were from oysters screened using qPCR and were not infected. In 2015, 84.7% of samples had no detectable infection, similar to 2014 (85.8%), but lower than for 2010 to 2013 (90 %, 88 %, 89 %, and 88% respectively).

Of the remaining 15.3% of oysters with detectable infections in 2015, 4.3 % had light category 1 and 2 infections (3–5% in 2010–2014), and 11.0% had category 3 and higher infections (7–9 % in 2010–2014) which are normally fatal. The prevalence of infection ranged from 0% to 56% in 2015 with no detectable infection at five of the 57 stations, and peak prevalence the highest since 2010. The median prevalence of 12% was the same as in 2014, but was higher than for 2011–2013 (5–8%).

Intensity of infection was determined from heart imprints to maintain the time series of bonamia survey data. The median infection for stations was category 3.1 (Table 13), slightly higher than in 2014 (3.0. Infection levels were generally high with 52% or more of infected oysters expected to die within a few weeks of sampling in 2015, similar to 2014 (50%). The mean intensity of infection at stations (3.2) was similar for the years 2009–2014. The percentage of stations in 2015 with category 3 and higher infections (90%) is higher than in 2014 (81%), and ranged from 67%–94% between 2009 and 2014 (the coverage of sampling and numbers of stations sampled differed between years). The intensity of infection was highly variable within stations, and patterns of variation were similar across the fishery area, in all years.

The prevalence of infection at all sample stations is similar and consistently variable between 2007 and 2012, with an increase in the lower quartile range of prevalence in 2013 and a further increase in prevalence in 2014 which remained similar in 2015 (Figure 23). In 2014, the upper 50% percentiles had a higher spread of infection, with a median prevalence at about 13%, and 25% of the stations had a prevalence of infection above 24%. In 2015, 61.4% of stations had a prevalence of infection above 12%, and 25% of the stations above 24%, similar to 2014. qPCR samples showed higher prevalence than histology, and qPCR gill samples higher than qPCR heart samples (Figure 23). Each of the three estimates (histology, qPCR analysis of heart and gill tissues) of the distribution of the prevalence in infection were similar for 2014 and 2015 (Figure 23). The prevalence of infection estimated from qPCR shows that 75% of stations had a prevalence of infection higher than 12% in 2014, and 79% of stations in 2015 (Figure 23). The range of mean intensity of infection (stations with bonamia infection only) in 2014 was similar to 2013, and to the longer inter-annual trend (Figure 24). The mean intensity of infection was generally higher in 2015, similar to that in 2009, suggesting higher levels of fatal infections (Figure 24).

The percentages of stations with no detectable infection decreased from 2009 to a six year low in 2015 (Figure 25). The percentage prevalence of infection by station was generally low in 2009, but increased markedly in 2014, and further in 2015: the distribution of prevalence shows a marked shift to the right showing more stations with higher prevalence (Figure 25). Mean intensity of infection has been generally high since February 2009 (Figure 26), and in 2014 and 2015 there was also a marked shift to the right from where it was over the last six years – stations generally had higher mean intensity of

infection (Figure 26). The differences in mean intensity between February 2009 and 2015 may reflect rapid seasonal intensification of infection rather than inter-annual differences, and may be associated with female oyster spawning cycles and the timing of the re-absorption of ova post spawning. The increased prevalence and high intensity of infection was likely to lead to higher levels of bonamia mortality over the summer of 2014 and 2015.

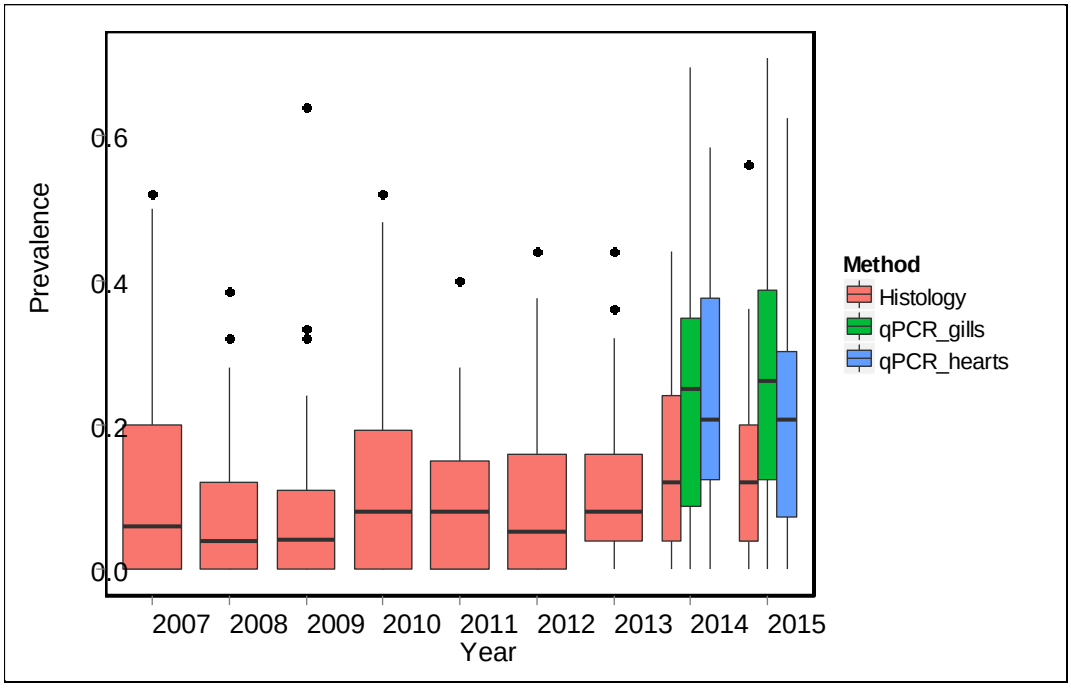

Figure 23: Boxplots of the mean prevalence of bonamia infection 2007–2015. The mean prevalence of infection at all stations determined from histology, and for qPCR heart tissues (qPCR_hearts) and gill tissues (qPCR_gills) in 2014 and 2015. Medians shown as solid lines, boxes represent 50 percentiles and whiskers 95 percentiles, and outliers as filled black circles.

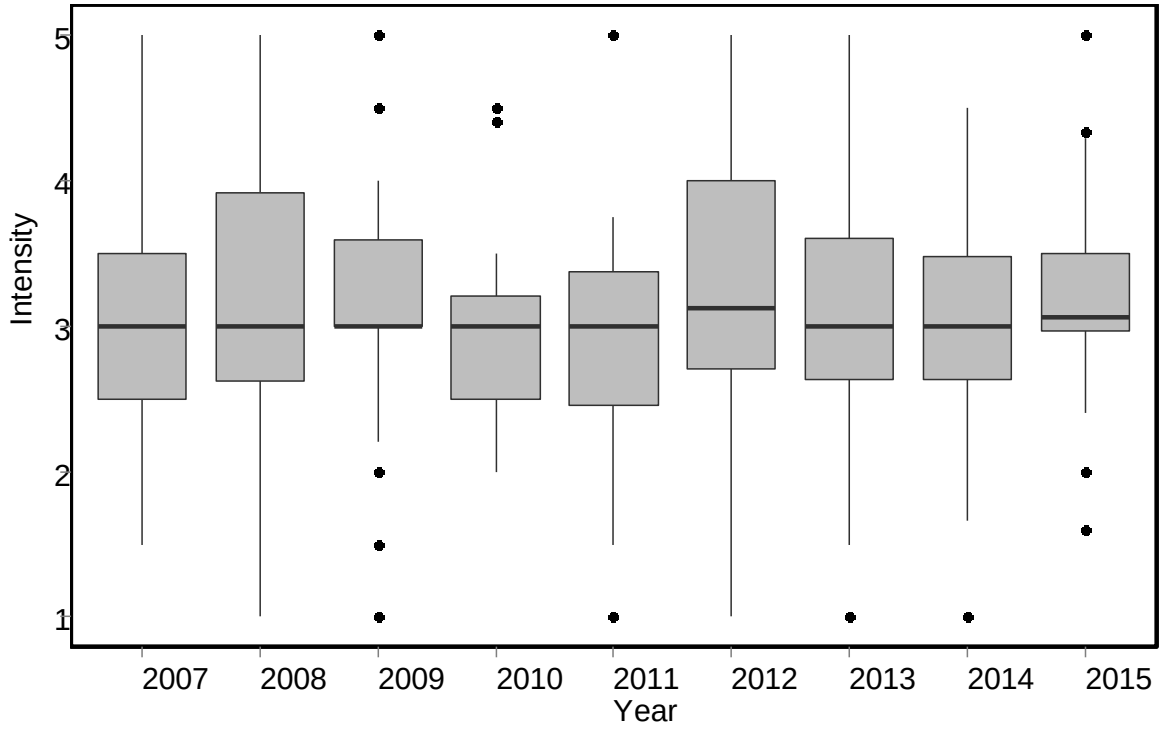

Figure 24: Boxplots of the mean intensity of bonamia infection 2007–2015. The mean intensity of infection at all stations determined from histology. Medians shown as solid lines, boxes represent 50 percentiles and whiskers 95 percentiles, and outliers as filled black circles.

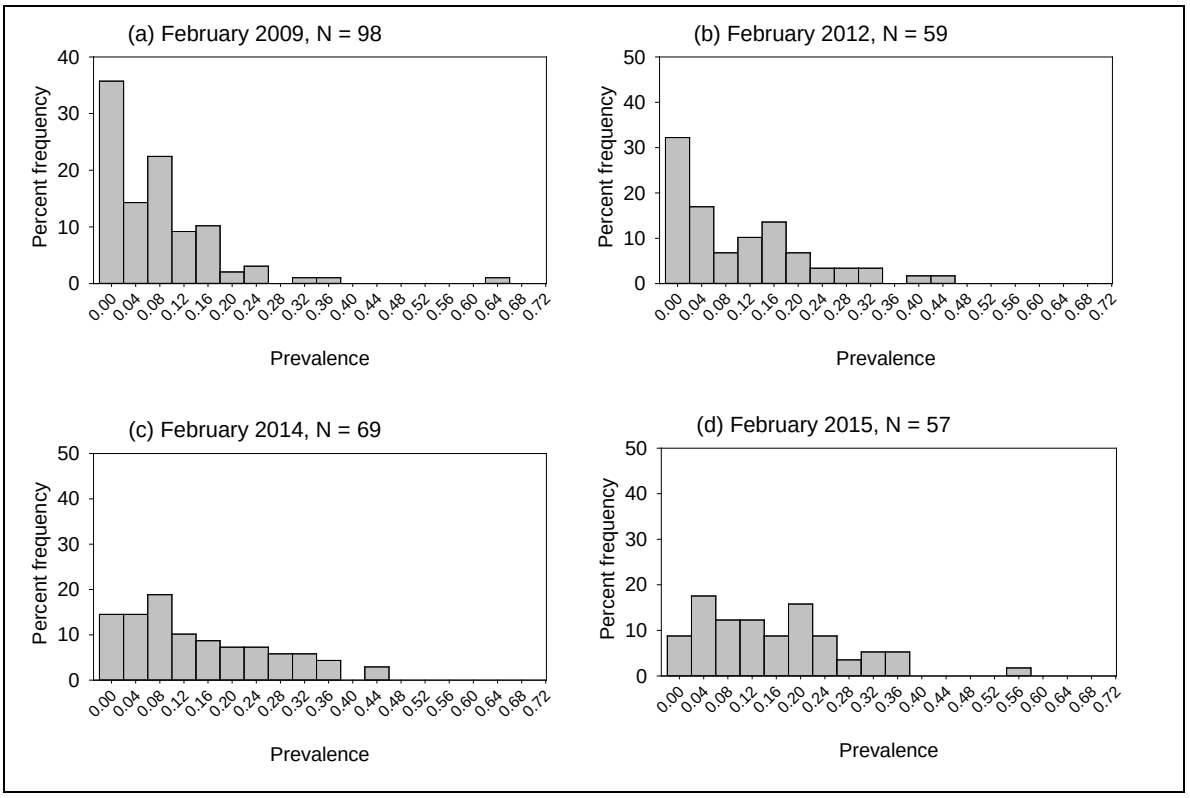

Figure 25: Percentage prevalence of bonamia infection at stations sampled in (a) February 2009, (b) February 2012, (c) February 2014, and (d) February 2015.

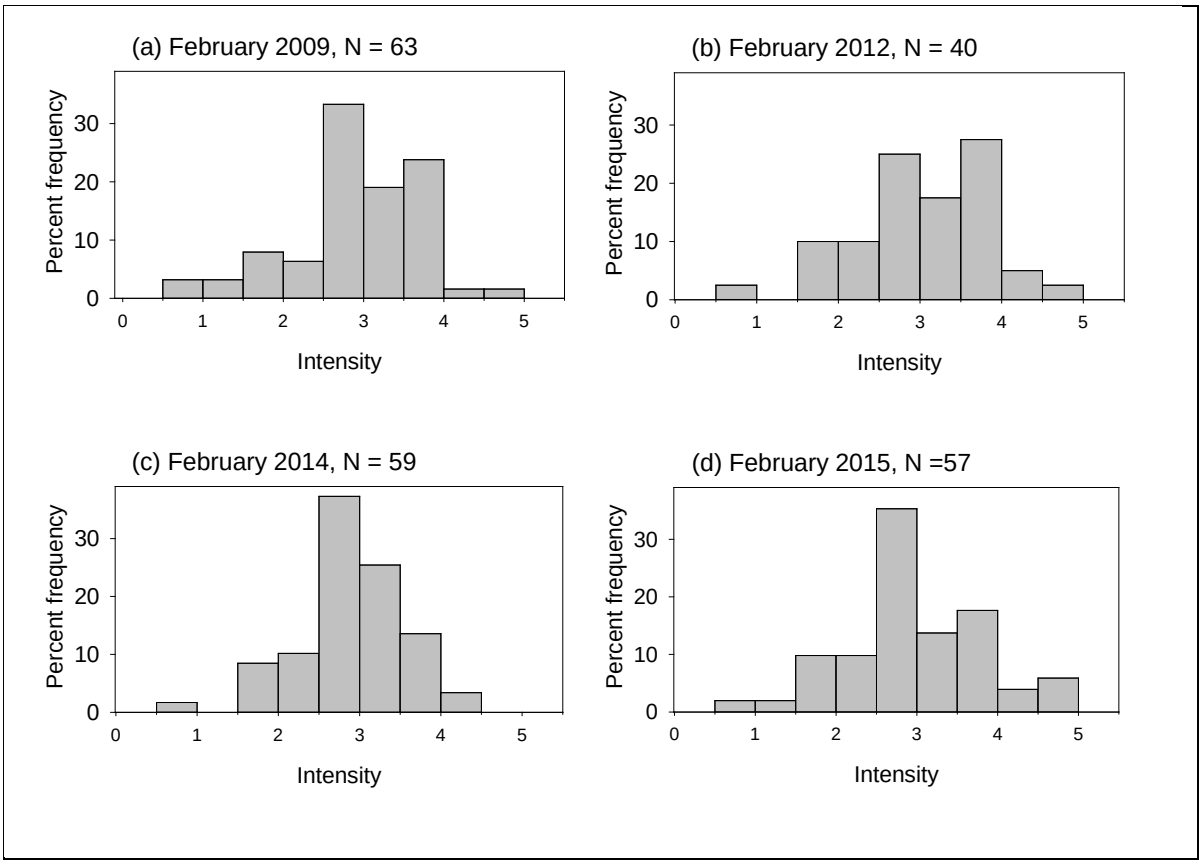

Figure 26: Percentage mean intensity of bonamia infection at stations sampled in (a) February 2009, (b) February 2012, (c) February 2014, and (d) February 2015.

Table 14: Details of recruit-sized oysters and densities by station; the numbers of histology samples (heart imprint slides) and numbers of uninfected (Un.inf) samples, samples with non-fatal infections (NF.inf) and fatal infections (Fatal.inf) based on category 3 and higher infections, and the prevalence and intensity of infection from heart imprints. The numbers of heart (Heart.No.) and gill (Gill.No) tissues where qPCR assays meet criteria for data inclusion and the prevalence of bonamia infection detected in heart (Prev.H (%)) and gill (Prev.G (%)) tissues from the February 2015 survey.

			Histology						qPCR assays			
Station	**Recruits**	**Density**	**Total**	**Un.inf**	**NF.inf**	**Fatal.inf**	**Prev (%)**	**Intensity**	**Heart.No.**	**Prev.H (%)**	**Gill.No**	**Prev.G (%)**
1	103	0.08	25	20	1	4	20.0	3.2	24	16.7	23	16.7
2	94	0.08	25	25	0	0	0.0	NA	24	0.0	23	4.2
3	68	0.06	25	22	1	2	12.0	3.0	24	16.7	23	20.8
4	337	0.28	25	25	0	0	0.0	NA	24	0.0	23	0.0
5	376	0.31	25	20	3	2	20.0	2.4	24	25.0	23	33.3
6	299	0.24	25	23	1	1	8.0	2.5	24	20.8	23	33.3
7	224	0.18	25	16	0	9	36.0	3.2	24	54.2	23	54.2
8	195	0.16	25	11	2	12	56.0	3.3	24	62.5	23	50.0
9	508	0.42	25	23	1	1	8.0	3.5	24	16.7	23	12.5
10	33	0.03	25	22	1	2	12.0	3.3	24	29.2	23	45.8
11	58	0.05	25	18	0	7	28.0	4.3	24	41.7	23	54.2
12	103	0.08	25	24	0	1	4.0	3.0	24	8.3	23	16.7
13	53	0.04	25	24	0	1	4.0	4.0	24	4.2	23	4.2
14	32	0.03	25	16	2	7	36.0	3.1	24	41.7	23	50.0
15	26	0.02	25	24	0	1	4.0	5.0	24	4.2	23	12.5
16	201	0.16	25	22	0	3	12.0	4.3	24	25.0	23	29.2
17	45	0.04	25	24	0	1	4.0	3.0	24	4.2	23	12.5
18	62	0.05	25	16	2	7	36.0	3.6	24	50.0	23	45.8
20	17	0.01	25	21	2	2	16.0	2.5	24	29.2	23	29.2
21	131	0.11	25	20	3	2	20.0	2.4	24	37.5	23	33.3
22	151	0.12	25	17	2	6	32.0	3.4	24	29.2	23	29.2
23	229	0.19	25	20	2	3	20.0	3.6	24	58.3	23	70.8
24	271	0.22	24	19	4	1	20.8	1.6	24	33.3	23	29.2

			Histology						qPCR assays			
Station	**Recruits**	**Density**	**Total**	**Un.inf**	**NF.inf**	**Fatal.inf**	**Prev (%)**	**Intensity**	**Heart.No.**	**Prev.H (%)**	**Gill.No**	**Prev.G (%)**
25	198	0.16	25	20	2	3	20.0	3.4	24	29.2	23	41.7
26	32	0.03	25	23	1	1	8.0	3.5	23	13.0	23	13.0
27	32	0.03	25	24	0	1	4.0	5.0	24	12.5	23	16.7
28	16	0.01	22	18	0	4	18.2	3.5	22	27.3	22	22.7
29	3	0.00	8	7	0	1	12.5	3.0	NA	NA	NA	NA
30	41	0.03	25	24	0	1	4.0	4.0	24	4.2	23	8.3
31	153	0.13	25	21	3	1	16.0	2.0	24	20.8	23	20.8
33	238	0.19	25	23	1	1	8.0	2.5	24	8.3	23	16.7
35	56	0.05	25	25	0	0	0.0	NA	24	0.0	23	0.0
36	164	0.13	25	24	1	0	4.0	2.0	24	4.2	23	4.2
37	399	0.33	25	22	1	2	12.0	3.0	24	33.3	23	29.2
38	183	0.15	25	21	2	2	16.0	3.0	24	20.8	23	37.5
39	24	0.02	25	23	1	1	8.0	2.5	24	4.2	23	4.2
40	55	0.04	25	21	1	3	16.0	3.0	24	16.7	23	12.5
41	491	0.40	25	25	0	0	0.0	NA	24	4.2	23	4.2
42	87	0.07	25	23	0	2	8.0	3.5	24	8.3	23	8.3
43	119	0.10	25	22	0	3	12.0	3.0	24	16.7	23	16.7
44	118	0.10	25	17	2	6	32.0	3.6	24	41.7	23	45.8
45	440	0.36	25	17	4	4	32.0	2.8	24	29.2	23	33.3
46	91	0.07	25	25	0	0	0.0	NA	24	8.3	23	8.3
47	642	0.53	25	20	2	3	20.0	3.0	24	20.8	23	29.2
48	599	0.49	25	22	1	2	12.0	3.0	24	20.8	23	20.8
49	24	0.02	25	24	0	1	4.0	5.0	24	8.3	23	12.5
50	134	0.11	25	19	0	6	24.0	3.5	24	25.0	23	29.2
51	56	0.05	25	18	3	4	28.0	2.9	24	33.3	23	41.7
52	192	0.16	25	20	1	4	20.0	3.0	24	25.0	23	41.7
53	2	0.00	2	2	0	0	0.0	NA	NA	NA	NA	NA
54	3	0.00	11	11	0	0	0.0	NA	NA	NA	NA	NA
55	80	0.07	25	19	1	5	24.0	2.8	24	45.8	23	41.7

			Histology						qPCR assays			
Station	**Recruits**	**Density**	**Total**	**Un.inf**	**NF.inf**	**Fatal.inf**	**Prev (%)**	**Intensity**	**Heart.No.**	**Prev.H (%)**	**Gill.No**	**Prev.G (%)**
64	46	0.04	25	22	1	2	12.0	3.3	23	26.1	23	26.1
65	211	0.17	25	24	0	1	4.0	5.0	20	15.0	20	10.0
75	146	0.12	25	23	1	1	8.0	3.0	24	12.5	23	16.7
82	170	0.14	25	20	2	3	20.0	2.8	24	20.8	23	25.0
94	49	0.04	25	21	0	4	16.0	4.0	24	33.3	23	50.0
95	66	0.05	25	19	2	4	24.0	3.0	24	33.3	23	29.2
99	283	0.23	25	24	0	1	4.0	3.0	24	8.3	23	8.3
100	51	0.04	25	19	1	5	24.0	3.2	24	37.5	23	45.8
T1	192	0.16	25	17	2	6	32.0	3.0	24	37.5	23	33.3
T2	348	0.28	25	24	0	1	4.0	4.0	24	8.3	23	8.3
T3	263	0.22	25	16	5	4	36.0	2.3	22	45.5	22	45.5
T4	313	0.26	25	23	1	1	8.0	3.0	24	45.8	23	29.2
T5	510	0.42	25	22	0	3	12.0	3.3	24	16.7	23	16.7
T6	51	0.04	25	24	1	0	4.0	1.0	24	4.2	23	4.2
T7	117	0.10	25	16	4	5	36.0	2.7	24	58.3	23	50.0
T8	446	0.36	25	17	1	7	32.0	3.8	24	45.8	23	58.3
T9	240	0.20	25	23	0	2	8.0	3.5	24	16.7	23	29.2
T10	17	0.01	19	19	0	0	0.0	NA	19	10.5	19	10.5
T11	24	0.02	25	24	0	1	4.0	4.0	24	8.3	23	8.3
T12	283	0.23	25	22	0	3	12.0	3.0	24	16.7	23	54.2

Changes in the distribution of prevalence and intensity of bonamia infection

The distribution of prevalence estimated from heart imprints and from qPCR analysis of heart and gill tissues in 2014 (Figure 27) and 2015 (Figure 28) shows similar patterns of distribution with the qPCR tissues showing higher sensitivity than heart imprints.

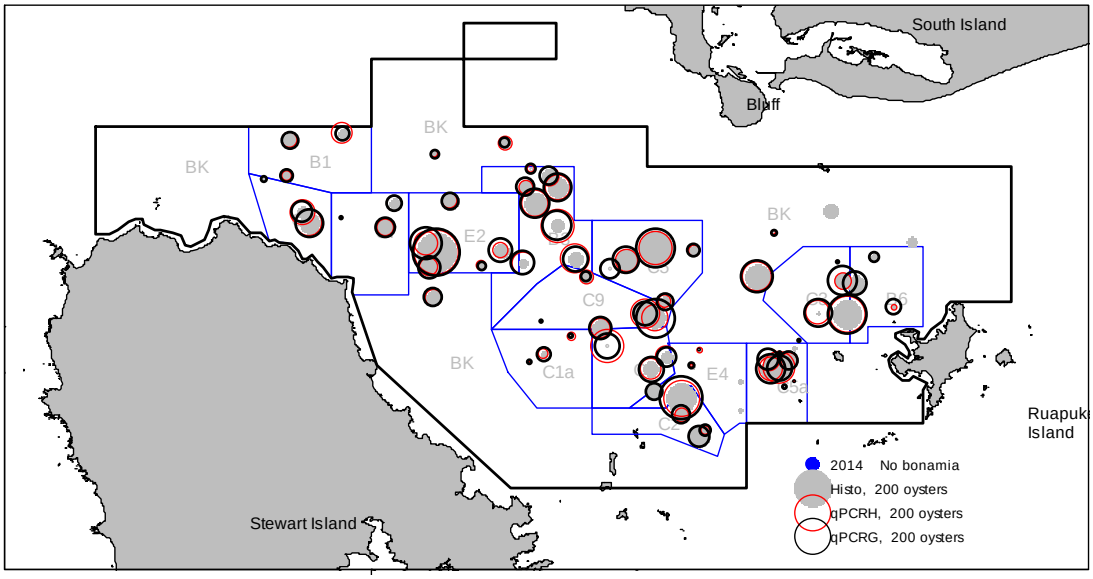

Figure 27: The distributions of bonamia infection in February 2014 estimated from heart imprints, and qPCR analysis of heart and gill tissues. Numbers of oysters with bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles), qPCR heart tissues (qPCRH, open red circles), and qPCR gill tissues (qPCRG, open black circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line), the February 2014 survey strata (blue lines), and the stratum labels in grey.

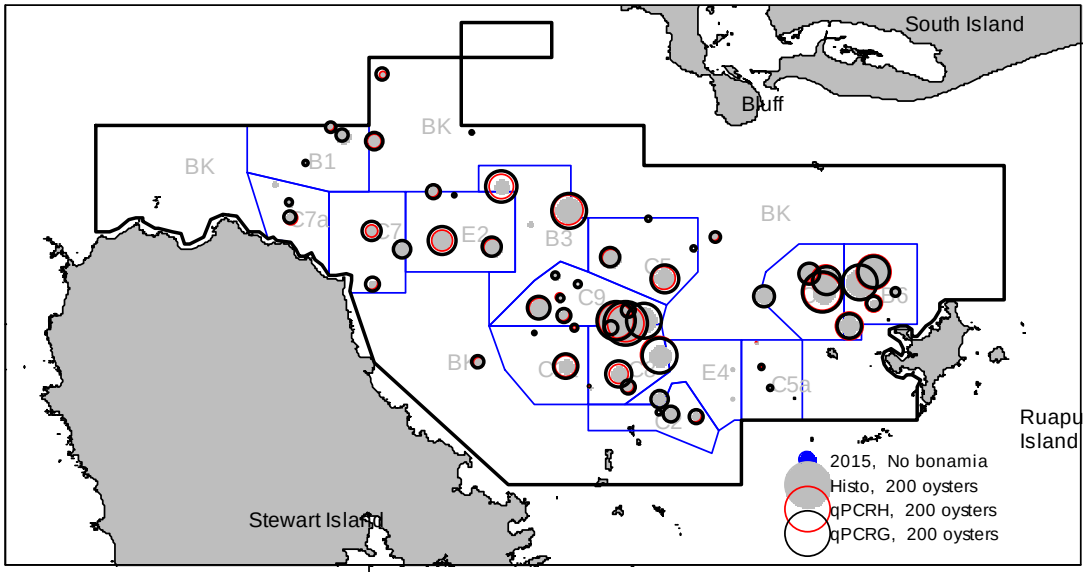

Figure 28: The distributions of bonamia infection in February 2015 estimated from heart imprints, and qPCR analysis of heart and gill tissues. Numbers of oysters with bonamia infection (intensity categories 1–5 combined) from heart imprints (Histo, filled grey circles), qPCR heart tissues (qPCRH, open red circles), and qPCR gill tissues (qPCRG, open black circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line), the February 2015 survey strata (blue lines), and the stratum labels in grey.

The prevalence of infection was highest in eastern, southern, and western fishery areas in February 2012, with little infection in the central fishery areas where oyster density was high (Figure 29). In areas with relatively high infection, bonamia infection was widespread and patchy, the prevalence and intensity of infection were highly variable at small spatial-scales. Stations with high prevalence and high intensity of infection in 2012 were interspersed amongst stations with no detectable infection. There was a marked increase in bonamia infection in February 2014 from 2012, prevalence of infection was higher and more widespread, and the intensity of infection had increased markedly, especially in the commercially important central fishery areas (Figure 30). The distribution of recruit-sized oyster density and infection in February 2014 (Figure 30) showed the marked effects of bonamia mortality between the 2012 and 2014 surveys. Prevalence of infection was widespread, and highly variable at small spatial-scales with some stations having a relatively high prevalence of infection. The intensity of infection also varied. Some stations showed high numbers of fatally infected oysters (Figure 30). The prevalence of infection was widespread, but variable at small spatial scales in 2015 (Figure 31) with high levels of fatal infection, especially in areas with relatively high density and areas with no detectable infection interspersed amongst areas with high levels of infection. Bonamia mortality had further reduced oyster density in 2015 (Figure 31). Oyster density had decreased in most areas between 2012 and 2015 (see Figure 10).

Patterns in the distribution of prevalence and intensity of infection between 2012 and 2014 were not consistent with patterns in the distribution of oyster dredging from fishers' logbook data or with oyster density from survey data; there were areas of high oyster density with a relatively high prevalence and intensity of infection in areas that were not fished since 2008 because of the low meat quality there.

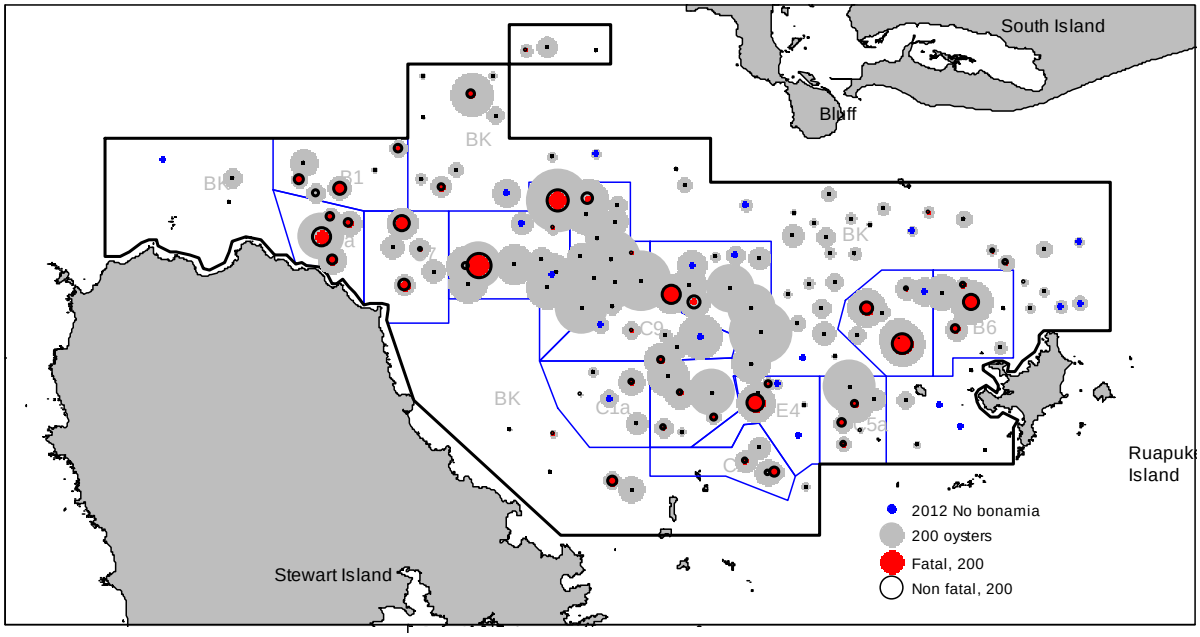

Figure 29: The distributions of oysters and bonamia infection in February 2012. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line), the February 2014 survey strata (blue lines), and the stratum labels in grey.

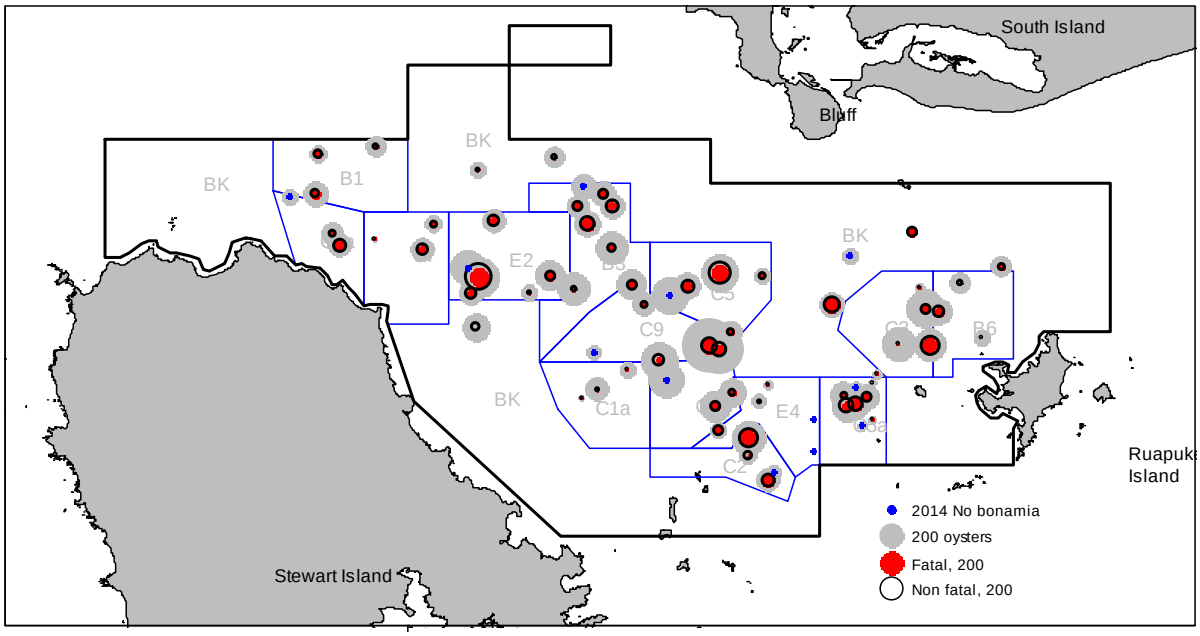

Figure 30: The distributions of oysters and bonamia infection in February 2014. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line), the February 2014 survey strata (blue lines), and the stratum labels in grey.

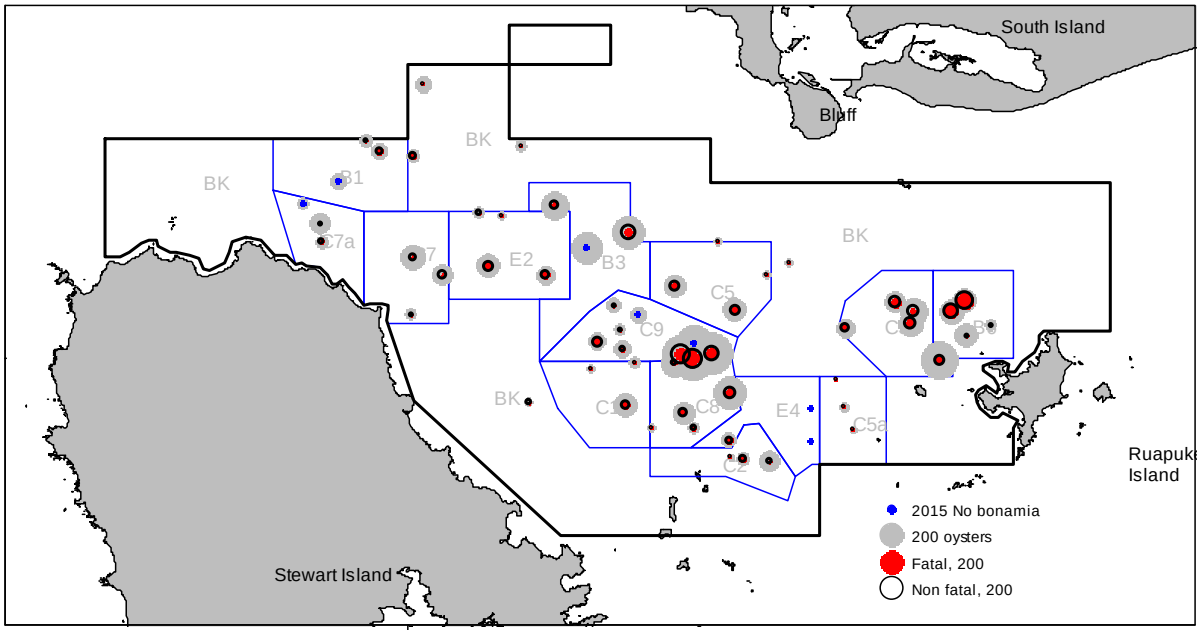

Figure 31: The distributions of oysters and bonamia infection in February 2015. Numbers of oysters (filled grey circles), numbers of oysters with bonamia infection (intensity categories 1–5 combined, open black circles); and fatal infections (intensity categories 3–5 combined, filled red circles). Stations with no bonamia (filled blue circles). The 2007 survey area (black outer line), the February 2015 survey strata (blue lines), and the stratum labels in grey.

The total numbers of recruit-sized oysters infected with bonamia

Estimates of the total numbers of recruit-sized oysters infected with bonamia from heart imprints were scaled up from the catches at randomly selected stations. Table 15 gives population estimates of recruit-sized oysters with bonamia infection (categories 1–5) in 2015, and Table A4.1 (Appendix 4) gives estimates for 2012 and 2014 for comparison. The estimate for core strata declined from 89.5 million (95% CI 50.8–146.1) in 2014 to 49.8 million (95% CI 29.7–78.2) recruit-sized oysters in 2015, and this decline of 44% is higher than that for the recruit-sized oyster population (35%) suggesting that prevalence may be waning. Estimates of the total numbers of recruit-sized oysters with non-fatal and fatal infections are given in Tables 16 and 17 respectively. In 2015, fatal infections were 63% of all infections and were estimated to reduce the recruit-sized oyster population by 31.6 million (95% CI 18.8–48.7). Four of the fourteen strata (B6, C9, E2, and C3) accounted for over half of the projected mortalities.

The estimate for infected oysters over the whole of the 2007 survey area in 2015 was 71.3 million (95% CI 42.6–113.3), lower than in 2014 with 176.1 million (95% CI 63.9–325.3). These figures should be viewed with caution as there were only five stations sampled in the background stratum that accounted for 54.0% of the survey area.

Table 15: Scaled up estimates of the population size of recruit-sized oysters with bonamia infection (prevalence) estimated by heart imprints for the core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

2015 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.05	0.03	0.62	3.6	0.0	8.9	78.2
B3	3	0.16	0.11	0.67	7.2	0.0	18.2	44.7
B6	5	0.24	0.10	0.42	7.1	1.2	14.4	30.0
C1a	3	0.04	0.04	0.84	1.3	0.0	3.9	31.3
C2	3	0.06	0.03	0.47	1.3	0.1	2.8	21.9
C3	4	0.22	0.03	0.15	7.0	4.2	11.0	32.7
C5	4	0.09	0.05	0.52	3.5	0.0	8.0	37.7
C5a	2	0.10	0.05	NA	NA	NA	NA	NA
C7	3	0.08	0.02	0.28	3.0	1.2	5.4	36.1
C7a	3	0.04	0.02	0.61	0.9	0.0	2.1	23.6
C8	5	0.10	0.04	0.39	2.6	0.6	5.2	26.8
C9	9	0.22	0.09	0.41	7.6	1.5	15.4	34.5
E2	4	0.10	0.04	0.39	4.5	1.0	9.0	42.8
E4	1	0.14	0.13	NA	NA	NA	NA	NA
Core total	49	0.11	0.02	0.15	49.8	29.7	78.2	440.3
Background strata	5	0.04	0.01	0.35	21.5	6.2	41.6	578.4
Survey total	54	0.07	0.01	0.15	71.3	42.6	113.3	1018.7

Table 16: Scaled up estimates of the population size of recruit-sized oysters with non-fatal infections (category 1 and 2) estimated by heart imprints for the core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

2015 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.01	0.01	0.53	0.9	0.0	2.0	78.2
B3	3	0.09	0.07	0.71	4.2	0.0	10.8	44.7
B6	5	0.04	0.02	0.57	1.1	0.0	2.6	30.0
C1a	3	0.00	0.00	0.00	0.0	0.0	0.0	31.3
C2	3	0.02	0.01	0.34	0.4	0.1	0.8	21.9
C3	4	0.11	0.04	0.34	3.6	1.2	6.9	32.7
C5	4	0.04	0.02	0.53	1.4	0.0	3.2	37.7
C5a	2	0.10	0.05	NA	NA	NA	NA	NA
C7	3	0.05	0.03	0.57	1.6	0.0	3.8	36.1
C7a	3	0.02	0.01	0.51	0.5	0.0	1.0	23.6
C8	5	0.03	0.02	0.52	0.9	0.0	1.9	26.8
C9	9	0.08	0.04	0.50	2.9	0.0	6.4	34.5
E2	4	0.02	0.01	0.58	0.7	0.0	1.7	42.8
E4	1	0.14	0.13	NA	NA	NA	NA	NA
Core total	49	0.04	0.01	0.21	18.2	9.7	30.3	440.3
Background strata	5	0.00	0.00	0.66	2.2	0.0	5.6	578.4
Survey total	54	0.02	0.00	0.20	20.4	10.9	33.5	1018.7

Table 17: Scaled up estimates of the population size of recruit-sized oysters with fatal infections (category 3–5) estimated by heart imprints for the core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

2015 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.04	0.02	0.66	2.8	0.0	7.0	78.2
B3	3	0.07	0.04	0.63	3.1	0.0	7.4	44.7
B6	5	0.20	0.09	0.46	6.0	0.6	12.7	30.0
C1a	3	0.04	0.04	0.84	1.3	0.0	3.9	31.3
C2	3	0.04	0.02	0.58	0.9	0.0	2.1	21.9
C3	4	0.10	0.03	0.30	3.4	1.3	6.1	32.7
C5	4	0.06	0.03	0.52	2.1	0.0	4.8	37.7
C5a	2	0.10	0.05	NA	NA	NA	NA	NA
C7	3	0.04	0.00	0.13	1.4	0.9	2.1	36.1
C7a	3	0.02	0.02	1.00	0.4	0.0	1.3	23.6
C8	5	0.07	0.02	0.36	1.8	0.5	3.4	26.8
C9	9	0.14	0.05	0.36	4.7	1.3	9.0	34.5
E2	4	0.09	0.04	0.43	3.8	0.5	7.8	42.8
E4	1	0.14	0.13	NA	NA	NA	NA	NA
Core total	49	0.07	0.01	0.16	31.6	18.8	49.7	440.3
Background strata	5	0.03	0.01	0.39	19.3	4.5	38.5	578.4
Survey total	54	0.05	0.01	0.18	50.9	29.0	82.7	1018.7

The population estimates of recruit-sized oyster with bonamia infection (categories 1–5) from the qPCR analysis of heart tissues in 2015 are given in Table 18 and for 2014 in Table A4.2 (Appendix 4). The estimates from qPCR for core strata in 2015 were higher (72.7 million, 95% CI 44.8–112.5) than from heart imprints (49.8 million, 95% CI 29.7–78.2), highlighting the greater sensitivity of the qPCR method in detecting infections. The numbers of infected oyster determined from qPCR were fewer in 2015 than in 2014 (146.9 million, 95% CI 88.2–234.5).

Table 18: Scaled up estimates of the population size of recruit-sized oysters with bonamia infection (prevalence) estimated by the qPCR analysis of heart tissues for the core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2015. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum.

2015 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.05	0.02	0.53	3.6	0.0	8.2	78.2
B3	3	0.25	0.14	0.53	11.4	0.0	25.5	44.7
B6	5	0.36	0.11	0.30	10.8	4.0	19.7	30.0
C1a	3	0.08	0.08	0.92	2.6	0.0	8.0	31.3
C2	3	0.09	0.04	0.42	1.9	0.4	4.0	21.9
C3	4	0.39	0.10	0.26	12.6	5.5	22.0	32.7
C5	4	0.12	0.06	0.51	4.6	0.0	10.4	37.7
C5a	2	0.10	0.05	NA	NA	NA	NA	NA
C7	3	0.11	0.02	0.20	4.0	2.1	6.6	36.1
C7a	3	0.05	0.03	0.67	1.1	0.0	2.8	23.6
C8	5	0.20	0.11	0.54	5.5	0.0	12.3	26.8
C9	9	0.27	0.09	0.34	9.3	2.9	17.7	34.5
E2	4	0.12	0.05	0.39	5.3	1.2	10.5	42.8
E4	1	0.14	0.13	NA	NA	NA	NA	NA
Core total	49	0.17	0.02	0.14	72.7	44.8	112.5	440.3
Background strata	5	0.06	0.02	0.31	32.8	12.0	60.6	578.4
Survey total	54	0.10	0.01	0.13	105.6	64.9	165.5	1018.7

The distribution of recruit-sized oysters with non-fatal bonamia infections

The distribution of non-fatal infections in February 2014 (Figure 32) was widespread and variable across the fishery. The prevalence of non-fatal, category 1 and 2 infections varied at small spatial-scales; stations with relatively high prevalence were often close to stations with low prevalence or no infection. Stations with high non-fatal prevalence are likely to be subjected to heightened bonamia mortality in the future. The distribution of non-fatal infections in February 2015 (Figure 33) showed similar spatial patterns, however, recruit-sized oyster densities were further reduced by bonamia mortality between 2014 and 2015, as had the numbers of oysters with non-fatal infections.

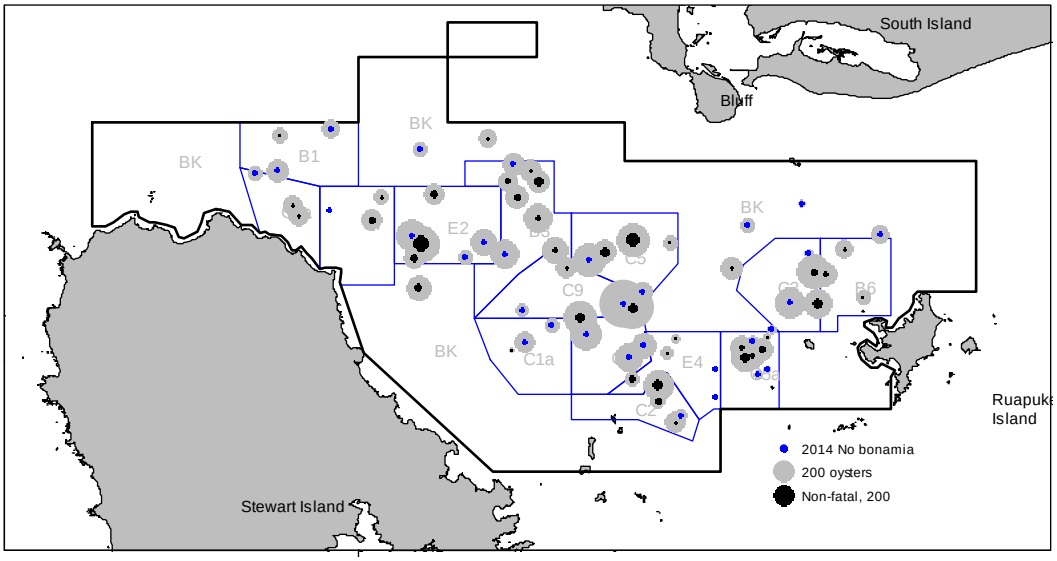

Figure 32: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with category 1 and 2 infections (open black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2014. Stations with no bonamia infection are shown by open blue circles.

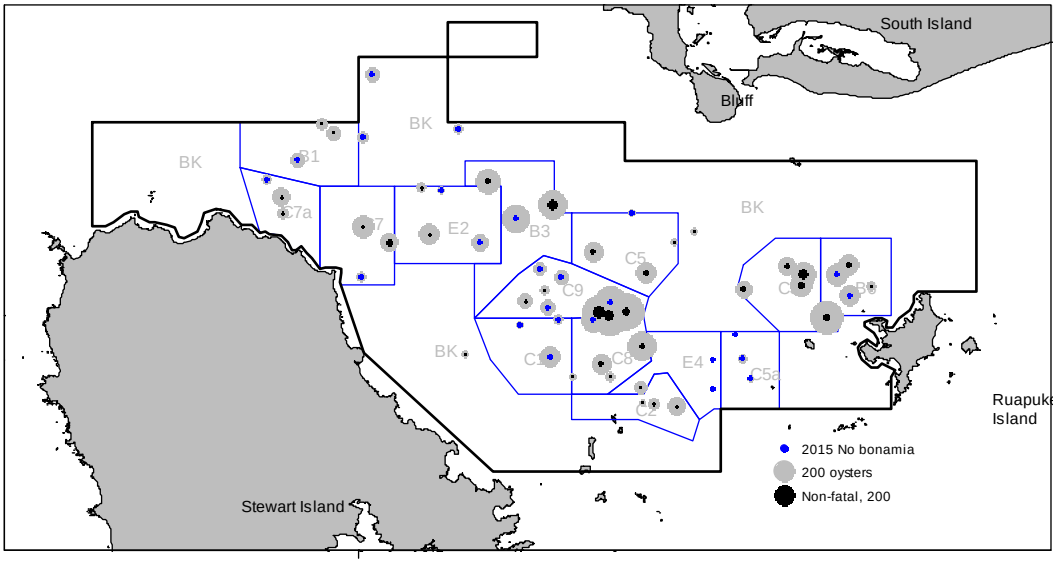

Figure 33: The distribution of recruit-sized oysters (filled grey circles showing numbers per standard tow) and oysters with category 1 and 2 infections (open black circles, the numbers of oysters scaled to the size of the catch with intensity of infection category 1 and 2) in February 2015. Stations with no bonamia infection are shown by open blue circles.

4.6 Estimate the summer mortality from Bonamia in the commercial fishery area (objective 4)

Pre-survey mortality was estimated from the population size of recruit-sized new clocks and gapers in Section 4.3. The pre-survey mortality in all core strata combined was estimated to be 13.5 million recruit-sized oysters (95% CI 13.1–13.9) in 2015, 3.6% of the recruited population. Projections of post-survey mortality (within about two months of sampling) from the proportion of oysters with categories three and higher (fatal) infections scaled-up to the size of the total recruit-sized oyster population are given below. We used two methods to crosscheck the scaled-up estimates of fatal infections: 1, by applying a correction factor to the population estimates derived from the average proportion of infected oysters in the stratum; and 2, post-survey mortality was estimated from the numbers of infected oysters at each sample station scaled to the catch, then to stratum, and to the survey area level.

Projected short-term mortality from bonamia infections

Post-survey mortality of recruit-sized oysters was estimated for core strata with three or more randomly selected stations. Because bonamia mortality had reduced oyster densities to low levels in some strata, some stations caught insufficient numbers of oysters for bonamia sampling. Strata C5a and E4 were omitted from the analysis for this reason. The mean proportion of oysters infected with category 3 and higher infections in the catch was used to calculate a correction factor for each stratum (1 (the total catch) less the mean proportion of oysters infected with bonamia, Table 19) and this correction factor was applied to the mean oyster density estimated from all random tows. The post-survey mortality of oysters was projected to reduce the recruit-sized oyster population in core strata from 345.2 million oysters at the time of the survey (February 2015) to 310.8 million oysters by early in the new oyster season (March 2015), a loss of 34.4 million oysters (10.0%), (Table 20). Post-survey mortality of recruit-sized oysters by stratum (Table 20) ranged from 0.3% in stratum C1a to 20.7% in B3. Of the core strata in 2015, B3, B6, B1, and E2 had a post-survey mortality greater than 10%, fewer than in 2014 (C2, E2, C5, B1, C7, C7a, E4, and C3). Estimates for 2012 and 2014 are given in Tables A4.3 and A4.4 (Appendix4).

The estimates of post-survey mortality from individual catches of fatally infected oyster within the core strata with three or more randomly selected stations were similar to that using averaged correction factors for strata, 31.6 million oysters (9.2%) (Table 19). Strata B6, B3, C8, and E2 had the highest mortalities (Table 19).

The percentage of oysters with fatal infections in commercial strata (2012 and 2013) and core strata in 2014 increased from 7.7% to 11.5% over the same period and declined to 10% in 2015. Of all the recruit-sized infected oysters in the strata surveyed, 68.8% were fatally infected in 2012, 72.9% in 2013, 70% in 2014, and 63% in 2015.

How quickly low level, category 1 and 2 infections progress to category 3+ infections, and the variance amongst individual oysters is not known. Where the prevalence of category 1 and 2 infections was high, and occurred in areas of relatively high oyster density, these areas may eventually be subjected to heightened mortality.

Pre-survey mortality in core strata of 13.5 million oysters and post survey mortality ranging between 31.6 million and 34.4 million oysters combined gave a summer mortality of 45.1 million to 47.9 million oysters. Summer mortality, estimated as the percentage of recruit-sized oyster deaths from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March), calculated as the percentage of all deaths (pre-survey mortality and post survey mortality combined) of the recruit-sized population at the beginning of summer (population size of recruit-sized new clocks and population size of recruit-sized oysters at the time of survey combined), was 12.4–13.1% of the recruit-sized population (Table 20).

Table 19: Absolute population estimates for recruit-sized oysters after projected mortality from bonamia based on category 3 and higher infections: the number of randomly selected stations sampled (No. stns), the correction factor applied to each stratum (Correction factor), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the oyster density, mean population size at the time of survey (Pop.n1), mean post mortality population size (Pop.n2) in millions of oysters, upper and lower 95% confidence intervals (CI) for the post-mortality estimate, the area of each stratum (Area.km^2), by stratum for the February 2015 survey.

2015 Core Strata	No. stns	Correction factor	Mean density	Density s.d.	CV	Pop.n1	Pop.n2	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.85	0.36	0.04	0.11	33.5	28.4	18.1	43.4	78.2
B3	3	0.90	1.49	0.10	0.07	73.6	66.5	44.1	98.3	44.7
B6	5	0.80	0.92	0.29	0.32	34.5	27.7	9.7	51.2	30.0
C1a	3	0.99	0.43	0.26	0.61	13.5	13.4	0.0	32.7	31.3
C2	3	0.93	0.34	0.17	0.50	8.0	7.5	0.2	16.6	21.9
C3	4	0.95	0.90	0.16	0.17	31.1	29.4	16.7	47.0	32.7
C5	4	0.88	0.46	0.19	0.41	19.7	17.4	3.1	35.5	37.7
C5a	2	NA	0.10	0.05	NA	NA	NA	NA	NA	NA
C7	3	0.94	0.68	0.25	0.37	25.8	24.4	6.2	47.7	36.1
C7a	3	0.92	0.43	0.16	0.36	10.9	10.1	2.8	19.6	23.6
C8	5	0.87	0.78	0.29	0.37	24.1	20.9	5.3	40.7	26.8
C9	9	0.97	1.38	0.38	0.28	49.3	47.7	19.8	84.9	34.5
E2	4	0.82	0.40	0.15	0.38	21.0	17.3	4.2	34.2	42.8
E4	1	NA	0.14	0.13	NA		NA	NA	NA	NA
Core total	49	"-	0.71	0.06	0.08	345.2	310.8	205.4	461.0	440.3
Background strata	5	0.91	0.25	0.06	0.23	158.5	144.0	70.7	245.3	578.4
Survey total	54	"-	0.45	0.04	0.09	503.8	454.8	295.8	687.1	1018.7

Table 20: Summer mortality estimated as the percentage of recruit-sized oyster deaths from the time mortality began at the beginning of summer to the end of the seasonal mortality (about mid-March), calculated as the percentage of all deaths (pre-survey mortality and post survey mortality combined) of the recruit-sized population at the beginning of summer (population size of recruit-sized new clocks and population size of recruit-sized oysters at the time of survey combined).

	Millions
Pre-survey mortality	**of oysters**
Recruit-sized new clocks (NC)	13.5
Post-survey mortality	
Correction factor	34.4
Scaled catch	31.6
Combined summer mortality	
Correction factor +NC	47.9
Scaled catch +NC	45.1
Population before summer mortality	
Recruit-sized oysters +NC	364.9
Percent summer mortality	**Percent**
Correction factor +NC	13.1
Scaled catch +NC	12.4

4.7 The current status of the OYU 5 fishery and future trends (objective 6)

Disease mortality and recruitment to the fishery appear to be the main drivers of future stock size in the OYU 5 fishery. The stock assessment for OYU 5 suggests that an annual commercial harvest of up to 20 million oysters is not likely to have a significant effect on the future (1–3 years) status of the stock (Figure 34).

Between 1993 and 1999, the fishery rebuilt rapidly from a historically low size, mainly due to low and non-detectable bonamia mortality and high recruitment to the fishery. After the second low point in the fishery in 2005, the fishery was again rebuilding rapidly with good spatfall and juvenile survival driving recruitment, with a bonamia mortality of about 10% of the recruit-sized population. Since 2009, the population size of recruit-sized oysters continued to increase and this high number of recruits should have led to increased recruitment, but instead recruitment has been low. The low recruitment to the fishery combined with a continuing bonamia mortality of about 10% flattened the stock trajectory between 2010 and 2013 (Figure 34).

Significant summer mortality from bonamia, 15.9% in 2013, 18.3% in 2014, and 13.6 % in 2015, along with the low recruitment to the fishery has led to a decline in the recruit-sized population since 2012. The recruit-sized population size declined from 538.0 million oysters in 2014 to 351.4 million in 2015, a decrease of about 34.7%.

Given the low recruitment to the fishery over the summer of 2014–15, and the heightened level of bonamia mortality over the summer of 2013–14, the current status of the fishery is likely to be best represented by series “c” in Figure 34 which assumes a bonamia mortality of 20% and incorporates the decreased recruitment between the 2009 and 2012 stock assessments.

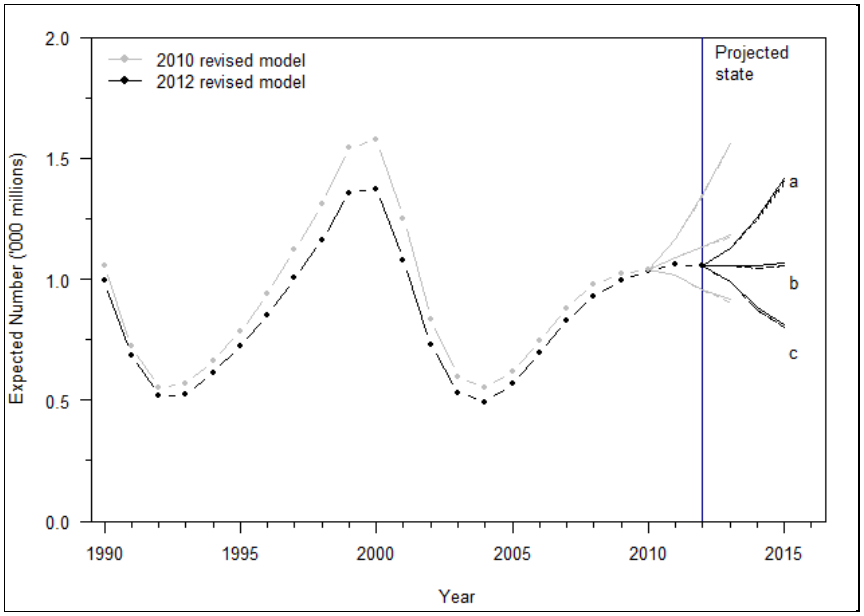

Figure 34: Model estimates of recruit-sized stock abundance (2012) and projected recruit-sized stock abundance for 2013–15 with a catch of 7.5 (solid line), 15 (dash dot), and 20 million oysters (dash line) under assumptions of (a) no disease mortality, (b) disease mortality of 0.10 per year, and (c) disease mortality of 0.20 per year, for the 2010 and 2012 revised models (figure reproduced from Fu 2013).

5. DISCUSSION

Annual bonamia surveys to monitor the status of bonamia infection and mortality in the oyster fishery have been undertaken since 2000. Bonamia surveys were undertaken concurrently with stock assessment surveys, and dedicated bonamia surveys were undertaken in alternate years to stock assessments. Occasionally more than one bonamia survey was undertaken in any given year.

The objectives of bonamia surveys changed over time, from station level comparisons of changes in bonamia status and related mortality, and in oyster density to determine spread of the epizootic to include information on the effects disease mortality has on oyster density at stratum level. The March 2000 bonamia survey focused on determining the spread of infection (Dunn et al. 2000). Sampling oysters to determine bonamia infection during stock assessment surveys (in the current epizootic) began in October 2001, and only a subset of the randomly selected stations that were sampled for oyster density were sampled for bonamia infection (Michael et al. 2004). The objective of bonamia surveys has typically been to survey a subset of strata within the stock assessment survey area considered important by oyster skippers, and to resample a randomly selected subset of stations sampled during stock assessment surveys to determine the distribution, prevalence and intensity of infection by bonamia, and to estimate the oyster density in the commercial areas sampled for bonamia. Bonamia surveys (2000–2004) were not intended to inform stock assessments or to cover the entire fishery area.

From March 2006 onwards, projections of disease mortality between the February surveys and the start of the oyster season were made from data on the intensity of bonamia infection. Three bonamia surveys since 2009 (2010, 2011, and 2013) provided a suite of information for management of the OYU 5 fishery and to the oyster industry, but were not directly comparable. The February 2010 survey (Michael et al. 2011) estimated total summer mortality based on pre-survey estimates of mortality from new clocks and gapers and the post-survey mortality estimated from fatal bonamia infections. It was acknowledged that estimates of pre- and post-survey mortality are not directly comparable. However, the combined totals of these two estimates provide the only estimate available for total summer mortality which is important in determining which projection from the previous stock assessment is best able to inform future stock status. The three projections of future stock status are based on 0%, 10%, and 20% disease mortality (Dunn 2007). This estimate of post-survey mortality became a key result for both the stock assessment surveys and bonamia surveys that followed; and there was acceptance that bonamia surveys whilst not representative of the whole fishery area, gave a good indication of what would be likely to occur in the commercial fishery areas important to industry and stakeholders. Projections of future stock status determined by the levels of summer disease mortality were consistent with recruit-sized population estimates from subsequent stock assessment surveys. A new time-series of bonamia and oyster surveys which have incorporated a fully randomised, two-phase sampling design and a standard bonamia survey area to make these surveys comparable from year to year was established in 2014.

The February 2015 survey is the second in this new time-series of bonamia and oyster surveys that incorporate a fully randomised, two-phase sampling design aimed at better estimating oyster density and population size in the three size groups of oysters surveyed, and for new clocks. Because both estimates of new clocks and fatal infections are scaled to the size of the oyster population, better estimates of oyster density are likely to give more precise estimates of total summer mortality. This time series of surveys also samples a standard bonamia survey area to make these surveys comparable from year to year. This area represents the core commercial fishery through the highs and lows in relative oyster abundance driven by bonamia mortality. Core strata comprised 14 of the 26 stock assessment survey strata from 2012 representing 75% of the recruit-sized oyster population and 46% of the stock assessment survey area. There is also some limited sampling in background strata so that the bonamia survey data could provide oyster density estimates for the whole stock assessment survey area and therefore allow these data to be incorporated into the planned five-yearly stock assessments.

5.1 Survey results

The 2015 survey was conducted in rougher sea conditions than previous years, but within the limits of conditions that were not expected to affect dredge efficiency. The winch used to deploy the sampling dredge changed from a traditional friction winch to a hydraulic winch for this survey. We cannot determine if these factors produced a downward bias in the population estimates, but this seems unlikely.

The qPCR method is more sensitive in the detection of bonamia than heart imprints. Gill tissues generally produced lower Cq values than heart tissues, which may either mean that they are more sensitive (provide for better amplification) or that they are amplifying external contamination of gill tissue by water-borne bonamia particles. Tissue-specific inhibition of DNA amplification is a common problem with qPCR assays and dark tissues such as heart muscle thought to inhibit amplification. We will further explore the differences in the amplification of heart and gill tissues. Heart tissues may provide better estimates of oyster infection and gill tissues better estimates of pathogen presence in the environment.

Recent surveys of the status of bonamia infection found that prevalence was generally low in February 2009, but the infection was widespread and variable; stations with no detectable infections were interspersed with stations with low and high intensity infections. The distribution of infection remained similar in the core commercial fishery areas between 2010 and 2012. The prevalence of infection was higher and infected stations generally had higher intensities of infection in 2013 than in previous years, especially in central fishery areas. The prevalence of infection was higher again in 2014 as were the numbers of fatally infected oysters. Mortality from bonamia was higher over the summers of 2012–13 and 2013–14 compared to previous summers. Summer mortality was estimated to be 15.9% in 2013 and 18.3% in 2014. The heightened bonamia mortality since 2012 has reduced oyster densities and population sizes in the fishery. The recruit-sized oyster population declined 34.7% between 2014 and 2015.

5.2 Status of the OYU 5 stock

The 2012 stock assessment suggested that the exploitation rates were low, and the stock continued to rebuild since the 2000 outbreak of bonamia reduced the stock to low levels in 2005. These estimates suggest that the spawning stock population in 2012 was about 35% (31–41%) B_0, and recruit-sized stock abundance (rB_{2012}) was about 30% (26–34%) of initial state (rB_{1907}). (Fu 2013).

By 2012, the trajectory of the future stock size was already starting to flatten due to the continuing low level mortality of between 8% and 12% from 2007 to 2012 and the reduced recruitment since 2009. At 10% bonamia mortality, long-term average recruitment, and a harvest level below 20 million oysters, there was no change expected in stock size between 2013 and 2015.

The increase in summer mortality from bonamia to 18.3% in 2014 and 13.6% in 2015, and the continued low recruitment to the oyster population is expected to further steepen the downward trend in the oyster population. Further, both the population sizes of pre-recruits and small oysters are low, and are likely to result in little recruitment to the fishery in the short to medium term. A significant increase in recruitment could have a major restorative effect, but there will be a 4–6 year lag before recruitment to the population increases recruitment to the fishery. The recruit-sized population in core strata was expected to be 303.5 million oysters at the beginning of the 2015 oyster season.

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8. APPENDIXICES

8.1 Appendix 1: Survey station form

FOVEAUX STRAIT OYSTER SURVEY, STATION DATA RECORD

Vessel name

Recorder .....

	Day	Month	Year	Time NZST	Station no.	Stratum	Depth (m)	Speed (knots)
Date								

	Latitude		Longitude		
Start position		S		E	
Finish position		S		E	

	Live	Gapers	New clocks*	Old clocks**	Number of live oysters 10-50 mm
Number of Oysters ≥58 mm					
Number of Oysters 50-57 mm					

% fullness of dredge including sediment

Live Bryozoa

Bycatch photo numbers ___ – ___

Wind force, beaufort

Did the dredge fish well? Y=1 or N=2

Bonamia sample?

Comments?

If N please repeat tow and record both tows. Strike out repeated tow with diagonal line across page

Sediment type

Circle the main type (one only)

Weed	Shell	Shell/sand	Shell/gravel	Pea gravel	Sand	Silt	Sponges	Bryozoa
0	1	2	3	4	5	6	7	8

Comments:

1 Nautical mile = 1.853 km

* New clocks are hinged shells of recently dead oysters, inner shell glossy with no fouling except the odd speck of coralline

** Old clocks are hinged shells of dead oysters with fouling inside

Counts of oysters and clocks to include samples taken for population size and *Bomania*

8.2 Appendix 2: Survey bonamia form

FOVEAUX STRAIT OYSTER BONAMIA DATA RECORD

Page ___ of ___

Date

Station no. | Day | Month | Year | Time NZST

Recorder

Comments

Oyster no	Length (mm)	Height (mm)	Size category [1]	Heat imprint score	Histology sample

1) Start a new form for each new station
2) Measure oysters to the nearest mm down
3) Check oysters for size; recruit (R), pre-recruit (P), and small recruit (O) size with 'oyster rings'.

8.3 Appendix 3: Population estimates for recruit-sized, pre-recruit, and small oysters from the 2012 and 2014 surveys. Comparisons between the population estimates for all background strata combined in 2012 and 2014 should be made with caution as there were only 5 stations sampled in total in 2014.

Table A3.1: Absolute population estimates from randomly allocated stations only for recruit-sized oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, the mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95% CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimates, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0.82	0.16	0.2	63.9	49.8	78.0	34.5	105.4	78.2
B3	8	1.31	0.13	0.1	58.7	54.7	62.7	37.8	88.2	44.7
B6	3	0.82	0.23	0.29	24.6	16.8	32.4	9.9	43.9	30
C1a	3	0.53	0.28	0.53	16.5	6.6	26.4	0	37.2	31.3
C2	3	1.2	0.47	0.39	26.4	14.7	38.1	5.9	52.2	21.9
C3	4	1.25	0.63	0.51	40.9	20.7	61.1	0	91.1	32.7
C5	4	1.25	0.42	0.34	46.9	31.5	62.3	14.9	88.5	37.7
C5a	7	0.7	0.25	0.36	16.4	12.1	20.7	4.6	31.8	23.5
C7	3	0.53	0.27	0.52	19	8.0	30.0	0	42.1	36.1
C7a	3	0.78	0.16	0.21	18.5	14.2	22.8	9.7	30.8	23.6
C8	3	1.77	0.24	0.14	47.6	40.3	54.9	28.8	74.6	26.8
C9	4	2.83	1.26	0.45	97.4	54.9	139.9	12.1	206.2	34.5
E2	4	1.33	0.49	0.37	56.8	36.3	77.3	15.7	112.1	42.8
E4	3	0.17	0.15	0.91	4.6	0.0	9.2	0	14.2	28
Core total	55	1.09	0.12	0.11	538.0	522.3	553.7	343.6	832.2	491.8
Background strata	5	0.83	0.18	0.21	482.9	391.1	574.7	250.7	806.2	578.4
Survey total	60	0.95	0.11	0.12	1020.9	991.0	1050.8	635.1	1554.2	1070.2

Table A3.2: Absolute population estimates from randomly allocated stations only for recruit-sized oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2012. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	0.7	0.24	0.34	54.6	38.2	71.0	17.3	103.2	78.2
B3	13	3.56	0.7	0.2	158.9	141.9	175.9	87.2	258.7	44.7
B6	6	1.15	0.5	0.44	34.4	22.4	46.4	4.7	70.8	30
C1a	5	0.58	0.23	0.39	18.2	11.9	24.5	3.9	36.4	31.3
C2	3	0.97	0.16	0.17	21.2	17.2	25.2	12.2	33.9	21.9
C3	6	1.44	0.41	0.29	47.1	36.4	57.8	19.1	84	32.7
C5	8	1.98	0.53	0.27	74.5	60.7	88.3	31.4	132.7	37.7
C5a	5	1.34	0.86	0.64	31.6	13.8	49.4	0	77.8	23.5
C7	5	1.01	0.18	0.18	36.4	30.7	42.1	20.5	58.6	36.1
C7a	4	1.78	0.76	0.43	42	24.4	59.6	6.1	86	23.6
C8	6	1.65	0.64	0.39	44.3	30.6	58.0	10.3	88.2	26.8
C9	6	1.04	0.49	0.47	35.8	22.3	49.3	2.5	76.9	34.5
E2	8	1.87	0.49	0.26	80.3	65.7	94.9	35.6	141.4	42.8
E4	4	0.31	0.22	0.7	8.8	2.7	14.9	0	23.2	28
Core total	84	1.4	0.13	0.09	688.1	674.4	701.8	449.2	1046.7	491.8
Background strata	62	0.4	0.08	0.2	230.3	218.8	241.8	125.2	376	578.4
Survey total	146	0.86	0.07	0.08	918.4	906.3	930.5	600.1	1383.7	1070.2

Table A3.3: Absolute population estimates from randomly allocated stations only for pre-recruit oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. Stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0.54	0.15	0.28	42.4	29.1	55.7	17.5	75.9	78.2
B3	8	0.13	0.02	0.17	6	5.4	6.6	3.5	9.5	44.7
B6	3	0.15	0.08	0.55	4.4	1.7	7.1	0	10.1	30
C1a	3	0.08	0.03	0.39	2.6	1.5	3.7	0.6	5.2	31.3
C2	3	0.71	0.26	0.36	15.5	9.1	21.9	4.4	30	21.9
C3	4	0.32	0.14	0.44	10.5	6.0	15.0	1.4	21.9	32.7
C5	4	0.24	0.08	0.34	9	6.1	11.9	2.8	17.1	37.7
C5a	7	0.1	0.03	0.32	2.3	1.8	2.8	0.8	4.3	23.5
C7	3	0.3	0.18	0.61	10.8	3.5	18.1	0	26	36.1
C7a	3	0.35	0.05	0.14	8.2	6.9	9.5	5	12.8	23.6
C8	3	0.37	0.05	0.13	10	8.5	11.5	6.1	15.5	26.8
C9	4	0.27	0.14	0.52	9.3	4.6	14.0	0	20.7	34.5
E2	4	0.31	0.07	0.22	13.2	10.3	16.1	6.6	22.4	42.8
E4	3	0.15	0.15	1	4.1	0	8.7	0	13.3	28
Core total	55	0.3	0.04	0.12	148.4	143.2	153.6	93.7	230.7	491.8
Background strata	5	0.13	0.05	0.35	77.9	51.6	104.2	22.6	150.3	578.4
Survey total	60	0.21	0.03	0.14	226.2	218.0	234.4	135.1	352.1	1070.2

Table A3.4: Absolute population estimates from randomly allocated stations only for pre-recruit oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2012. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	0.6	0.21	0.35	46.6	32.3	60.9	14	89	78.2
B3	13	0.94	0.14	0.15	41.8	38.4	45.2	25.3	65.3	44.7
B6	6	0.52	0.29	0.55	15.5	8.6	22.4	0	35.4	30
C1a	5	0.19	0.05	0.26	6	4.6	7.4	2.6	10.5	31.3
C2	3	0.65	0.2	0.31	14.3	9.3	19.3	5.3	26.4	21.9
C3	6	0.53	0.16	0.29	17.5	13.3	21.7	6.9	31.3	32.7
C5	8	0.58	0.13	0.22	21.8	18.4	25.2	11	36.9	37.7
C5a	5	0.36	0.24	0.67	8.4	3.5	13.3	0	21.2	23.5
C7	5	0.87	0.15	0.17	31.6	26.8	36.4	18	50.6	36.1
C7a	4	1.39	0.52	0.37	32.9	20.8	45.0	8.4	64	23.6
C8	6	0.51	0.13	0.26	13.7	10.9	16.5	6	24.1	26.8
C9	6	0.26	0.1	0.4	9	6.2	11.8	1.9	18.1	34.5
E2	8	0.85	0.31	0.37	36.2	27.1	45.3	9.4	71.1	42.8
E4	4	0.08	0.04	0.54	2.1	1.1	3.1	0	5	28
Core total	84	0.6	0.06	0.1	297.4	291.0	303.8	192.6	454.4	491.8
Background strata	62	0.2	0.05	0.23	116.9	109.6	124.2	57.6	196.9	578.4
Survey total	146	0.39	0.04	0.1	414.3	407.4	421.2	267.8	629	1070.2

Table A3.5: Absolute population estimates from randomly allocated stations only for small oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. Stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0.32	0.09	0.29	25.3	17.2	33.4	9.9	45.7	78.2
B3	8	0.22	0.04	0.19	9.7	8.5	10.9	5.3	15.7	44.7
B6	3	0.22	0.09	0.42	6.5	3.5	9.5	1.1	13.1	30
C1a	3	0.2	0.12	0.6	6.3	2.0	10.6	0	15.2	31.3
C2	3	0.72	0.12	0.17	15.9	12.9	18.9	9.2	25.3	21.9
C3	4	0.37	0.17	0.47	12	6.6	17.4	0.9	25.6	32.7
C5	4	0.29	0.16	0.56	10.9	5.0	16.8	0	25.4	37.7
C5a	7	0.29	0.11	0.37	6.8	4.9	8.7	1.8	13.2	23.5
C7	3	0.16	0.06	0.39	5.6	3.2	8.0	1.3	11.2	36.1
C7a	3	0.62	0.24	0.39	14.6	8.2	21.0	3.1	29	23.6
C8	3	0.51	0.05	0.1	13.8	12.3	15.3	8.8	20.9	26.8
C9	4	0.35	0.14	0.4	11.9	7.2	16.6	2.4	24.1	34.5
E2	4	0.31	0.09	0.29	13.2	9.4	17.0	5.3	23.9	42.8
E4	3	0.14	0.13	0.97	3.9	0	8.0	0	12.3	28
Core total	55	0.32	0.03	0.1	156.3	152.4	160.2	101.1	239.4	491.8
Background strata	5	0.25	0.05	0.2	146.3	120.7	171.9	78.1	242	578.4
Survey total	60	0.28	0.03	0.11	302.6	294.4	310.8	189.2	459.2	1070.2

Table A3.6: Absolute population estimates from randomly allocated stations only for small oysters in the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2012. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	1.13	0.4	0.36	88	60.7	115.3	24.2	169.9	78.2
B3	13	0.73	0.12	0.17	32.8	29.9	35.7	19.2	52	44.7
B6	6	0.74	0.34	0.45	22.1	14.0	30.2	2.2	46.3	30
C1a	5	0.32	0.07	0.24	9.8	7.9	11.7	4.8	16.8	31.3
C2	3	1.38	0.58	0.42	30.4	15.9	44.9	5.3	62.1	21.9
C3	6	0.49	0.16	0.32	16.1	11.9	20.3	5.6	29.6	32.7
C5	8	0.55	0.12	0.22	20.9	17.7	24.1	10.7	35.3	37.7
C5a	5	0.52	0.28	0.53	12.2	6.4	18.0	0	27.7	23.5
C7	5	1.36	0.29	0.21	49.2	40.0	58.4	25.7	81.7	36.1
C7a	4	4.04	2.56	0.63	95.5	36.2	154.8	0	234.2	23.6
C8	6	0.43	0.12	0.27	11.7	9.1	14.3	5	20.6	26.8
C9	6	0.3	0.09	0.3	10.3	7.8	12.8	3.9	18.9	34.5
E2	8	1.11	0.36	0.33	47.5	36.8	58.2	15.7	89.4	42.8
E4	4	0.18	0.08	0.46	4.9	2.8	7.0	0.6	10.6	28
Core total	84	0.92	0.15	0.16	451.3	435.6	467.0	261.5	731.7	491.8
Background strata	62	0.28	0.08	0.29	160.9	149.5	172.3	64.2	286.8	578.4
Survey total	146	0.57	0.08	0.14	612.2	598.3	626.1	370.3	967.9	1070.2

Table A3.7: Absolute population estimates from randomly allocated stations only for the size of the recruit-sized oyster population above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) in the 2014 core strata (N = 14), all background strata (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where an S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0	0	0	0	0	0	0	0	78.2
B3	8	0	0	0	0	0	0	0	0	44.7
B6	3	0	0	0	0	0	0	0	0	30
C1a	3	0	0	0	0	0	0	0	0	31.3
C2	3	0.7	0.7	1	15.4	-2.0	32.8	0	49.4	21.9
C3	4	1.16	0.69	0.59	37.8	15.8	59.8	0	89.4	32.7
C5	4	0.6	0.6	1	22.8	0.5	45.1	0	72.6	37.7
C5a	7	0	0	0	0	0	0	0	0	23.5
C7	3	0	0	0	0	0	0	0	0	36.1
C7a	3	0	0	0	0	0	0	0	0	23.6
C8	3	0.75	0.75	1	20	-2.6	42.6	0	64.3	26.8
C9	4	2.49	1.45	0.58	85.7	36.8	134.6	0	202	34.5
E2	4	0.69	0.69	1	29.6	0.6	58.6	0	94.6	42.8
E4	3	0	0	0	0	0	0	0	0	28
Core total	55	0.43	0.14	0.34	211.3	193.1	229.5	69	396.2	491.8
Background strata	5	0	0	0	0	0	0	0	0	578.4
Survey total	60	0.2	0.07	0.34	211.3	192.6	230.0	69	396.2	1070.2

Table A3.8: Absolute population estimates from randomly allocated stations only for the size of the recruit-sized oyster population above a density of 400 oysters per survey tow (equivalent to about 6–8 sacks per hour in commercial dredging) for the 2014 core strata (N = 14), all background strata (N = 12), and for the whole 2007 stock assessment survey area sampled in 2012. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	0	0	0	0	0	0	0	0	78.2
B3	13	3.22	0.81	0.25	143.7	124.0	163.4	66.3	248.9	44.7
B6	6	0.88	0.57	0.65	26.4	12.7	40.1	0	65.2	30
C1a	5	0	0	0	0	0	0	0	0	31.3
C2	3	0	0	0	0	0	0	0	0	21.9
C3	6	0.89	0.57	0.63	29.2	14.2	44.2	0	71.2	32.7
C5	8	1.33	0.68	0.51	50.1	32.3	67.9	0	112	37.7
C5a	5	0.95	0.95	1	22.3	2.8	41.8	0	70.8	23.5
C7	5	0	0	0	0	0	0	0	0	36.1
C7a	4	1	1	1	23.6	0.5	46.7	0	75.4	23.6
C8	6	1.16	0.74	0.64	31.2	15.3	47.1	0	77.5	26.8
C9	6	0.58	0.58	1	19.9	4.0	35.8	0	63.3	34.5
E2	8	1.55	0.6	0.39	66.4	48.6	84.2	15.5	132.1	42.8
E4	4	0	0	0	0	0	0	0	0	28
Core total	84	0.84	0.15	0.17	412.7	396.9	428.5	236.9	660.4	491.8
Background strata	62	0.11	0.07	0.62	61.2	51.5	70.9	0	146.4	578.4
Survey total	146	0.44	0.08	0.17	473.9	459.9	487.9	274.4	759.6	1070.2

Table A3.9: Recruit-sized new clocks estimated from randomly selected stations from the 2014 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0.06	0.01	0.21	4.7	3.6	5.8	2.5	7.8	78.2
B3	8	0.11	0.03	0.24	5.1	4.3	5.9	2.5	8.7	44.7
B6	3	0.09	0.05	0.6	2.6	0.8	4.4	0	6.1	30
C1a	3	0.04	0.02	0.39	1.2	0.7	1.7	0.3	2.4	31.3
C2	3	0.04	0.01	0.28	1	0.7	1.3	0.4	1.7	21.9
C3	4	0.15	0.07	0.49	4.9	2.5	7.3	0.2	10.7	32.7
C5	4	0.12	0.06	0.51	4.5	2.3	6.7	0	10.1	37.7
C5a	7	0.06	0.02	0.38	1.4	1.0	1.8	0.3	2.8	23.5
C7	3	0.01	0.01	0.5	0.5	0.2	0.8	0	1	36.1
C7a	3	0.1	0.05	0.5	2.3	1.0	3.6	0	5	23.6
C8	3	0.1	0.04	0.41	2.7	1.4	4.0	0.5	5.4	26.8
C9	4	0.11	0.05	0.43	3.9	2.3	5.5	0.6	8.1	34.5
E2	4	0.09	0.02	0.23	3.8	2.9	4.7	1.9	6.5	42.8
E4	3	0.03	0.02	0.91	0.8	0	1.6	0	2.3	28
Core total	55	0.08	0.01	0.12	39.4	38.2	40.6	24.7	61.4	491.8
Background strata	5	0.08	0.03	0.39	44.7	29.4	60.0	10.3	89	578.4
Survey total	60	0.08	0.02	0.21	84.1	79.6	88.6	42.5	140.6	1070.2

Table A3.10: Recruit-sized new clocks estimated from randomly selected stations from the 2012 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	0.05	0.02	0.5	3.9	2.2	5.6	0.1	8.6	78.2
B3	13	0.11	0.02	0.2	4.7	4.2	5.2	2.6	7.7	44.7
B6	6	0.03	0.01	0.45	0.9	0.6	1.2	0.1	1.9	30
C1a	5	0.01	0.01	0.63	0.4	0.2	0.6	0	0.9	31.3
C2	3	0.02	0.01	0.21	0.5	0.4	0.6	0.3	0.9	21.9
C3	6	0.05	0.01	0.31	1.6	1.2	2.0	0.6	2.8	32.7
C5	8	0.02	0.01	0.27	0.8	0.7	0.9	0.4	1.5	37.7
C5a	5	0.02	0.01	0.51	0.5	0.3	0.7	0	1	23.5
C7	5	0.1	0.07	0.72	3.5	1.3	5.7	0	9.1	36.1
C7a	4	0.08	0.07	0.81	2	0.4	3.6	0	5.6	23.6
C8	6	0.02	0.01	0.45	0.4	0.3	0.5	0	0.9	26.8
C9	6	0.02	0.01	0.31	0.7	0.5	0.9	0.2	1.2	34.5
E2	8	0.06	0.02	0.31	2.4	1.9	2.9	0.8	4.4	42.8
E4	4	0.01	0.01	1	0.2	0.0	0.4	0	0.8	28
Core total	84	0.05	0.01	0.17	22.4	21.6	23.2	12.8	36.6	491.8
Background strata	62	0.01	0	0.21	7.6	7.2	8.0	4	12.6	578.4
Survey total	146	0.03	0	0.14	30	29.3	30.7	18.4	46.8	1070.2

Table A3.11: Pre-recruit-sized new clocks estimated from randomly selected stations from the 2014 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	3	0.02	0	0.2	1.3	1.0	1.6	0.7	2.1	78.2
B3	8	0.01	0	0.46	0.2	0.1	0.3	0	0.5	44.7
B6	3	0.01	0.01	0.55	0.4	0.2	0.6	0	0.9	30
C1a	3	0	0	0.57	0.2	0.1	0.3	0	0.4	31.3
C2	3	0.01	0	0.5	0.1	0.0	0.2	0	0.3	21.9
C3	4	0	0	1	0.1	0.0	0.2	0	0.3	32.7
C5	4	0.01	0	0.6	0.2	0.1	0.3	0	0.5	37.7
C5a	7	0	0	0.4	0.1	0.1	0.1	0	0.2	23.5
C7	3	0	0	1	0.1	0.0	0.2	0	0.2	36.1
C7a	3	0.02	0.01	0.6	0.4	0.1	0.7	0	1.1	23.6
C8	3	0	0	0.01	0.1	0.1	0.1	0.1	0.2	26.8
C9	4	0	0	0.58	0.1	0.0	0.2	0	0.2	34.5
E2	4	0	0	0.63	0.2	0.1	0.3	0	0.4	42.8
E4	3	0	0	1	0.1	0.0	0.2	0	0.3	28
Core total	55	0.01	0	0.14	3.6	3.5	3.7	2.2	5.7	491.8
Background strata	5	0	0	0.67	1.7	0.7	2.7	0	4.3	578.4
Survey total	60	0	0	0.24	5.3	5.0	5.6	2.5	9	1070.2

Table A3.12: Pre-recruit-sized new clocks estimated from randomly selected stations from the 2012 survey. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters where a S prefix denotes the sampling CI calculated from the mean, standard deviation and sample size alone, and a B prefix denotes the bootstrapped estimate, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 Foveaux Strait oyster survey.

2012 Core Strata	No. stns	Mean density	Density s.d.	CV	Pop.n	S.lower 95%CI	S.upper 95%CI	B.lower 95%CI	B.upper 95%CI	Area.km^2
B1	5	0.03	0.01	0.39	2.5	1.6	3.4	0.5	4.9	78.2
B3	13	0.03	0.01	0.23	1.3	1.1	1.5	0.6	2.1	44.7
B6	6	0.01	0	0.53	0.3	0.2	0.4	0	0.6	30
C1a	5	0.01	0	0.67	0.2	0.1	0.3	0	0.5	31.3
C2	3	0	0	1	0.1	0	0.2	0	0.3	21.9
C3	6	0.01	0.01	0.37	0.5	0.4	0.6	0.1	0.9	32.7
C5	8	0.01	0	0.39	0.3	0.2	0.4	0.1	0.7	37.7
C5a	5	0.01	0	0.43	0.2	0.1	0.3	0	0.4	23.5
C7	5	0.05	0.03	0.5	1.9	1.1	2.7	0	4.2	36.1
C7a	4	0.03	0.01	0.35	0.6	0.4	0.8	0.2	1.2	23.6
C8	6	0.01	0	0.29	0.3	0.2	0.4	0.1	0.5	26.8
C9	6	0.01	0	0.54	0.2	0.1	0.3	0	0.5	34.5
E2	8	0.01	0.01	0.55	0.5	0.3	0.7	0	1.1	42.8
E4	4	0	0	1	0.1	0	0.2	0	0.2	28
Core total	84	0.02	0	0.17	8.9	8.6	9.2	5.1	14.4	491.8
Background strata	62	0.01	0	0.34	3.1	2.8	3.4	1	5.9	578.4
Survey total	146	0.01	0	0.15	12.0	11.7	12.3	7.2	18.9	1070.2

8.4 Appendix 4: Estimates of the total numbers of recruit-sized oysters infected with bonamia scaled up from the catches at randomly selected stations in 2012 and 2014.

Table A4.1: Scaled up estimates of the population size of recruit-sized oysters with bonamia infection estimated by heart imprints for the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The 2012 stock assessment survey did not sample for bonamia infection from all strata or the whole survey area, strata sampled for bonamia accounted for 628.6 km^2 (58.7%) of the survey area. Only stratum C6a was sampled in 2012 and not in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 and February 2014 Foveaux Strait oyster surveys.

	2012							2014							
Core Strata	**No. stns**	**Mean density**	**Density s.d.**	**CV**	**Pop.n**	**Lower 95%CI**	**Upper 95%CI**	**No. stns**	**Mean density**	**Density s.d.**	**CV**	**Pop.n**	**Lower 95%CI**	**Upper 95%CI**	**Area.km^2**
B1	3	0.21	0.06	0.28	16.1	6.6	28.8	3	0.13	0.03	0.26	10.2	4.5	17.9	78.2
B3	3	0.37	0.26	0.70	16.4	0.0	42.1	8	0.18	0.04	0.24	7.8	3.8	13.3	44.7
B6	3	0.24	0.13	0.57	7.1	0.0	16.4	3	0.11	0.07	0.67	3.3	0.0	8.2	30.0
C1a	3	0.02	0.02	0.88	0.6	0.0	1.8	3	0.02	0.01	0.42	0.7	0.1	1.5	31.3
C2	NA	NA	NA	NA	NA	NA	NA	3	0.38	0.15	0.40	8.4	1.8	16.9	21.9
C3	3	0.11	0.10	0.85	3.7	0.0	10.5	4	0.18	0.11	0.64	5.9	0.0	14.4	32.7
C5	3	0.10	0.10	1.00	3.7	0.0	11.7	4	0.35	0.18	0.52	13.1	0.0	29.4	37.7
C5a	3	0.06	0.03	0.53	1.5	0.0	3.3	7	0.10	0.06	0.59	2.4	0.0	5.6	23.5
C7	3	0.24	0.13	0.53	8.8	0.0	19.7	3	0.12	0.06	0.56	4.2	0.0	9.6	36.1
C7a	4	0.27	0.12	0.45	6.4	0.7	13.5	3	0.14	0.09	0.67	3.2	0.0	8.1	23.6
C8	3	0.07	0.01	0.13	1.8	1.1	2.8	3	0.10	0.06	0.56	2.7	0.0	6.2	26.8
C9	3	0.01	0.01	1.00	0.2	0.0	0.7	4	0.23	0.10	0.44	7.8	1.0	16.4	34.5
E2	3	0.37	0.36	0.98	15.8	0.0	50.3	4	0.43	0.26	0.61	18.4	0.0	45.0	42.8
E4	NA	NA	NA	NA	NA	NA	NA	3	0.05	0.05	1.00	1.5	0.0	5.0	28.0
Core total	37	0.19	0.05	0.26	*82.0	36.6	142.3	55	0.18	0.03	0.17	89.5	50.8	146.1	491.8
Background strata	9	0.01	0.00	0.69	**1.3	0	3.3	5	0.15	0.09	0.60	86.5	0.0	209.9	578.4
Survey total	46	0.13	0.03	0.26	***83.3	37.5	147.6	60	0.16	0.05	0.31	176.1	63.9	325.3	1070.2

*** (90.4%), ** (32.3%), *** (58.7%) of the survey area respectively.**

Table A4.2: Scaled up estimates of the population size of recruit-sized oysters with bonamia infection estimated by heart tissues using qPCR for the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The number of stations sampled (No. stns), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n, shaded grey), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2014 Foveaux Strait oyster survey.

2014 Stratum	No. stns	Mean density	Density s.d.	CV	Pop.n	Lower 95%CI	Upper 95%CI	Area.km^2
B1	3	0.24	0.07	0.29	18.4	7.4	33.1	78.2
B3	8	0.44	0.10	0.22	19.5	10.1	32.5	44.7
B6	3	0.20	0.14	0.69	6.0	0.0	15.4	30.0
C1a	3	0.05	0.02	0.41	1.6	0.3	3.4	31.3
C2	3	0.52	0.27	0.51	11.5	0.2	25.6	21.9
C3	4	0.27	0.19	0.73	8.7	0.0	23.1	32.7
C5	4	0.39	0.18	0.47	14.6	1.0	31.4	37.7
C5a	7	0.15	0.08	0.49	3.6	0.1	7.9	23.5
C7	3	0.15	0.07	0.47	5.4	0.4	11.6	36.1
C7a	3	0.34	0.17	0.51	7.9	0.0	17.5	23.6
C8	3	0.50	0.16	0.33	13.5	4.6	25.3	26.8
C9	4	0.35	0.16	0.46	12.1	1.2	25.6	34.5
E2	4	0.50	0.33	0.65	21.6	0.0	54.4	42.8
E4	3	0.08	0.08	1.00	2.3	0.0	7.5	28.0
Core total	55	0.30	0.04	0.15	146.9	88.2	234.5	491.8
Background strata	5	0.22	0.11	0.52	127.8	0.0	287.6	578.4
Survey total	60	0.26	0.07	0.25	274.8	124.2	480.4	1070.2

Table A4.3: Absolute population estimates for recruit-sized oysters after projected mortality from bonamia based on category 3 and higher infections: the number of randomly selected stations sampled (No. stations), the correction factor applied to each stratum (Corr. factor), the mean oyster density per m^2 (Mean density), standard deviation (s.d.) of the density estimate, coefficient of variation (CV) of the oyster density, mean post mortality population size (Post mort pop.n) in millions of oysters (Mean population), upper and lower 95% confidence intervals (CI), the area of each stratum (Area.km^2), by stratum for the February 2014 survey. The population size at the time of the survey and mortality in millions of oysters, and percentage mortality.

Stratum	Factor	No. stns	Mean density	Density s.d.	CV	Post mort pop.n	Lower 95%CI	Upper 95%CI	Area.km^2	Pop.n at survey	Mortality	Percentage mortality
B1	0.85	3	0.69	0.14	0.20	54.2	29.2	89.3	78.2	63.9	9.8	15.3
B3	0.91	8	1.20	0.12	0.10	53.6	34.5	80.5	44.7	58.7	5.1	8.7
B6	0.91	3	0.74	0.21	0.29	22.3	8.9	39.8	30.0	24.6	2.3	9.4
C1a	0.95	3	0.50	0.27	0.53	15.7	0.0	35.5	31.3	16.5	0.8	4.6
C2	0.78	3	0.94	0.37	0.39	20.5	4.6	40.7	21.9	26.4	5.8	22.1
C3	0.90	4	1.12	0.57	0.51	36.7	0.0	81.8	32.7	40.9	4.2	10.2
C5	0.84	4	1.04	0.35	0.34	39.3	12.5	74.1	37.7	46.9	7.6	16.2
C5a	0.93	7	0.65	0.23	0.36	15.2	4.3	29.5	23.5	16.4	1.2	7.3
C7	0.85	3	0.45	0.23	0.52	16.1	0.0	35.9	36.1	19.0	2.8	14.9
C7a	0.85	3	0.67	0.14	0.21	15.8	8.2	26.3	23.6	18.5	2.7	14.6
C8	0.94	3	1.66	0.23	0.14	44.7	27.1	70.1	26.8	47.6	2.9	6.0
C9	0.94	4	2.65	1.18	0.45	91.4	11.4	193.5	34.5	97.4	6.0	6.1
E2	0.82	4	1.09	0.41	0.37	46.6	12.9	92.1	42.8	56.8	10.1	17.9
E4	0.89	3	0.15	0.13	0.91	4.1	0.0	12.6	28.0	4.6	0.5	11.3
Core total						476.3				538.0	61.7	11.5
Background strata	0.87	5	0.73	0.16	0.21	422.2	219.2	704.9	578.4	482.9	60.7	12.6
Survey total		60	0.84	0.10	0.12	898.5	558.9	1367.7	1070.2	1020.9	122.4	12.0

Table A4.4: Scaled up estimates of the population size of recruit-sized oysters with fatal bonamia infections estimated by heart imprints for the 2014 core strata (N = 14), all background strata combined (N = 12), and for the whole 2007 stock assessment survey area sampled in 2014. The 2012 stock assessment survey did not sample for bonamia infection from all strata or the whole survey area, strata sampled for bonamia accounted for 628.6 km^2 (58.7%) of the survey area. Only stratum C6a sampled in 2012 and not in 2014. The number of stations sampled (no. stn), the mean oyster density per m^2 (Mean density), the standard deviation of the mean density estimate (Density s.d.), the coefficient of variation (CV) of the density estimate, mean population size in millions of oysters (Pop.n), upper and lower 95% confidence intervals (95%CI) in millions of oysters, and the area of each stratum (Area. km^2) in square kilometres, by stratum for the February 2012 and February 2014 Foveaux Strait oyster surveys.

	2012								2014							
Core Strata	**No. stns**	**Mean density**	**Density s.d.**	**CV**	**Pop.n**	**Lower 95%CI**	**Upper 95%CI**		**No. stns**	**Mean density**	**Density s.d.**	**CV**	**Pop.n**	**Lower 95%CI**	**Upper 95%CI**	**Area.km^2**
B1	3	0.14	0.06	0.41	10.6	2.0	21.3		3	0.12	0.03	0.27	9.7	4.2	17.0	78.2
B3	3	0.23	0.17	0.75	10.4	0.0	27.8		8	0.11	0.03	0.25	5.0	2.4	8.6	44.7
B6	3	0.19	0.09	0.48	5.8	0.3	12.4		3	0.08	0.06	0.83	2.3	0.0	6.6	30.0
C1a	3	0.02	0.02	1.00	0.6	0.0	1.8		3	0.02	0.01	0.46	0.7	0.1	1.5	31.3
C2	NA	NA	NA	NA	NA	NA	NA		3	0.25	0.11	0.44	5.5	0.8	11.5	21.9
C3	3	0.11	0.10	0.85	3.7	0.0	10.5		4	0.11	0.07	0.66	3.5	0.0	8.7	32.7
C5	3	0.02	0.02	1.00	0.9	0.0	2.9		4	0.20	0.09	0.47	7.5	0.5	16.2	37.7
C5a	3	0.04	0.02	0.53	1.0	0.0	2.2		7	0.05	0.03	0.59	1.2	0.0	2.8	23.5
C7	3	0.18	0.09	0.50	6.5	0.2	14.3		3	0.08	0.04	0.56	2.7	0.0	6.3	36.1
C7a	4	0.16	0.06	0.38	3.7	0.9	7.3		3	0.11	0.08	0.73	2.6	0.0	6.9	23.6
C8	3	0.05	0.01	0.29	1.3	0.5	2.3		3	0.10	0.06	0.56	2.7	0.0	6.2	26.8
C9	3	0.01	0.01	1.00	0.2	0.0	0.7		4	0.17	0.09	0.54	5.9	0.0	13.6	34.5
E2	3	0.27	0.26	0.97	11.4	0.0	36.2		4	0.23	0.14	0.61	10.0	0.0	24.4	42.8
E4	NA	NA	NA	NA	NA	NA	NA		3	0.02	0.02	1.00	0.5	0.0	1.7	28.0
Core total	NA	NA	NA	NA	NA	NA	NA		55	0.12	0.02	0.16	59.9	34.9	96.7	491.8
Background strata	NA	NA	NA	NA	NA	NA	NA		5	0.10	0.09	0.85	59.6	0.0	173.2	578.4
Survey total	46	0.09	0.02	0.27	*56.9	24.4	102.2	628.6	60	0.11	0.05	0.43	119.4	19.4	247.3	1070.2

*** The 2012 stock assessment survey did not sample for bonamia infection from all strata or the whole survey area, strata sampled for bonamia accounted for 628.6 km^2 (58.7%) of the survey area. Only stratum C6a sampled in 2012 and not in 2014.**